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Abstract

The objective of this study is to evaluate the antioxidant and antidiabetic activity of *Olea europea*, *Trigonella foenum-graecum* and *Eucalyptus globulus* extracts, which were popular through the survey, by Streptozotocin- induced diabetes in normal male adult rats via comparison of changes in body weight, biochemical and histopatholoangical parameter. Intravenous injection of 50 mg/kg of Streptozotocin in rats, makes pancreas swell and causes degeneration of beta cells in Langerhans islet and induces experimental diabetes in 2 days. In this study, the results showed that methanolic extract (ME) and chloroformic extract (ChE) of *Eucalyptus globulus* leaves contained high polyphenolics and flavonoids contents, respectively, whereas ethyl acetate extract (EAE) of *Olea europea* leaves and *Trigonelle foenum-graecum* seeds contain the most important quantity in polyphenols and flavonoids. The obtained results from HPLC technique allowed the detection of 36 phenolic compounds in MEO, 16 in MET and 40 in MEE. In addition, ME of *E.globulus* showed a strong ABTS radical scavenging activity, and inhibited the linoleic acid oxidation in ferric thiocyanate method and TBA. While, the EAE of *O.europea* and *T.foenum-graecum* exhibited a good activity in reducing power, ABTS and TBA assays. After Induction of diabetes, the volume of urine and glucose increased in the untreated animals in comparison with normal ones, but the body weight decreased in the untreated animals. The administration of MEO and MET at doses of 200 and 600 mg/kg and MEE at doses of 150 and 500mg/kg increased catalase activity, GSH level and decreased lipid peroxidation in the tissues of liver and kidneys, serum total cholesterol and triglycerides levels. Furthermore, histological damages in pancreas, kidney and liver tissues were reduced. These results indicated a good hypoglycemic and antioxidant activity of the three studied plants, could explain their use in folk medicine in the control of diabetes and preventing diabetic complications by scavenging free radicals.

Key words :

Olea europea L, *Trigonella foenum-graecum*, *Eucalyptus globulus*, antidiabetic, antioxidant, polyphenols

Résumé

L'objectif de cette étude est d'évaluer l'activité antioxydante et antidiabétique des extraits des *Olea europea*, *Trigonella foenum-graecum* et *Eucalyptus globulus*, ce qui étaient populaires par le questionnaire, en induissant expérimentalement le diabète par streptozotocine chez des rats adultes mâles normaux par l'intermédiaire de la comparaison des changements du poids corporel, les paramètres biochimiques et histopatholoangical. L'injection intraveineuse de 50 mg / kg de streptozotocine chez le rat, fait gonfler le pancréas et enfin, provoque une dégénérescence des cellules bêta des îlots de Langerhans et un diabète sucré en 2 jours. Dans cette étude, les résultats ont montré que l'extrait méthanolique (EM) et l'extrait chloroformique (ECh) des fleurs *Eucalyptus globulus* ont contenu des teneurs élevées en polyphénols et flavonoïdes, respectivement, tandis que l'extrait ethyl acetate (EEA) des fleurs *Olea europea* et les graines *Trigonelle foenum-graecum* étaient les meilleurs en polyphénols et flavonoïdes. Les resultats obtenus par la méthode HPLC permettent de détecter 36 des composés phénoliques dans EMO, 16 dans EMT et 40 dans EME. En outre, EM d'*E.globulus* a montré une forte activité de piégeage des radicaux ABTS, et a inhibé l'oxydation de l'acide linoléique par le test de thiocyanate ferrique et TBA. Alors que, EEA d'*O.europea* et *T.foenum-graecum* montraient une bonne activité dans les tests pouvoir réducteur, ABTS et TBA. Après l'induction du diabète, le volume d'urine et de glucose ont augmenté chez les animaux non traités en comparaison avec les animaux normaux, mais le poids corporel a diminué chez les animaux non traités. L'administration de EMO et EMT aux doses de 200 et 600 mg / kg et EME à des doses de 150 et 500mg / kg a entrainé d'augmentater l'activité de catalase, le niveau de GSH et une diminution de la peroxydation lipidique dans les tissus du foie et des reins, le taux de cholestérol total et triglycérides dans le serum. En outre, les dommages histologiques des tissus de pancréas, de rein et de foie ont été réduits. Ces résultats indiquent une bonne activité hypoglycémique et antioxydante des trois plantes étudiées, peuvent ainsi expliquer leurs utilisations dans la médecine traditionnelle dans la lutte contre le diabète et la prévention des complications du diabète en piégeant les radicaux libres.

Mots clés :

Olea europea L, *Trigonella foenum-graecum*, *Eucalyptus globulus*, antidiabétiques, antioxydant, les polyphénols

ملخص

الهدف من هذه الدراسة هو تقييم النشاط المضاد للأكسدة والمضادة لداء السكري لمستخلصات *Olea europea*، *Trigonella foenum-graecum* و*Eucalyptus globulus* ، والتي حظيت بشعبية كبيرة من خلال دراسة للاستبيان، عن طريق إحداثه تجريبيا بحقن جرذان بالغين بالإستربتوزوتوسين و ذلك بمقارنة التغيرات في وزن الجسم، والكيمياء الحيوية والمرضية. الحقن الوريدي لجرعة 50 ملغ / كغ من الإستربتوزوتوسين، يؤدي إلى انتفاخ البنكرياس ويسبب انحلالا في خلايا بيتا في جزر لانجرهانس و بالتالي إلى احداث الداء السكري التجريبي في يومين. في هذه الدراسة، أظهرت النتائج أن المستخلص الميثانولي (ME) و المستخلص الكلوروفورمي (ChE) لأوراق نبتة *E.globulus* يحتويان على نسبة عالية من عديدات الفينول و الفلافونويدات بالترتيب. في حين، مستخلص خلات الإثيل (EAE) لأوراق نبتة *O.europea* وبذور نبتة *T.foenum-graecum* يحتوي على كمية معتبرة من عديدات الفينول و الفلافونويدات. النتائج المتحصل عليها في HPLC أظهرت 34 مركب فينولي في MEO ،16 في MET و 40 مركب في MEE . كذلك، مستخلص ME لنبتة *E.globulus* أظهر قوة إزاحة لجذر ABTS، ويمنع أكسدة حمض اللينوليك وثيوسيانات الحديد وكذا TBA.في حين EAE لنبتتي *O.europea*،*T.foenum-graecum* أبدى قوة تثبيطية لتقنية القدرة الإرجاعية، ABTS وTBA. بعد تحريض السكري، نشهد زيادة في حجم البول وإرتفاع السكر في الدم في الحيوانات غير المعالجة مقارنة مع الحيوانات الطبيعية، ولكن نلحظ انخفاض في وزن الجسم للحيوانات غير المعالجة. أدت معالجة الجرذان بمستخلصي MEO وMET بجرعات 200 و600 ملغ / كغ ومستخلص MEE بجرعات 150و 500ملغ / كغ إلى زيادة في نشاط الكاتلا ز، مستوىGSH وانخفاض مستوى MDA في أنسجة الكبد والكلى، مستوى الكوليسترول والدهون الثلاثية المستويات في البلازما. وعلاوة على ذلك، تم تخفيض الأضرار النسيجية في البنكرياس والكبد والكلى. إذا فهذه النتائج أشارت إلى نشاط جيد لتخفيض نسبة السكرفي الدم ونشاط مضاد للأكسدة لمختلف النباتات الثلاثة المستعملة، مما قد يفسر استخدامها في الطب الشعبي في السيطرة على داء السكري ومنع مضاعفات هذا الداء من خلال تثبيط الجذور الحرة.

الكلمات المفاتيح :

Olea europea، *Trigonella foenum-graecum*، *Eucalyptus globulus*، مضاد السكري، مضادات الأكسدة، عديدات الفينول.

List of abbreviations

ABTS : 2,2'-azino-bis (3-ethylbenzenothiazoline acid)

ALcl3 : aluminium trichloride

ALT : alanine aminotransferase

AST : Aspartate aminotransferase

BHT : butylated hydroxytoluene

CAT : catalase

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl

DM : diabetes mellitus

EDTA : ethylenediamine tetraacetic acid

Fe2+ : ferrous iron

FTC : ferric thiocyanate

GDM : gestational diabetes

GPx : glutathione peroxidase

GSH : reduced glutathione

HDL : high density lipoprotein

HPLC: high performance liquid chromatography

H_2O_2 : hydrogen peroxide

HO_2^- : perhydroxyl radical

I%: Inhibition percentage

IC_{50}%: Inhibitory concentration for 50% of activity

LDL : Low density lipoprotein

MeOH: Methanol

MDA : malondialdehyde

MEO : methanolic extract of *Olea europea*

MET : methanolic extract of *Trigonella foenum-graecum*

MEE : methanolic extract of *Eucalyptus globulus*

Mrp2 and 3 : multidrug resistance protein 2 and 3

NIDDM : non-insulino dependent diabetes mellitus

NO^- : nitric oxide

O_2^- : superoxide radical

OH^- : hydroxil radical

$OONO^-$: peroxynitrite anion

ROS : reactive oxygen speices

SEM: Standard error of the mean

SOD : superoxide dismutase

STZ : streptozotocin

TBA : thiobarbituric acid

T1D : type 1 diabetes mellitus

T2D : type2 diabetes mellitus

VLDL : very low density lipoprotein

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Introduction

Diabetes is a common disease. World Health Organisation (WHO) estimates that 30 million people had diabetes in 1985, and this number increased to 171 million people in 2000. In that year, an estimated 2.9 million people died of diabeted, representing 5.2% of all deaths, probably the fifth largest cause of mortality in the word (Roglic *et al*., 2005). It is estimated that in 2030, people with diabetes will reach 366 million, most of them from developing countries, especially among people from45 to 64 years of age (Roglic, 2004).

Diabetes mellitus (DM) is a chronic endocrine disorder of glucose characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Wild *et al*., 2004).

The importance of oxidation in the body and in foodstuffs has been widely recognized. Oxidative metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes. There is increasing evidence for the involvement of such species in a variety of normal *in vivo* regulatory systems (Winrow *et al*., 1993). When an excess of free radicals is formed, they can overwhelm protective enzymes such as superoxide dismutase, catalase and peroxidase and cause destructive and lethal cellular effects (e.g., apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration (Bae *et al*., 1999 ; Bauer *et al*.,1999).

But, the chronic hyperglycemia was found to increase the production of free radicals that is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Baynes, 1991 ; Mohamed *et al*., 1999). Several hypotheses have been reported to explain the genesis of free radicals in diabetes. These include oxidation of glucose, the nonenzymatic and progressive glycation of proteins with consequently

increased formation of glucose-derived advanced glycation end products (AGEs) (Booth *et al*., 1997; Vlassara and Palace, 2001). Evidences indicate that free radicals, membrane lipid peroxidation and protein oxidation are significantly increased in diabetic patients and in experimental diabetic animals (Gallou *et al*., 1993; Telci *et al*., 2000).

The method for creating diabetes in animals is injecting drugs such as alloxan or Streptozotocin. These materials inflate and ultimately degenerate the Langerhans islets beta cells (Ikebukuro *et al*., 2002) where, Streptozotocin or Streptozocin or Izostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classifically an anti-tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy (Akbarzadeh *et al*., 2007).

Marles and Farnsworth (1995) listed 1200 species of plants that have been used to treat diabetes worldwide. They mostly belong to the families of *Fabaceae*, *Asteraceae*, and *Lamiaceae*. A wide array of plant have limitless ability to synthesize substances such as polyphenols, mainly flavonoids and phenolic acids which exhibit antioxidant properties due their hydrogen-donating and metal-chelating capacities (Weijl *et al*., 1997) and their possible use in the treatment of non-insulino dependent diabete mellitus (NIDDM) (Ivorra *et al*., 1988 ; Bailey and Day, 1989; Marles and Farnsworth, 1995).

In this work, a survey of hypoglycemic plants was conducted in Setif region, where *Olea europea*, *Trigonella foenum-graecum* and *Eucalyptus globulus* presented the higher proportion. The present study aimed to evaluate the antioxidant activity of the three plants studies, their phenolic contents and the relationship between the oxidative stress and diabete mellitus. It is also aimed to understand of the pathophysiology and natural history of diabetes.

The principal objectives are :

-Determination of polyphenols and flavonoids in plant extracts

- Phytochemical analysis of methanolic extracts of studied plants

- Evaluatation of the antioxidant activity of extracts by different methods *in vitro*

- Studying the antidiabetic activity of extracts using streptozotocin-induced diabetes in rats

- Determination of biochemical parameters and hematobiochemical in treated and untreated animals

- Evaluatation of plasma antioxidant activity using DPPH assay and reducing power

- Studying the antioxidant properties of plant extracts *in vivo* (MDA, GSH, catalase activity)

- Studying the possible alterations in tissues (pancrea, liver and kidney) by plant extracts treatement

- Evaluatation of the diuretic effect of extracts in streptozotocin-induced diabetes rats

I.1. The implication of the free radicals in the pathogenecity of diabetes

I.1.1. Oxidative stress

The term “stress” was first used in the biomedical literature as a description of hyperactivity in the hormone system, in particular concerning corticosteroids of the adrenal cortex (Seyle,1936). “Stress” is primarily as a factor causing disease, and even today, as exemplified by this thematic issue, modern stress research is still largely concerned with pathomechanisms of human disease. Today we know that in many of those stress sitations, in fact redox processes play a major role. The concept of physiological stress in general for a long time was ill-defined in physicochemical terms. It took decades before a clearer picture could be established by delineating the molecular mechanisms of stress generation, stress defense and stress signaling (Sies, 1985).

I.1.1.1. Definition

The term “oxidative stress” was coined only 30 years ago (Sies, 1985). The concept is inspired by early publications related to oxygen toxicity, often connected with the problem of aging (Gerschman *et al*.,1954; Orgel, 1963), the metabolism of oxygen (and other) radicals in biological systems (Harman, 1956), the gradual development of our understanding of mitochondrial physiology (Mitchell,1961; Racker,1977), “mitochondrial” aging research (Harman,1972 ; Chance and Sies,1979), and the study of redox imbalance in cells and organisms (Schafer and Buettner, 2001). Redox imbalance according to one definition is another name for oxidative stress which is based on the Nernst equation taking into account all the redox couples present in the cell or in the different cellular subcompartments (Aung-Htut *et al*., 2012). Another more practical and operational definition of oxidative stress is given by

Lushchak: “Oxidative stress is a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents” (Lushchak, 2014).When, more formation of free radicals or levels of antioxidants are diminished, the cell enters a state called “oxidative stress”(Lian *et al*., 2008).

I.1.2.Reactive oxygen speices (ROS)

I.1.2.1.Definition

Free radicals are atoms or groups of atoms containing at least one unpaired electron in their orbital and can be formed when oxygen interacts with certain molecules (Lien *et al*., 2008). free radical is any chemical species capable of independent existence and possessing one or more unpaired electron, an unpaired electron being one that is alone in an orbital. Radicals, often denoted by the insertion of the superscript dot (.), are generally less stable than non-radicals, although their reactivities vary (Hey and Waters, 1937 ; Moad and Solomon, 1995).

The most popular ROS are superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and (OH^-) originating from one, two or three electron transfers to dioxygen (O_2). Under physiological conditions O_2^- is not very reactive against the biomolecules of the cell and in aqueous solutions at neutral or slithly acidic pH disproportionates to H_2O_2 and O_2. H_2O_2 is relatively stable and not very reactive, electrically neutral ROS, but is very dangerous because it can pass through cellular membranes and reaches cell compartments far from the site of its formation (Wojktaszek, 1997).

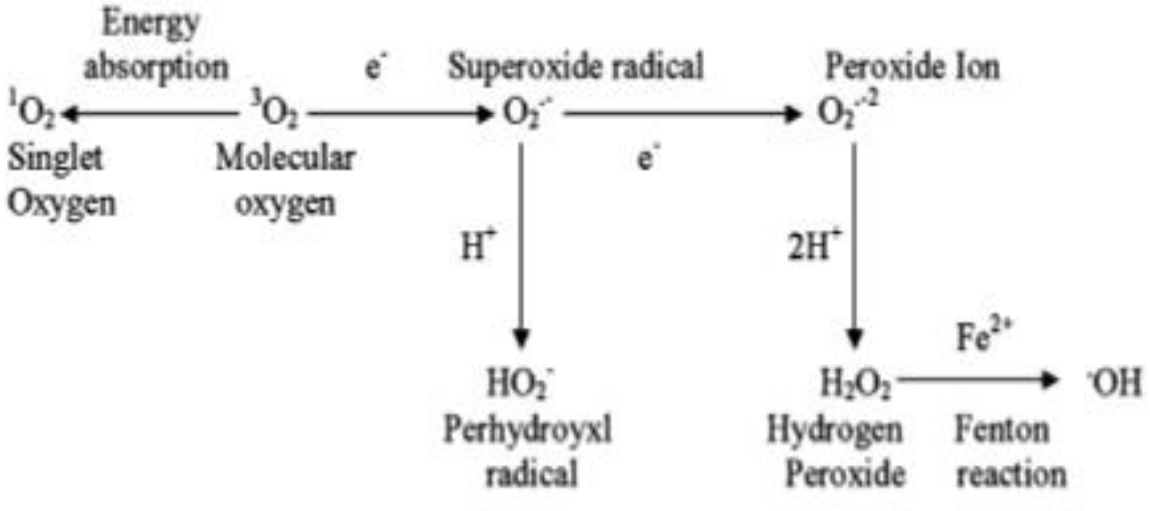


Fig.1 : Schematic representation of ROS generation. The single-electron reduction of $\mathbf{O_2}$ results in the generation of the $\mathbf{O_2^{\cdot-}}$. At low pH, dismutation of $\mathbf{O_2^{\cdot-}}$ is unavoidable, with one $\mathbf{O_2^{\cdot-}}$ giving up its added electron to another $\mathbf{O_2^{\cdot-}}$ and then with protonation resulting in the generation of $\mathbf{H_2O_2}$. Again, $\mathbf{O_2^{\cdot-}}$ can be protonated to form the $\mathbf{HO_2^-}$. Additionally, in the presence of transition metals such as copper and iron, further reactions take place, e.g., through the Haber–Weiss mechanism or the Fenton reaction to give up **• OH**. $\mathbf{O_2^-}$ can also react with another very influential signaling-free radical species, $\mathbf{NO^-}$, to give up peroxynitrite ($\mathbf{OONO^-}$). (Susinjan, 2015).

I.1.2.2. Sources of ROS

1.Endogenous sources

In chronic infections and inflammation, as well as in other disorders, release of leukocytes and other phagocytic cells readily defends the organism from further injury. The cells do this by releasing free oxidant radicals, and these by-products are generally reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxide, which result from cellular redox processes (Ames *et al*.,1993; Mongelli *et al*.,1997). At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function (Mongelli *et al*.,1997 ; Wang *et al*.,1999). At high levels, however, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy *et al*., 2008).

1.1.Mitochondria as a source of ROS

Mitochondrial energy metabolism is also recognized as the main source of cellular reactive oxygen species (ROS) in most eukaryotic cells (Boveris and Chance, 1973; Boveris *et al*., 1972 ; Turrens, 2003). However, mitochondria also have the highest antioxidant capacity, making them a player not only as a superoxide anion (O_2^-) source but also as a cellular redox sink (Kowaltowski *et al*., 2009; Peng and Jou, 2010 ; Dikalov, 2011). The initial concept that mitochondrial ROS were essentially an undesirable metabolic by product generated by the mitochondrial respiratory chain has changed. Based on alarge body of experimental evidence, it is now recognized that, under physiological conditions, mitochondrial ROS generation is a continuous and tightly adjusted process required for the regulation of many cellular processes (Hamanaka and Chandel, 2010; Dikalov, 2011; Toledo and Augusto, 2012). In addition to the physiological processes controlled by mitochondrial ROS, a large body of evidence indicates that mitochondrial oxidative imbalance is responsible for the development and progression of a series of abnormalities such as cancer, diabetes, inflammatory diseases, hypertension,neurodegenerative and ischemia-related diseases, as well as aging (Hamanaka and Chandel, 2010 ; Dikalov, 2011).

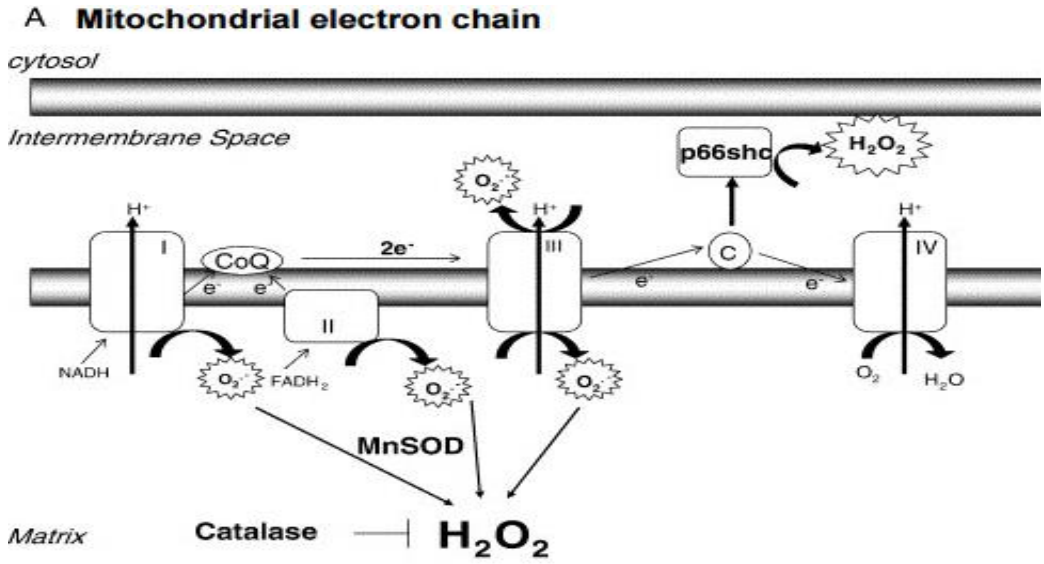


Fig. 2 : Mitochondria Produce ROS (Chandel and Budinger.2007).

1.2. Peroxisomes as a source of ROS and RNS

The term 'peroxisome' was introduced by Nobel Laureat Christian de Duve in 1965 to define a cell organelle which contains at least one H_2O_2-degrading enzyme (Duve, 1965). This implies that peroxisomes generate ROS as an integral feature of their normal metabolism. This is further exemplified by the fact that peroxisomes in rat liver may be responsible for as much as endogenous stress generators comes from the finding that a long-term administration of peroxisome proliferators to rodents induces oxidative stress in liver cells (Kasai *et al*., 1989). This is most likely due to the fact that these compounds, which activate the nuclear receptor PPARα (peroxisome proliferator-activated receptor-alpha), lead to many-fold induction of H_2O_2-producing enzymes without a concomitant increase in catalase activity (Reddy *et al*., 1986). Numerous observations indicate that peroxisomes can also protect cells from oxidative stress.For example, the absence of functional peroxisomes causes increased apoptosis in the developing mouse cerebellum (Krysko *et al.*, 2007) ; human patients suffering from an inherited deficiency of catalase, the most abundant peroxisomal antioxidant enzyme, face an

increase risk of developing age-related diseases including diabetes, arthrosclerosis, and cancer (Goth and Eaton, 2000).

1.3. NADPH oxidase as a source of ROS

NAD(P)H oxidase is a membrane-bound enzyme complex which represents a major source of $O_2^{\bullet-}$ in the body. It is present in various cells, e.g., the endothelial cells, smooth muscle cells, fibroblasts, monocytes, and macrophages. Although NAD(P) H oxidases were originally considered as enzymes expressed only in the phagocytic cells, the recent evidence indicates that there is an entire family of NAD(P) H oxidases (Miyano *et al*., 2005).

1.4. Prostaglandin H Synthase (PHS) as a source of ROS

Prostaglandin H synthase (PHS), also known as cyclooxygenase (COX), is a heme-containing enzyme that catalyzes the initial steps in the production of prostaglandins and thromboxanes. PHS is a bifunctional enzyme that has COX and peroxidase activities (Kaufmann *et al*., 1997). The COX component converts arachidonic acid (AA) to the endoperoxide-hydroperoxide prostaglandin G2 (PGG2). The peroxidase component reduces the hydroperoxide to prostaglandin H2, and in the process, a cosubstrate can be oxidized. It is during this step that endogenous compounds or xenobiotics can serve as cosubstrates, which form free radicals that can generate reactive oxygen species (ROS) which oxidatively damage macromolecules such as protein, lipids, and DNA (Marnett, 1990; Wells *et al*., 2009).

1.5. Cytoplasmic Cytoplasmicsources of ROS and RNS

Xanthine oxidoreductase (XOR) is another important enzymatic source of ROS which belongs to metallofl avoprotein family. XOR (EC 1.17.1.4) catalyzes the oxidation of hypoxanthine and xanthine to form uric acid. XOR is shown to exist in two forms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH). The enzyme catalyzes the reduction of O_2, leading to the formation of superoxide ($O_2^{\bullet-}$) and H_2O_2 ; it is proposed as a central mechanism of oxidative injury (Nishino *et al*., 2008).

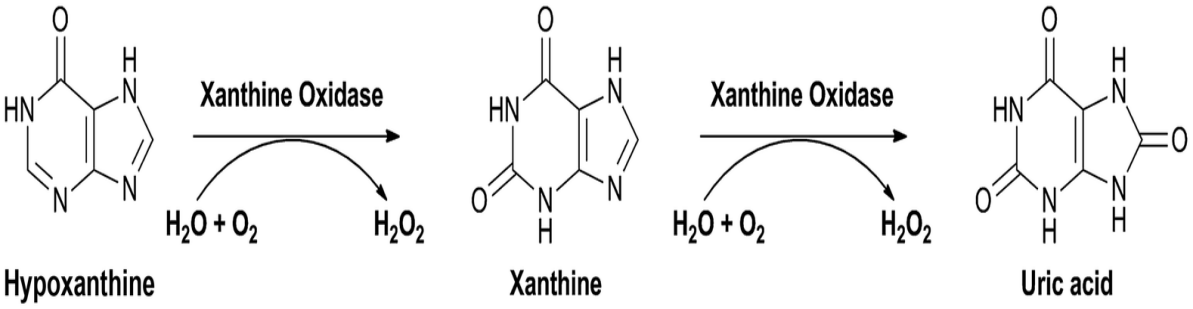


Fig. 3: Principle reaction catalyzed by xanthine oxidase (XO) (Rodrigues *et al*., 2015).

2.Exogenous sources

Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes. They can also be formed in response to environmental factors such as excess pollution, excessive UV rays, and exposure to cigarette smoke, automo- bile exhaust, and pesticides. Inadequate rest or sleep, inability to manage stress responses, and unhealthy eating habits can also cause free radical damage (Oboh$_b$, 2004; Oboh$_a$, 2005).

I.1.2.3. ROSs and cellular damage

ROSs are known to damage cellular membranes by inducing lipid peroxidation (Ramadevi and Prasad, 1998). They also can damage DNA, proteins, lipids and chlorophyll (Mittova *et al*., 2000). Free radical damage is one of the most prominent causes of devastating diseases that are responsible for killing many people in the world, such as cardiovascular disease, which can manifest as heart attacks, and cancer (Amic *et al*., 2003). The aging process has been linked by some researchers to free radical damage in the body (Oboh$_a$, 2005). Also, oxidative stress plays a major role in the development of chronic and degenerative ailments such as cancer, auto-immune disorders, rheumatoid arthritis, cataracts, aging, cardiovascular, and neurodegenerative diseases (Willcox *et al*., 2004; Pham-Huy *et al*., 2008).

1. Oxidative damage to proteins

Oxidative damage to proteins results in modifications of site-specific amino acid, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis ((Farr and Kogama, 1991). Also, oxidative modification of enzymes can have either mild or severe effects on cellular or systemic metabolism, depending on the percentage of molecules that are modified and the chronicity of the modification. Several groups have demonstrated that certain enzymes become oxidatively modified during aging (Oliver *et al*., 1987). Modification of structural proteins can also lead to a loss of function. For example, when the plasma protein fibrinogen is oxidized either by treatment with an iron/ascorbate radical-generating system or with ionizing radiation, it loses its ability to form a solid clot (Shacter *et al*., 1995).

2. Oxidative damage to lipids

Formation of peroxides, especially lipid ones, is a consequence of the activation of O_2, the interconversion of reactive species and natural systems protection overcoming. In biological environments, the most favorable substrate for peroxidation is represented by polyunsaturated fatty acids (PUFA), components of cell and subcellular membranes. Peroxidation is a complex process that includes three phases: initiation, propagation, end-decomposition, which interpose, so that only end products can be determined chemically: aldehydes (malondialdehyde), polymerized carbonyl compounds (lipofuscin) (Holley and Cheesman, 1993).

3. Oxidative damage to DNA

Although DNA is a stable and well-protected molecule, ROS can interact with it and cause several types of damage: modification of DNA bases, single- and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system (Kohen and Nyska, 2002). Of the ROS, the highly reactive OH reacts with DNA by addition to double bonds of DNA bases and by abstraction of an H

atom from the methyl group of thymine and each of the CH bonds of 2'-deoxyribose. Addition of OH to the C5-C6 double bond of pyrimidines leads to C5-OH and C6-OH adduct radicals of cytosine and thymine and H atom abstraction from thymine results in the allyl radical. Adduct radicals differ in terms of their redox properties, C5-OH- and C6-OH-adduct radicals of pyrimidines possess reducing and oxidising properties, respectively (Sonntag, 1987, Cooke *et al*., 2003).

I.1.3. Antioxidant defense system

I.1.3.1. Introduction

Antioxidants are powerful free radical scavengers in the body, while free radicals are highly reactive chemical substances that travel around in the body and cause damage to body cells (Alia *et al*., 2003). So, antioxidants are the substances that may protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage that free radicals might otherwise cause. However, these antioxidants whenever are consumed in large doses can act as prooxidants (Seifried *et al*., 2007). The antioxidants depending on their source of availability can be endogenous or exogenous in nature. The endogenous antioxidants can either be enzymatic, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) (Mates, 2000), or nonenzymatic in nature. The nonenzymatic antioxidants can be further grouped to metabolic antioxidants, such as lipoic acid, glutathione, L-arginine, uric acid, bilirubin (Kohen and Nyska, 2002), and nutrient antioxidants. Some of the nutrient antioxidant can be exogenous in nature as they cannot be produced in the body and must be provided through foods such as vitamin E, vitamin C, carotenoids, and trace elements (Se, Cu, Zn, Mn) (Willett and Macmahon, 1984; Radimer *et al*., 2004).

I.1.3.2. Sources of antioxidants

I.1.2.3.1. Endogenous sources

1. Enzymatic antioxidant

The most important antioxidant enzymes are: superoxide dismutase (SOD Ec 1.15.1.1), catalase (CAT Ec 1.11.1.6), ascorbate peroxidase (apX Ec 1.11.1.11), monodehydroascorbate reductase (MDAR Ec 1.1.5.4), dehydroacscorbate reductase (DHAR EC 1.8.5.1) and glutathione reductase (GR EC 1.6.4.2). At least four of them participate in a highly developed detoxification system named the ascorbateglutathione cycle (halliwell-asada cycle) (knÖrzer *et al*., 1996 ; Mittova *et al*., 2000 ; Morabito and Guerrier, 2000).

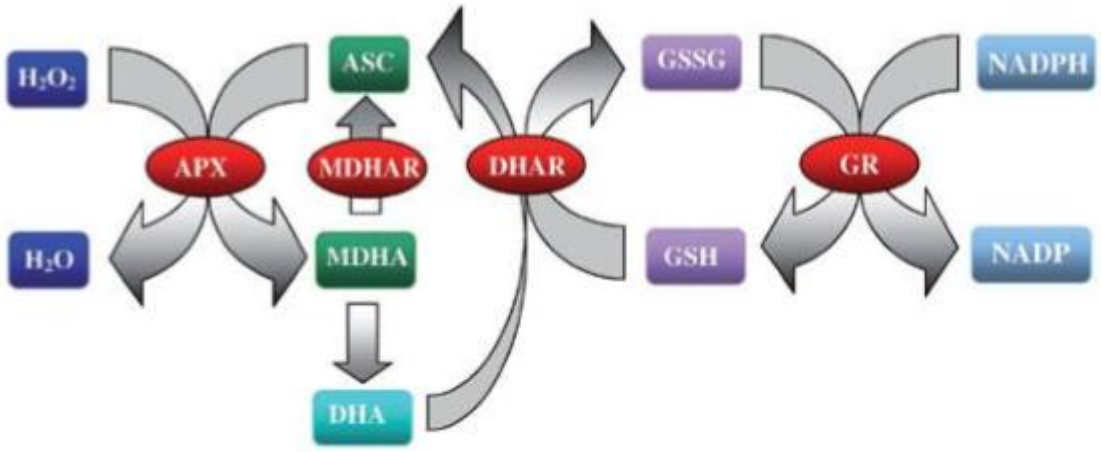


Fig.4 : Detoxification of ROS via glutathione-ascorbate cycle (Saruhan *et al*., 2009) ASC: Ascorbate; APX: Ascorbate peroxidase; GSH Reduced glutathione; GSSG: Oxidized glutathione; GR: Glutathione reductase; DHA Dehydroascorbate; DHAR: Dehydroascorbale reductase; MDHA: Monodehydroascorbale MDHAR: Monodehyroascorbale reductase.

- **Manganese superoxide dismutase (MnSOD)**

Manganese superoxide dismutase (MnSOD, also called SOD2) is a homotetrameric enzyme located in the mitochondrial matrix near the electron transport chain (Fridovich, 1998).Or Manganese superoxide dismutase is a ubiquitous metalloenzyme found in virtually all aerobic organisms from bacteria to humans, and even anaerobes (Ravindranath and Fridovich ., 1975).

Manganese superoxide dismutase is uniformly distributed throughout the cytoplasm in procaryotic cells (Steinman *et al*., 1994). So, Manganese superoxide dismutase (MnSOD) is a very important antioxidant enzyme that catalyzes the conversion of superoxide radicals ($O_2^{\bullet-}$) to hydrogen peroxide and molecular oxygen in the mitochondria (Weisiger and Fridovich, 1973). Under normal physiological conditions, mitochondria are the major source of $O_2^{\bullet-}$ production. Numerous studies have indicated that MnSOD plays an important role in preventing cells from oxidative stress and inhibiting tumorigenicity (Oberley and Buettner, 1979).

- **Catalase**

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*., 2004). It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004). Catalase can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction: $H_2O_2 + H_2R \rightarrow 2H_2O + R$

The exact mechanism of this reaction is not known. (Murthy *et al*., 1981).

2. Nonenzymatic antioxidant

- **Glutathione as an antioxidant**

The most important endogenous antioxidant defence systems are composed of the thiol-containing tripeptide glutathione and small thiol-containing proteins, such as thioredoxin, glutaredoxin, and peroxiredoxin. Of these, glutathione is found at millimolar concentrations in most cells and is the major contributor to the cell's redox state. Glutathione occurs in cells in

both reduced (GSH) and oxidized (GSSG) forms. It may also covalently bind to proteins through glutathionylation (Thomas *et al*., 1995 ; Huang and Huang, 2002). One important task of cellular glutathione is to scavenge free radicals and peroxides produced during normal cellular respiration, which would otherwise oxidize proteins, lipids and nucleic acids (Hayes and Pulford, 1995 ; Wild and Mulcahy, 2000). GSH reacts non-enzymatically with superoxide, nitric oxide, hydroxyl radical, and peroxynitrite as an antioxidant (Aoyama *et al*., 2008).

- **N-acetylcysteine**

N- acetylcysteine (NAC) is a metabolite of the sulphur-containing amino acid cysteine. It has the molecular formula $HSCH_2CH$ ($NHCOCH_3$) CO_2H and formula weight 163.19. In humans it can be administered orally or by intravenous infusion and can also be inhaled using a nebuliser. NAC exhibits direct and indirect antioxidant properties. Its free thiol group is capable of interacting with the electrophilic groups of ROS (Moldeus *et al*., 1986 ; Aruoma *et al*.,1989). This interaction with ROS leads to intermediate formation of NAC thiol, with NAC disulphide as a major end product (Cotgreave, 1997). In addition, NAC exerts an indirect antioxidant effect related to its role as a GSH precursor. GSH is a tripeptide made up of glutamic acid, cysteine and glycine. It serves as a central factor in protecting against internal toxic agents (such as cellular aerobic respiration and metabolism of phagocytes) and external agents (such as NO, sulphur oxide and other components of cigarette smoke, and pollution). The sulphydryl group of cysteine neutralises these agents (Moldeus *et al*., 1986) .

N-acetylcysteine (NAC) is a promising compound to increase GSH synthesis in the brain. NAC stimulates GSH synthesis not only by providing a source of cysteine, but also by activating GR (De Flora *et al*., 1984). N-acetyl-cysteine (NAC) is an acetylated cysteine residue. An optimal thiol redox state has been demonstrated to be of primary importance if attempting to optimize the protective ability of the cell to oxidative stress. Among the most widely used agents to

maintain the cysteine pool is NAC in addition to α-lipoic acid. While other agents have been used, NAC and α-lipoic acid are the most commonly utilized and discussed as a result of their proven safety and efficacy. In addition to the role glutathione and other thiols have on maintaining the cellular redox state, many studies have begun to explore if NAC supplementation can actually improve performance due to its ability to promote a more favorable cellular environment to achieve higher levels of performance (Sen, 2001).

- **Uric Acid**

Uric acid (UA) is an important antioxidant in blood. UA scavenges singlet oxygen, hydroxyl radicals, and peroxynitrite in blood at its physiological concentration (Ames *et al*., 1981). Uric acid is a final enzymatic product in the degradation of purine nucleosides and free bases in humans. The pathway of purine catabolism in humans is shortest among vertebrates because about 8–20 million years ago during primate evolution the activity of urate oxidase (uricase, an enzyme catalyzing conversion of uric acid to allantoin) was lost in a two-step mutation process (Wu *et al*., 1992; Oda *et al*., 2002). In other mammals, the last enzymatic product of purine degradation chain is allantoin, which is excreted in the urine. Lower vertebrates (e.g., fish) have enzymes that further degrade allantoin to allantoic acid and glyoxylic acid and finally to urea. As a consequence, humans have to cope with relatively higher levels of uric acid in the blood (200–400 μM) and are prone to hyperuricemia and gout (Johnson *et al*., 2005).

I.1.2.3.2. exegenous sources

- **Vitamins**

Ascorbate (Vitamin C) and α-tocopherol (Vitamin E) are also important antioxidants in the brain (Perry *et al*., 1985; Gilgun-Sherki *et al*., 2001). The concentration of ascorbate in the human brain ranges from 1 to 2.6 mM (Grunewald, 1993), similar to that the concentration of GSH in the brain. However, humans cannot produce ascorbate and the BBB almost completely

blocks ascorbate penetration into the brain (Agus, 1997). Alpha-tocopherol is the most potent antioxidant in the lipid part of the biological membrane (Gilgun-Sherki *et al*., 2001). However, the α-tocopherol level in the brain is relatively lower than those of ascorbate and GSH (Metcalfe *et al*., 1989; Grunewald, 1993; Cooper and Kristal, 1997; Gilgun-Sherki *et al*., 2001). In addition, the oral administration of α-tocopherol did not increase its concentration in the central nervous system (CNS) due to its limited penetration of the BBB (Pappert *et al*.,1996).

- **Plants phenolic compounds**

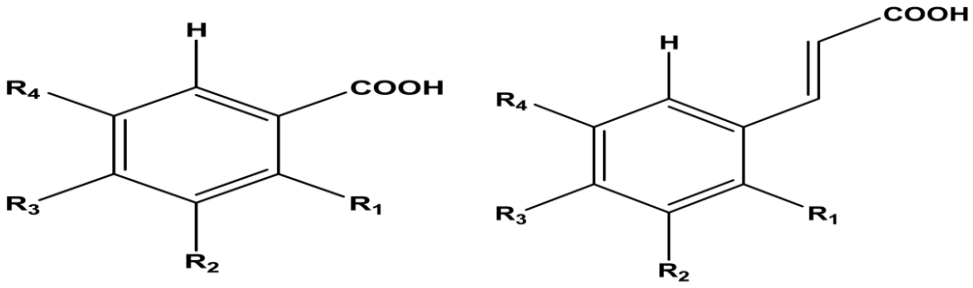
Plants produce a great variety of organic compounds as a response to environmental stresses like microbial attack, insect/animal predation and ultraviolet radiations. The role of these metabolites is to increase plants resistance to these stresses. They can be classified into three major groups according to their biosynthetic route and structural features: terpenoids, alkaloids, and phenolic compounds.

1. Phenolic acids

All plants produce an amazing diversity of secondary metabolites.One of the most important groups of these metabolites are phenolic acid. Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups. They are mainly synthetized from cinnamic acid, which is formed from phenylalanine by the action of l-phenyloalanine ammonia-lyase PAL (Ec 4.3.1.5), the branch point enzyme between primary (shikimate pathway) and secondary (phenylopropanoid) metabolism (Dixon and Paiva, 1995). The significance of this route can be supported by the fact that, in normal growth conditions, 20% of carbon fixed by plants flows through this pathway (Diàz *et al*., 2001).

Phenols are divided into several different groups, distinguished by the number of constitutive carbon atoms in conjunction with the structure of the basic phenolic skeleton (simple phenols,

benzoic acids, phenylopropanoids and flavonoids) (Harborne, 1964; Rice-Evans *et al*.,1997; Chaudiere and Ferrari-iliou, 1999 ;).



Hydroxybenzoic Acids Hydroxycinnamic Acids

Fig.5 : Structures of phenolic acids (Harborne, 1986).

1.1. Flavonoids

Flavonoids and ubiquinol are considered potential antioxidants, but their role is less certain (Halliwell, 1991). So, flavonoids are secondary plant metabolites, which together with other plant phenols share a common origin : the amino acid phenylalanine (Parr and Bolwell, 2000).

These phenols are derived from a common building block in their carbon skeleton : the phenylpropanoid unit, C6-C3. Biosynthesis according to this pathway produces the large variety of plant phenols: cinnamic acids (C6-C3), benzoic acids (C6-C3, or C6-C1), flavonoids(C6-C3-C6), proanthocyanidins (C6-C3-C6)n, stilbenes(C6-C2-C6), coumarins (C6-C3), lignans (C6-C3-C 3-C6), and lignins (C6-C3)n. Within each family of plant phenols many compounds may exist. Thousand different flavonoids have been described as occurring in plants (Harborne and Baxter, 1999).

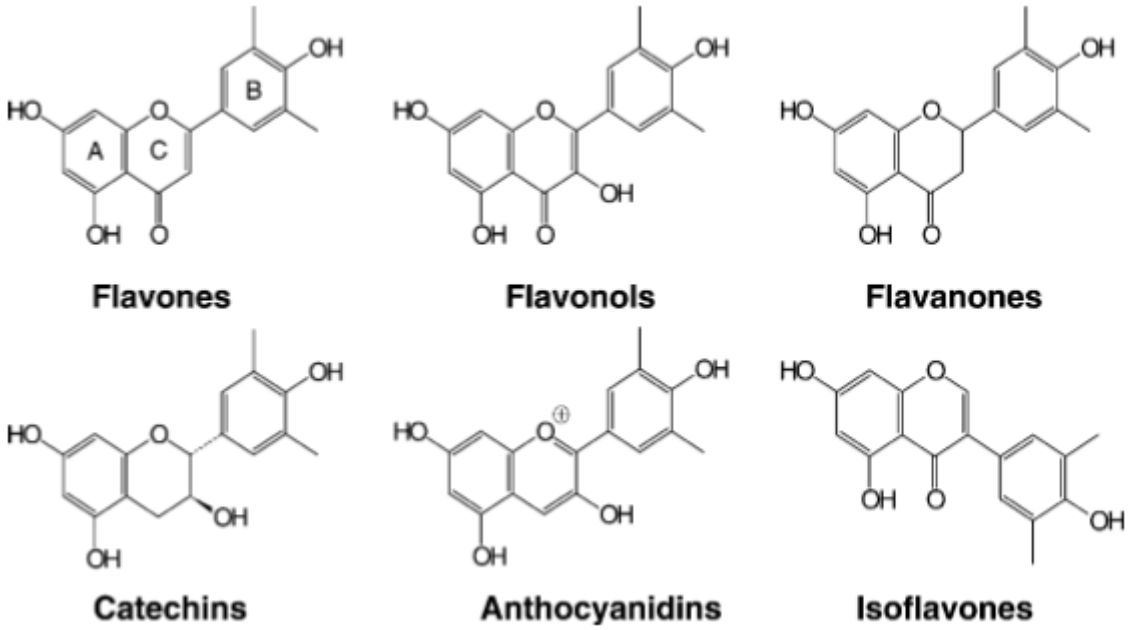


Fig.6 : Subclasses of flavonoids. Classification is based on variations in the heterocyclic C-ring (Harborne and Baxter, 1999).

1.2. Polyphenols

polyphenols are classified into :

1.2.1. Proanthocyanidin derivatives

These are oligomers containing two to six units of flavan-3-ol or high molecular weight polymer of flavan-3-ol (Haslam, 1998).

1.2.2. Galloyl and hexahydroxydiphenyl ester derivatives

In this class, different gallic and hexadiphenic acid derivatives are present as esters of a polyol (usually D-glucose) at the core of the polyphenolic ester (Haslam, 1998).

1.2.3. Hydroxy cinnamic acid derivatives

These are the condensation oligomers of mono lignols, namely, p-coumaryl alcoholconiferyl alcohol and sinapyl alcohol (Tuckmantel *et al*., 1999 ; Dean, 2001; Elias,1995). Lignin is one

such example formed from the oxidative polymerization of coniferyl alcohol. Lignin is a reticulated polyphenol, having three main functions in plants. They provide mechanical support, play role in conduction of water and also provide protection against biodegradation in plants (Haslam, 1998).

1.2.4. Phloroglucinol derivatives

These oligomers are derived from phloroglucinol subunits. They are formed by oxidative C–C and C–O coupling reactions of phloroglucinol (Haslam, 1998).

1.3. Tannins

Many tannins can be fractionated hydrolytically into their components, for example by treatment with hot water or with tannases, led to the classification of such tannins as 'hydrolysable tannins'. Non-hydrolysable oligomeric and polymeric proanthocyanidins were classified as condensed tannins (Würdig and Woller, 1989).

1.3.1. Hydrolysable tannins

the term 'hydrolysable tannins' includes both the gallotannins and the ellagitannins (Kashiwada *et al*., 1992; Weinges and Plieninger, 1999). It should also be mentioned here that there are ellagitannins that are not hydrolysable, because of a further C–C coupling of their polyphenolic residue with the polyol unit, but are nevertheless for historical reasons classified as hydrolysable tannins (Yoshida *et al*., 1999).

1.3.2. Complex tannins

In 1985 the first tannins were described that contained, in addition to the hexahydroxydiphenoyl (HHDP) units (the characteristic structural element of the monomeric ellagitannins), also C-glycosidic catechin units (Nonaka *et al*., 1985; Miyamoto *et al*., 1987) .These tannins were originally classified as 'non-classified tannins', because they are only partially hydrolysable due

to the C–C coupling of their catechin unit with the glycosidic part (Nishimura *et al*.,1986). To properly place these 'non-classified tannins' in some scheme, the terms 'complex tannins' (Haslam, 1989 ; Porter, 1989; Kashiwada *et al*., 1992). and flavanoellagitannins (Kashiwada *et al*., 1992; Ferreira and Bekker, 1996) were established over the following years. These examples clearly show that the division of the tannins into two groups, hydrolysable and non-hydrolysable or condensed tannins, (Würdig and Woller, 1989; Griffiths, 1991). cannot do justice to the structural diversity of the tannins.

1.3.3. Condensed tannins

The terms 'flavanotannins' or 'condensed flavanoid tanning substances' (Weinges and Plieninger, 1999). that are occasionally found in the literature denote tannins consisting of catechin units. The polymeric flavanotannins, constructed from coupled flavan-3-ol (catechin) units, belong to the condensed tannins (oligomeric and polymeric proanthocyanidins). On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins.

(1) Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.

(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin.

I.1.3.3. Antioxidant effects of phenolic compounds

Antioxidants may act as physical barriers to prevent ROS generation or ROS access to important biological sites (UV filters, cell membranes); chemical traps/sinks that "absorb" energy and electrons, quenching ROS (carotenoids, anthocyanidins); catalytic systems that neutralize or divert ROS [antioxidant enzymes SOD (superoxide dismutase), catalase, and glutathione peroxidase]; binding/inactivation of metal ions to prevent generation of ROS (ferritin, ceruloplasmin, catechins); and chain-breaking antioxidants which scavenge and destroy ROS (ascorbic acid, tocopherols, uric acid, glutathione, flavonoids) (Karadag *et al*., 2009).

I.1.3.3.1. Antioxidant activity of flavonoids

The most described property of flavonoids is their capacity to protect the organism against free radicals and oxygenated reactive species (ORS) produced during the metabolism of oxygen. The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation (Grace, 1994).

***Free radicals trapping**

The flavonoids can prevent the damage caused by the free radicals according to various ways; one of them is the direct trapping of the radicals. In this case, the flavonoids are oxidized by the radicals (R) leading to less reactive and more stable species according to the following mechanisms (Halliwell, 1995):

The formed flavonoxy radical (flavonoid (O•)) is stabilized by resonance. The non-paired electron can be delocalized on the whole of the aromatic cycle. But, it can continue to evolve

according to several processes (dimerisation, dismutation, recombination with other radicals, oxidation in quinon) either while reacting with radicals or other antioxidants, or with biomolecules. The flavonoxy (FL-O) radical can react with another radical to form stable quinine.

The flavonoxy radical can interact with oxygen to give a quinone and a superoxide anion. This reaction is responsible for an undesirable prooxidant effect of flavonoids. So the capacity of flavonoids to act as antioxidant depends not only on the redox potential of the couple Flavonoid (O)/ Flavonoid (OH), but also on the reactivity of generated flavonoxy radical (Van Acker *et al*., 1995).

*** Effect on the mediator of nitric oxide synthesis**

Several flavonoids reduce the cellular lesions related to ischaemia, by interfering with the activity of nitric oxide synthase. The nitric oxide is produced by various types of cells such as the endothelium cells and the macrophages. The nitric oxide is produced through the constitutive activity of nitric oxide synthase. It plays a role for the maintenance of the dilation of the blood-vessels, the relaxation of the smooth muscles, the signal of transduction and the inflammation (Valko *et al*., 2007; Parihar *et al*., 2008). However, at high concentrations it induces an irreversible oxidative damage on cellular walls; because the activated macrophages increase their simultaneous productions of nitric oxide and the superoxide anions. The nitric oxide reacts with the free radicals producing peroxynitrite anion ($ONOO^-$), a more reactive species:

$$H^+ + O^-\cdot_2 + NO\cdot \rightarrow ONOO^- + H^+ \leftrightarrow ONOOH$$

When the flavonoids are used as antioxidants, the free radicals are trapped thus reducing the conversion of nitric oxide into peroxynitrite (Shutenko *et al*., 1999). Flavonoids can also react with nitric oxide directly. Therefore, it was speculated that the trapping of nitric oxide by the

flavonoids is in the origin of their protective effect of the cardiovascular system (Van Acker *et al*., 1995).

*** Inhibition of the enzymes activities**

It is well known that flavonoids are able to inhibit the activities of several enzymes implicated in radical's generation. Among these enzymes, the xanthine oxidase, lipoxygenase, cyclo-oxygenase, peroxidase and tyrosin kinase are the most studied.

The xanthine dehydrogenase and the xanthine oxydase are implied in the metabolism of the xanthine into uric acid. The xanthine deshydrogenase is the configuration available under the normal physiological conditions, but it changes into xanthine oxydase during cells reperfusion (re-oxygenation) and reacts with molecular oxygen to release the superoxyde radical (O_2^-). The flavonoids act as a strong inhibitor of the xanthine oxydase and as a trapper of the superoxide radical (Sanhueza *et al*., 1992).

The flavonoids have also the capacity in one hand, to inhibit the metabolism of the acid arachidonic (Ferrandiz and Alcaraz, 1991) by inhibiting the lipooxygenase and thus preventing the production of the chimiotactic compounds from this acid. This characteristic gives to the flavonoids the anti-inflammatory and anti-thrombogenic properties. In the other hand, flavonoids have also the capacity to reduce the release of peroxidases and proteolytic enzymes and thus the production of the ROS (Middleton and Kandaswami, 1992).

The activity of the tyrosin kinase is affected by the presence of the flavonoids (Nijveldt *et al*., 2001). This enzyme is implied in several cellular functions such as the enzymatic catalysis, the transport through the membrane, the transduction of the signals for hormones or growth factors and the transfer of energy in the synthesis of ATP. So, the inhibition of this enzyme by the flavonoids interferes with the way of transduction of the signals controlling the cellular proliferation.

*** Chelation of the metal ions**

The ferrous ions (Fe^{2+}) and copper (Cu^{+}), are essential for certain physiological functions of living cells (Van Acker *et al*., 1995). They can be, either as components of hemoproteins, or of cofactors of various enzymes implicated in antioxidant defense system of cells. Besides their beneficial role, they are also responsible for the production of the hydroxyl radical by the reduction of hydrogen peroxide (·OH) according to the following reaction :

$$H_2O_2 + Fe^{2+} (Cu^{+}) \rightarrow \cdot OH + \cdot OH + Fe^{3+} (Cu^{+})$$

I.1.3.4. Oxidant effect of phenolic compounds

Flavonoids and phenolic acids are receiving increased attention as potential antioxidants, primarily due to their wide presence in a large number of widely consumed foods. Different experimental methods developed for this purpose represent a major critical factor when it comes to strategies for developing fortified foods, or the characterization of a functional food and/or the formulation of an antioxidant supplement (Antolovich *et al*., 2002).

Currently, the exogenous antioxidants, including polyphenols were considered "doubleedged swords" in the cellular redox state. Several studies of exogenous antioxidants showed controversial results, especially when administered at high doses. The type, dosage and matrix of exogenous antioxidants may be determining factors impacting the balance between beneficial or deleterious effects of these natural compounds (Bouayed and Bohn, 2010). From epidemiological and dietary intervention studies, it appears, however, that exogenous antioxidants at physiologic (nutritional) doses play an important role in the maintenance or re-establishment of redox homeostasis, an essential state in maintaining healthy biological systems (Bouayed and Bohn, 2010; Valko *et al*., 2007).

I.2. Diabetes

The word ‘diabetes’ is derived from the Greek word “Diab” (meaning to pass through, referring to the cycle of heavy thirst and frequent urination); ‘mellitus’ is the Latin word for “sweetened with honey” (refers to the presence of sugar in the urine). Greeks had knowledge of a disease accompanied by polyurea and wasting of body, whereas Aretaeus of Cappadocia mentioned a disease characterized by thirst and polyurea. Subsequently, the knowledge spreaded to Chinese, Iranians and Arabians. From the Middle East, the knowledge of diabetes mellitus had spread to Spain as a disease characterized by polyurea, polydipsia with sugary flavoured urine. With the discovery of sugar in urine and its detection by laboratory test, the knowledge permeated into the 18^{th} century. The estimated burden of diabetes in India was 22 millions in 1990, 28 million in 1995 and 33 millions in 2000. It is the most common metabolic associated disease in the world. Non insulino-dependent diabete mellitus (NIDDM) is the most common form of diabetes constituting nearly 90% of the diabetic population in any country with varying numbers in different geographical regions (Warjeet Singh, 2011).

Diabetes mellitus is a chronic metabolic disorder of carbohydrates, proteins and fat metabolism which can be due to absolute or relative deficiency of insulin secretion or insulin resistance. It is characterised by high blood glucose level, which can cause various type of secondary complication associated with morbidity and mortality. The number of people suffering with diabetes worldwide is increasing at an alarming rate. It is predicated that, the number of diabetes person could reach upto 366 million by the year 2030 (Oyedemi *et al*., 2011).

I.2.1. Structure of the pancrea

The pancreas (meaning all flesh) lies in the upper abdomen behind the stomach. The pancreas is part of the gastrointestinal system that makes and secretes digestive enzymes into the intestine, and also an endocrine organ that makes and secretes hormones into the blood to control energy metabolism and storage throughout the body (Daniel Longnecker, 2014).

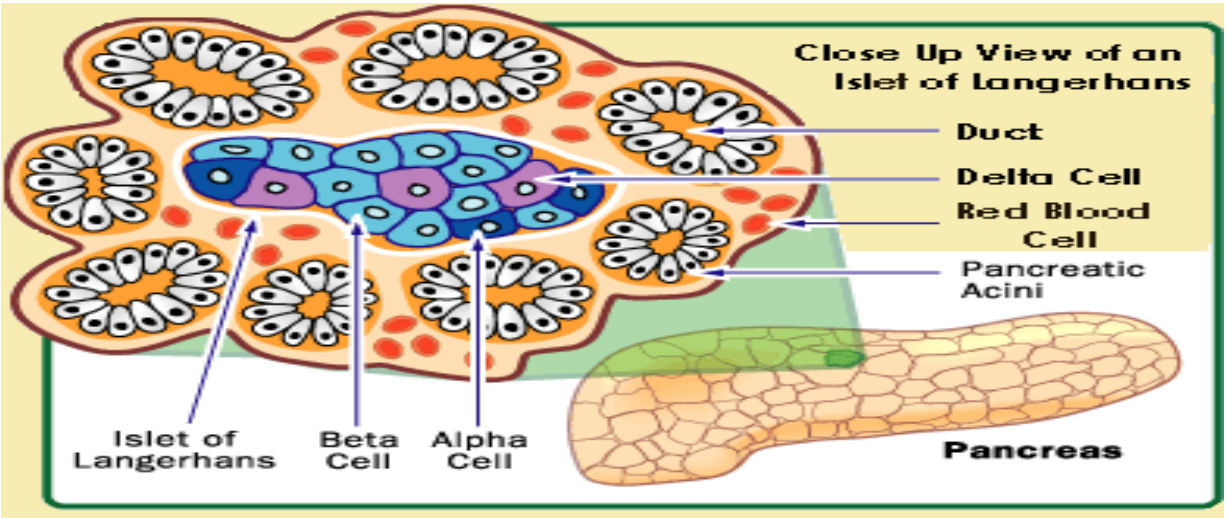


Fig.7 : Structure of pancrea **(http://health.howstuffworks.com/diabetes1.htm).**

It is worthwhile to mention a few definitions for key terms as used in the context of the pancreas :

I.2.1.1. Exocrine pancreas

The portion of the pancreas that makes and secretes digestive enzymes into the duodenum. This includes acinar and duct cells with associated connective tissue, vessels, and nerves. The exocrine components comprise more than 95% of the pancreatic mass (Daniel Longnecker, 2014).

I.2.1.2. Endocrine pancreas

The portions of the pancreas (the islets) that make and secrete insulin, glucagon, somatostatin and pancreatic polypeptide into the blood (Daniel Longnecker, 2014). Islets (numbering approximately one million in an adult human pancreas) comprise 1-2% of pancreatic mass and are present throughout the organ. Each islet is composed of about 2000 cells, of which insulin secreting β-cells comprise about 60% of the total. Glucagon secreting α- cells, somatostatin-releasing δ-cells and polypeptide P- (PP) cells make up the majority of the rest of the islet (Bergman, 1989).

I.2.1.2.1. Pancreatic β-cell

The role of the β-cell is to sense an increase in the concentration of nutrients (notably glucose) in the blood and to deliver an appropriate quantity of insulin into the systemic circulation. This ensures that the sugar is efficiently taken up and stored (as glycogen or triglyceride) by peripheral tissues (liver, muscle, and adipose). Combined with insulin resistance (Bergman, 1989), the loss of responsiveness to glucose of β-cells (Polonsky *et al*., 1988), with little or no change in the total β-cell mass (Guiot *et al*., 2001), leads to type II diabetes mellitus (DeFronzo *et al*., 1992).

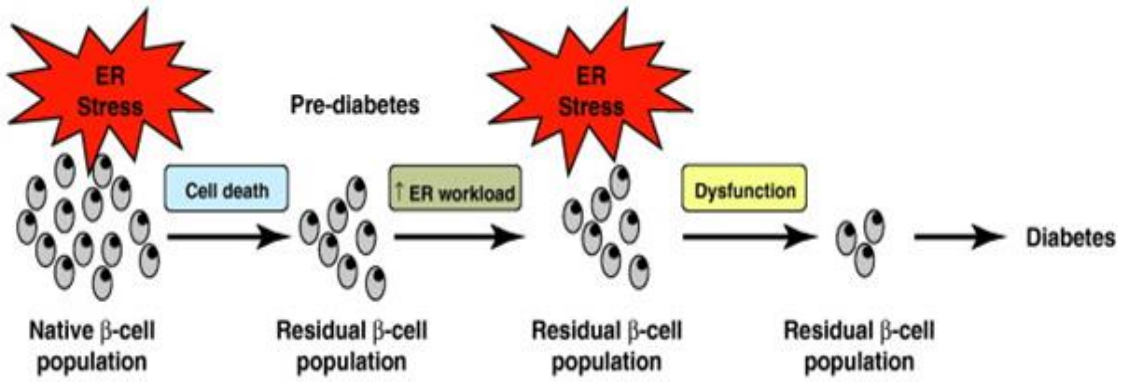


Fig.8 : Effect of stress on diabetes (Sonya *et al*., 2011).

I.2.1.2.2. Insulin

Insulin is synthesised and stored in a specialised micro-organ, the pancreatic islet of Langerhans (Ashcroft and Ashcroft, 1992).

Insulin biosynthesis

The islets of langerhans are the clusters of the endocrine tissue that scatter among the exocrine cells in the pancreas. The islets occupy approximately 1-2% of the total pancreatic tissue. Approximately, 1 million islets are scattered in the 25 cm long human pancreas. The insulin-producing cells make up 80% of each islet, while the remaining includes glucagon-producing α-cells, somatostatin-producing δ-cells and the pancreatic polypeptide (PP) F-cells (Unger *et al*., 1978). Insulin is first synthesized as a 110-amino acid polypeptide chain known as pre-proinsulin. This precursor form contains a hydrophobic 24-amino acid at its N-terminus known as the signal peptide. This signal peptide is removed during translocation from the cytoplasm to the endoplasmic reticulum, producing the proinsulin which comprises of chains A, B and C with three disulfide bonds. Further proteolysis of proinsulin in the secretory vesicles by the prohormone convertases (PC1 and PC2) and the carboxypeptidase E, removes the C peptide from the rest of the molecule while still retains three disulfide bonds. This remaining part or mature insulin contains 21 amino acids on chain A and 30 amino acids on chain B (Steiner, 1969).

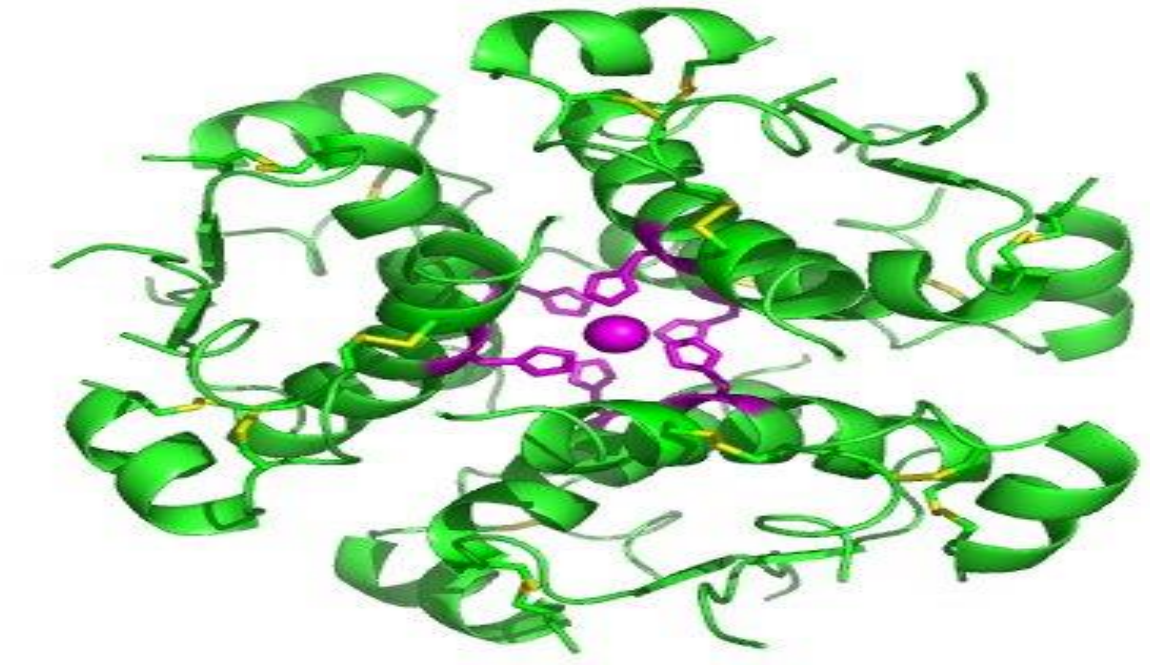


Fig. 9 : High-resolution model of six insulin molecules assembled in a hexamer, highlighting the threefold symmetry, the zinc ion holding it together (pink sphere), and the histidine residues (pink sticks) involved in zinc binding. Inactive insulin is stored in the body as a hexamer, while the active form is the monomer (Chang *et al*., 1997).

Insulin secretion

The mature insulin is stored in the secretory granules which can be divided into two distinct pools, i.e. the reserve pool (RP) and the readily releasable pool (RRP) (Barg *et al*., 2002; Bratanova-Tochkova *et al*., 2002). The RRP is located close to the plasma membrane and is a rather small pool of insulin, comprising only 1-10% of total insulin in the cell. In contrast, the RP is located intracellularly and is a largest insulin pool. Once insulin granules in the RRP are released, the RP moves close to the plasma membrane to replenish the RRP (Barg *et al*., 2002; Bratanova-Tochkova *et al*., 2002). Unlike other endocrine cells in the pancreas, β-cells secrete insulin. This occurs not only under low glucose conditions (non-stimulatory conditions) (3-5 mM glucose) but also when the glucose concentration in plasma is high during the postprandial period (10-25 mM glucose) when β-cells secrete much larger amounts of insulin into the

circulation. Although several nutrients including glucose, some amino acids and non-esterified fatty acids can stimulate insulin secretion, glucose appears to be the most potent insulin secretagogue. The mechanism of glucose-induced insulin secretion (GSIS) is the most extensively studied. Secretion of insulin in response to the elevated levels of glucose in plasma is rapid and occurs in a two-step process known as biphasic insulin secretion (Straub and Sharp, 2002; Straub and Sharp, 2004). The first phase occurs very rapidly within a first few minutes upon stimulation. At this stage, the insulin granules in the RRP are fused very rapidly with the plasma membrane, resulting in a sharp release of insulin in the blood circulation. The first phase lasts only for a few minutes before the second phase begins and is sustained to the peak at 30-40 min or longer, depending on whether the concentration of plasma glucose is still high. The amount of insulin released during the second phase is much higher than the first phase (Barg *et al*., 2002). It is estimated that 99% of total insulin is secreted in this second phase, with an approximate release rate of 5-40 granules/cell/minute (Barg *et al*., 2002; Straub and Sharp, 2004). Therefore the second phase of insulin secretion is more physiologically important. Not unexpectedly, this biphasic insulin secretion appears to be impaired in the patients with type 2 diabetes. The translocation of the insulin granules in the RP to become the RRP, as well as the docking of secretory vesicles to the plasma membrane are dynamic processes, requiring the rearrangement of cytoskeleton proteins inside the β-cell (Wang and Thurmond, 2009).

I.2.2.Classification of diabetes

Diabetes mellitus is mainly classified into : type 1 (T1D), type 2 (T2D) Diabetes and Gestational Diabetes.

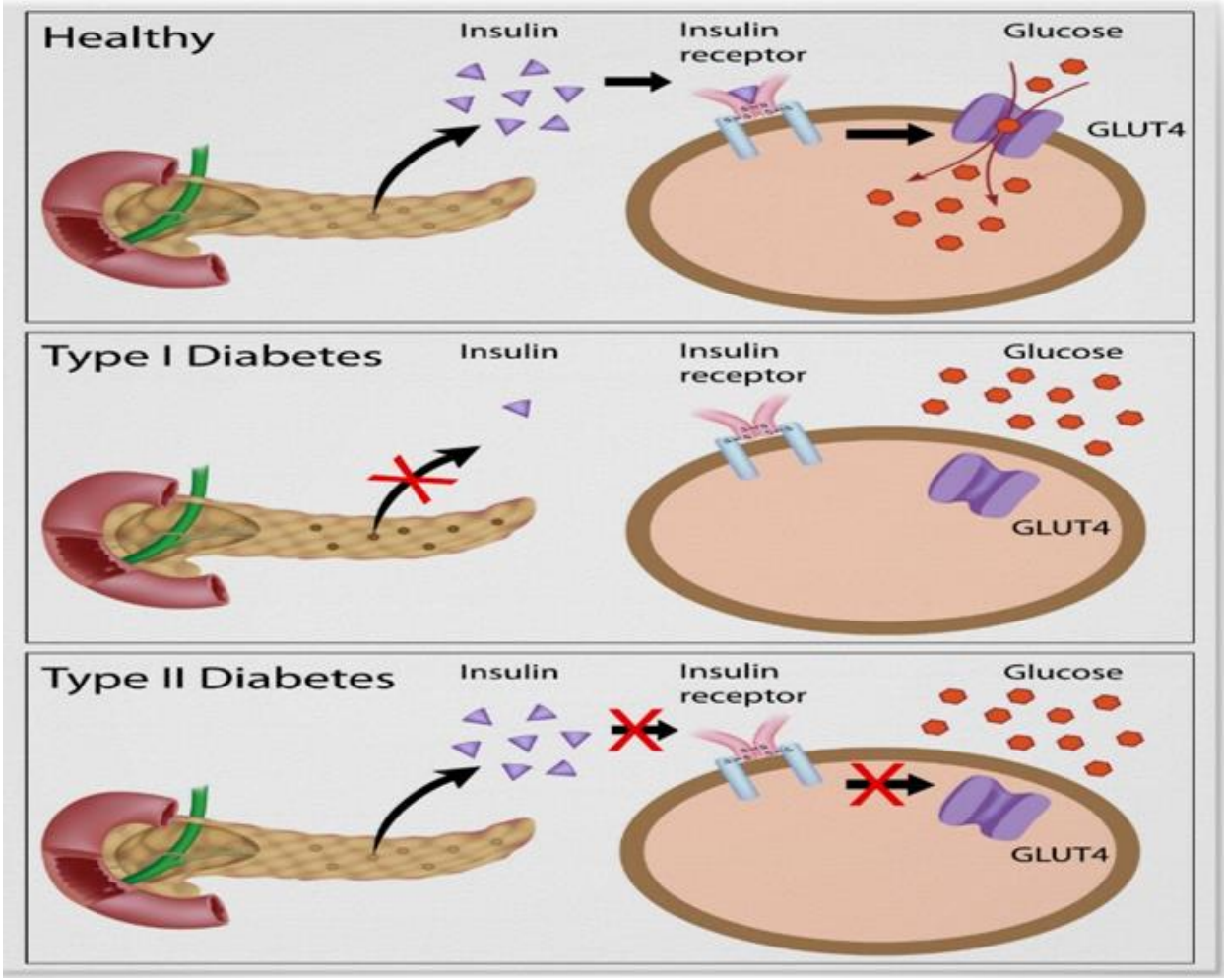


Fig. 10 : Insulin action and diabetes type1 and 2 **(http://science-tuition.co.uk/diabetes/).**

I.2.2.1. Type1 diabetes (T1D)

T1D involves the autoimmune destruction of insulin-producing pancreatic beta-cells via auto-aggressive T-cells and pancreatic macrophage infiltration (Achenbach *et al*., 2005 ; Reimann *et al*., 2009). Genetic and environmental factors, which both remain undefined, are thought to act together to trigger the autoimmune disease (Baisch *et al*., 1990). Variations in the human leukocyte antigen (HLA) region located on chromosome 6 may account for more than 50% of familial aggregation in T1D (Todd, 1995; Reimann *et al*., 2009). When the two haplotypes DR4-DQ8 and DR3-DQ2, are present in the same individual it increases the risk for T1D and

the gene encoding insulin, located on chromosome 11, is another susceptibility factor, which contributes to 10% of genetic susceptibility to T1D (Reimann *et al*., 2009). There are several short versions of tandem repeats in the insulin promoter that are associated with a higher risk for T1D, while the long versions seem to provide protection (Bennett *et al*., 1995). Whole genome scans have led to the identification of two important negative regulators of T-cell activation, cytotoxic T lymphocyte antigen4 (CTLA4) and the PTPN22 variant of the lymphoid tyrosine phosphatase encoding gene (Reimann *et al*., 2009).

I.2.2.2. Type2 diabetes (T2D)

Type 2 diabetes (T2D) is the most common endocrine disorder worldwide, covering 90-95% of all diabetes cases.The classification and pathogenesis of T2D involves abnormalities in glucose and lipid metabolism, inadequate insulin secretion from pancreatic beta-cells and resistance to insulin activity (Goldstein, 2007). Insulin resistance and impaired glucose tolerance (IGT), both conditions preceding the development of T2D are closely related to obesity (Sharma, 2007). The contribution of excess visceral fat to the development of insulin resistance, due to pronounced lipolysis and the subsequent release of free fatty acids that can directly block insulin signaling pathways, is well established (Mlinar *et al*., 2007; Reimann *et al*., 2009). Increasing environmental pressure may widen the susceptibility profile of T2D (Reimann *et al*., 2009).

I.2.2.2.1. Type 2 DM and lipid

Lipid abnormalities occur most commonly in type 2 diabetic subjects, even in those who have reasonable glycaemic control (Bopanna *et al*., 1997). The characteristic pattern of blood lipids in type 2 diabetes is called 'diabetic dyslipidaemia' and consists of elevated serum total VLDL (very low-density lipoprotein), triglyceride, low HDL (high-density lipoprotein), and essentially normal LDL (lowdensity lipoprtein) and cholesterol concentrations. The distribution of LDL subfractions, however, is altered, with a predomination of small dense LDL particles

which are strongly related to vascular disease in the general population. Dysipidaemia is also present in patients with impaired glucose tolerance. Diabetic dyslipidaemia is a component of the insulin resistance syndrome (syndrome X), i.e. central or truncal obesity, hypertension, glucose intolerance, accelerated atherosclerosis, dyslipidaemia and insulin resistance (Reaven, 1998).

I.2.2.2.2. Role of Insulin in lipids metabolism

The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma (Briones *et al*., 1984, Nikkila, 1984). In addition, it leads to a variety of derangements in lipid metabolism, which in turn leads to accumulation of lipids such as total cholesterol and triglycerides in diabetic patients (Goldberg, 1981; Shukla *et al*., 1995). However, Bopanna *et al* (1997) reported that abnormal high concentration of serum lipids in the diabetic subject is due mainly to increase in the mobilization of free fatty acids from the peripheral fat depots.

I.2.2.3. Gestational diabetes (GDM)

GDM defined as carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy (Metzger, 1991), becomes an important public health issue. The etiopathogenesis of glucose intolerance that develops in women with GDM could be the result of their inability to increase insulin secretion enough to overcome insulin resistance that occurs even in non diabetic pregnancy (Kuhl *et al*., 1985).

The establishment of pregnancy requires a receptive uterus able to respond to a variety of biochemical and molecular signals produced by the developing conceptus, as well as specific interactions between the uterine endometrium and the extra-embryonic membranes. Therefore, placental development and function are prerequisites for an adequate supply of nutrients and oxygen to the fetus and successful establishment of pregnancy (Behrman *et al*., 2001).

Oxidative stress has been proposed as the causative agent of several pathologies such as female sterility, recurrent pregnancy loss and several pregnancy-related disorders as preeclampsia, intra-uterine growth restriction (IUGR) and gestational diabetes. (Levente, 2012).

Pregnancy is a state of oxidative stress arrising from the increased metabolic activity in the placenta and reduced scavenging power of antioxidants (Riley *et al*., 1991). Normal pregnancy is associated with physiological hyperlipidemia (Paszkovski *et al*., 1995). Physiological alterations are manifested by increased levels of triglycerides and cholesterol in pregnancy, which decreases rapidly after delivery. Preeclampsia is characterized by further elevation of serum triglycerides and serum free fatty acids. Increased lipid peroxidation has been reported in preeclampsia, IUGR (Levente, 2012).

I.2.3. Free radicals and diabetes

Increased oxidative stress has been proposed to be one of the major causes of diabetes complications. Hyperglycemia in an organism stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose autooxidation, NAD(P) H oxidase, lipooxygenase, cytochromes P450 monooxygenases, and nitric oxide synthatase (NOS) (Valko *et al*., 2007). Under normal conditions, the key sites of superoxide formation in the mitochondrial membrane are complex I and the ubiquinone-complex III interface, where the presence of long lived intermediates allows reaction of electrons with molecular dioxygen (Kwong and Sohal, 1998 ;Valko *et al*., 2007). However, diabetes alters the primary sites of superoxide generation so that complex II becomes the primary source of electrons that contribute to superoxide formation under diabetic conditions (Nishikawa *et al*., 2000; Valko *et al*., 2007). Another source of ROS in diabetes is NAD(P)H. Several lines of evidence support that NAD(P)H oxidases are a major source of glucose induced ROS production in the vasculature and kidney cells, confirming thus NAD(P)H as a mediator of diabetic complications

(Li and Shah, 2003). Since hyperglycemia-induced oxidative stress occurs in non nucleated cells lacking mitochondria and the NAD(P)H oxidase (erythrocytes), another mechanism of ROS formation in such cells must exist. A possible explanation for such behaviour is glucose auto-oxidation, glucose itself, as well as its metabolites, is known to react with hydrogen peroxide in the presence of iron and copper ions to form hydroxyl radical (Valko *et al*., 2007). In addition to ROS, RNS have been implicated as one of the sources of nitrosative stress in diabetes. NO can react with superoxide forming peroxynitrite, a highly reactive oxidant linked with many disease states including diabetes (Zou and Shi, 2002). In addition to that, Xanthine oxidase (XO) has been proposed to be a major source of ROS in diabetes mellitus , treatment of non-insulin dependent diabetes patients with the XO inhibitor allopurinol reduces the level of oxidised lipids in plasma and improves blood flow. Lipooxygenases catalyse the conversion of arachidonic acid into a broad class of signalling molecules, such as leukotrienes, lipoxins, and hydroxyeicosatetraenoic acid. Thus diabetes is also associated with increased lipoxygenase expression, resulting in eicosanoid formation (Valko *et al*., 2007).

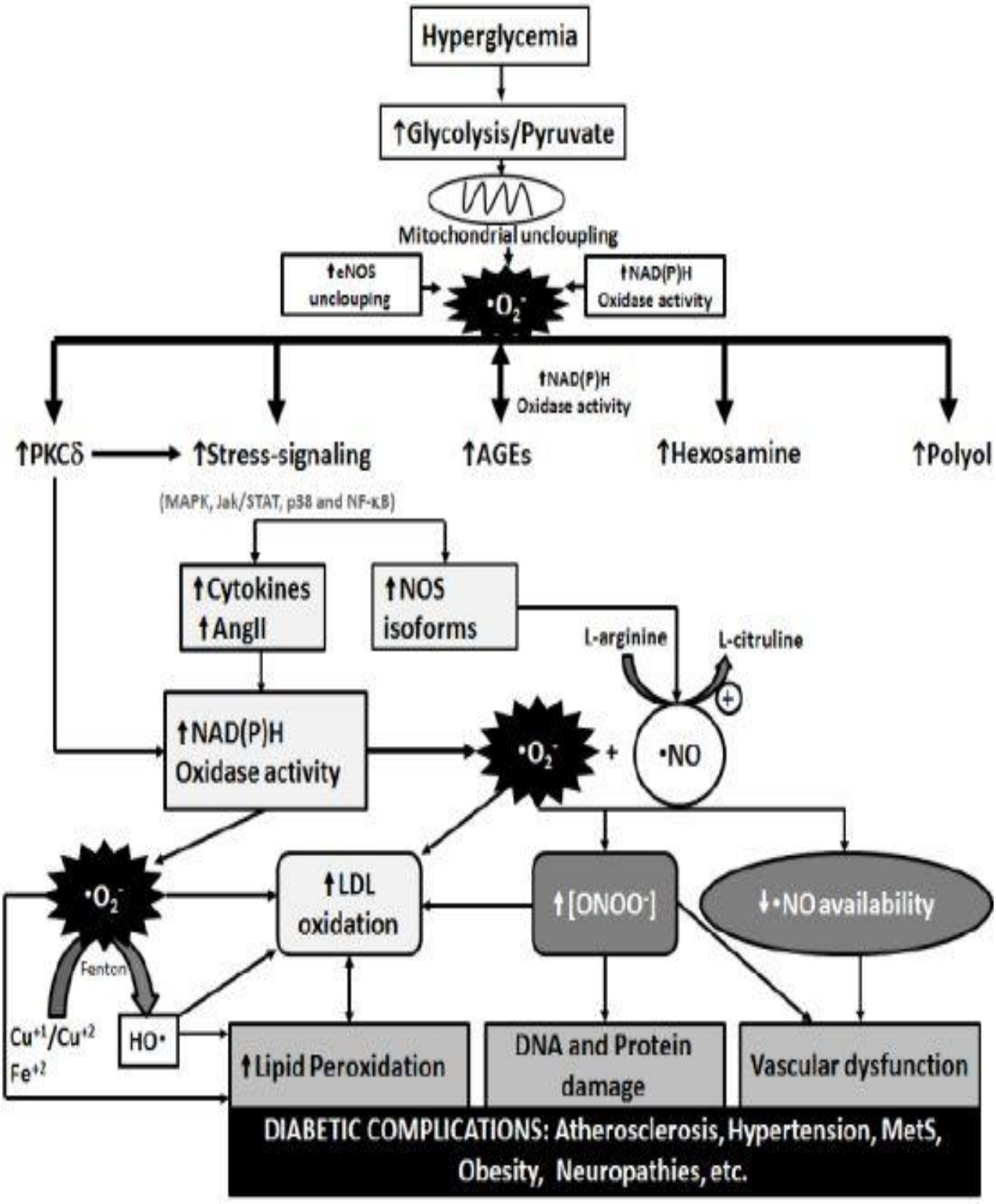


Fig.11 : Participation of hyperglycemia in triggering the multiple oxidative stress pathways in the course of diabetes. AngII: angiotensin II; eNOS: endothelial nitric oxide synthase; Jac/STAT: janus kinase (Jac)-signal transducer and activator of transcription (STAT); LDL: low density lipoprotein cholesterol; MAPK: mitogen-activated protein kinase; MetS: metabolic syndrome; NF-κb: nuclear transcription factor κb; •NO: nitric oxide; NOS: nitric oxide synthase; •O2−: superoxide anion radical; •OH: hydroxyl radical; ONOO−: peroxynitrite; PCKδ: protein kinase C δ. Adapted from Johansen *et al*., 2005 (Suziy *et al*., 2013).

I.2.4. Experimental diabetes induced by Streptozotocin

I.2.4.1. Definition of Sterptozotocin

Streptozotocin (STZ) is a glucose analogue that is selectively toxic to β cells in the pancreatic islets and specifically damages pancreatic βcells (Ohly and Gleichmann, 1995). This compound has been used extensively to produce an experimental model of diabetes mellitus in animals (Meyerovitch *et al*., 1987; Pederson *et al*., 1989; Poucheret *et al*., 1995; Dehghani *et al*., 1997; Cam *et al*., 1999).

Streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of $C_{14}H_{27}N_5O_{12}$ (Dorr and Fritz, 1980). It is widely used to induce insulin-dependent diabetes in experimental animals because of its toxic effects on islet beta cells (Fadillioglu *et al*., 2008; Punithavathi *et al*., 2008). The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Gu *et al*., 1997). The effects of STZ on different organs have been extensively studied. STZ has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis (Magee and Swann, 1969). STZ is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration (Piyachaturawat *et al*., 1988; Piyachaturawat *et al*., 1990).

It has been reported that the increased STZ toxicity towards beta cells depends on the expression of GLUT2 receptors which preferentially facilitate the uptake of STZ (Hosokawa *et al*., 2001).

I.2.4.2. Diabetes treatment

The present treatment of diabetes is focused on controlling and lowering blood glucose to a normal level. The mechanisms to lower blood glucose aimed to :

- stimulate β-cell of pancreatic islet to release insulin

- resist the hormones which rise blood glucose

- increase the number or rise the appetency and sensitivity of insulin receptor site to insulin

- decrease the leading-out of glycogen

- enhance the use of glucose in the tissue and organ

- clear away free radicals, resist lipid peroxidation and correct the metabolic disorder of lipid and protein

- improve microcirculation in the body.

Based on the above-mentioned mechanisms, the drugs clinically used to treat diabetes can be mainly divided into insulin, insulin-secretagogues, insulin sensitivity improvement factor, insulin-like growth factor, aldose reductase inhibitor, α-glucosidase inhibitors and protein glycation inhibitors (Liu and Wang, 1996; Zhao, 1999). The effect of these drugs is only aimed to lower the level of blood glucose. Moreover, in most cases, side-effect such as hypoglycemia, lactic acid intoxication and gastrointestinal upset appear after patients took these medicines. The drugs commonly used in clinic to treat or control diabetes are: (Li *et al*.,2004).

- **Insulin:** There are many kinds of preparations.

- **Sulfonylureas (SU): Insulin-secretagogues:** Tolbutamide (D860, Orinase), Glibenclamide (Glyburide, HB419, Micronase, Daonil), Gliclazide (Diamicron), Glibenese (Minidiab), Glurenorm (Gliquidone), Glutril (Glibornuride) and Glimepiride.

- **Biguanide (BG):** Phenformin (Phenethyldiguanidi Hydrochloridum, Diabenide, DBI), Dimethylbiguanide (FluamineMetformin, Diaformin, Diabex, Mellitin, Obin, Melbine, Metformin, Hydrochloride, Glucophage, DMBG).

- **α-Glucosidase inhibitors (α-GDI):** Glucobay (Acarbose), Voglibose, Miglitol, Emiglitate, Glyset, Precose.

- **Aldose reductase inhibitor (ARI):** Tolrestat, Alredase, Epslstat, Kinedak, Imirestat, Opolrestat.

- **Thiazolidinediones (TZD):** Troglitazone, Rosigitazone, Pioglitazone, Englitazone.

- **Carbamoylmethyl benzoic acid (CMBA):** Repaglinide.

- **Insulin-like growth factor (IGF):** IGF-1.

- **Others:** Dichloroacetic acid.

- **The metformin :** The metformine is a hypoglycemia drug used in the treatment of the diabetes type 2 with an aim of decreasing the rate of blood glucose. It is prescribed when the diet, the physical exercise and a loss of weight do not manage to lower the rate of blood glucose sufficiently (Shaw *et al*., 2005).

I.3. Herbal therapy of diabetes

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara *et al*., 1998). Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques (Saifi *et al*., 1971; Mukherjee *et al*., 1972; Coimbra *et al*., 1992; Ajit kar *et al*., 1999; Jafri *et al*., 2000). A wide array of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabete mellitus (Ivorra *et al*., 1988; Bailey and Day, 1989; Marles and Farnsworth, 1995). Among these are alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates,

glycopeptides, terpenoids, amino acids and inorganic ions and phenolic acid (Rang and Dale, 1991; Dixon and Paiva, 1995). Over 4000 different flavonoids have been described as occurring in plants where polyphenol play role in conduction of water and also provide protection against biodegradation in plants (Harborne and Baxter, 1999).

The family *Lamiaceae* occupies a significant place in many ethnobotanical surveys, because of its aromatic characteristics and high phytochemical diversity. Importantly, hypoglycemic activities attributed to flavonoids in leaves of *Origanum majorana L*. and phenolic compounds from the leaves of *Hyssopus officinalis L*. have been found (Jung *et al*., 2006).

Also, the species most frequently mentioned for use with diabetes were *Syzygium cumini* (*Myrstaceae*), *Bauhinia forficata* (*Fabaceae*), *Sphagneticola trilobata* (*Asteraceae*), *Baccharis trimera* (*Asteraceae*), *Cynara scolymus* (*Asteraceae*) and *Leandra australis* (*Melastomataceae*) (Trojan-Rodrigues *et al*., 2012).

I.3.2. Botanical identification and description of plant species chosen for this study

I.3.2.1. *Olive tree* (*Olea europaea L.*) :

Popular names: زيتون zitone, أزمور azemmour.

Kingdom : *Plantae*

Class : *Equisetopsida*

Subclass : *Magnoliidae*

Order : *Lamiales*

Family : *Oleaceae*

Genus : *Olea*

Species : *Olea europaea labill.*

I.3.2.1.1. Botanical description

The Olive tree (*Olea europaea L.* [Family : *Oleaceae*]) has been more than a thousand years. Not only the olive, but also the leaves have been used for medical purposes, and were introduced recentry (El and Karakaya, 2009).

Olive tree have white greyish, flexible, almost cylindrical ; leaves elongated from 4 to 7 cm, narrow sheets from 2 to 4 cm, greens then black (according to the variety). Mediterranean species cultivated in Algeria in the willayas of Blida, Tizi-Ouzou, Bejaia, Jijel, in Aures and with the West in particular in the area of Sig. The olive tree is of an exceptional longevity, it is one of the oldest cultivated plants (since more than 5000 years, in Crete) according to authors (Baba aïssa, 2011).

Fig.12 : Photograph of *Olea europaea L* (self photograph).

I.3.2.1.2. Phytochemical composition

the principal components of olive and oil are : unsaturated fatty acids (acid oleic…), proteins, carbohydrates, cellulose, glucosidic flavones (oleoside, oleuro-peine), carotene, enzymes, many mineral elements (calcium, suffers, phosphorus…), vitamins A, C… ; whereas the sheets

have tanins, triterpenes (mannitol…), glucosides, saponins, lactone, choline, ole-astrerol, oleoropine, leine… (Baba aïssa, 2011).

It is well known that oleuropein and its derivatives such as hydroxytyrosol and tyrosol are the main phenolic constituents of olive leaves, which is thought to be responsible for their pharmacological effects. Furthermore, olive leaves contain caffeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Bianco and Uccella, 2000 ; El and Karakaya, 2009). Olive leaf contains the active substances oleuropein (a polyphenolic iridoid glycoside) (Panizzi *et al*., 1960), oleacein (Somova *et al*.,2003) and oleanolic acid (Hansen *et al*., 1996).

I.3.2.1.3. Medicinal use of the plant

In many countries, it is known as a folk remedy for hypertension and diabetes (El and Karakaya, 2009). Like many natural herbs, olive leaves are also known to be an antioxidant and contain some of the most powerful known antioxidants (Benavente-Garcia *et al*., 2000; Lee and Lee, 2010). The results of previos studies have also demonstrated that application of olive leaves leads to alleviation of disease symptoms (Park *et al*., 2009 ; Pattern *et al*., 2009).

I.3.2.2. Fenugreek (*Trigonella foenum-graecum L.*)

Other name: Trigonelle , Fenugreek

Popular names: حلبة helba

Kingdom : *Plantae*

Subkingdom : *Tracheobionta*

Division : *Magnoliophyta*

Class : *Magnoliopsida*

Subclass : *Rosidae*

Order : *Fabales*

Family : *Fabaceae*

Genus : *Trigonella*

Species : *Trigonella foenum-graecum labill.*

I.3.2.2.1. Botanical description

*Trigonella foenum-graecum L.*or Fenugreek is an annual plant of the *Fabaceae* family. It is one of the oldest medicinal plants, originating from India and Northern Africa and is indigenous to wastern Asia and south Eastern Europe. It has long been cultivated in the Mediterranean area, in India and in North Africa (Rosengaren, 1969).

Fenugreek is an annual grass with round stem, smooth, upright, from 20 to 50 cm; sheets made up of 3 oval leaflet; whitish flowers, blunt at the top, or obovales, whitish flowers, often laid out per pairs axillaires, sessile; pods lengthened, narrow, drawn up, arched, finished by a point, being able to contain to 20 polyhedral seeds of yellow ochre color, characteristic odor (Baba aïssa, 2011).

Fig.13 : Photograph of *Trigonella foenum-graecum L* (self photograph).

I.3.2.2.2. Phytochemical composition

Principal components of Fenugereek are mucilage, saponins, diosgenin, flavonoides, protids, prolamin, essential oil, nicotinic acid, mineral elements: iron, phosphorus, calcium., vitamins, A, B1, C, alkaloids (trigonelline…), choline… (Baba aïssa, 2011). Fenugreek seeds contain 40-50% galactomannan (soluble and insoluble) (Sirajudheen *et al*., 2009). Previos studies highlighted the presence of polysaccharides (Petropoulos, 2002), steroidal sapogenins (Fazli and Hardman,1968; Sauvaire *et al*.,1991; Taylor *et al*., 1997), triterpenoids (Shangs *et al*.,1998), alkaloids (Petropoulos,2002), flavonids and phenolic acids (Wagner *et al*., 1973; Huang and Liang, 2000; Petropoulos,2002; Rayyan *et al*., 2010; Liu *et al*.,2012; Kenny *et al*., 2013). Trigonelline is the major component of alkaloids in fenugreek (Mishkinskey *et al*., 1967)

I.3.2.2.3. Medicinal use of the plant

This plant regarded as a panacea on popular medical. The use of fenugreek is common in various forms: seed decoctions, flour mixed with honey, or the vinegar. The most properties are polishing substance, anabolisant, aperitif, emollient, febrifuge, galactagogue, hypoglycemic, tonic. The anabolic and tonic actions are due to the diosgenin, substance used formerly for the preparation of cortisone and certain genital hormons (Baba aïssa, 2011). Other therapeutic actions have been reported for this plant such as antimicrobial (Bhatti *et al*.,1996), antioxidant (Mansour and Khalil, 2000 ; Raskin *et al*., 2002 ; Madhava *et al*., 2011), anti-allergic (Thiel, 1997), anti-inflammatory (Liu *et al*., 2012), antipyretic (Ahmadiani *et al*., 2001) and anti-cancer effects (Devasena and Menon, 2003). Trigonelline protects β-cells of the pancreas and increase insulin sensitivity index as well as insulin content (Zhou *et al*., 2013).

I.3.2.3. Eucalyptus (*Eucalyptus Globulus Labill.*)

Other name: blue gum tree.

Popular names: كاليتوس kalitous, كافورة kafoura.

Kingdom : *Plantae*

Subkingdo : *Tracheobionta*
Superdivision : *Spermatophyta*
Division : *Flowering plants*
Class : *Dicotyledons*
Subclass : *Rosidae*
Order : *Myrtaceae*
Genus : *Eucalyptus*
Species : *Eucalyptus globulus Labill*.

I.3.2.3.1. Botanical description

Large tree with a right trunk, with smooth bark of gray color and red wood, sheets following age: sickle-shaped, leathery, alternate, pendulous, stalked (of the elderly), and oval, waxy, light green, opposite, sessile (in younger subjects and releases), whitish flowers in top surmounted by a pseudo- corolla shaped cap falls during the flowering, letting appear a plume of stamens; fruit shaped capsules, hard, angular, warted, 4 boxes containing several seeds; balsamic odor (Baba aïssa, 2011).

Fig.14 : Photograph of *Eucalyptus globulus L* (self photograph).

I.3.2.3.2. Phytochemical composition

Tannins, pigments, flavonoiques, resin, Eucalyptine, essential oil (cineol, eucalyptol, geraniol, alpha-pinene, terpineol, phélandrène, limonene, cymene, piperitone), alcohols ... (Baba aissa, 2011). The leaf chemistry of *E. globulus* has been carefully investigated. Guenther (1950) states that cineole, a-pinene, and camphene are the important terpenes. In addition there is an array of phenolic acids present in the leaves. These include ellagic, gallic, caffeic, gentistic, p-coumarylquinic, and chlorogenic acids (Hillis, 1966). We have shown that some of these compounds are toxic to a variety of seed plants (Muller and del Moral, 1966). The terpenes are very slightly soluble in water and the phenolic acids are water soluble.

I.3.2.3.3. Medicinal use of the plant

The use of eucalyptus is common in folk medicines which one of the active substances is eucalyptol. Dried or fresh leaves are used in fumigations to disinfect homes in times of flus, and infusion against affections of the respiratory tract (Barton, 2000). The bark of twigs contains a balsamic resin. Eucalyptus oil is reputed to ward off mosquitoes (Lis-Balchin *et al*.,

2000). Combined with olive oil, it has a calming action against rheumatic pain and burns. It has an antibiotic, antiseptic, antibacterial, carminative, diaphoretic, expectorant, antipyretic, stimulating actions (Baba aïssa, 2011). Eucalyptus has been used in traditional medicine for the management of diabetes mellitus in South America, Africa and Iran (Ahlem *et al*., 2009). Studies on streptozotocin-induced diabetes in animal models confirmed the anti-hyperglycemic action of eucalyptus (Ahlem *et al*., 2009; Eidi *et al*., 2009) and antiinflammatory effects (Vigo *et al*., 2004). Eucalyptus oil is used as an anti-microbial element in different kinds of cream, soap and toothpaste (Lis-Balchim *et al*., 2000). Laboratory studies have revealed that eucalyptus oil contains substances having strong anti-bacterial and anti-fungal properties (Ramezani *et al*., 2002; Sartorelli *et al*., 2007). The leaves of eucalyptus also contain substances that have anti-bacterial properties (Erdogrul, 2002; Schelz *et al*., 2006).

Materials and methods

II.1. Preparation of a survey on the hypoglycemic plants used in Setif region

II.1.1. Ethnobotanic investigations

Ethnobotanical survey is one of the reliable sources to natural and synthetic drug discovery (Fabricant and Farnsworth, 2001). It aims at confronting the knowledge to make traditional in its contemporary form and research in pharmacology. This study surveyed the plants species mentioned for the treatment of diabetes in ethnobotanical surveys performed in Setif and Bordj-Bou-Arreridj, and evaluated the current status of knowledge related to antidiabetic activity of these plants.

Investigations based on the direct questions related to the use of plants quoted in the traditional medecine in Setif region. This survey was cinducted during 2013 in many cities of Setif region (Elbabour ; Setif ; Ras-El-Oued ; Bougaaa ; El-elma ; Ain-azal ; Ain-oulman). The approach of the 325 vendor interviewed was based on the dialogue in local language, accompanied sometimes by the purchase of the medicinal plants. Botanical descriptions and the various uses of the medicinal plants were enriched by the information received from the vendor more accessible and motivated in the circumstance.

As a whole, this ethnobotanic investigation consists of :

• A preliminary investigation near the herbalists who allows to draw up a first list of the medicinal spontaneous plants of the area.

• A systematic statement of pharmacological knowledge of the plants near the populations which makes it possible to release the basic concepts of the perception of the natural environment and the description of the diseases.

• Collections of the specimens, in order to make its the botanical identification.

II.1.2. The questions

Main themes addressed by the study survey :

1. Demographic details of the patient	* Gender, age group
2. Disease details (obtained from patient file)	* Type of diabetes : Type I, type II, .. and period of treatment
3. Information about the use of herbs or herbal preparation	* Plant Part used, Forms of use (Raw, Cooked, Infusion, Decoction, juice), Mode of Preparation, Administration, Dose, period of use.
4. Purpose of the use of herbs	*Did the patient achieve the sought effect and reduction in side effects from prescribed medication.

II.1.3. Data analysis

Responses were coded and entered into SPSS for Windows Integrated Student, version 19, for statistical analysis. Analysis tables, reports and delivery of code were used to make percentage for obtained results. Graphs, old version, sector and summary for groups of observation were used to draw sector for question 10.

II.2. The effects of the plant extracts on experimental diabetes in rats

II.2.1. Materials

II.2.1.1. Plants materials

Olea europea L and Eucalyptus globulus L leaves, Trigonella foenum-graecum L seeds were collected in September,2013 from were collected in the end of May and the beguining of June from Res-El-Oued-Ain Ouelman. Setif. So from each plants the parts used (leaves, seeds) were seperated from the other parts and dried at room temperature. The plants (fenugreek, Olive tree and eucalyptus) were identified by Professor Pr. Laouer Hocine from the Faculty of Sciences of the Nature and life. Department of Ecology. University Ferhat Abbass, Setif-1, Algeria. The plant samples were air dried in shadow and finely powdered in a rotating knife grinder. The powder was sieved through a 1 mm mesh to remove large fragments. Each plant powder was then used for the extraction.

Healthy male adult albino rats weighing 170–280 g were purchased from Pasteur Institute, Algiers. Animals were kept for one week and housed in an air-conditioned animal room, with light/dark cycle photoperiod, and given free access to water and feeding ***ad libitum.***

II.2.1.2. Chemicals

Animals, methanol (MeOH) , Hexan , Ethyl acetate , Chloroform , Tannic acid, aluminium trichloride (AlCl3) , Butylated hydroxytoluene (BHT) , Tween 40 , and Carbonate, Tween20, Sodium phosohate monohydrate, Sodium phosphate dibasique, Ammonium thiocyanate, Ascorbic acid, Iron(II)chloride tetrahydrate, The various products used were purchased from Merck and Sigma.

β-carotene, Linoleic acid, Ammonium thiocyanate, butylated hydroxytoluene (BHT) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The chemicals such as 2,2-diphenyl-1picryl-hydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), Gallic acid, 6-hydroxy-2, 5,7,8-tetramethyl chroman-2-carboxylic acid (trolox), 2.2´-Azino-bis(3-ethylbenzenothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Potassium persulphate, potassium ferricyanide, trichloroacetic acid, thiobarbituric acid (TBA), ferrozine, ferrous and ferric chloride were obtained from Merck. Streptozotocin from Sigma, St. Louis, MO, USA. All other reagents were of analytical grade.

II.2.2. Methods

II.2.2.1. Preparation of plant extracts

The different extract of plants were obtained following the extraction method described by (Markham, 1982) with slight modification.

100g of plants powder are macerated in 1 liter of 85% methanol, the mixture was shaking during 72h. The whole solution was filtered thereafter, methanol is eliminated by rotary evaporation in Rotavapor (BÜCHI). The extraction is remade for the second time using 50% methanol. The second filtrate is mixed with the first. Extraction was made first with hexane, to remove lipids and then with chloroform and finally with ethyl acetate to obtain five fractions; the crude methanolic extract (ME), the hexane extracts (HxE), chloroform extracts (ChE), the ethyl acetate extracts (EAE) and aqueous extracts (AqE).

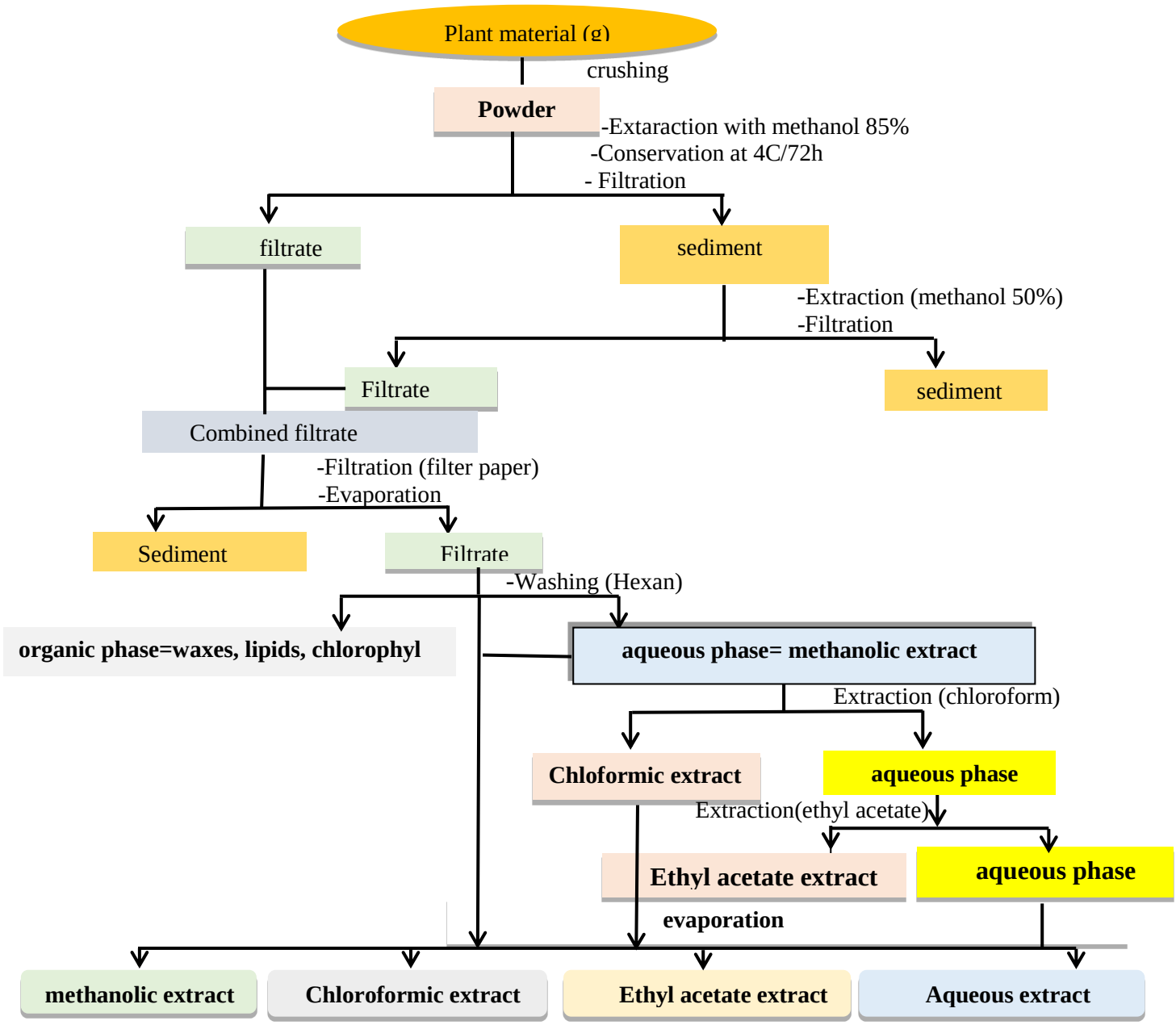


Fig. 15: Schematic diagram showing the process of extraction.

II.2.2.2. Assessment of total polyphenols in the extracts

According to the Folin–Ciocalteu assay and with the method of Li *et al* (2007), the proportioning of the flavonoids is carried out after slight modifications, 100 µ L of each extract of the plant (ME, AqE, ChE, EAE) diluted and is mixed with 500 µl folin-ciocalteu (2M) diluted 10 times and 400 µl of sodium carbonate (Na_2CO_3) with concentration of 7.5g/ 100ml. The absorbance is measured at 765 nm, after incubation for 1 hour and 30 min at ambient temperature against a methanol blank. The color produced is proportional to the amount of polyphenols present in the extract analyzed (kassemi, 2006).

A standard curve of gallic acid was created using an adequately range of gallic acid concentration from 150 to 5 µg/ml. The results were expressed as µg gallic acid equivalent/ milligram of extract. All measurements are repeated 3 times.

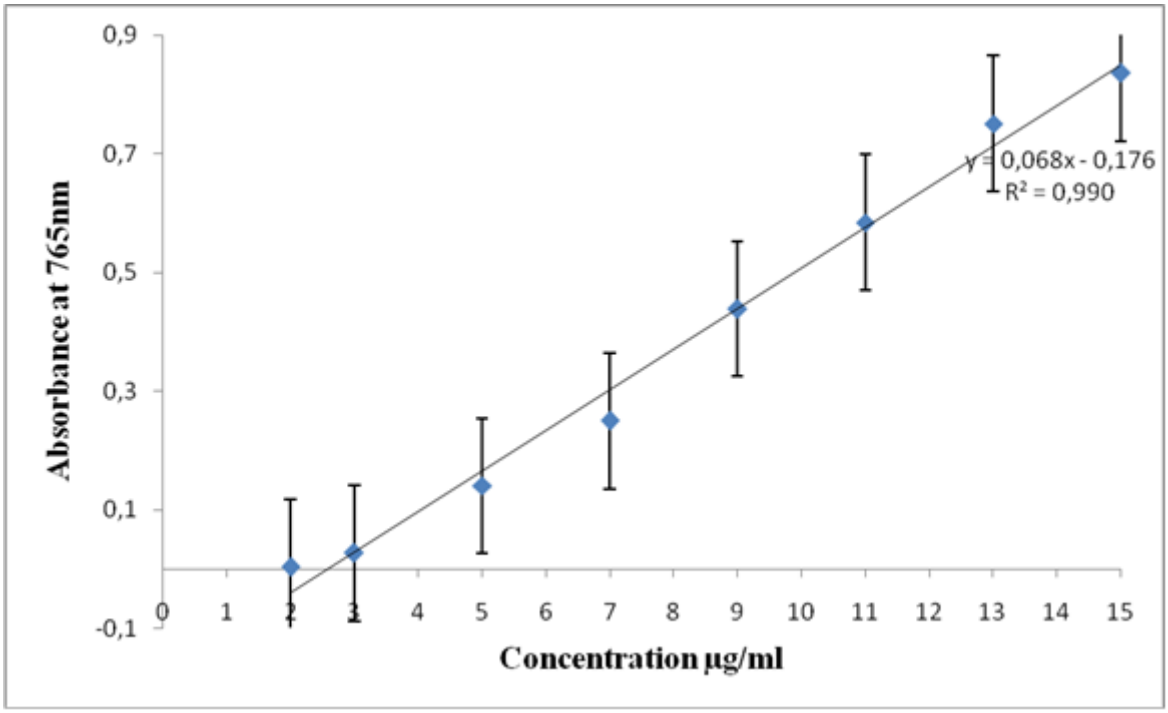


Fig. 16: Standard curve of gallic acid for the determination of total polyphenols. (mean ± SD).

II.2.2.3. Assessment of flavonoids in extracts

In order to quantify flavornoids in plant extracts aluminium trichloride ($AlCl_3$) was used (Bahorum *et al.*, 1996); 1ml of each extracts was added to 1ml of $AlCl_3$ solution (2% in methanol). After 10 minutes of incubation, the absorbance was measured at 430 nm. By using a typical standard curve for the reactivity of quercetin and rutin (0-40 µg/ml) with $AlCl_3$ solution, concentration of the flavonoids is established (figure18) and values are expressed as milligram of Quercetin or Rutin equivalent per gram of extract.

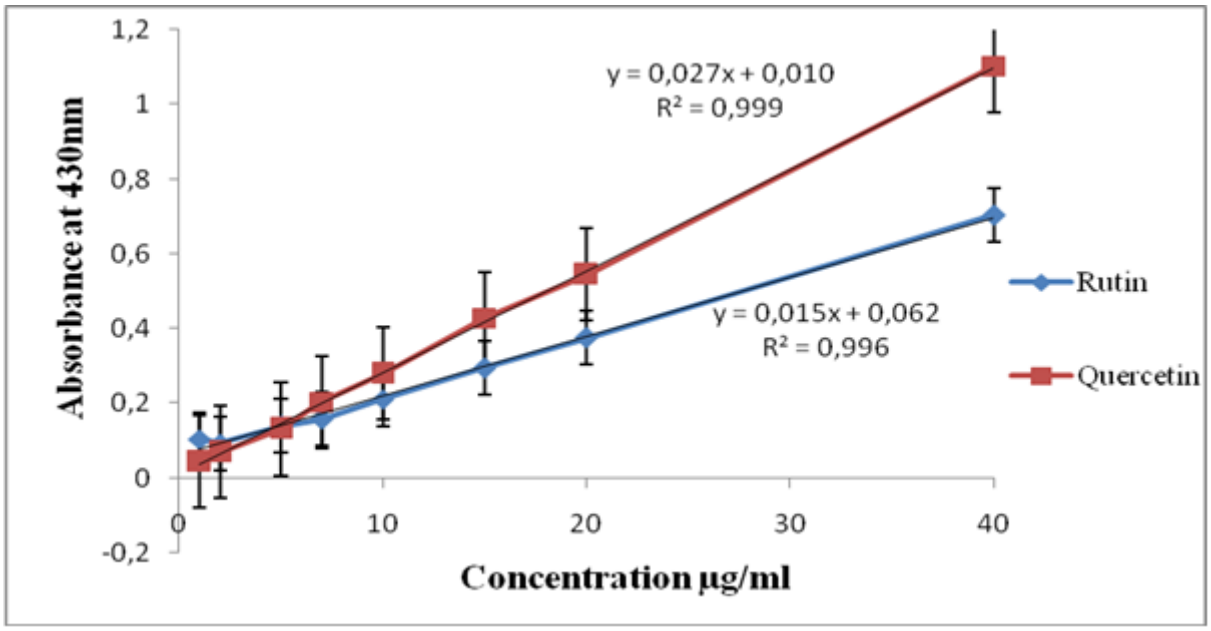


Fig.17: Standard curve of quercetin and rutin for the determination of total flavonoids. (mean ± SD of three measurements).

II.2.2.4. HPLC analysis of methanolic extracts of plants

The HPLC technique has been shown to be a very efficient system for separating complex mixtures of plants. HPLC methods have been reported for the determination of substances and for monitoring variation in the composition of samples (Coutinho *et al.*, 2008). HPLC of plant

extracts were conducted at the Laboratory of Department of Chemistry, Faculty of Science, Çankırı Karatekin University, Çankırı, Turkey.

The HPLC was carried out as described by Abay *et al*. (2015). Agilent Technology of 1260 Infinity HPLC System was coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x 100 mm, 3.5 µm) column. Mobile phases A and B were ultra-pure water with 0.1% formic acid and acetonitrile, respectively. Flow rate was 0.6 mL min^{-1} and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0-1 min 10% B; 1-20 min 50% B; 20-23 min 80% B; 23-25 min 10% B; 25-30 min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C, nitrogen gas flow of 10.0 L min^{-1}, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered through a PTFE (0.45 µm) filter by an injector to remove particulates.

II.2.2.5. *In vitro* antioxidant and antiradicalar activities of plants extracts

II.2.2.5.1. Test of DPPH

The experiment was carried out according to the method described by Benabadji *et al* (2004). 50µl of various concentrations of extracts wass added to 5ml solution of DPPH (0,004%). The absorbance is read at 517nm, after 30 minutes of incubation at ambient temperature and in darkness, Negative control was represented by the methanolic solution of the DPPH and the positive control wass represented by the BHT.

The percentage of discolouration of the DPPH in solution in methanol (Inhibition % or I%) expresses the capacities to trap the free radical and is estimated according to the formula:

Inhibition % = (ABS control - ABS test) x 100 / ABS control.

Where:

ABS control: Absorbance of control at the wavelength 517nm;

ABS test: Absorbance of the sample at the wavelength 517nm.

The IC_{50} value is the concentration of the sample required to give a reduction of 50% of the absorbance of the solution controls to constitute methanol and DPPH. The IC_{50} values were calculated by the linear regression where the X-coordinate is represented by the concentration of the compounds tested and ordered by (I %) the percentage of inhibition (Mensor *et al*., 2001).

II.2.2.5.2. ABTS radical scavenging activity assay

The spectrophotometric analysis of $ABTS^+$ radical scavenging activity was determined according to method reported by Re *et al* (1999) with some modifications. The $ABTS^+$ solution was produced by the reaction of 7 mmol/L of ABTS solution in 2.45 mmol/L potassium persulfate (final concentration). The mixture was kept in the dark at room temperature for 24 h before use. The solution was diluted with methanol and equilibrated at room temperature to give an absorbance of 0.70±0.02 at 734 nm in a 1 cm cuvette. Then, 50 µL of the extract dilutions (0.012 5, 0.025, 0.05, 0.1, 0.2, 0.3, 0.5 mg/ mL) was mixed with 1 mL $ABTS^+$ solution and kept for 30 min at room temperature; the absorbance of reaction mixture was measured at 734 nm. Trolox was used as a positive control. The $ABTS^+$ radical scavenging ability was calculated according to the same equation in the DPPH assay.

II.2.2.5.3. Ferrous ion chelating activity

The metal chelating, such as iron, is considered freely strong stimulition the oxidation reactions and the production of free radicals. Following the method of Decker and Welch (1990), 500 µl of different concentrations of plant extracts or EDTA, 100 µl $FeCl_2$ samples (0.6 mM) and 900 µl of methanol were mixed, after shaking the mixture and leaving it for 5 minutes, and then 100 µl of ferrozine (5 mM) was added. The absorbence of the complex Fe^{+2}-Ferrozine formed was

read at 562 nm after 10 minutes of the reaction. The control solution contains all the reactants except the extracts or EDTA. The chelating effect was calculated as follows:

(%) = (ABS control - ABS test) x 100 / ABS control.

Where :

ABS control: Absorbance of control at the wavelength 562nm;

ABS test: Absorbance of the sample at the wavelength 562nm.

The effective concentration corresponding is to account the 50% inhibition of free-radical (IC_{50}) from the standard curve (I%) with concentrations of extracts and the lowest IC_{50} value represent the highest scavening effect of the extract.

II.2.2.3.4. Reducing power

The return Fe^{+3} reagent is consedred as granting electrons, which represent an important mechanism in the antioxidant activity of phenolic compounds (Ozsoy *et al*., 2008). The reducing power of the plant extracts can be measured using the method of Chung *et al* (2005). Breifly, 0.1 ml of various concentrations of the sample was mixed with the same volume of phosphate buffer (0.2 M, pH=6.6) and 0.1 ml of K_3FeCN_6. The mixture was incubated for 20 min at 50° C for the return of ferricyanide to ferrocyanide, after that, 0.25 ml (1%) of acid trichloroacetic was added to stop the reaction and the mixture was centrifuged at 3000 /minutes for 10 minutes. Then, 0.25 ml of the supernatant was added to 0.25 ml of distilled water and 0.5ml $FeCl_3$ (0.1%), the absorbance at 700 nm was cheked to estimate the amount of ferric ferrocyanide formed.

II.2.2.5.5. Test of β-Carotene/Linoleic acid

The mechanism of bleaching of β-carotene is a free-radical mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. β-carotene in this model system undergoes

rapid discoloration in the absence of an antioxidant. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and charachteristic orange color, which can be monitored spectophotometrically. The presence of different extracts can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system (Madhava *et al*., 2011).

According to the method described by Kartal *et al* (2007), the inhibition of the oxydative decomposition of β-carotene (discolouration) by the products of oxidation of the linoleic acid was determined. 0.5 mg of β-carotene in 1ml of chloroform was solubilized to prepare the emulsion of β-carotene/ linoleic acid, 25µl of the linoleic acid and 200 mg of Tween 40 are added, chloroform was completely evaporated by rotary evaporation. After that, 100ml of distilled water saturated with oxygen were added with vigorous shaking. 350µl of solution of extracts or antioxydants (BHT) solubilized in methanol (2mg/ml) were added to 2.5ml of the preceding emulsion.

The kinetics of discolouration of the emulsion in the presence and absence of antioxidant (negative control in which the sample is replaced by 350µl methanol and distilled water) was followed at 490 nm with intervals of regular times during 48 heurs (after : 1h, 2h, 3h, 4h, 6h, 24h, and 48h) of incubation at ambient temperature and in darkness.

The percentage of inhibition ofdecoloration by extracts was measured as follows:

$$\mathbf{AA\% = ABS_{test} / ABS_{BHT} \times 100}$$

AA%: Percentage inhibition (antioxidant activity)

ABS $_{test}$: Absorbance in the presence of the extract

ABS $_{BHT}$: Absorbance in the presence of positive control BHT.

II.2.2.5.6. Ferric thiocyanate test (FTC)

A modified method of Yen *et al* (2003) was adopted for the FTC assay. In this method, the concentration of peroxide decreases as the antioxidant activity increases. 2.5 ml of 0.02M linoleic acid emulsion pH=7 ; the emulsion was prepared by mixing 0.284 mg of linoliec acid and 0.2804 g of Tween 20 and 50 ml of phosphate buffer and 2 ml of 0.2 M phosphate buffer (pH= 7) were added to 0.5 ml of extract sample. The incubation was during five days. After that, 0.1 ml of the reaction mixture at 24 h intervals was added to 4.7ml (75% ethyl alcohol), 0.1 ml of ammonium thiocyanate (30%), and 0.1ml (0.02 M ferrous chloride in 3.5% HCl). After 3 min, the absorbance was measured at 500 nm. Each 24 h, the absorbance was taken until the absorbance of the control reached its maximum value. BHT was used as a positive control.

II.2.2.5.7. Thiobarbituric acid (TBA) method

This was conducted according to the method described by Kikuzaki and Nakatani (1993). The same preparation method as described in the FTC method was used. To 1ml of sample solution, 20% trichlroacetic acid (2ml) and thiobarbituric acid solution (0.67%) (2ml) were added. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. The absorbance of the supernatant was measured at 532nm. Antioxidant activity was recorded based on the absorbance of the final day of FTC assay. Both methods (FTC and TBA) were expressed as percent inhibition :

$$\textbf{\% Inhibition} = 100 - [(A_{sample}/A_{control}) \times 100]$$

Where, Acontrol and Asample are the absorbances of the control (without sample) and the experimental (with sample) reactions, respectively.

II.2.2.6. Antidiabetic and antioxidant activity of plant extracts *in vivo*

1. Treatment protocol

Diabetes was induced in rats as described by Adolfo Andrade Cetto *et al* (2000) with some modifications ; a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/kg in cold sodium nitrate (0.9%) was made to overnight fasted rats. Animals, which did not develop more than 250 mg/dl blood glucose levels, were rejected.

The rats were divided into 9 groups of six animals each, and the treatment was given every day via orogastric tube for 18 days as the following:

Group1: was received normal saline (0.9%) and served as control group.

Group2: was received ME of *O.europea* at the dose of 200 mg/kg.

Group 3: received ME of *O.europea* at the dose of 600 mg/kg.

Group4 : was treated with ME of *F. foenum-graecum* at the dose of 200 mg/kg.

Group5 : was treated with ME of *F. foenum-graecum* at the dose of 600 mg/kg.

Group6 : was treated with ME of *E.globulus* at the dose of 150 mg/kg.

Group7 : received ME of *E.globulus* at the dose of 500 mg/kg.

Group8 : received Glibinclamide (100 mg/kg) and served as drug control or reference control.

Group9 : diabetic but received only normal saline (0.9%).

After18 days of treatement, the rats were sacrificed. Blood was collected into 2 tubes : the first tubes, containing heparin and the second tubes containing EDTA, the blood in tubes containing heparin was used to obtain the plasma which was seperated by centrifugation (15 min, 3000rpm) and was separeted into two one portion was analysed for : Triglycerides, total cholesterol, LDL, HDL, Albumin, Uric acid, Totale bilirubin, TGO or AST, TGP or ALT and

Creatinine levels using an automate apparatus and the second was used to assess the reducing power and DPPH scavenging activities. Whereas tubes containing EDTA were used to analyse the hematological parameters. The pancreas, liver and kidney were removed from each animal for further studies.

1.1. Assessment of DPPH scavenging activity of plasma

According to the method of Burits and Bucar (2000) with some modifications, the capacity of plasma to trap the DPPH radical was avaluated. In brief, a volume of plasma was added to solution of the DPPH at a concentration of 0,004%. After incubation for 30 min in the darkness followed by a centrifugation, the absorbance is measured at 517 nm and the plasmatic antioxydant capacity was calculated as follows :

% scavenging activity = [(Abs control-Abs sample)/Abs controls] X 100

A control : is the absorbance of the blank solution

A sample : is the absorbance in the presence of the test compound.

1.2. Assessment of reducing power in plasma

According to the method of Chung *et al* (2005), the reducing power was determined. Briefly, 0.1 ml of plasma was mixed with 0.1 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.1 ml of potassium ferricyanide (1 %). The mixture was incubated for 20 min at 50°C. After that, 0.250 ml of trichloroacetic (1%) were added. The mixture was then centrifuged for 10 min at 3000 rpm. An aliquot of (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. A higher absorbance indicated a higher reducing power.

1.3. Histological sections studies

After dissection of animals (through longitudinally cutting in the abdominal region), the abdominal and thoracic cavity were opened immediately then the liver, kidney and pancrea were removed and examined macroscopically. Tissues were cleaned several times with distilled water then fixed in formalin 10% (Sigma-aldrich). The samples were preceded for histological section in the laboratory of histopathology at the University Hospital in Setif.

1.4. Preparation of homogenate

4.5 ml (1.15M KCl buffer) were added to 0.5g of liver or kidney tissues, and homogenized using dounce homogenizer in ice-cold condition. The obtained homogenate was centrifuged at 4°C and 4000 rpm for 15 min and the supernatant collected was used for the determination of lipid peroxidation (MDA), catalase activity, reduced glutathione (GSH) and total protein. All experiment were conducted on ice.

1.4.1. Determination of total protein level

Protein concentration was measured according to the method described by Gornall *et al* (1949) using the Biuret reagent and bovine serum albumin as a standard. Briefely, 1 ml biuret reagent was mixed with 25 µl sample or standard (albumin), and the absorbance of mixture was measured after 10 min of incubation at 37 °C at 540nm. Total protein level was calculated as follows :

$$\textbf{Total protein (mg/ml)} = (\textbf{Abs of sample} / \textbf{Abs of standard}) \times \textbf{n}$$

Where n is standard concentration

1.4.2. Determination of catalase activity

The activity of catalase (CAT) (EC.1.11.1.6) was assayed according to Clairborne (1985). In a cuvette containing 2950 µl of 19 mM H_2O_2 prepared in 0.1M phosphate buffer (pH 7.4), 50 µl

of each supernatant was added. The rate of the breakdown of H_2O_2 by the catalase was measured spectrophotometrically in an interval of two minutes time against a blank at 240 nm. Catalase activity was determined as µmole/min/mg protein.

1.4.3. Determination of lipid peroxidation (MDA)

MDA levels were measured according to Ohkawa *et al* (1979). Briefly, 0.5 ml of each homogenate, 0.5 ml of TCA (20 % w/v) and 1ml of TBA (0.67 % w/v) were mixed. After that, the mixture was boiled in a water bath for 15 min. After cooling at a room temperature, 4 ml of n-butanol were added to each sample and centrifuged at 3000 rpm for 15 min. The absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was determined from a standard curve of 1, 1, 3,3 tetraethoxypropane in the same conditions and expressed as n mol/ g tissue.

1.4.3. Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) forms a characteristic compound with 5-5'dithiobis 2-nitro benzoic acid (DTNB) and was measured following the method of Ellman's (1959). 50 µl of supernatant were deluted in 10 ml phosphate buffer (0.1 M, pH 8). After diluation, 3 ml of the mixture were added to 20µl of DTNB (0.01 M). The absorbance was read at 412 nm after 5 min. A series of standards GSH (0.4-20 µmol/ml) were treated in a similar manner along with blank. Reduced glutathione was expressed as µmol/ g tissue.

1.5. Determination of the diuretic activity

The diuretic activity in rats was evaluated following the method reported by Zhang *et al* (2010), with some modifications. Each group was individually placed in a metabolic cage, and the cumulative urine output was determined at the end of the experimental period for 5 h.

1.6. Statistical analysis

Statistical analysis was performed using the Graph Pad Prism (version 5.01 for Windows). All *in vitro* results were calculated as mean±SD for three measurements and were analyzed by One-way analysis of ANOVA followed by Tukey's test. Whereas *in vivo* experiments were calculated as mean ± S.E.M and were analysed by Student t-test. The $P< 0.05$ was considered statistically significant.

Results and discussion

III.1. Hypoglycimic plants used in Setif region

The survey focuses on plants belonging to several different families from around Setif to understand their therapeutic uses and their potential antioxidant activities. The vendors ignore the weight, specific measures and dosage of drugs in the preparation. The accuracy is lacking on several plants such quantities of plant to prepare, the solvent or vehicle used, the time required for preparation of solutions (decoction, infusion, maceration) and dose to prescribe. However, different antioxidant chemical compounds isolated from some of these plants were also discussed in order to know the active constituents responsible for the antioxidant potential of the plants.

Table1 : Hypoglycimic plants used for the treatment of diabetes by population in Setif region, results of survey.

العربية	الاسم الشائع	Francais	Englais	Nom scientifique	Familly
الباذنجان	badendjan	Aubergine	Aubergine	*Solanum Melongena L.*	*Solanacees*
البرتقال المر	bourtouqal	Oranger doux	Soft orange tree	*Citrus sinensis L.*	*Rutacees*
الترفاق	terfaq	Aigremoine	Aigremoine	*Agrimonia eupatoria L.*	*Rosacees*
التوت البري	toute	Mûrier noir	Black mulberry tree	*Morus nigra L.*	*Moracees*
التيفاف، توفالت	touffalt	Thapsia velue	Villous Thapsia	*Thapsia villosa L.*	*Apiacees*
الثوم	thoum	Ail	Garlic	*Allium sativum L.*	*Liliacees*
الجعدة	djaâda	Polium	Polium	*Teucrium Polium L.*	*Labiees*
الجزر	djazr boustani	Carotte	Carrot	*Daucus carota ssp.sativus Hayek*	*Apiacees*
جوزة	djouza	Noyer	walnut	*Juglans regia L.*	*Juglandacees*
حب الرشاد	habb errachad	Cresson alénois	Cresson	*Lepidium sativum L.*	*Brassicaceae*
الحبة السوداء	el-haba essouda	Nigelle	Nigella	*Nigella sativa L.*	*Renonculacees*
حبق	h'baq	Basilic	Basil	*Ocimum baslicum L.*	*Labiees*
الحريق	harraïq	Ortie	Nettle	*Urtica pilulifera L.*	*Urticacees*
الحلبة	helba	Fenugrec	Fenugrec	*Trigonella foenum-graecum L.*	*Fabaceae*
الخبيزة	khoubeïza	Mauve	Mauve	*Malva sylvestris L.*	*Malvacees*
الخرشف	kherchef	Cardon	Cardoon	*Cynara cardunculus L.*	*Composees*
خياطة الأجراح	khayat el-adjrah	Phlomis	Phlomis	*Phlomis crinita Cav. Et Ph. Bovie de Noé*	*Labiees*
الريحان	rihan	Myrte	Myrtle	*Myrtus communisL.*	*Myrtacees*
الزرية	zeria	Galéga	galega	*Galega officinallis L.*	*Papilionacees*
زهور الأقحوان	oqhouane	Chrysanthème des fleuristes	Chrysanthemum of the florists	*Chrysanthemum morifolium*	*Composees*

زهور البابونج	baboundj	Camommile romaine	Roman Camommile	*Anthemis nobilis L.*	*Asteraceae*
الزيتون	zitoune	Olive	Olive	*Olea europaea L.*	*Oleacees*
الشعير	chair	Orge	Barley	*Hordeum vulgare L.*	*Poaceae*
سواك النبي	siouak en'bi	Sauge officinale	Officinal sage	*Salvia officinalis L.*	*Labiees*
الشيح	chih	Armoise blanche	White Armoise	*Artemisia herba alba Asso*	*Asteraceae*
العرعار	aâr-âar	Genévriers	Juniper	*Juniperus phoenica L.*	*Cupressacees*
عرق السوس	arq essouse	Réglisse	Liquorice	*Glycyrrhiza glabra L.*	*Papilionacees*
عرق غريس	Arq ghriss	Azereu	blue broom	*Erinacea pungens L.*	*Fabaceae*
عقاية	âgaïa	Zygophylle cornu	Horned Zygophylle		
علك اللبان					
علك صنوبر					
الفاصوليا		Haricot	Beans	*Phaseolus vulgaris L.*	*Papillonacees*
فتتة الحجر	fetatet el-hadjar	Pariétaire	Pellitory	*Parietaria officinalis L.*	*Urticacees*
الفراولة	faraoula	Fraisier	Strawberry plant	*Fragaria vesca L.*	*Rosacees*
فقوس حمير	fegouss el-hamir	Comcombre d'ane	Momordique	*Ecballium Elaterium A. Rich.*	*Cucurbitacees*
القرفة	qorfa	Cannelle	Canella	*Cinnamomum zeylanicum L.*	*lauraceae*
القرنفل	qranfel	Œillet	Eyelet	*Dianthus caryophyllus L.*	*Caryophyllaceae*
القرنون	qarnoun	Artichaut	Artichoke	*Cynara scolymus L.*	*Asteraceae*
قضاب	qadhab	Pervenche bleue	Blue periwinkle	*Vinca difformis Pourr.=V.media Link. Et Hoffm.*	*Apocynacees*
الكاليتوس	kalitouss	Eucalyptus	Eucalyptus	*Eucalyptus Globulus L.*	*Myrtacees*
الكسبر	kesbar	Coriandre	Coriandre	*Coriandrum sativum L.*	*Apiaceae*
لسيقة	loussayqa	Bardane	Burdock	*Arctium minus(Hill.) Bern.= Lappa minor Hill.*	*Asteraceae*
الليمون	laymoun	Citronnier	Lemon tree	*Citrus limonum Risso.*	*Rutacees*
مرارة الحنش	meraret el-hanech	Petite Centaurée	Centaury	*Erythraea centaurium Pers.=Centraurium umbellatum (Gibb) Beck.*	*Gentianacees*
المرة					
مروصبر	marou'esabr	Aloès	Aloe	*Aloe socotrina L.*	*Liliacees*
المريوت	marriouet	Marrube	Marrube	*Marrubium vulgare L.*	*Labiees*

النعناع	nânâ	Menthe verte	Spearmint	*Mentha spicata L. (M. virdis L.*	*Labiees*
الهندي	hendi	Opuntia	Opuntia	*Opuntia ficus indica(L). Mill.*	*Cactacees*
اليوسفي	youssoufi	Mandarinier	Mandarin tree	*Citrus reticulata= C. nobilis*	*Rutacees*

III.1.1. Statistical study by SPPS for the survey results

The results indicated that interviewed patients who used medicinal plants to treat diabetes mellitus declared that they used phytotherapy because it is cheaper (47.1%), more efficient (61.9%) and better (71.9%) than modern medicine. In addition, the patients included in this study were generally illiterate. In all groups, the number of plant users was very important and did not depend on sex and age. In addition, the patients did not respect both the precision of doses and duration of the use. This could explain some accidental intoxication by medicinal plants (Eddouks *et al*., 2002).

The oral administration, which gathers the majority of the modes of preparation: infusion, maceration, decoction, herb tea, powder, is recommended and this results are in accordance with other researchers (Jean and Jiri, 1983) and Soltener (1989). The parts used in descending order, are sheets, stems, fruits, the roots and the inflorescences. The prevalence of use of a body compared to another in the therapeutic field derives from the concentration in active ingredients in this body (Babba aïssa, 1999) .

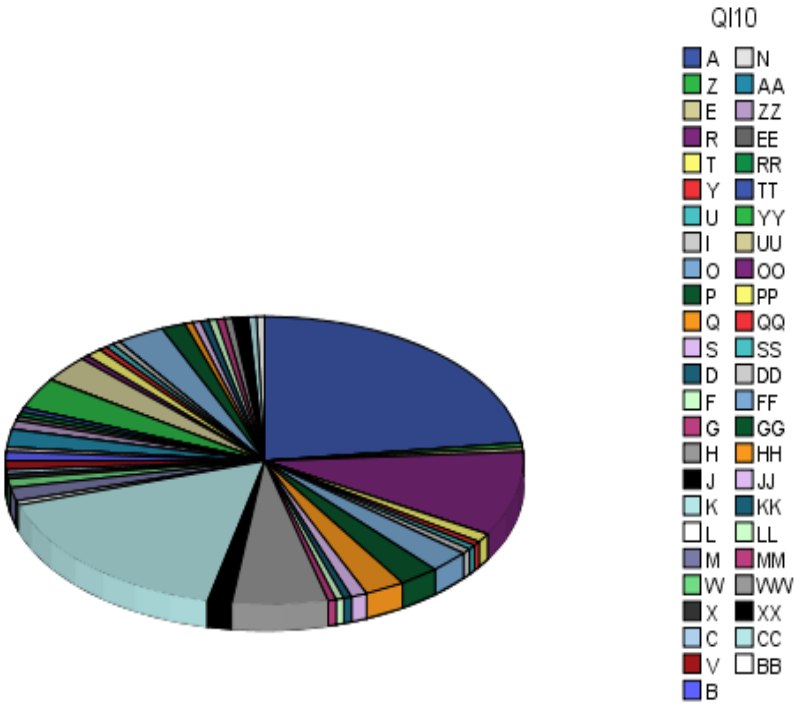


Fig.18 : Statistical study by SPPS : results of question 10 (**A :** *Trigonella foenum-graecum L.*, **Z :** *Cinnamomum zeylanicum L.*, **E :** *Dianthus caryophyllus L.*, **R :** *Eucalyptus Globulus L.*, **T :** *Coriandrum sativum L.*, **Y :** *Myrtus communisL.*, **U :** *Glycyrrhiza glabra L.*, **I :** *Agrimonia eupatoriaL.*, **O :** *Allium sativum L.*, **P :** *Daucus carota*, **Q :** *Cynara cardunculus L.*, **S :** *Erythraea centaurium L.*, **D :** *Opuntia ficus indica L.*, **F :** *Galega officinallis L.*, **G :** *Phaseolus vulgaris L.*, **H :** *Marrubium vulgare L.*, **J :** *Malva sylvestris L.*, **K :** *Olea europaea L.*, **L :** *Juglans regia L.*, **M :** *Hordeum vulgare L.*, **W :** *Cynara scolymus L.*, **X :** *Urtica pilulifera L.*, **C :** *Thapsia villosa L*, **V :** *Salvia officinalis L.*, **B :** *Juniperus phoenica L.*, **N :** *Solanum Melongena L.*, **AA :** *Citrus sinensis L.*, **ZZ :** *Citrus limonum L.*, **EE :** *Citrus reticulata L.*, **RR :** *Fragaria vesca L.*, **TT :** *Morus nigra L.*, **YY :** *Mentha spicata L.*, **UU :** *Nigella sativa L.*, **II :** *Lepidium sativum L.*, **OO :** *Phlomis crinita*, **PP :** المرة , **QQ :** *Erinacea pungens L.*, **SS :** *Chrysanthemum morifolium L.*, **DD :** *Anthemis nobilis L .*, **FF :** *Artemisia herba* , **GG :** *Teucrium Polium L.*, **HH :** *Parietaria officinalis L.*, **JJ :** علك لبان , **KK :** علك صنوبر , **LL :** *Aloe socotrina L.*, **MM :** *Vinca difformis L.*, **WW :** عقاية , **XX :** *Ocimum baslicum L.*, **CC :** *Ecballium Elaterium*, **BB :** *Arctium minus L.*).

The sheets are used because, are at the same time central of photochemical reactions and organic matter which derive from it. They provide the majority of alkaloids, heterosides and essential oils. The fruits find their importance by the concentrations of certain bitter, glucidic or aromatic substances associated certain pigments giving them a characteristic colouring. Finally the flowers find their use by the essential oil concentration; it is the same for the roots and seeds which were rich in sugars and vitamins (Babba Aïssa, 1999).

III.2. Total polyphenols and flavonoids in plants extracts

The metabolism of plants is divided into primary and secondary. The substances that are common to living organisms and essential to cells maintenance (lipids, proteins, carbohydrates, and nucleic acids) are originated from the primary metabolism. On the other hand, substances originated from several biosynthetic pathways and that are restricted to determined groups of organisms are results of the secondary metabolism (Vickery and Vickery, 1981). Phenolic compounds constitute in one of the biggest and widely distributed groups of secondary metabolites in plants (Scalbert and Williamson, 2000). Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Grace, 1994). Based on the absorbance value of the plants extracts solution reacting with Folin-Ciocalteu, phenol reagent and compaired with the absorbance values of standard solutions of gallic acid which was expressed the total phenolics content as µg gallic acid/mg of extract. Unlike the phenolics content, the total flavonoid content of different fractions of plants were reported as mg Quercetin equivalent per g of extract (mg QE/g of extract). Although in this studies have shown few statistically significant correlations between the levels of total phenolics and antioxidant capacity, in others the content of total phenolic compounds was highly correlated with the antioxidant power of samples.

As shown in table 2, total phenolics content of *O.europea* and *T. foenum-graecum* extracts was estimated highly in EAE and lowly in AqE. Whereas in *E.globulus*, the high content of polyphenols was found in ME with 1093.77±0.016 µg GAE/mg of extract and low in EAE with 807.11±0.029 µg GAE/mg of extract.

The flavonoid results showed that the EAE of *O.europea* and *E.globulus* were high (122. 91±0.028 mg QE/gE; 225.58±0.084 mg QE/gE). While, ChE of *T. foenum-graecum* contains the highest value of flavonoid (65.77±0.012 mg QE/gE). However, the lowest value was recorded forAqE of the three plants.

Table 2: Total polyphenols, flavonoids in plants extracts.

Extract	***O. europea***		**T.*foenum-graecum***		***E. globulus***	
	Flavonoid [(a)]	**Polyphenol [(b)]**	**Flavonoid [(a)]**	**Polyphenol [(b)]**	**Flavonoid [(a)]**	**Polyphenol [(b)]**
ME	52.54±0.018	403.72±0.022	38.12±0.006	161.66±0062	90.86±0.025	**1093.77±0.016**
ChE	41.33±0.014	572.94±0.008	**65.77±0.012**	154.01±0.101	62.22±0.009	96355±0.060
EAE	**122.91±0.028**	**611.37±0.087**	17.03±0.002	**177.45±0.173**	**225.58±0.084**	80711±0.029
AqE	20.54±0.002	219.70±0.047	15.16±0.005	125.68±0.002	31.40±0.027	996.88±0.006

[(a)] mg Quercetin equivalent per gramme of extract.

[(b)] µg Gallic acid equivalent per milligramme of extract.

The values present the mean of three measurements ± SD.

The diversity of phenolic compounds and their different distribution in the plant may explain the different ranges obtained for the total phenolic contents (Silva *et al*., 2006). Where the leaves had the highest values of phenolic compounds which were in accordance with this study.

A comparative study of different extraction techniques, each technique seemed to be more adequate than others for the extraction of each particular class of phenolic compounds (Japón-Luján *et al*., 2006). The results obtained in this study of total flavonoids and phenol content

were higher than with the findings of another studies of Caroline *et al*. (2015) where the extraction was made from the dried leaves of *Olea europea L.* applying an ethanol (80%m/m). The total concentration of phenolics was evaluated using two identical sequential extraction in order to achieve an exhaustive extraction of compounds from leaves of Grappolo, Koroeiki, Ascolano, Negrinha do Freixo and Arbosana, which were varieties of olive leaves from Southern Brazil. The results were statistically different from all the varieties, which explain the importance of the use of this complementary step in order to achieve reliable results (Caroline *et al*., 2015). One similar study was performed by Abaza *et al.* (2011) who extracted the phenolic compounds from olive leaves of the Chetoui variety. They found a total phenolic content similar to that found in the work when one extraction was performed. These results are in accordance with the present study.

The total phenolic content of the aqueous extract was estimated to be twice more than to of Kaviarasan *et al*. (2007) who find that the total polyehenol from seeds were 78.6±1.2 mg GAE/g. Early study by Gupta and Nair (1999) had shown that the fenugreek seeds are rich in flavonoids (>100mg/ml). The scavenging activities of the phenolic substances are attributed to the active hydrogen-donating ability of the hydroxyl subtitutions (Bors *et al*., 1996).

Similar to what was found in the present study, Ângelo *et al.* (2014) declared that the difference between the results of flavonoids extracted from *E.globulus* with n-hexane and by the other polar solvents (ethanol, methanol and 75% ethanol), may be explained by the fact that flavonoids can be linked to sugar molecules in the form of heterosides. Heterosides were more easily extracted by polar solvents and free-flavonoids (aglycones) can be simply extracted by non-polar solvents like n-hexane. Therefore, the n-hexane extracts are rich in aglyconic flavonoids contrariwise to the other polar extracts which present more heterosidic flavonoid molecules.

So, differences in polyphenols and flavonoids contents could be due to extraction conditions, the solvent used as well as the geographical origin, plant species and the part of the plant tissue to be used, in which could find various contents in the same extracts (Skotti *et al*., 2014).

III.3. HPLC analysis of methanolic extracts of studied plants

The beneficial health effects of the plants prompted us to explore its secondary metabolites in order to provide major information about its chemical content. The increasing interest of nutritional and pharmacological power of different parts of the plant motivated this investigation (Mital and Sirimavo, 2014). The individual peak resolution showed their respective retention time which are depicted. The obtained results showed that the identified compounds are present at different concentrations, with high peak as a major compound whereas the other compounds were presented at lower relative concentrations and were presented as shorten peak.

The HPLC separation profile revealed the presence of various peaks in the studied sample extracts (figure 19. A, B, C). Where methanilic extract of *O.europea* (MEO) revealed 36 phenolic compounds among them Fumaric acid and Ellagic acid which were presented the highest amount (RT=2.58; RT=9.76, respectively), Hydroxycinnamic acid derivatives and flavonoids were detected in ME of fenugreek seeds (MET) among them the flavonoid glucoside Apigetrin (RT=11.36) and were in total 16; and 40 phenolic acids were detected in methanolic extract of *E.globulus* (MEE) where Quercetin-3-β-D-glucoside was detected as a major compound (RT=9.58) as shown in Table 3. A, B, C.

The componds in extracts were: Gallic acid, fumaric acid, gentisic acid, chlorogenic acid, catechin, 4-hydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, syringic acid,

rutin, 4-hydroxybenzaldehyde, polidatine, ellegic acid, scutellarin, quercetin-3-β-D-glycoside, sinapic acid, naringin, diosmin, taxifolin, hesperidin, apegetrin, neohesperidin, myricetine, baicalin, p-coumaric acid, morin, salicylic acid, cinnamic acid, apegenin, naringenin, diosmetin.

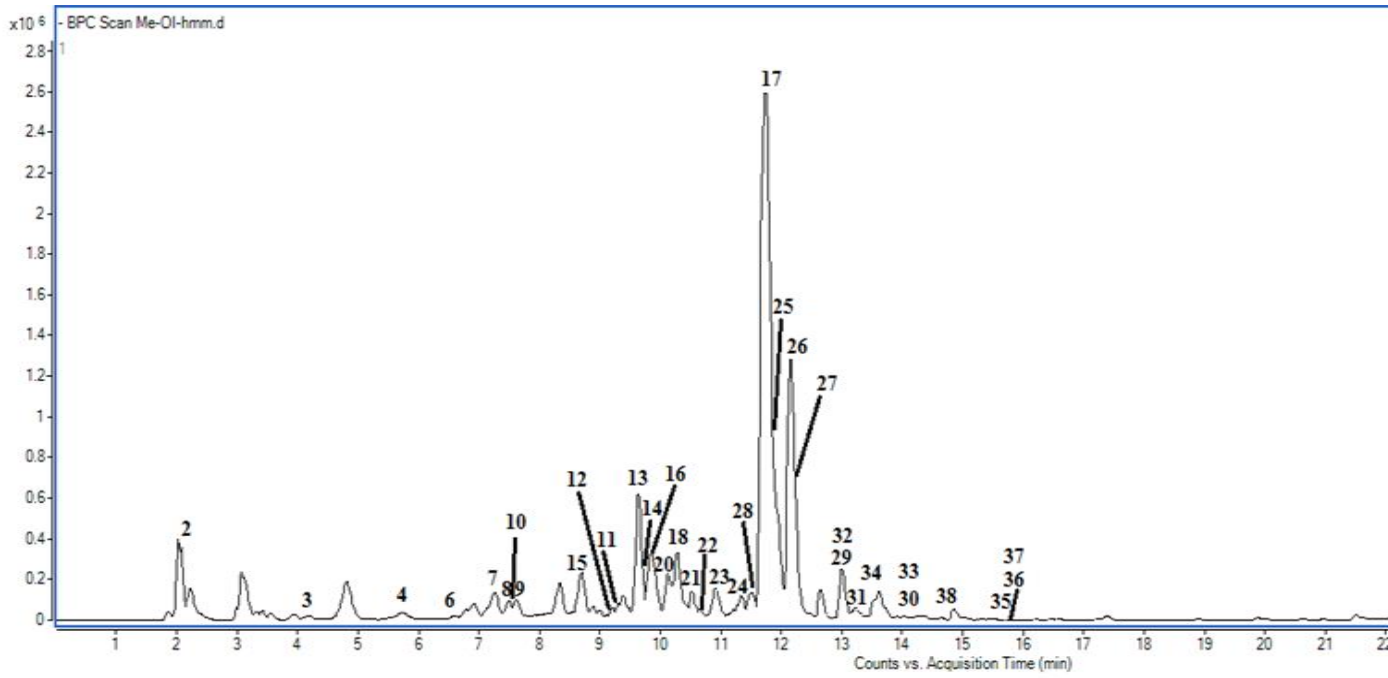


Fig.19.A: HPLC chromatograms of the methanolic extracts of *O.europea* (MEE)

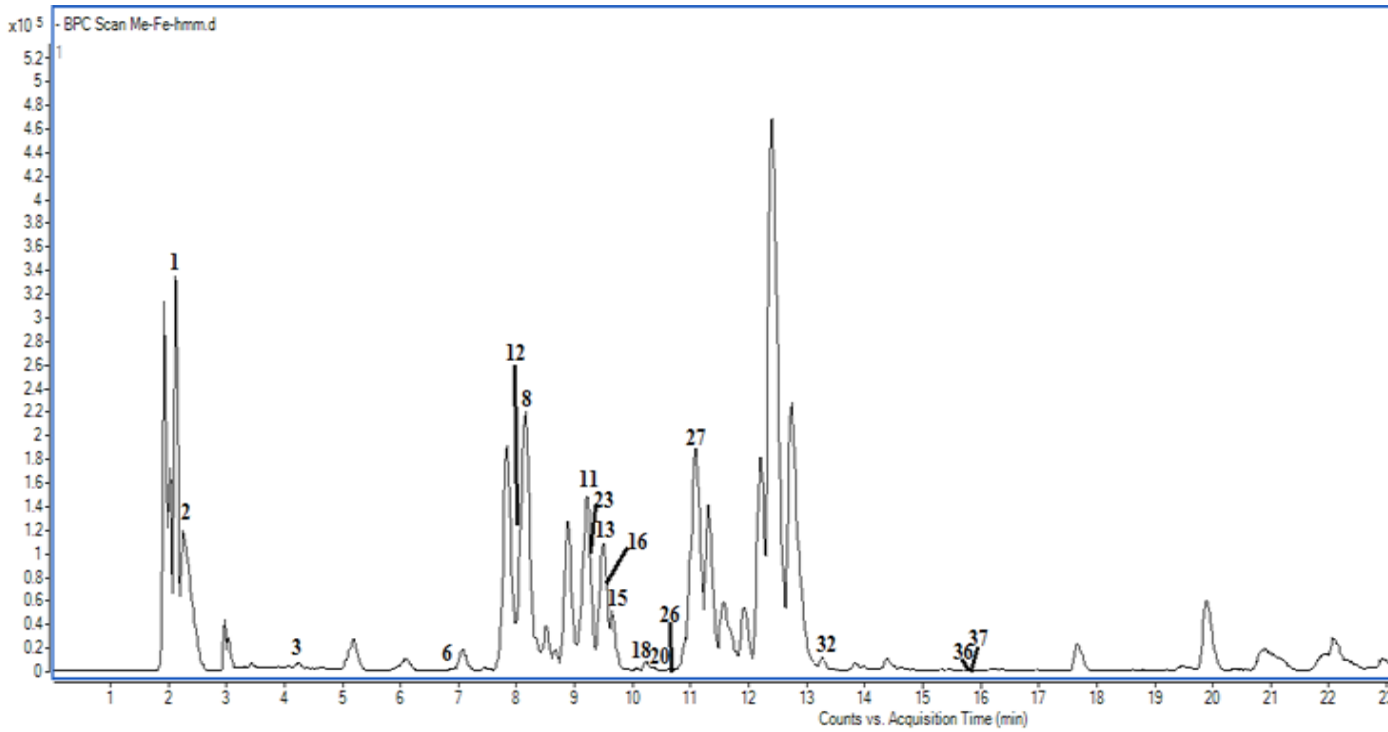


Fig.19.B: HPLC chromatograms of the methanolic extracts of *T.foenum-greacum* (MET)

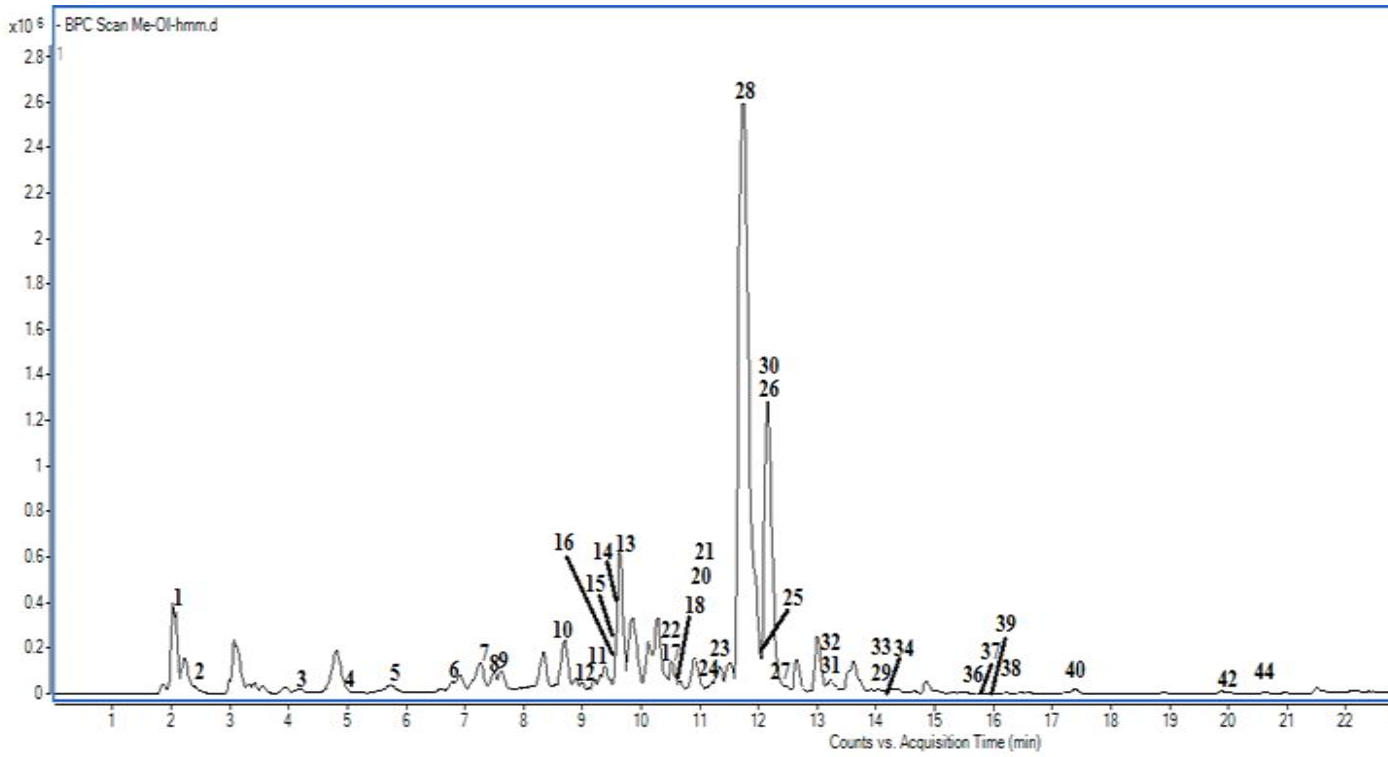


Fig.19.C: HPLC chromatograms of the methanolic extracts of *E.globulus* (MEE)

Table 3. A: Chromatograph analysis of MEO

Name	**RT**	**MEO (mg/kg)**	**Structure**
1.Gallic acid	2,23	tr	$C_7H_6O_5$
2.Fumaric acid	2,58	2274,10	$C_4H_4O_4$
3.Gentisic acid	4,37	287,00	$C_7H_6O_4$
4.Chlorogenic acid	5,02	27,97	$C_{16}H_{18}O_9$
5.Catechin	5,76	88,47	$C_{15}H_{14}O_6$
6.4-hydroxybenzoic acid	6,80	37,30	$C_7H_6O_3$
7.protocatechuic acid	7,30	25,25	$C_7H_6O_4$
8.caffeic acid	7,57	25,32	$C_9H_8O_4$
9.Vanillic acid	7,70	414,38	$C_8H_8O_4$
10.Syringic acid	8,62	329,02	$C_9H_{10}O_5$
11.rutin	9,29	tr	$C_{27}H_{30}O_{16}$
12.4-hydroxybenzaldehyde	9,11	tr	$C_7H_6O_2$

13.Polydatine	9,77	tr	$C_{20}H_{22}O_8$
14.Ellagic acid	9,76	3376,06	$C_{14}H_6O_8$
15.Scutellarin	9,61	34,90	$C_{21}H_{18}O_{12}$
16.Quercetin-3-β-D-glucoside	9,58	419,53	$C_{21}H_{20}O_{12}$
17.sinapic acid	10,57	152,49	$C_{11}H_{12}O_5$
18.naringin	10,62	2013,58	$C_27H_{32}O_{14}$
20.diosmin	10,61	27,64	$C_{28}H_{32}O_{15}$
21.Taxifolin	10,80	14,26	$C_{15}H_{12}O_7$
22.hesperidin	10,59	134,75	$C_{28}H_{34}O_{15}$
23.Apigetrin	11,36	1,10	$C_{21}H_{20}O_{10}$
24.Neohesperidin	11,18	tr	$C_{28}H_{36}O_{15}$
25.Myricetine	12,00	tr	$C_{15}H_{10}O_8$
26.Baicalin	12,21	tr	$C_{21}H_{18}O_{11}$
27.p-coumaric acid	12,31	tr	$C_9H_8O_3$
28.Fisetin	11,95	tr	$C_{15}H_{10}O_6$
29.Protocatechuic acid ethyl ester	14,15	63,35	$C_9H_{10}O_4$
30.Morin	12,23	tr	$C_{15}H_{10}O_7$
32.Resveratrol	13,35	tr	$C_{14}H_{12}O_3$
32.Salicylic acid	13,35	tr	$C_7H_6O_3$
33.quercetin	14,15	tr	$C_{15}H_{10}O_7$
34.Silibinin	14,26	tr	$C_{25}H_{22}O_{10}$
35.Cinnamic acid	15,86	17,95	$C_9H_8O_2$
36.Apigenin	15,89	tr	$C_{15}H_{10}O_5$
37.Naringenin	15,90	tr	$C_{15}H_{12}O_5$
38.kaempferol	16,24	tr	$C_{15}H_{10}O_6$
39.diosmetin	16,00	nd	$C_{16}H_{12}O_6$
40.Neochanin	17,49	nd	$C_{22}H_{22}O_9$
42.Wogonin	19,98	nd	$C_{16}H_{12}O_5$
44.Biochanin A	20,60	nd	$C_{16}H_{12}O_5$

Table 3.B : Chromatograph analysis of MET

Name	**RT**	**MET (mg/kg)**	**Structure**
1.Gallic acid	2,23	tr	$C_7H_6O_5$
2.Fumaric acid	2,58	17,72	$C_4H_4O_4$
3.Gentisic acid	4,37	7,88	$C_7H_6O_4$
4.Chlorogenic acid	5,02	nd	$C_{16}H_{18}O_9$
5.Catechin	5,76	nd	$C_{15}H_{14}O_6$
6.4-hydroxybenzoic acid	6,80	2,22	$C_7H_6O_3$
7.protocatechuic acid	7,30	nd	$C_7H_6O_4$
8.caffeic acid	7,57	tr	$C_9H_8O_4$
9.Vanillic acid	7,70	nd	$C_8H_8O_4$
10.Syringic acid	8,62	nd	$C_9H_{10}O_5$

11.rutin	9,29	tr	$C_{27}H_{30}O_{16}$
12.4-hydroxybenzaldehyde	9,11	tr	$C_7H_6O_2$
13.Polydatine	9,77	13,85	$C_{20}H_{22}O_8$
14.Ellagic acid	9,76	nd	$C_{14}H_6O_8$
15.Scutellarin	9,61	8,60	$C_{21}H_{18}O_{12}$
16.Quercetin-3-β-D-glucoside	9,58	tr	$C_{21}H_{20}O_{12}$
17.sinapic acid	10,57	nd	$C_{11}H_{12}O_5$
18.naringin	10,62	2,34	$C_27H_{32}O_{14}$
20.diosmin	10,61	38,11	$C_{28}H_{32}O_{15}$
21.Taxifolin	10,80	nd	$C_{15}H_{12}O_7$
22.hesperidin	10,59	nd	$C_{28}H_{34}O_{15}$
23.Apigetrin	11,36	123,71	$C_{21}H_{20}O_{10}$
24.Neohesperidin	11,18	nd	$C_{28}H_{36}O_{15}$
25.Myricetine	12,00	nd	$C_{15}H_{10}O_8$
26.Baicalin	12,21	0	$C_{21}H_{18}O_{11}$
27.p-coumaric acid	12,31	0	$C_9H_8O_3$
28.Fisetin	11,95	nd	$C_{15}H_{10}O_6$
29.Protocatechuic acid ethyl ester	14,15	nd	$C_9H_{10}O_4$
30.Morin	12,23	nd	$C_{15}H_{10}O_7$
31.Resveratrol	13,35	nd	$C_{14}H_{12}O_3$
32.Salicylic acid	13,35	tr	$C_7H_6O_3$
33.quercetin	14,15	nd	$C_{15}H_{10}O_7$
34.Silibinin	14,26	nd	$C_{25}H_{22}O_{10}$
35.Cinnamic acid	15,86	nd	$C_9H_8O_2$
36.Apigenin	15,89	tr	$C_{15}H_{10}O_5$
37.Naringenin	15,90	tr	$C_{15}H_{12}O_5$
38.kaempferol	16,24	nd	$C_{15}H_{10}O_6$
39.diosmetin	16,00	nd	$C_{16}H_{12}O_6$
40.Neochanin	17,49	nd	$C_{22}H_{22}O_9$
42.Wogonin	19,98	nd	$C_{16}H_{12}O_5$
44.Biochanin A	20,60	nd	$C_{16}H_{12}O_5$

Table 3.C : Chromatograph analysis of MEE

Name	**RT**	**MEE**	**Structure**
1.Gallic acid	2,23	247,87	$C_7H_6O_5$
2.Fumaric acid	2,58	366,56	$C_4H_4O_4$
3.Gentisic acid	4,37	405,26	$C_7H_6O_4$
4.Chlorogenic acid	5,02	1358,64	$C_{16}H_{18}O_9$
5.Catechin	5,76	2142,84	$C_{15}H_{14}O_6$
6.4-hydroxybenzoic acid	6,80	4,85	$C_7H_6O_3$
7.protocatechuic acid	7,30	64,36	$C_7H_6O_4$
8.caffeic acid	7,57	tr	$C_9H_8O_4$

9.Vanillic acid	7,70	437,70	$C_8H_8O_4$
10.Syringic acid	8,62	192,92	$C_9H_{10}O_5$
11.rutin	9,29	23,03	$C_{27}H_{30}O_{16}$
12.4-hydroxybenzaldehyde	9,11	tr	$C_7H_6O_2$
13.Polydatine	9,77	14,96	$C_{20}H_{22}O_8$
14.Ellagic acid	9,76	154,58	$C_{14}H_6O_8$
15.Scutellarin	9,61	23,12	$C_{21}H_{18}O_{12}$
16.Quercetin-3-β-D-glucoside	9,58	7508,39	$C_{21}H_{20}O_{12}$
17.sinapic acid	10,57	tr	$C_{11}H_{12}O_5$
18.naringin	10,62	18,91	$C_27H_{32}O_{14}$
20.diosmin	10,61	187,02	$C_{28}H_{32}O_{15}$
21.Taxifolin	10,80	24,15	$C_{15}H_{12}O_7$
22.hesperidin	10,59	tr	$C_{28}H_{34}O_{15}$
23.Apigetrin	11,36	tr	$C_{21}H_{20}O_{10}$
24.Neohesperidin	11,18	tr	$C_{28}H_{36}O_{15}$
25.Myricetine	12,00	tr	$C_{15}H_{10}O_8$
26.Baicalin	12,21	11,95	$C_{21}H_{18}O_{11}$
27.p-coumaric acid	12,31	tr	$C_9H_8O_3$
28.Fisetin	11,95	tr	$C_{15}H_{10}O_6$
29.Protocatechuic acid ethyl ester	14,15	tr	$C_9H_{10}O_4$
30.Morin	12,23	54,86	$C_{15}H_{10}O_7$
31.Resveratrol	13,35	tr	$C_{14}H_{12}O_3$
32.Salicylic acid	13,35	tr	$C_7H_6O_3$
33.quercetin	14,15	tr	$C_{15}H_{10}O_7$
34.Silibinin	14,26	tr	$C_{25}H_{22}O_{10}$
35.Cinnamic acid	15,86	nd	$C_9H_8O_2$
36.Apigenin	15,89	tr	$C_{15}H_{10}O_5$
37.Naringenin	15,90	tr	$C_{15}H_{12}O_5$
38.kaempferol	16,24	tr	$C_{15}H_{10}O_6$
39.diosmetin	16,00	tr	$C_{16}H_{12}O_6$
40.Neochanin	17,49	tr	$C_{22}H_{22}O_9$
42.Wogonin	19,98	tr	$C_{16}H_{12}O_5$
44.Biochanin A	20,60	tr	$C_{16}H_{12}O_5$

High performance liquid chromatography (HPLC) analysis of *O. europea L* revealed a complex mixture of phenolic compounds: oleuropein (19,8%), luteolin-7-O-glucose (0,04%), apigenine-7-O-glucose (0,07%), quercetin (0,04%) and 0,02% of caffeic acid (Dekanski *et al*., 2009) where the caffeic acid was lower than in the present study.

Whereas for fenugreek, Shang *et al* (1998) identified five different flavonoids namely vitexin, tricin, naringenin, quercetin and tricin-7-O-β-σglucopyranoside to be present in fenugreek

seeds extract which showed significant antiradical and antioxidant properties, and were found to be concentrate-dependent. Also, four flavonoids and four phenolic acids were identified in the ethyl acetate extract by LC/MS analysis: kaempferol 3-O-glucoside, apeginin7-O-rutinoside, naringenin, luteolin, gallic acid, caffeic acid, p-coumaric acid and chlorogenic acid (Belguith-Hadriche *et al*., 2010). These results are in someway similar to the results of the present study in naringenin and quercetin.

Also, Benayad *et al* (2014) showed that the quantitative composition of the studied of fenugreek crude seeds was dominated by non-acylated flavonoid glycosides (48.60%), followed by acylated flavonoid glycosides (45.86%) and finally phenolic acids (5.55%). Apigenin was the major constitutive aglycon (61.30%), followed by luteolin (21.15%) and kaempferol (11.22%). This showed that the studied sample was dominated by flavone derivatives (83.20%). The results obtained demonstrated that acylated and non-acylated flavone derivatives, with apigenin as the main aglycon, dominate the phenolic composition of crude fenugreek seeds. Another research revealed that *Trigonella foenum-graecum* seeds contain considerable amount of isoflavones, lignans and coumestrol (Mital and Sirimavo, 2014). The different plant speices of the *T. foenum-graecum* were rich in flavones were in accordance with the plant studied.

In the present study, the dominant flavonoid in *E.globulus* were quercetin and catechin. Whereas, Dezsi *et al*. (2015) reported only phenolic acid in the *E. globulus* extract which was chlorogenic acid. Whereas, the patterns of flavonoids and flavonols indicate large quantitative differences. Hyperoside also, isoquercitrin, rutin and quercitrin were found in extract with highest amount.

III.4. Antioxidant activity of extracts: *In vitro* assays

III.4.1. DPPH scavenging activity of extracts

The extract is capable of donating an H atom to free radical this is determined by the decrease in its absorbance at 517nm. It is very convient to follow the DPPH reactions and it has often been used to estimate the antiradical activity of the natural products. The decrease in DPPH absorption in the presence of varing concentration of extract monitored gives the changes in the absorbance at 517nm, at different concentration of extract. The lower of IC_{50} value is the higher of free radical scavenging activity of a sample (Kaviarasan *et al*., 2007).

The free radical scavenging activities of all extracts of *Olea europaea L*. were in the following order: ethyl acetate extract (0.016±0.0012mg/ml)> methanolic extract (0.040±0.00010 mg/ml)> chloroform extract (0.052±0.0166 mg/ml)> aqueous extract (0.111±0.00092 mg/ml) compared to BHT (0.0318±0.00064 mg/ml). The ethyl acetate extract, which contained the highest amount of flavonoids and polyphenols, had the highest free radical scavenging activity. The scavenging effect of ME, ChE and EAE extracts showed no significant difference from BHT ($p>0.05$) this is probably attributed to their high phenolic componuds and flavonoids.

It was reported that the EC_{50} value of ethanolic olive leaf extract was 231.62mg of extract /ml. The effects of different concentration of extract were also approximately equal to that of butylated hydroxytoluene (ranging from 20 to 100 µg/ml) (Kaeidi *et al*., 2011). Talhaoui *et al*. (2014) studied DPPH scavenging activity of different varieties of olive leaves. They reported differences between these vareity. Moreover a high correlation was shown between radical scavenging activity and total phenolic content of olive leaves ($r=-0.9525$;$p<0.05$).

In the present study, *Trigonella foenum-graecum L* showed high activity of chloroformic extract with : IC_{50}=0.106± 8.88334E-05 mg/ml. Interestingly, free radical-scavenging activities of the ethyl acetate extract with 0.466± 0.0148mg/ml, aqueous extract were 1.092± 0.0120 mg/ml and the methanolic extract with 1.192± 0.0194 mg/ml (figure 20). Therefore, it is found that the effect of DPPH radical scavenging extracts of the plant is inferior to that of standards: BHT.

It was reported that the ability of the aqueous extract of fenugreek to neutralize the free radicals such as DPPH radicals. The extract at high concentrations showed significant decrease in the absorbance of DPPH radical. The IC_{50} value for the extract was found to be 350µg/ml (Kaviarasan *et al*., 2007). The presence of phenolic groups especially naringin and quercetin in fenugreek seeds could be responsible for radical scavenging activity (Kaviarasan *et al*., 2007). The antioxidant activity of aqueous, methanolic, hexanic, dichlro-methanic and ethyl acetate extracts of fenugreek were tested, but the ethyl acetate is not directly correlated with the total polyphenolics and flavonoid content of the extract, as both the methanol and ethyl acetate contained similar amounts of these compounds. Therefore, the total phenolic content is not the key to the "lipid effect". However, there might be qualitative differences in the composition of phenolic compounds in these extracts that could explain the differences. However, Belguith-Hadriche *et al*. (2010) reported four flavonoids and four phenolic acids in the ethyl acetate extract by LC/MS analysis: kaempferol 3-O-glucoside, apeginin7-O-rutinoside, naringenin, luteolin, gallic acid, caffeic acid, p-coumaric acid and chlorogenic acid. These results suggested that the protective effects, exhibited by ethyl acetate, are probably due to their flavonoid content, and particularly naringenin, which is the most abundant flavonoid in the ethyl acetate extract (38% of the total flavonoid).

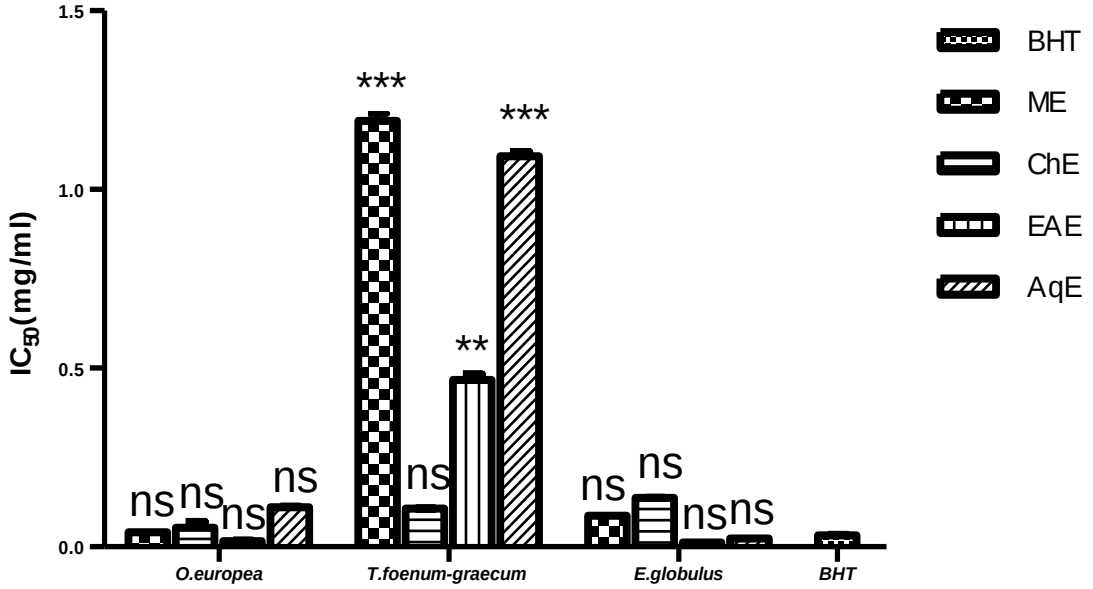


Fig.20 : IC_{50} values of different plants extracts in DPPH assay. Results are expressed as mean± SD (n = 3). (ns: no significant difference ; ** p<0.01; *** p < 0.001) compared to BHT as standard.

Concerning *Eucalypus Globulus L.*, results showed that an increase in extracts concentration resulted an increase in free radical-scavenging activity. For example, the ethyl acetate had the highest value of DPPH scavenging activity followed by aqueous extract, methanolic extract and chloroformic extract (figure 20). Therefore, free radical-scavenging activities of the extracts were comparable to the BHT.

Akolade *et al* (2012) showed that the oil exerted a concentration dependent radical scavenging activity and at lower concentrations, the activity of the oil was significantly lower when compared to the standard drug ascorbic acid. Boulekbache-Makhlouf *et al* (2012) reported that *E. globulus* bark (in Bejaia) at different concentrations of extracts (25, 50, 75, 100, 125 µg/ml), both crude extract and its fractions showed a dose-dependent anti-radical activity towards DPPH radical.

The antioxidant activity profiles obtained show that extracts of plants had a dose-dependent antioxidant activity, all the extracts and the commercial standards (BHT) depleted the initial

DPPH concentration by 50% within 1h especialy the ethyl acetate extract this due to their phenolic compounds such as flavonoids which can exhibit their antioxidant activity (AO) in several ways (Bombardelli and Morazzoni, 1993) :

(i) Radical scavenging activity toward either reactive species (e.g., reactive oxygen species: ROS) such as $^{\bullet}OH$, $O2^{\bullet -}$, $^{1}O_2$, or toward lipid perodizing radicals such as $R^{\bullet}$, $RO^{\bullet}$and $ROO^{\bullet -}$, radical scavenging action generally proceeds via hydrogen atom transfer or electron donation;

(ii) Prevention of the transition metal− catalyzed production of reactive species (i.e., via Fenton-type reactions) through metal chelation;

(iii) Interaction with other antioxidants (such as cooperative actions), localization, and mobility of the antioxidant at the microenvironment (Niki and Noguchi, 2000).

III.4.2. ABTS scavenging activity of extracts

ABTS assay is widely used for assessing the antioxidant capacity of both hydrophilic and lipophilic compounds, food products, extracts and biological fluids. The method is rapid and easy to perform, avoids unwanted reactions and does not require drastic conditions to generate radicals (Singh and Singh, 2008).

Figure 21, showed that EAE, AqE and ChE of *O.europea* exhibited the strongest antioxidant activity when compared to trolox (0.00258±0.00278) as a positive control (ns : not significant ; $p > 0.05$) while the ME showed a significant difference ($p < 0.05$): Me (0.088±0.081 mg/ml) ; ChE (0.034±0.034 mg/ml) ; EAE (0.0024±0.0019 mg/ml) ; AqE (0.0014±0.0065 mg/ml).

All the extracts of *T. foenum-graecum L* showed antioxidant activity (significant difference) comparing with the standard Trolox with IC_{50} values as follows : ME (0.196±0.245 mg/ml); ChE (0.205±0.092 mg/ml); EAE (0.09±0.005 mg/ml); AqE (0.40±0.054 mg/ml) (figure21).

Concerning the *E.globulus*, all the fractions are able to turn off radical ABTS or had a good ABTS radical-scavenging activities as shown in figure 21 wich are strong as the standard Trolox (0.0025±0.0027) with IC_{50}: ME (0.039±0.0007 mg/ml); ChE (0.040±0.035 mg/ml); EAE (0.047±0.0032 mg/ml); AqE (0.52±0 mg/ml) where the AqE are the powerful extract.

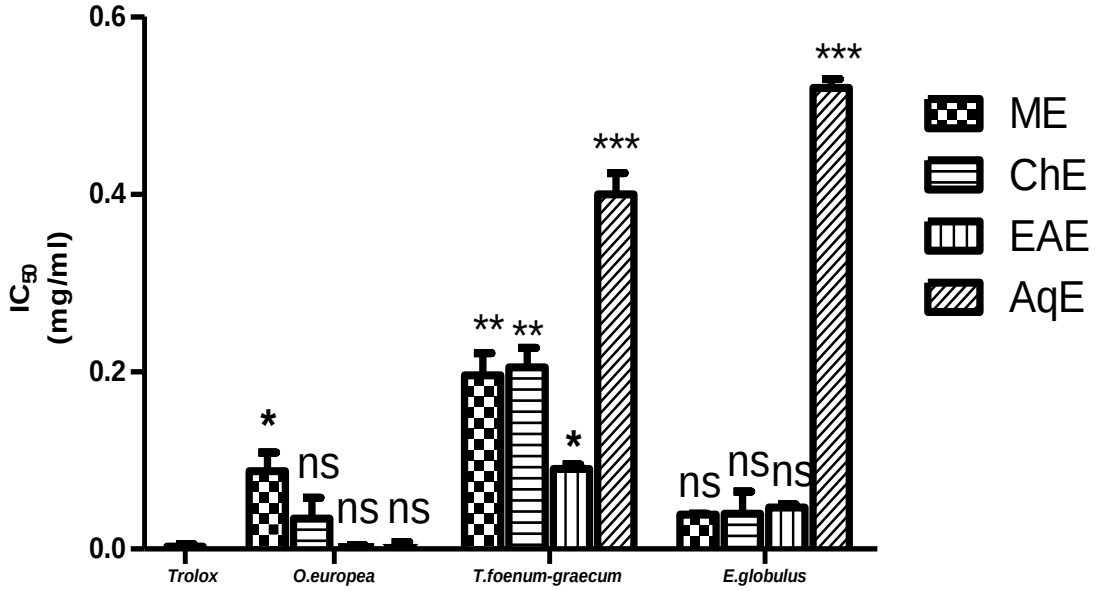


Fig.21 : Comparison between different plant extract and Trolox in ABTS free radical scavenging activity. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC50 means ± SD (n = 3). (ns: no significant difference; *p <0.05 ; ** p<0.01 ; ***p < 0.001 ; ns : not significant) compared to Trolox as standard.

As far as the results of the ABTS assay is concerned, olive leaf extracts submitted to the ABTS assay showed a value of 1.67 ±0.04 mM trolox equivalents/g dried leaves. This value compares well with that reported (1.52 mM trolox equivalents/g dried leaves) in previous study (Botsoglou and Botsoglou, 2010). It is olso in agreement with the study of Benavente-Garcia *et al.* (2000) who found a value of 1.58 mM trolox E/g dried olive leaves. According to these authors, this value was 72% higher than that theoretically calculated from the pooled average

ABTS values of the individual constituents of olive leaf polyphenolics, a finding suggesting that olive phenolics show a synergic behaviour in their radical scavenging capacity when mixed, as occurs in olive leaves. It may be of value to note that Benavente-Garcia *et al* (2000) found that olive leaves had an antioxidant activity higher than that of vitamin C and E with ABTS value of 1.12 and 1.10 mM trolox equivalents/g respectively and attributed this effect to synergy between of flavonoids, oleuropeosides and substituted phenols (Botsoglou *et al*., 2014).

Vani and Rajinder (2014) reported that methanolic extracts of fenugreek leaves of India have shown the maximum scavenging activity proving the samples to be excellent antioxidants with 0.85 mg/ml which was near to what be found in the present study.

Whereas for *E.globulus*, antioxidant molecules obtained from natural sources (plant extracts or essential oils), typically molecules with phenolic structures, are more often extracted by polar solvents, like alcoholic or aqueous mixtures of solvents. This fact can explain why the polar extracts of *E. globulus* presented higher antioxidant activity when compared to the ones obtained using n-hexane, a non-polar solvent (Ângelo *et al*., 2014).

III.4.3. Ferrous ion chelating activity of extracts

There are several ways of testing antioxidant activity of natural products, but it is believed that radical scavenging via hydrogen atom donation is the predominant mode. Other established antioxidant mechanisms involve radical complexing of prooxidant metals as well as quenching through electron donation and singlet oxygen quenching (Carlo *et al*., 1999).

Chelating agents may stabilize pro-oxidative metal ions in living systems by complexing them (Prieto *et al*., 1999 ; Pereira *et al*., 2009). Ferrozine can quantitatively form complexes with Fe^{2+} (Gülçin, 2006 ; Ak and Gülçin, 2008).

Our data indicated that the three studied plants are potential sources of secondary metabolites where methanolic extracts possess a good antioxidant activity. As seen in Figure 22, all extracts interfered with the formation of ferrous and ferrozine complex, suggesting that the extracts exhibited appreciable chelating activity. For the Olive-tree extracts, the chelating activities decreased as follows : methanol extract 0,066± 0,002 mg/ml> ethyl acetate extract 1,54± 0,357 mg/ml> aqueous extract 1,58± 1,27 mg/ ml> chloroform extract 6,425±0,029) mg/ml. Methanol, aqueous and ethyl acetate extracts were found to be as stronger as positive control, EDTA IC_{50}= 0.0073 ± 0.0001 mg/ml (no significant difference, P>0.05).

Concerning Fenugreek, it was observed that all extracts have the ability to chelate the ferrous ion less than EDTA (Figure 22). ME had an IC_{50} of 0,181± 0,039 mg / ml, followed by AqE extract (IC_{50} = 0,273± 0,012mg / ml), the ethyl acetate extract (IC_{50} = 0,593± 0,017 mg / ml). and the chloroformic extract (IC_{50} = 4,009±3,091mg / ml) compared to EDTA values (IC_{50} = 0.0073 ± 0.0001 mg / ml).

The chloroformic extract of the *Eucalypus Globulus L,* also showed greatest ability to chelate the ferrous ion (IC_{50} = 0,093±0,083mg/ml), followed by ME (IC_{50} = 0,258± 0,149mg/ml) and AqE, EAE (IC_{50}=0,573± 0,001mg/ml ; 6,144± 2,02mg/ml), respectively.

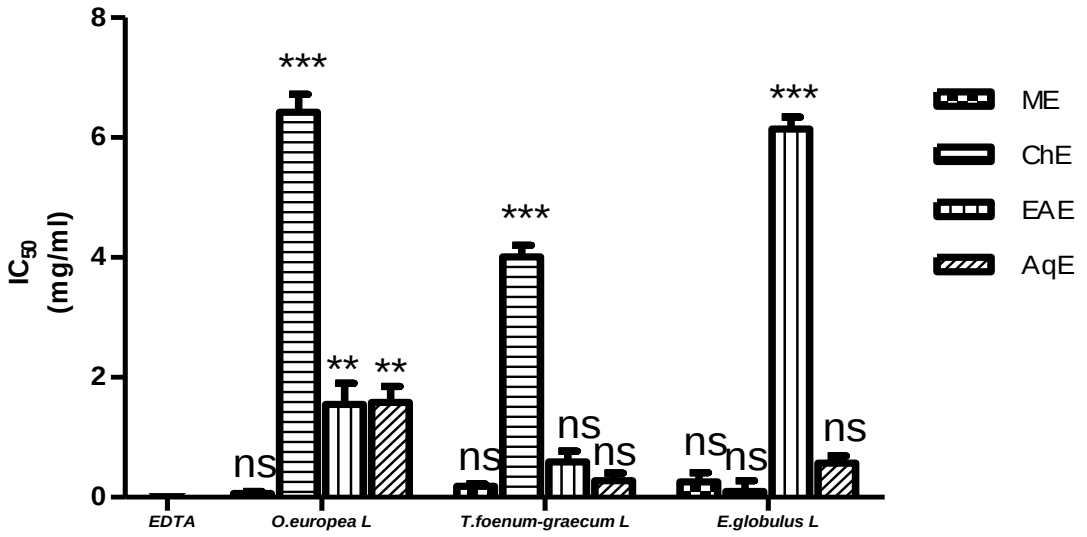


Fig.22 : Metal chelating activity of different plant extracts. Data were presented as means±SD (n=3). (ns: no significant difference; ***: P<0.001 ; **: P<0.01) compared to EDTA as standard; ME: Methanol extract; ChE: Chloroform extract; EAE: Ethyl acetate extract; AqE: Aqueous extract.

The IC_{50} value obtained for EDTA is very low (0.0073 ± 0.0001 mg / ml), which reflects the great ability to chelating the ferrous ion, this value is close to the value (0.0068 ± 0.13 mg / ml) obtained by Meziti *et al* (2012).

The difference in chelating activity was observed among the extract of fenugreek. The highest chelating activity was observed in ethanolic extract. Metal chelating capacity is significant since it reduces the concentration of catalyzing transition metal in lipid peroxidation. Moreover, the chelating agents, which form -bonds with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion, therefore it is an important parameter. The results from this parameter were in agreement with total phenolic contents, the highest chelating activity were found in ethanolic extract (1098 g/g of extract) while the lowest was for hexane extract (557 g/g of extract) (Syeda *et al*., 2008).

Our results showed that all extracts demonstrated an ability to chelate Fe^{2+} which means that they interfered with the formation of ferrous and ferrozine complex by capturing ferrous ion and form a complex with ferrozine. ME and AqE of extracts are lower than EAE in phenolic contents, but they appeared to be a considerably better chelator than of EAE and this finding was supported by the correlation coefficient (R^2=0.98) emphasizing its huge antioxidant potential.

Chelating agents may inhibit radical generations by stabilizing transition metals consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions (Zhao *et al*., 2008) for instance, flavonoids which form a stable complex with transition metals (Fe^{3+}, Al^{3+}, Cu^{2+}, Zn^{2+}); the stoichiometry of the complex and the site of chelation depend on the nature of the flavonoid mainly the presence of the catechol part (Le Nest *et al*., 2004) and the pH (Cornard and Merlin, $2002_{a,b}$). Moreover, this phenomenon of chelation is accompanied sometimes by the oxidation of the flavonoid (Cu^{2+}, Fe^{3+}). The chelation is occurred generally on the hydroxyl groups in position 3' and 4' of the B cycle, on the position 3 of hydroxyl group of A cycle and on the positions 3 and 4 of carbonyl group of C cycle (Fraga *et al*., 2010).

When flavonoids have several chelating metal sites, they can be polymerized. The copolymerization of the flavonoids and iron is responsible for anemia disease observed in large consumers of tea (Damas *et al*., 1985). The capacity of the flavonoids to complex metals is probably at the origin of the inhibition of many enzymes whose active site contains metals (Nijveldt *et al*., 2001).

III.4.4. Reducing power of plants extracts

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron

donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to ferrous form. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts.

Figure 23 showed the dose-response curves for the reducing powers of the extracts from *Olea europea L*. In this assay, the extracts showed a promising result. Reducing power of extracts and standard compound (BHT) were in the following order : BHT < ethyl acetate extract <methanolic extract< chloroformic extract< aqueous extract.

The reducing power of fenugreek showed that the chloroformic extract (ChE) (0.441±0.029 mg/ml) exihibited linear behaviour with concentration. The aqueous extract (AqE) was less active than ethyl acetate extract (EAE) (0.87± 0.011 mg/ml <0.603± 0.03 mg/ml respectively). The methanolic extract was less active than the others (1.429± 0.069 mg/ml) (figure 23).

Moreover, the extracts of the leaves of *E.globulus* showed a promising result in this assay (Figure 23), and the IC_{50} of extracts exhibited the following order: EAE (0,054± 0,001 mg/ml) < AqE (0,073± 0,0001mg/ml) < ME (0,08± 0,0005 mg/ml) <ChE (0,415±0,001 mg/ml). The EAE exhibited not significantly potent ($p>0.05$) reducing power when compared to BHT.

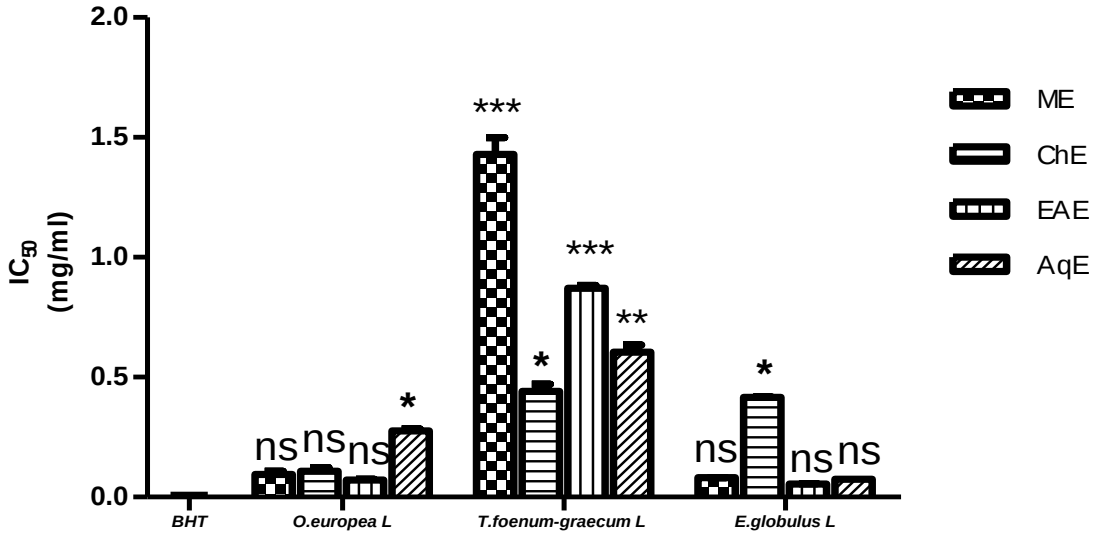


Fig.23 : IC_{50} of different extracts in reducing power assay. (ns: no significant difference; ***: P<0.001 ; **: P<0.01 ; *: P<0.05) compared to BHT as standard; ME: Methanolic extract; ChE: Chloroformic extract; EAE: Ethyl acetate extract; AqE: Aqueous extract.

An almost linear behaviour of the reducing power of *O.europea* with the ethyl acetate (EA) extract concentration was observed (Makris *et al*., 2007). Methanolic extracts from olive leaves showed a similar activity even when used at high concentration (Ferreira *et al*., 2007).

The reducing ability of the extract served as a significant indicator of its potential antioxidant activity. The ethanolic extract of *Trigonella foenum-graecum L* (EETFG) (wich contains alkaloids flavonoids, saponins, carbohydrates, proteins, and tannins) and standard (BHT) were used at dose range of 50- 800 µg/ ml. The reducing power of EETFG increased concentration dependently (Subhashini *et al*., 2011).

The reducing power of all the extracts increased with the increase in their concentration. However, among these extracts the ethanol extract of fenugreek has shown the highest reducing power. According to Syeda *et al* (2008) the fenugreek contained volatile oil, phenolic acids and flavonoids; therefore it is a potent source of antioxidants.

The reducing power of different concentration of *E.globulus* (25, 50, 75, 100, 125 µg/ml) compared with BHA and α-tocopherol. Reducing power of crude extract was found to be significant and dose dependent. All of the amounts of eucalyptus extract showed higher activities than that of α- tocopherol (0.53±0.01µg/ml) and these differences were statistically significant ($p<0.05$). Crude extract exhibited high reducing power (1.28±0.02 µg/ml) (Bourgou *et al*., 2008). Boulekbache-Makhlouf *et al* (2012) showed that $RC_{0.5}$ value of crude extract is about 40.74 µg/ml, significantly lower ($p<0.05$) than that of the standard α-tocopherol (117.76 µg/ml). However, our finding were higher than these results with (ME= 0.08mg/ml).

III.4.5. Antioxidant capacity of plants extracts using β-Carotene bleaching assay

In the β-carotene/linoleic acid assay, the antioxidant capacity is determined by measuring the inhibition of the organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. This assay has been used to simulate the oxidation of the membrane lipid components in the presence of antioxidants inside the cell (Coutinho *et al*., 2008).

The antioxidant activity of samples was reflected in their ability to inhibit the bleaching of β-carotene. In this assay, the chloroformic extract of *O.europea* possessed better antioxidant activity (73.67± 0.332 % of inhibition) but it did not reach BHT (85.78± 0.033%). Other extracts were also effective in inhibiting lipid peroxidation in this order: aqueous extract (67.72± 1.039 %), ethyl acetate extract (64.64± 0.1663%) and methanolic extract (54.17± 0%) (Figure 24).

The results obtained from extracts of *Trigonella foenum-graecum L.* seeds exhibited an antioxidant activity compared to BHT as a standard and presented in figure 24. The ethyl acetate extract was powerful than the other extracts with 70.98± 1164 % but lower than BHT 85.78± 0.033 %.

According to the the results which shows inhibition on lipid peroxidation in response to extracts of *E.globulus*. Both chloroformic and ethyl acetate extracts effectively inhibited the linoleic acid oxidation by 61.57±0.665 and 66.28±0%, respectively. In addition, methanol extract showed the higher inhibition percentage (84.14±0 %) compared to the BHT (85.78±0.0338%). Aqueous extract was not able to effectively inhibit the oxidation as much as the others extracts. It made only 38.52± 0.665% inhibition (Figure 24).

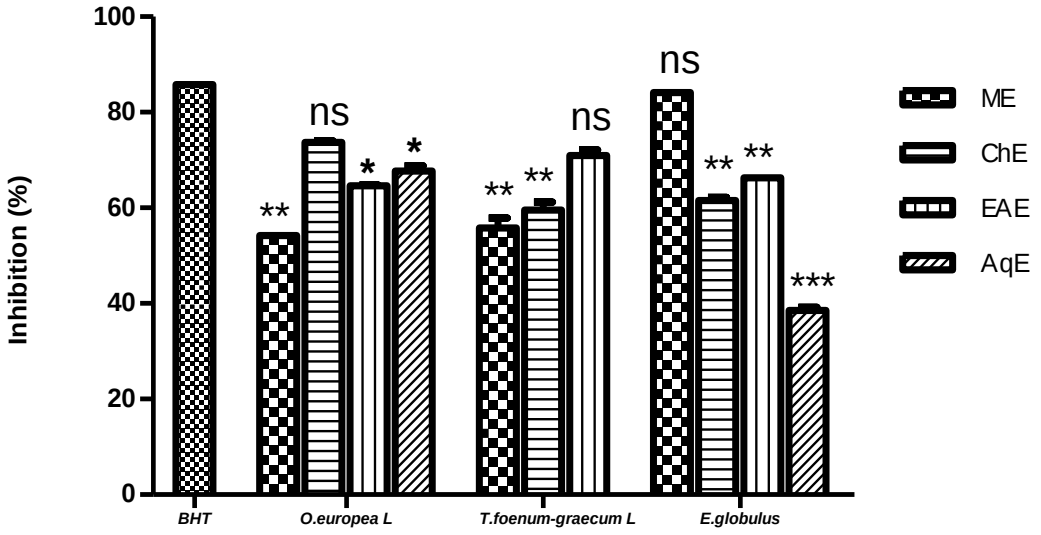


Fig.24 : Inhibition percentage of different extracts of plants in β-carotene/ linoleic acid assay after 24h (using BHT as standard).

Similar study shows that the hydrolysate of the ethyl acetate extract from olive leaves contained a relative high amount of hydroxytyrosol and was as effective as pure hydroxytyrosol and BHT as radical scavenger (Bouaziz and Sayadi, 2015), Synergistic action among flavonoids, oleuropeosides and substituted phenols present in olive leaf extract was reported (Benavente-Garcia *et al*., 2000).

Moreover, the antioxidant activity of fenugreek seed extract was evaluated by β-carotene method (Adegako *et al*., 1998). At 200µg, fenugreek seeds exhibited lower activity in

comparison to BHT. The bleaching capacity varied among different fenugreek fractions. IC_{50} values of fenugreek fractions were found to be 454µg (whole fenugreek extract). The differences among the samples were found to be extremely significant ($P<0.001$) (Madhava *et al*., 2011). These results were lower than our finding which could be explained by the difference between extracts and fractions and related in part to the type of compounds.

Generally, phenolic compounds with o-dihydroxyl group in aromatic ring possess stronger antioxidant activity than monophenolics (Rice-Evans *et al*., 1996). For example, caffeic acid reacted better with oxygen centered radicals than *p*-coumaric acid. Also, methoxy substitution of the hydroxyl group in the ortho position of the diphenolics, as in ferulic or vanillic acid, resulted into a decrease in the free radical scavenging reaction (Čanadanović-Brunet *et al*., 2007). According to literature data, (+)-catechin and (-)-epicatechin possess marked free radical scavenging activity. In both compounds the presence of *o*-dihydroxyl group in B-ring is fundamental for their scavenging properties, but *m*-hydroxyl groups at C5 and C7 in ring A also contribute to scavenging activity (Bouchet *et al*., 1998)

III.4.6. Antioxidant activity of extracts using Ferric thiocyanate (FTC) method

The Ferric thiocynate method determines the amount of peroxide produced at the beginning stage of Linoleic acid emulsion during incubation, which reacted with ferrous chloride to form ferric chloride which further formed ferric thiocynate (red pigment) upon reaction with ammonium thiocynate (Kim and Kim, 2010).

Iron(II) ion is known as a potent inducer of lipid peroxidation. It accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton type reaction (Gülçin, 2006; Ak and Gülçin, 2008). Using FTC method, the inhibition capacity of our extracts was measured against the oxidation of linoleic acid.

ME of *O.europea* exhibited good antioxidant potential with percent inhibition of (80.76 ±2.03%) compared with BHT (89.18± 1.88%). The result indicated that ME exerted marked effect on inhibition of linoleic acid oxidation, which was as strong as the positive reference, BHT. Other extracts showed good inhibition percentage ChE (77.53± 2.38%) ; EAE (78.11± 2.31%) ; AqE (62.67± 0.99%).

However, extracts of fenugreek exhibited an antioxidant activity ranged from 18.04 % to 73.97 %. For instance, ME and AqE showed a moderate inhibition (23.48±8.10 % ; 42.04±1.55 %) respectively. Whereas, EAE exhibited the powerful inhibition (73.97±2.75 %) while ChE showed significantly lower percent inhibition (18.04±8.68 %) (Figure 25).

In addition, the best activity of *E.globulus* extracts on lipid peroxidation inhibition was observed with ME, ChE and AqE (86.42± 1.43%; 88.96± 1.16%; 89.23± 0.28%) which were as strong as the positive control, BHT (no significant difference, p>0.05) (Figure 25).

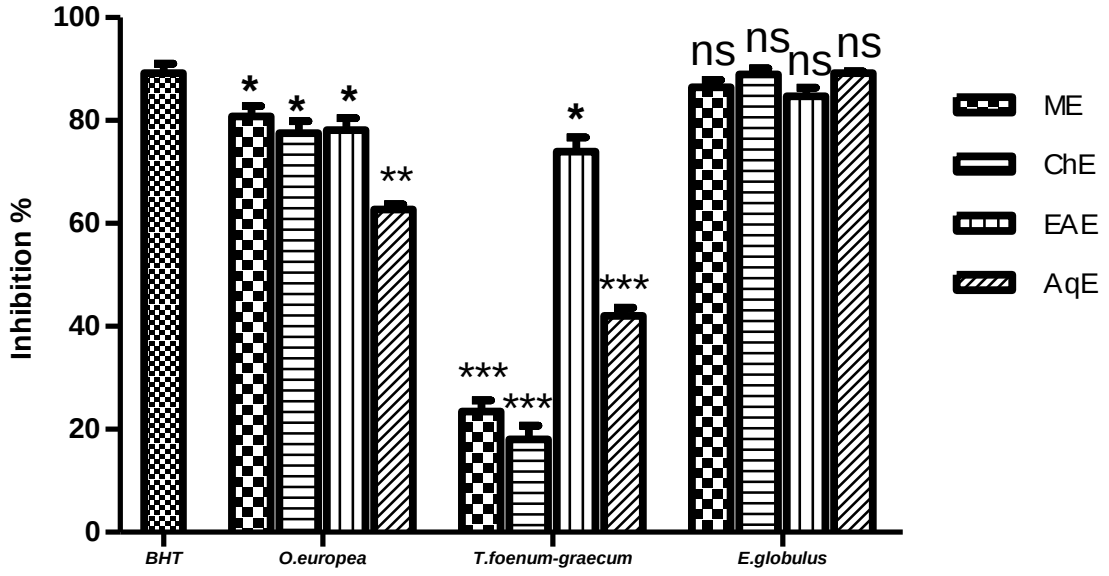


Fig.25 : Lipid peroxidation inhibition of extracts using FTC method. ME: methanolic extract, CHE: chloroformic extract, EAE: ethyl acetate extract, AqE: aqueous extract. BHT was used as reference antioxidant. Values are % means ± SD (n = 3). (ns: no significant difference ;*p < 0.05 ; ** : p<0.01; *** p < 0.001) compared to BHT as standard.

In this method, the concentration of peroxide decreases as the antioxidant activity increases. The highest percent inhibitions was shown in EAE for the three studied plants which means that EAE had a good antioxidant activity. Further more, flavonoids are known to be related to antioxidant activity (Stankoviec, 2001 ; Rezaeizadeh *et al*., 2011 ; Kaliq *et al*., 2015). Additionally, ME of *O.europea* and *E.globulus* exhibited a good antioxidant activity which have been reported to contain a significantly higher concentration of flavonoids (Stankoviec, 2001 ; Rezaeizadeh *et al*., 2011), especialy gallic acid which *In vitro* study also revealed the potent antioxidant effect of gallic acid (Punithavathi *et al*., 2011).

III.4.7. Antioxidant activity of extracts using TBA test

FTC is used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of oxidation. During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method (Mohd Zin *et al*., 2002).

This test is very commonly used both *in vivo* and *in vitro*. It involves reacting thiobarbituric acid with malondialdehyde produced by lipid hydroperoxide decomposition to form a red chromophore with peak absorbance at 532nm. This colored complex results in condensation of 2 moles of TBA and 1 mole of malondialdehyde (figure 26), under the joint effect of the medium temperature and pH. The first, is that malondialdehyde only forms from fatty acid chains containing at least three double bonds, like linolenic acid, to the eclusion of linoleic and oleic acid peroxide decomposition products (Dahle *et al*., 1962). Secondly, TBA is not specific to malondialdehyde because it can react with other aldehydes, browning reaction products, protein and sugar degradation products, amino acids and nucleic acid (Janero, 1990; Frankel, 1998).

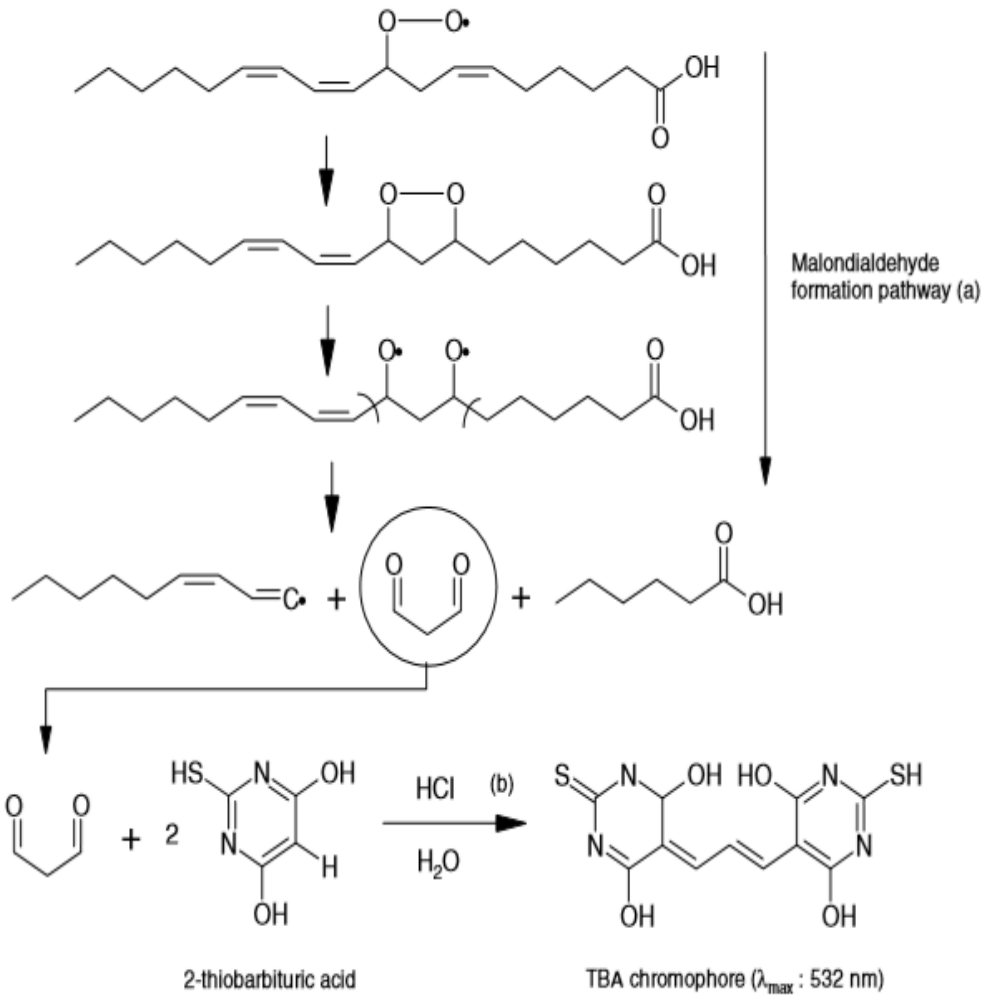


Fig.26 : Malondialdehyde formation pathway from peroxyl radical of triunsaturated C18 fatty acid (a) and formation of TBA chromophore from TBA and malondialdehyde (b) (Laguerre *et al*., 2007).

The inhibition of lipid peroxidation by *O. europea* extracts decreased in the following order: EAE (96.09 ± 1.89%) > CHE (94.26 ± 1.46%)>ME (94.26 ± 1.72%)> AqE (81.29 ± 0.83 %) compared to BHT (73.20±1.87%) as positive control which have a significant difference ($p < 0.05$; $p<0.001$) and more effective than BHT.

For fenugreek, all extracts inhibited the MDA formation in the following order : EAE (89.69±1.11 %)> ME (84.31±3.35%) > ChE (81.17±4.70%) > AqE (80.62±1.42%) (Figure 27).

Moreover, *E.globulus* and their percentage inhibition of MDA formation were approximately equal with the following percentage : ME 97.37±0.63% ; ChE 97.20±0.23% ; EAE 97.47±0.97% and AqE 96.24±0.39% (Figure 27).

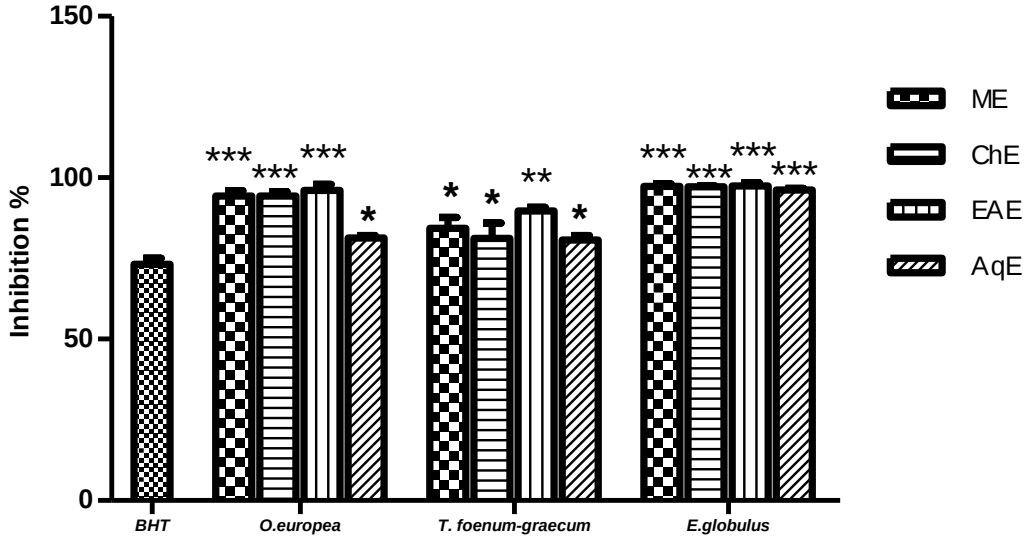


Fig. 27: Antioxidant activities of different plant extracts (2 mg/ml) measured by TBA method. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. BHT was used as reference antioxidant. Values are % means ± SD (n = 3). (ns: no significant difference ,*p < 0.05, ** p < 0.01, *** p < 0.001) compared to BHT as standard.

In contrast, the antioxidant activities of standards (BHT) seen to be higher in FTC assay compared to TBA method. This may indicate that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide in the secondary stage as reported by Rahmat *et al*. (2003). However, the result negatively correlated to samples extract. This also shows that the antioxidant activity detected with the FTC method was lower than that detected with the TBA method for samples extract. It is highly possible that several compounds of different polarity may contribute to the antioxidant activity of plants extracts (Mohd Zin *et al*., 2002). In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad

spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 1995).

III.5. Antidiabetic and antioxidant activity : *in vivo assays*

It has been reported that, ingestion of medicinal plants or drugs can alter the normal hematological values (Ajagbonna *et al*., 1999). Therefore, hematological parameters could be an important tool in the assessment of deleterious effect of drugs, as well as medicinal plants (Yakubu *et al*., 2007). Mansi and Lahham (2008) revealed that various hematological parameters and the immune system were reported to be altered during the course of diabetes. Thus, the intention of the present study was to scrutinize the influence of oral administration of the three plants extracts on the levels of hematological parameters including: hemoglobin (Hb), white blood cells (WBC), platelets (PLT), red blood cells (RBC)).

RBC count of untreated or diabetic rats ($7.53 \pm 0.58 \times 10^6/mm^3$) showed a high significant decrease ($P < 0.001$) as compared to normal control rats ($9.22 \pm 0.9 \times 10^6/mm^3$). All the treatment with different extracts showed a detectable elevation of RBC count in diabetic rats (Table 4). The recorded values of diabetic rats showed a significant ($P < 0.05$) decrease in blood hemoglobin content (11.7 ± 0.8 g/dl) as compared with the normal control rats (14.3 ± 0.02 g/dl). Treatment with the three extracts produced no significant change of the lowered blood hemoglobin content of diabetic rats; the values being (13.6 ± 0.27 and 13.8 ± 0.2 g/dl ; 13.75±0.55 and 13.8±1.10 g/dl ; 13.98±2.04 and 14±0.88 g/dl) for *O. europea ; T.foenum-graecum* and *E.globulus* respectively (Table 4). Concerning white blood cell count the present data showed a notable significant decrease ($P < 0.01$) in untreated group when compared with control group (Table 4).

The results showed that PLT changed slightly compared to control group. Glibil treated group were reported to have measurable effect on blood PLT (185 ± 0.72 $x10^3/mm^3$), Whereas,

Untreated group remained the lower values (175±2.88 x10^3/mm^3). As conclusion, the inclusion of the different extracts resulted in increased levels near to normal level (Table 4).

Table 4: Mean values of blood parameters in treated and untreated rats

	Control	*O.europea* (200 mg/kg)	*O.europea* (600 mg/kg)	*T.foenum-graecum* (200 mg/kg)	*T.foenum-graecum* (600 mg/kg)	*E.globulus* (150 mg/kg)	*E.globul* (500 mg
RCB 10^6/mm^3	9.22 ± 0.9	8.11±0.56**	8.63± 2.96**	8.49±0.23**	8.88±0.67 ns	8.95±1.36 ns	9.06±2.7
PLT 10^3/mm^3	230± 0.08	184± 0.5 **	193.45±0.65*	180± 1.8 **	196± 0.4 *	204± 1.87 ns	207.4±1.
GB 10^3/mm^3	12.1 ± 0.14	10.4±0.23 ns	11.87±1.02 ns	10.7 ± 0.09 ns	11 ± 1.8 ns	11.5±1.03 ns	11.96±1.9
HGB g/dl	14.3 ± 0.02	13.6 ± 0.27 ns	13.8 ± 0.2 ns	13.75±0.55 ns	13.8 ± 1.10 ns	13.98±2.04 ns	14 ± 0.88

Value are mean ± SEM (n=6) (ns: no significant difference,*p < 0.05, ** p < 0.01, *** p < 0.001) compared to control group.

The link between chronic diseases and anemia is well characterized (Weiss and Goodnough, 2005). The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (Oyedemi *et al*., 2011). Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun and Ramesh, 2002). The major pathological consequences of free radical induced membrane lipid peroxidation include increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity (Kolanjiappan *et al*., 2002). The decrease in MCH and MCHC values, observed after administration of STZ, is an indication of abnormal

hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolarity (Stookey *et al*., 2007). Following the three plants extracts administration, the level of RBCs and its related indices were appreciably improved. This gives an indication that flavonoids in this extracts can stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2012). The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC (Abu-Zaiton, 2010). These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. It has previously reported that the action mechanism of flavonoids may be attributed to their ability to lower lipid peroxidation level that causes haemolysis of erythrocytes (Mahmoud *et al*., 2012).

Gallic acid was a compound of the studied plants where Punithavathi *et al* (2011) demonstrated that the oral treatment with gallic acid (10 and 20mg/kg) daily for a period of 21days showed significant ($P<0.05$) protective effects on all the biochemical parameters studied.

III.5.1. Extracts effects on blood biochemical parameters

Increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltranspeptidase (GGT) are indicators of hepatocellular injury. Increased activity of these markers is associated with insulin resistance (Marchesini *et al*., 2001), metabolic syndrome, and type 2 diabetes (Sattar *et al*., 2004; Nakanishi *et al*., 2005; Wannamethee *et al*., 2005).

In the present study, we noticed high levels of cholesterol and triglycerides in untreated rats (0.67 ± 0.09 g/l and 1.67 ± 0.25g/l) respectively (Table 5). Chronic hyperglycemia induces carbonyl stress, which in turn can lead to increased lipid peroxidation. The same thing, was

observed for ALT where it increased significantly ($P < 0.001$) in rats untreated (79.3 ±0.46 UI/l). Rats treated with extract (200 mg/kg) of *O.europea* showed no significant in total cholesterol and ALT (0.54 ± 0.06 g/l and 51.65±0.21 UI/l) respectively. The olive leaves extract was found to be more effective than glibenclamide.

Concerning triglycerides, both doses of *E.globulus* and *O.europea* showed no change. Whereas, in cholesterol level, each of 200 mg/kg of olive tree and 600mg/kg of fenugreek and also dose of 150 mg/kg of *E.globulus* decreased the level (Table 5).

Morever, the both doses of *E.globulus* decreased the level of HDL and LDL. Also, both doses of *E.globulus* and *T.foenum-graecum* decresed Albumin level. Each of AST and Uric acid were decreased by the 600mg/kg of *O.europea* and dose of 500 mg/kg of *E.globulus* (Table 5).

Table 5 : Biochemical parameters in blood of treated and untreated animals

	Control	***O.europea*** **(200 mg/kg)**	***O.europea*** **(600 mg/kg)**	**T.*foenum-graecum*** **(200 mg/kg)**	**T.*foenum-graecum*** **(600 mg/kg)**	***E.globulus*** **(150 mg/kg)**	***E.glob*** **(500**
Lipidic metabolism							
Triglycerides g/l	1.26 ± 0.28	1.26± 0.33 ns	1.29 ± 1.07 ns	1.32 ±0.41**	1.36±0.74***	1.27± 0.46 ns	1.28 ±
Total cholesterol g/l	0.53 ± 0.02	0.54 ±0.06 ns	0.58 ± 0.08 **	0.58 ± 0.05**	0.55±0.12 ns	0.54 ±0.08 ns	0.59 ±
LDL g/l	0.05 ± 0.01	0.07±0.03 ns	0.08 ± 0.01 *	0.06 ± 0.01 ns	0.08 ± 0.02 *	0.05 ±0.03 ns	0.065
HDL g/l	0.64± 0.03	0.78 ±0.16 **	0.72 ±0.10 **	0.67 ± 0.08 *	0.68 ±0.09 *	0.65 ±0.10 ns	0.66 ±
Protein							
Albumin g/l	37.6 ± 0.72	40.66±0.11 *	39.4 ± 0.12 *	37.8 ± 0.12 ns	38 ± 0.33 ns	38 ± 0.6 ns	37.66
Hepatic Bilan							
AST UI/l	128.06±0.21	134.5±0.32 **	129.33±0.31 ns	136.54±0.008***	133.36±0.1**	132.32±1.13**	129.9
ALT UI/l	51.48 ± 0.93	51.65±0.21ns	56.35± 0.91 **	51.5 ± 0.63 ns	56.86 ±1.86 **	57.26 ±1.72 **	53.43
Renal fonction							
Uric acid mg/l	4.2 ± 1.04	4.38 ± 1.28 ns	4.3 ± 1.6 ns	4.5 ± 0.66 *	4.31 ± 0.16 ns	4.28 ± 0.72 ns	4.26 ±

Creatinine mg/l	2.4 ± 0.48	3.4 ± 0.48 **	3.66 ± 0.44 ***	2.8 ± 0.32 ns	3 ± 0.02 ns	3.4 ± 0.48 **	3.33 ±

Values are mean ± SEM (n=6) (ns : no significant difference ; ,*p < 0.05, ** p < 0.01, *** p < 0.001).

Similar studies indicated that the olive leaves extract decreased significantly serum glucose, triglycerides, cholesterol, urea, uric acid, creatinine, AST and ALT while it increased the serum insulin levels in treated diabetic rats compared with the control diabetic rats (Jouad *et al*., 2001; Alarcon-Aguilar *et al*., 2002; Verspohl, 2002).

The levels of plasma lipids are usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease (Prince *et al*., 1999). Lowering of plasma lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Rhoads *et al*., 1976). The results showed that the extracts exhibited a significant decrease in the level of serum lipids in diabetic rats. Consistently, other studies have reported that *Olea europaea* has hypolipidemic effects in diabetic rats (Somova *et al*., 2003). Some studies have demonstrated that oral administration of eucalyptus exhibited a significant, dose-dependent hypoglycemic effect in streptozotocin diabetic rats (Jouad *et al*.,2003; Abeeleh *et al*., 2009).

The data showed that the uric acid levels were increased in diabetic rats. This may be due to a metabolic disturbance in diabetes reflected in the high activities of xanthine oxidase, lipid peroxidation and increased triglycerides and cholesterol (Madinov *et al*., 2000). Moreover, protein glycation in diabetes may lead to muscle wasting and an increased release of purine, the main source of uric acid as well as in the activity of xanthine oxidase (Anwar and Meki, 2003). Creatinine is a metabolite of muscle creatine, whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant, so that elevated levels indicate diminished renal function only, since it is easily excreted by the kidneys (Enogieru *et al*., 2015). It has been hypothesized that a low muscle mass reflects a low serum creatinine levels in diabetics (Harita *et al*., 2009; Hjelmesath *et al*., 2010).

The present data showed that plant extracts especially *E.globolus* extracts decreased the serum urea and creatinine levels in diabetic rats. Elevation of the serum urea and creatinine, as significant markers, are related to renal dysfunction in diabetic hyperglycemia (Almadal and Vilstrup, 1988). Serum enzymes including AST and ALT are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels (Hultcrantz *et al*., 1986).

In accordance with these findings, streptozotocin treatment has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal values.

Intraperitoneal (IP) injection of quercetin, which found in the studied plants, into STZ-induced diabetic rats led to decreased hyperglycemia and improved glucose tolerance, increasing hepatic glucokinase activity and reducing plasma cholesterol and triglycerides (Vessal *et al*., 2003). As a result, it may be concluded that the extracts is more effective in comparison with glibenclamide in attenuating the increased serum parameters resulting from damage of STZ-induced diabetic rats.

III.5.2. Plasma antioxidant capacity using DPPH radical

DPPH is very useful tool for the study of natural antioxidants (Villano *et al*., 2007). DPPH or other simple test system for screening of a set of compounds or products (for example, plant extracts) can be used and an active compound (extract) chosen for a final test on the real product. Analysis of clinical samples (usually blood plasma) requires more caution. The results obtained in simple as well as complicated antiradical and antioxidative activity test systems usually correlate poorly with the data on the physiological activity of the compounds. A hot current question is whether or not the radical-scavenging (or antioxidant) activity is responsible

for the action of many drugs as well as for the activity of health improving products, or is it only a side effect of these compounds of no relevance to their biological effects ? In many cases the latter possibility appears to be true, as demonstrated by large epidemiologic studies (Huang *et al*., 2006; Bardia *et al*., 2008).

The administration *per os* of ME of *O.europea* at doses of 200 and 600 mg/Kg in rats increased significantly the plasma antioxidant capacity with values of 22.95 ± 1.19 % and 23.46 ± 0.96 %, respectively compared to control group (14.19 ±1.57 %).

However, each of ME of Fenugreek and *E.globulus* showed significant differences wether the percentage were increased (23.74±1.11 : 18.78±0.16 ; 26.64±0.42 %) or at the dose 600mg/kg of fenugreek which increased (16.34±0.16%) and they were found that these doses were as stronger ($p > 0.05$) to raise the antioxidant capacity in plasma comparing with the control (14.19±1.57%).

The group treated by Glibil showed no significant difference with inhibition percentage of 15.43±0.85% where untreated group showed 7.25±0.66%

Predominately, methonolic extracts of plants have good antioxidant capacity to increase plasma DPPH radical scavenging capacity rather than the drug Glibil.

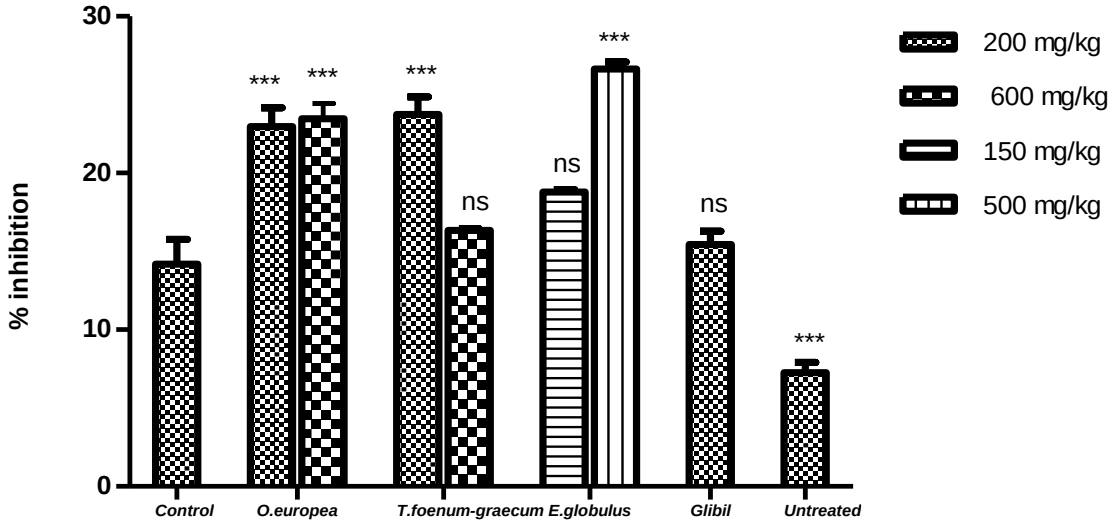


Fig.28 : Antioxidant capacity of plant extracts using DPPH radical in plasma. Data were presented as % means ± SEM (n = 6). (ns: no significant difference ; *** $p < 0.001$) compared to control group.

III.5.3.Assessment of plasma reducing power in treated and untreated animals

The reducing power, an other assay to determine the antioxidant activity in plasma. As shown in the figure 29, all extracts had dose-dependent activity. Both doses of *O.europea* and *E.globulus* showed alterations of plasma reducing power (significant difference $p < 0.001$) with values of 0.96±0.02 ; 0.97±0.02; 0.89±0.07 ; 1.04±0.03 respectively even when compared to untreated group 0.35±0.05 which had the lowest value of reducing power and were so significantly difference with control group (1.04±0.03).

However, the administration of doses fenugreek of increased the plasma reducing power with the values : 0.78±0.08 ; 0.91±0.01. Also the Glibil showed high diference (0.53±0.07).

It wa reported that the relation ship between DPPH assay, reducing power and plant extracts are related to the contents of phenolic compounds in plant extracts (Rebaya *et al*., 2014). In our study, we could classify plants effectiveness *in vivo* assays on DPPH radical and reduving power as follows : *E.globulus* > *O.europea* > *T.foenum-graecum.*

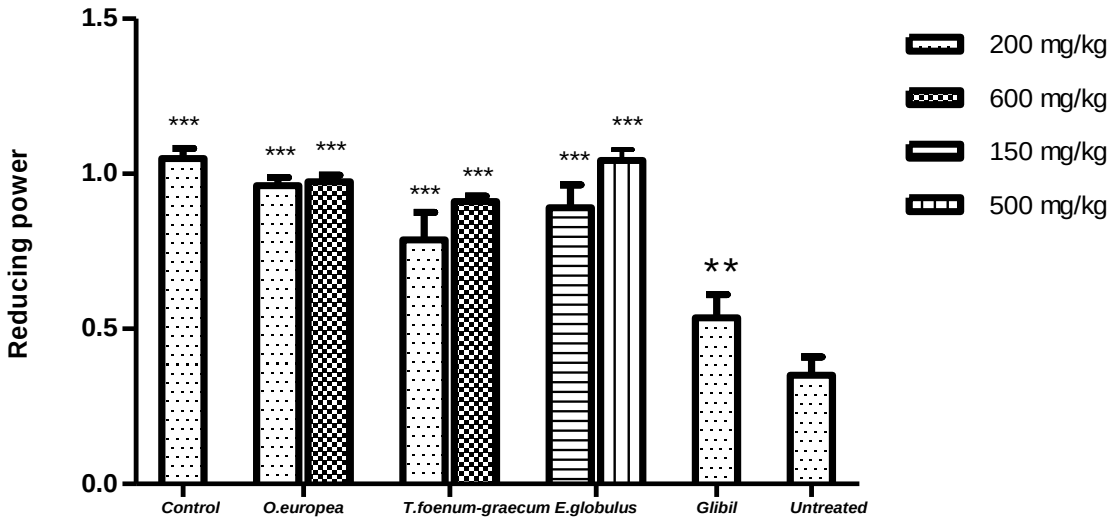


Fig.29 : Antioxidant capacity of plant extracts using reducing power in plasma. Data were presented as means ± SEM (n = 6). (** $p < 0.01$; *** $p < 0.001$) compared to untreated group.

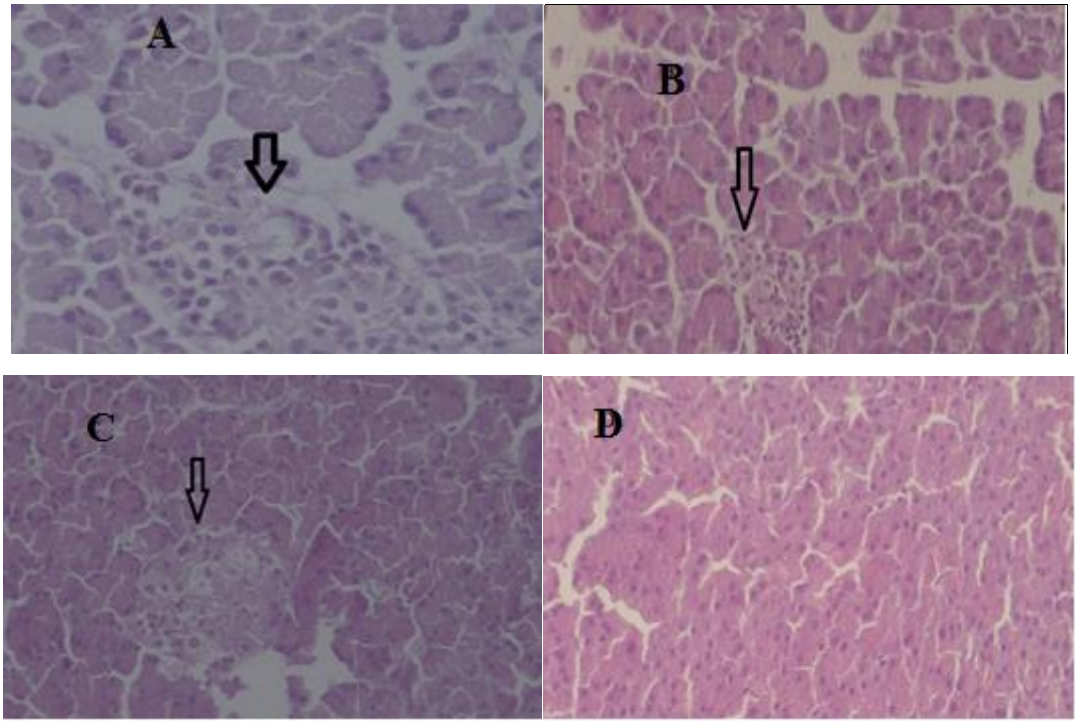
III.5.4. Histopathological examination of Liver, Kidney and Pancreas

Present observations on the kidney and liver sections showed progressive damage associated with the severity of hyperglycaemia. Severe hyperglycaemia induced by the streptozotocin caused the renal and hepatic damage. Diabetic kidneys and livers as observed in this study were prone to derangements. The changes at the molecular level impacted on the gross architecture of the kidney and liver tissues.

As shown in figure 30, no histological changes showed in pancreatic tissue of control group (figure 30-E), however the pancreas were disappeared in groups (figure 30: A and D) were the destruction of Islets of Langerhans. Whereas, sections of pancreas from the treated animals groups (figure 30: B and C) showed stearky inflammation by lymphocytes in the Islets region. In addition for the groups (figure30: F and G), there was some dystrophic Islets of Langerhans.

Streptozotocin induces various pathological alterations in the liver of rats. These alterations were characterized by centrilobular congestion, portal vein congestion, destruction of

membrane cells, lymphocyte infiltration, sinusoidal congestion (Figure 31-3). In combination group were the three plants extracts was administered, showed reparative changes (figure31 : A, B, F, G, H and I) where liver showed prominent recovery in the form of normal hepatocytes and very less centrilobular congestion and pronounced sinusoid with granular hepatocytoplasm were also evident. Liver of the control group had a regular histological structure with a characteristic pattern of hexagonal lobules (Figure 31-E). Furthermore, the high histological alterations were observed in the liver of the untreated group (Figure 31-D) where focal lymphocyte cells infiltration was abundant.



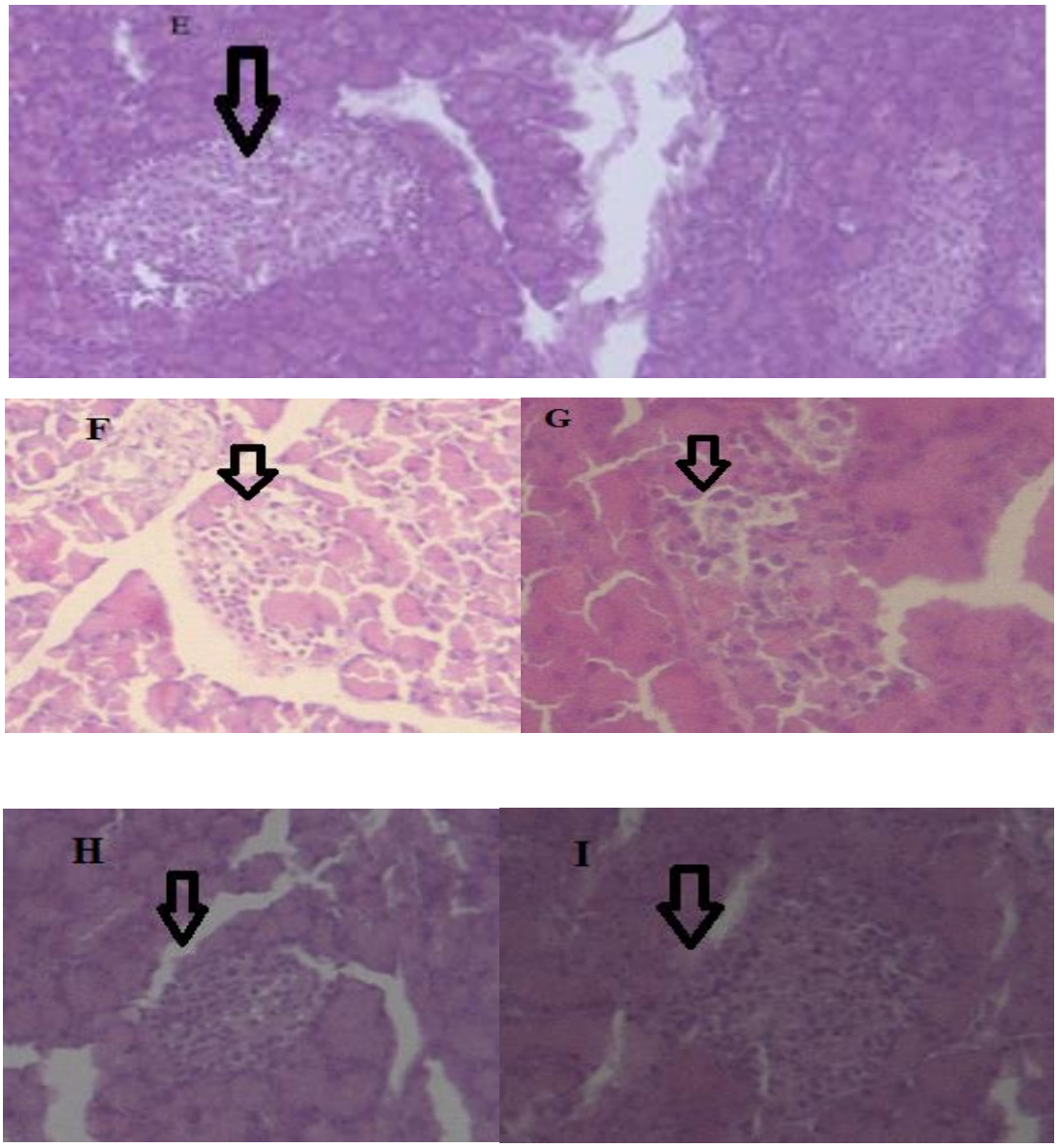
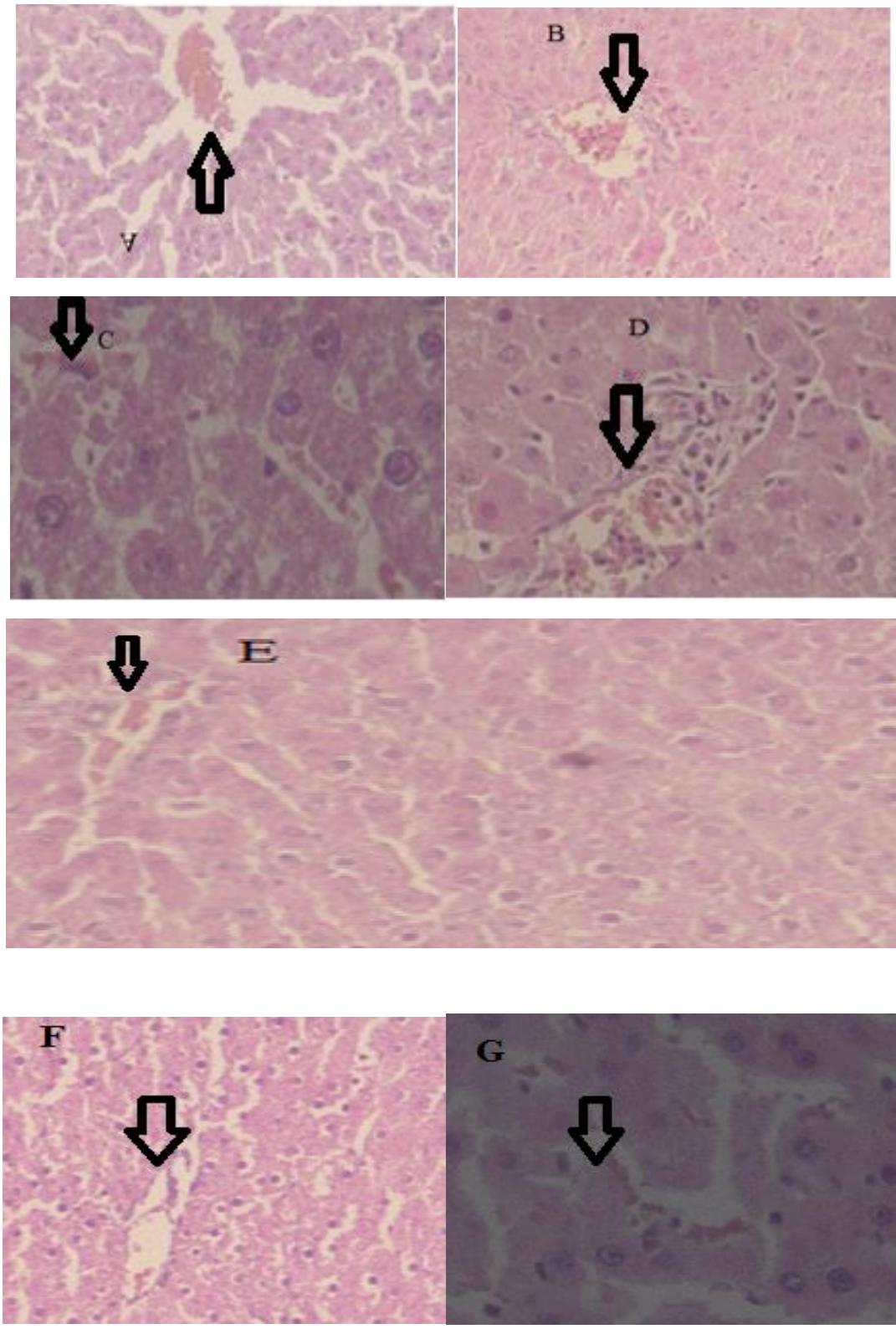


Fig. 30: Microphotographs of pancreatic islets in rats treated with MEO after 18 days of the treatment. Severe islet cell necrosis is observed, X20. A : IL infiltrated by lymphocytes from the group (MEO: 200mg/kg+STZ).B : seige inflammatory cells infiltration from group (MEO: 600mg/kg+STZ). C: IL infiltrated by lumphocytes group (Glibil+STZ). D : Absence of IL (Untreated group). E : Normal IL (Control group). F : some dystrophic IL (MET : 200 mg/kg+STZ). G : some IL (MET : 600 mg/kg+ STZ). H : dystrophic IL (MEE :150mg/kg+STZ). I : only one IL (MEE :500mg/kg+STZ).


B
C
D
E
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G

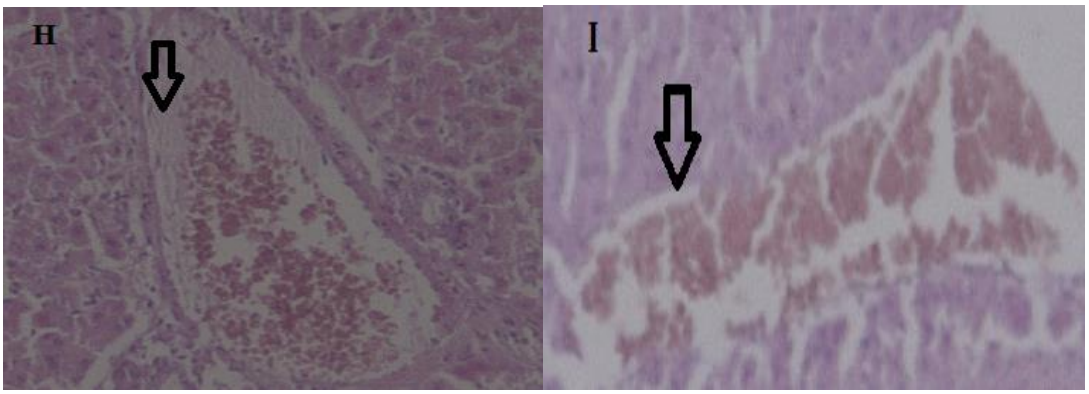
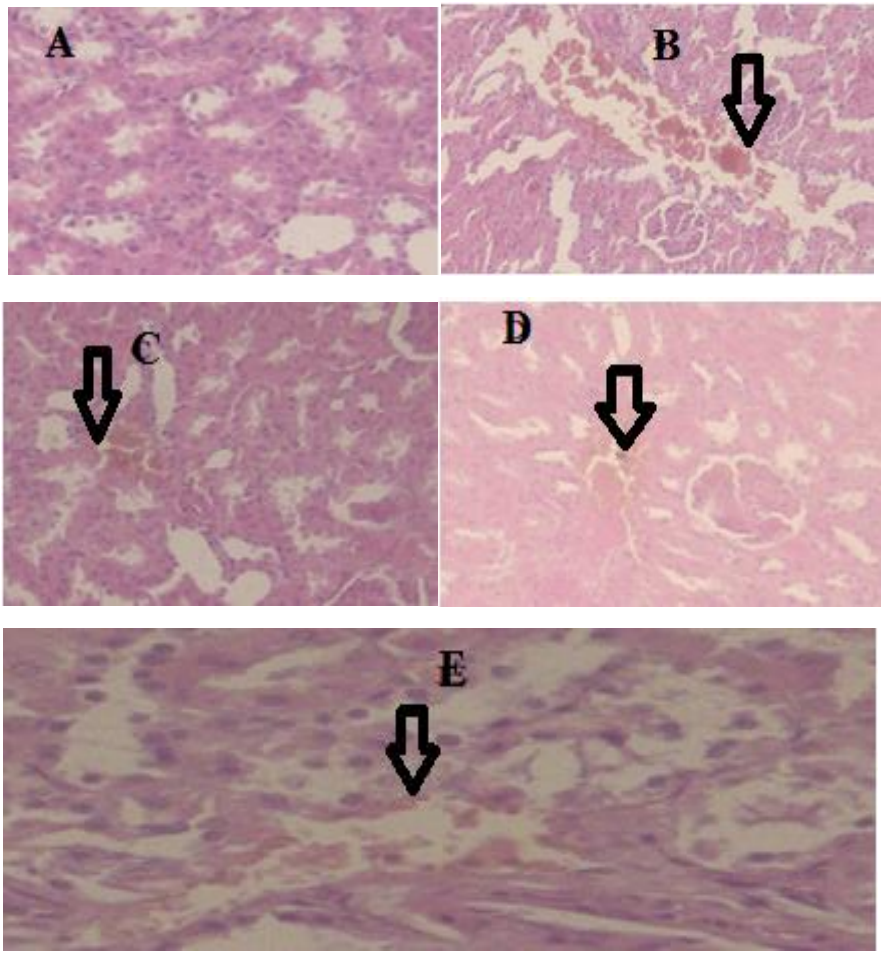


Fig.31: Photomicrographs of liver sections, X 10. A: normal hepatocytes showing normal architecture congestion in VCL (MEO: 200mg/kg +STZ group). B: Congetion in portal vein (MEO: 600mg/kg +STZ group). C: Congestion in VCL (Glibil +STZ group). D: lymphocyte infiltration (Untreated group). E: normal hepatocytes congested (Control group). F: Congestion (MET: 200 mg/kg+STZ). G: sinusoidal congestion (MET: 600 mg/kg+STZ). H: Congestion in portal vein (MEE: 150mg/kg+STZ). I: Congestion in VCL (MEE: 500mg/kg+STZ).



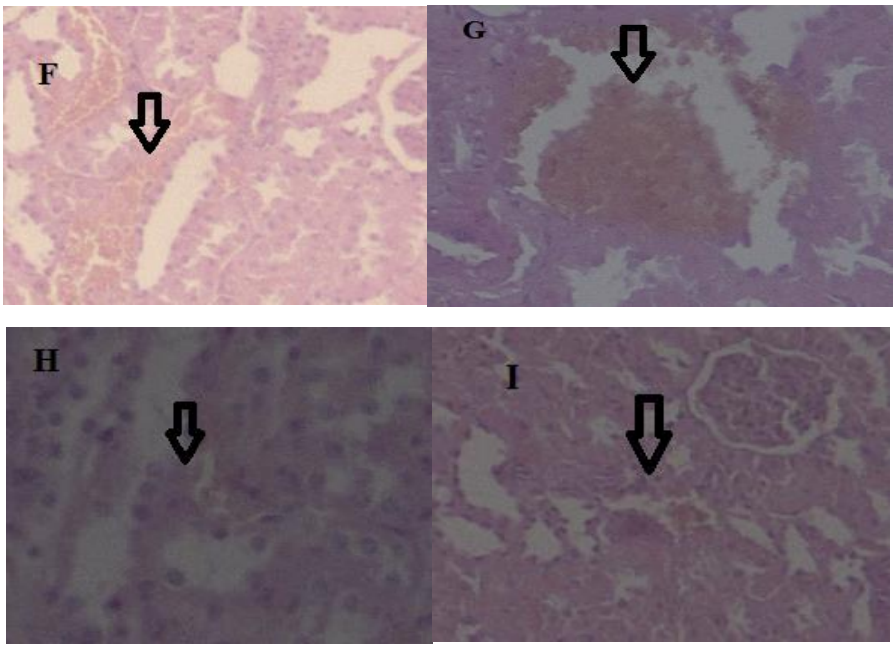


Fig.32 : Photomicrographs of kidney sections, X10. A : retain normal (MEO: 200mg/kg +STZ group), B : cortical edema and congestion (MEO: 600mg/kg +STZ group), C : cortical congetion (Glibil +STZ group), D : cortical congestion (Untreated group). E : discrete cortical congestion (Control group). F : Cortical congestion (MET : 200mg/kg+STZ). G : Cortical glomerular congestion (MET: 600mg/kg+STZ). H: Modular congestion (MEE: 150mg/kg+STZ). I : Cortical congestion (MEE: 500mg/kg+STZ).

The examination of rat kidney revealed the degeneration of the glomerular capsule and cortical congestion in the untreated diabetic group. These changes were reversed following plants extracts treatment. These pathologic changes were probably induced by the oxidative stress associated with diabetes. Jimoh and Odutaga (2004) reported alterations and disintegration of the glomeruli of kidneys as a consequence of free radicals generated by thermo-oxidised lipids. It is likely that these pathological changes led to disruption in filtration and concentration of urine, as well as fluid and electrolyte balance, as was observed in biochemical parameters. Administration of plants extracts in this study, reversed the antioxidant and biochemical changes associated with oxidative stress-hyperglycemia, hence the corrective measure observed in the histology of the kidney.

In diabetic rats, as result of acute hyperglycemia, kidney damage was observed through various degrees of congestions, hemorrhage, and necrosis in glomeruli, tubules and interstitial matrix with dilatation. This is because of several metabolic, functional and structural changes of

kidneys in streptozotocin induced-diabetic rats were found to have many fundamental similarities to those occurring during human diabetic nephropathy and this model has been used extensively in diabetes research to evaluate the pathogenesis of kidney in diabetic (Gaed *et al*., 2004). These structural changes in kidneys of diabetic rats are supported by others studies, where researchers observed that the first histopathological changes of kidneys during acute hyperglycemia are characterized by enlargement of glomerular mesangium due to increase of extracellular matrix synthesis that leads to glomerulisclerosis and tubule-interstitial fibrosis, basement membrane thickening, increasing endothelial cell permeability to albumin and increasing hyper-filtration that cause damage to tissues and blood vessels as end stage in diabetic renal disease (Masons and Wahab, 2003).

Histopathological examinations demonstrated mild to moderate inflammation of the hypotocytes. ROS and lipid peroxidation cause direct damage to hepatocytes by disrupting membranes, protein, and DNA (Peterson *et al*., 1971). In the STZ-induced diabetic animals, lower levels of activities of endogenous antioxidant enzymes such as SOD and CAT were noticed. Subsequently, these reductions can cause tissue degradations (Chang and chuang, 2010). These findings provid preliminary biochemical and histological support to the ethno medicinal uses of plants extracts in the management and/or control of diabetes mellitus. Where Histopathology of pancreas confirmed the protective effects of gallic acid in diabetic rats (Punithavathi *et al*., 2011).

A number of studies demonstrated the direct action of quercetin, which is a compound of the studied plants, on insulin-secreting β cells (Dai *et al*., 2013). Both quercetin and its glycoside derivatives improved glucose-stimulated insulin secretion and repressed oxidative stress and nitric oxide accumulation by regulating NF-κB and ERK 1/2 to protect INS1 cells and clonal pancreatic β cells. Taken together, quercetin is an effective biomolecule that acts on obesity and diabetes by inhibiting the digestion of intestinal starch and hepatic glucose production,

increasing glucose uptake in the skeletal muscle, and protecting against pancreatic islet damage (Mohammed Kawser *et al*., 2016).

III.5.5. Effect of different extracts on glucose loaded rats

As shown in table 6, we recorded an increase in the blood glucose in the first hour after the treatment with the two doses of 200 mg/kg and 600mg/kg of MEO (4.53 to 4.75g/dl and 4.33 to 4.52 g/dl) and decreased at the second hour (from 4.75 to 3.08 g/dl and 4.52 to 3.94 g/dl). A diminution in blood glucose level was noticed after 24 h to stabilize around 3.20 and 3g/dl respectively for the both dose. Whereas, untreated rats showed no change in their blood level (> 6 g/dl). Rats treated with Glibinclamide showed an increase until the 2^{nd}h and then decreased where it regulated after 24h to reach 3.98 g/dl.

The same effect of other plant extracts (METand MEE) was noted on glucose level where MET stabilized the level at 2.55 and 3.11 g/dl for the two doses respectively. While, MEE reached the level of 1.70 and 1.82 g/dl for both doses respectively. Comparison of plasma glucose levels between treated groups and untreated ones showed a highly significant difference ($P < 0.001$) as shown in Table 6. Statistically, a slight significant difference was observed in the final values of plasma glucose levels between the treated groups and control one (Table 6) .As a conclusion, it was found that the differents doses (200 mg/kg and 600 mg/kg) had a remarkable effect on glucose uptake by cells after 24h when compared with the standard drug Glibenclamide but the two doses of *E.globulus* showed the highest hypoglycemic effect in induced hyperglycemia in rats even at 24h and after 10 days of treatment. The antidiabetic effect of the extracts was more effective than that observed with glibenclamide.

Table 6: Glucose levels changes in the blood of treated and untreated animals

Hours	Control [(b)]	MEO: 200mg/kg	MEO: 600mg/kg	MET: 200mg/kg	MET: 600mg/kg
MEE: 150mg/kg	MEE: 500mg/kg	Glibil	Untreated		
0	1.19±0.13[a]	4.53 ± 0.34*** [a]	4.33 ± 1.05*** [a]	5.12±0.38*** [a]	3.43±1.67***[a]
4.83±0.01*** [a]	4.47±0.68*** [a]	4.13 ± 1.05** [a]	>6***		
1h	1.27 ± 0.15[a]	4.75 ± 0.83*** [a]	4.52 ± 1.2***[a]	4.69±0.49*** [a]	5.58±0.11***[a]
5.45±1.05*** [a]	5.1±0.1*** [a]	4.31±0.87*** [a]	>6***		
2h	1.32 ± 0.17[a]	3.08 ± 1.88*** [a]	3.94 ± 1.35***[a]	4.24±0.52***[a]	4.83±1.94***[a]
3.18±1.30*** [a]	3.71±0.70***[a]	4.70 ±1 *** [a]	>6**		
3h	1.41 ± 0.10[a]	3.13 ± 1.42*** [a]	2.74 ± 1.54* [a]	3.63±0.12*** [a]	3.88±1.94*** [a]
2.56±1.50*** [a]	3.61±0.25*** [a]	4.6± 0.35*** [a]	>6***		
24h	1.30 ± 0.12[a]	2.97±0.83**[a]	2.73±0.76* [a]	3.11±0.54***[a]	3.28±1.58***[a]
1.07±0.05ns [a]	1.48±0.38ns[a]	4.29±0.55*** [a]	>6***		
10 days after	1.11 ± 0.09[a]	3.20±0.15*** [a]	3 ± 2.05* [a]	2.55±1.05*[a]	3.11±0.16***[a]
1.70±0.82 ns [a]	1.82±0.01ns[a]	3.98± 1.74*** [a]	>6***		

(b): dl/l. Value are mean ± SEM (n=6), (ns: not significant, *<0.05, **P<0.01, ***P<0.00; when compared to control group; [a]: P<0.001 when compared to untreated group).

Zafar and Naeem (2010) analyzed the mean plasma glucose levels of rats after administration of STZ at variable time intervals and found that it may be concluded that the plasma glucose levels were significantly higher and plasma glucose levels were significantly increased in groups increasing with time.

Our results were in agreement with other findings on the antihyperglycemic effect of olive leaf extract due to its antioxidative properties. It has been observed that decreased activities of hepatic antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) found in diabetic rats were restored by the use of oleuropein and hydroxytyrosol, thereby attenuating the oxidative stress associated with diabetes (Jemai *et al*., 2008; Cumaoğlu *et al*., 2011). An other study indicated that the oral administration of the alcohol extract from *Olea europaea* produced significant hypoglycemic effects only in streptozotocin-induced diabetic rats not in normal rats.

Also, the extract affected insulin release from the pancreas of the diabetic group. In agreement with the present results, a few reports are available on the hypoglycemic effects of the leaves of *Olea europaea* (Jouad *et al*., 2001; Alarcon-Aguilar *et al*., 2002; Verspohl, 2002). One of the compounds responsible for this activity is oleuropoeside. The hypoglycemic activity of this compound may result from two mechanisms: (a) potentiation of glucose-induced insulin release, and (b) increased peripheral uptake of glucose (Gonzalez *et al*., 1992).

Houacine *et al* (2012) indicated that the induced of *E.globulus* had significant dose-dependent reduction in blood glucose of hyperglycemic rats ($P < 0.05$ to 0.003) and could occur partly by stimulating insulin production from the pancreatic islets, or it could stimulate insulin production and glucose utilization similar to glibenclamide due to the presence of certain hypoglycemic bioactive components in its extract.

The hypoglycaemic effect of fenugreek is thought to be largely due to its high content of soluble fibre which acts to decrease the rate of gastric emptying, thereby delaying absorption of glucose from the small intestine (Yin *et al*., 2003). The seeds of fenugreek contain about 50% fibre, of which 20% is fibre similar to guar gum which is known as a hypoglycaemic agent. The high level of fibre interferes with carbohydrate absorption. Saponin compounds diosgenin, alkaloids and trigonelline were shown to be associated with inhibition of intestinal glucose uptake *in vitro* (Al-Habori *et al*., 2001). The portion fraction of the seeds contains the amino acid 4-hydroxyisoleucine which stimulates insulin production (Souvaire *et al*., 1996). Owing to its particular insulinotropic action, 4-hydroxyisoleucine might be considered as a novel secretagogue with potential role in the treatement of diabetes (Baquer *et al*., 2011). The hypoglycaemic effect of fenugreek seeds is modiated, at least in part, by the activation of an insulin signaling pathway in adipocytes and liver cells (Vijayakumar *et al*., 2005).

Rutin (a glycosylated quercetin, also known as rutoside, quercetin-3-*O*-rutinoside, and sophorin), which is a compound of the studied plants, was reported to have anti-obesity and

anti-diabetic functions (Kreft *et al*., 1999 ; Huang *et al*.,2012). Diabetic mice fed with 100 mg/kg rutin in the diet showed significant reductions in plasma glucose levels and increased insulin levels along with the reestablishment of glycogen content and the activities of carbohydrate metabolic enzymes (Prince and Kamalakkannan, 2006). Rutin was also found to activate liver enzymes linked with the gluconeogenic and lipid metabolic processes. The flavonoid also reduced the levels of fasting blood glucose, blood urea nitrogen, and creatinine and the intensity of oxidative stress, with a significant increase in phosphorylation of mothers against decapentaplegic homolog 7 (SMAD7), an inhibitory SMAD, I-SMAD. SMAD7 belongs to the transforming growth factor β (TGFβ) superfamily of ligands and is a TGFβ type 1 receptor antagonist that blocks the association of the TGFβ type 1 receptor and SMAD2, a receptor-regulated SMAD, R-SMAD. Rutin was shown to influence glucose uptake in the rat soleus muscle through the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (Kappel *et al*., 2013). Rutin was also reported to reduce the levels of plasma glucose, hemoglobin A1C (HbA1c, a glycated (beta-*N*-1-deoxy fructosyl) hemoglobin), and cytokines, including IL-6 and TNF-α.

Quercetin is one of the most plentiful flavonoids in human dietary nutrition and forms the skeletons of other flavonoids, such as hesperidin, naringenin, and rutin.(45-47) quercetin was shown to induce the insulin-independent 5′ adenosine monophosphate-activated protein kinase (AMPK) signaling pathway in muscle cells and slow adenosine diphosphate-stimulated oxygen consumption in isolated mitochondria (Eid *et al*., 2010). Notably, this mechanism is analogous to that of metformin (*N*, *N*-dimethylimidodicarbonimidic diamide), the first-line medication used to treat T2DM.

Additionally, quercetin derivatives such as isoquercetin (quercetin-3-*O*-glucoside) and hyperoside (quercetin-3-*O*-galactoside) as well as quercetin aglycone may also improve insulin-independent glucose uptake and stimulate AMPK in muscle cells. Therefore, quercetin

and its derivatives, are thought to be the major bioactive components of the plants sutdies, that activate AMPK and stimulate glucose uptake in muscle cells. The anti-diabetic effect of quercetin was also investigated in streptozotocin (STZ)-induced diabetic mice; treatment of quercetin resulted in the reduction of hyperglycemia-stimulating GLUT4 and glucokinase, increased liver glucose uptake, and decreased hepatic glycogenolysis and gluconeogenesis (Kobori *et al*., 2009 ; Alam *et al*., 2014; Xu *et al*., 2014) . Dietary supplementation of 0.5% quercetin in the diet for two weeks enhanced serum insulin concentrations and lowered blood glucose in STZ-induced diabetic mice. Moreover, a diet supplemented with quercetin caused upregulation of the expression of genes associated with cell proliferation and survival in the liver (Kobori *et al*., 2009). Another study showed that supplementation with quercetin at 30 mg/kg body weight (approximately equivalent to 0.045% quercetin in the diet) per day for six weeks in 6-week-old male Wistar rats fed a high-fat high-sucrose diet, significantly reduced basal levels of glucose and insulin (Arias *et al*., 2014). Additionally, supplementation of 0.04% quercetin in the diet decreased blood glucose and improved insulin resistance in obese diabetic mice (Jeong *et al*., 2012).

III.5.6. Body weight alteration

The present study was designed to observe the effects of streptozotocin (STZ)-induced diabetes and studying the association between the reduction of body weights and diabetes. As shown in figure 33, the group treated with MET decreased the body weight with very highly significant difference ($P < 0.001$) when compared to control group and near to untreated group. While groups treated by MEE and MEO decreaced the body weight significantly ($P<0.01$).

Similar study showed that the decrease in body weight observed in uncontrolled diabetic might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source. Where, body weight enhanced significantly ($P<0.01$) in eucalyptus treated diabetic rats

when compared with untreated-diabetic ones. Likewise, eucalyptus decreased significantly (P<0.01) polyphagia and polydipsia in treated-diabetic rats when compared with diabetic group (Alireza *et al*., 2009).

The appearence ill-looking with loss of their body weights because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. Our present observations are in agreement with the findings of Piyachaturawat *et al*. (1988), Habibuddin *et al*. (2008) and Lee *et al*. (2008).

It may also be stated that streptozotocin by producing diabetes (hyperglycaemia) and hypoinsulinemia causes reduction in the body weight of diabetic animals. Zafar and Naeem (2010) concluded that the reduction in body weight was associated with increase in the relative weight of kidney and liver while the pancreas weight was unaffected. The decrease in the weight of pancreas could be attributed to the disruption and disappearance of pancreatic islets and selective destruction of insulin-producing cells (Kim *et al*. 2006; Heidari *et al*. 2008).

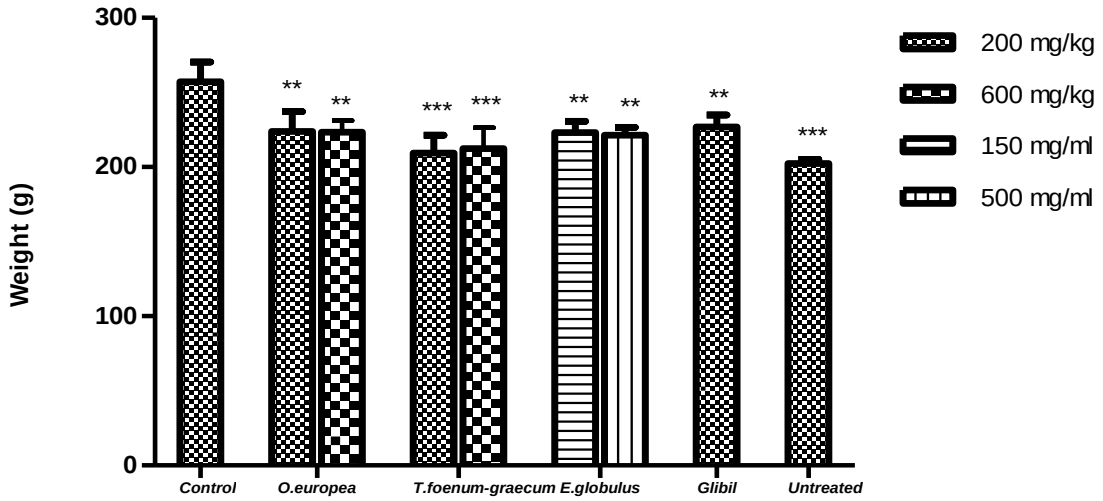


Fig.33 : Changes in the mean body weight (g). Results are expressed as means± SD. (*** : P<0.001, ** : P<0.01).

III.5.7. Effect of treatment with plant extracts on antioxidant parameters in liver and kidney

Oxidative stress in the kidney of diabetics is usually associated with tissue damage that interferes with proper organ function, causing an increase in urinary protein excretion and blood urea nitrogen (BUN) (Montero *et al*., 2000).

Liver also is a major organ attacked by ROS (Sanchez-Valle *et al*., 2012) .Antioxidants may help to ameliorate liver damage and hepatic oxidative stress which are common in diabetes mellitus. Considering the roles of the liver in glucose homeostasis and in mediating the hypoglycemic effect of antidiabetic agents such as glibenclamide, antioxidant therapy may improve liver function and contribute to improved glycemic control (Dias *et al*., 2005 ; Erejuwa *et al*., 2012).

Our studies have demonstrated that enzymatic as well as non-enzymatic systems which maintaining cellular homeostasis are remarkably affected by diabetes. In particular, the activities of SOD, CAT, GSH-Px, as well as the level of lipid peroxidation were changed in animals treated with STZ. The degree of tissue damage persuaded by free radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism (Davi *et al*., 2005).

III.5.7.1. Effect of extracts on catalase activity

Hydrogen peroxide plays an important role in the immune system and acts either directly or indirectly as a messenger molecule in the inflammation events and cells signaling pathways (Auroma *et al*., 1989). However, overproduction of H_2O_2 can lead to cell death (Butterfield and Kanski, 2001). Thus, endogenous and/or exogenous antioxidants would be capable of

protecting biological systems thats are exposed to high levels of H_2O_2. Figure 34 showed that the extracts are capable of scavenging H_2O_2 in a dose-dependent manner.

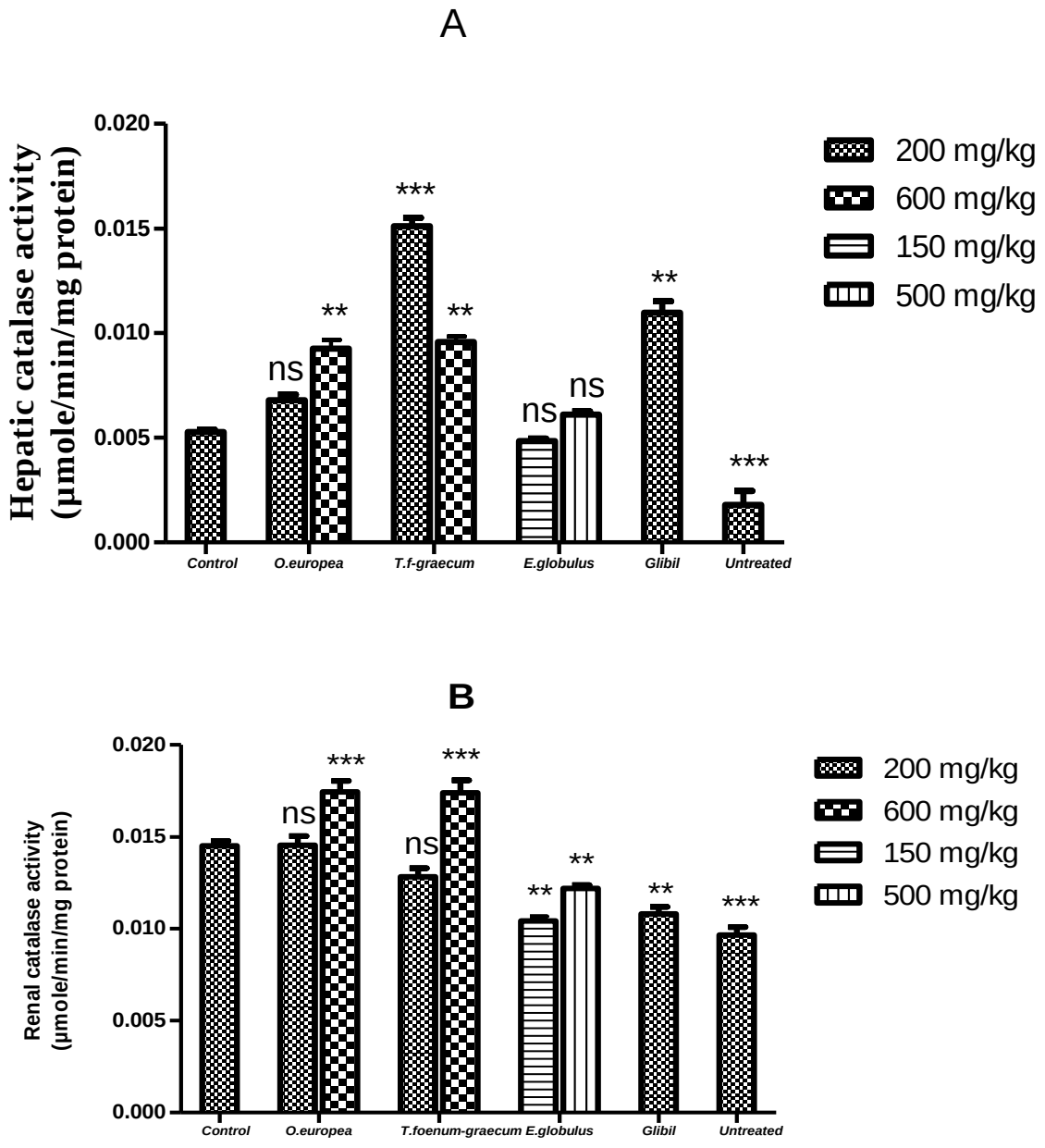


Fig.34 : Effect of methanolic extract of *O.europea*, *T.foenum-graecum*, *E.globulus* and untreated on catalase activity in liver (A) and kidney (B) of rats. Values are given as means ± SEM (n=6). (ns: no significant difference) compared to control group.

In present study, the activities of CAT in liver extracts of the STZ-diabetic rats were differ from control group. While the activity of CAT in liver treated with *E.globulus* were near to control

animals (Figure 34). The others extracts (*O.europea* and *T.foenum-graecum*) increased the activity of catalase but statistically were significant difference with values of 0.0067±0.0002 U/mg protein ; 0.0092±0.0004 U/mg protein) and (0.0151±0.0003 U/mg protein ; 0.0095±0.0002 U/mg protein) respectively, whereas the Glibil caused an activity higher than that of control group (0.0109±0.005 U/mg protein) and untreated group showed the lowest activity (0.0071±0.0006 U/mg protein).

In kidney, the treatement with *E.globulus*, and Glibil increased the level of catalase compared to control group (0.014±0.0002 U/mg protein) with the values : (0.010±0.0002 U/mg protein ; 0.012±0.0001 U/mg protein) ; and (0.010±0.0003 U/mg protein). But, the administration of both doses 200mg/kg of *O.europea* and *T.foenum-graecum* not significantly elevated the catalase activity when compared to control group (Figure 34) while untreated group showed 0.009±0.0004 U/mg protein.

Chiang *et al*. (2006) declared that the increase in the antioxidant capacity include the increase of antioxidant activity enzymes, such as superoxide dismutase and catalase.

It was also determined whether the change in the antioxidant enzymes activities was correlated with their respective mRNA expression in the phenolic acids-supplemented rats. The mRNA expression of hepatic CuZnSOD, GPx, and catalase in liver tissues were higher in the phenolic acids supplemented groups than in the control group (Yeh and Yen, 2006). Multidrug resistance protein 2 and 3 (Mrp2 and Mrp3) have gained significance during the last few years because of their function as transporters of organic anions and conjugates, and their involvement in hepatic detoxification and tissue-specific distribution of drugs (Smitherman *et al*., 2004). Many genes encoding detoxification and antioxidant proteins are regulated by Nrf2 (NF-E2-related factor-2). Recently, Nrf2, a member of the basic-region leucine zipper transcription factor family, was identified as an ARE-binding protein so the increase expression of Nrf2 protein may be related to the phenolic acids-induced Mrp gene activation (Kobayashi and Yamamoto, 2005).

III.5.7.2. Effect of extracts on MDA level

As shown in Figure 35, high significant ($p<0.001$) reduction in the production of malondialdehyde (MDA), which is a measure of lipid peroxidation in all groups. So in liver, the administration of doses 200mg/kg, 600mg/kg from both *O.europea* and *T.foenum-graecum* reduced MDA formation level (41.40±3.82 nmol/ g tissue; 29±3.34 nmol/ g tissue) ;(34.97±2.4 nmol/ g tissue ; 36.10±2.03 nmol/ g tissue) compared to untreated group (93.32±3.76 nmol/ g tissue) while Glibil 45.17±2.26 ; control group 38.62±2.16 nmol/ g tissue. Whereas, *E.globulus* decreased MDA formation with level of 40.59 ± 3.78 nmol/ g tissue and 43.67 ± 3.16 nmol/ g tissue for the doses.

However in kidney, There was significant decrease in the levels of MDA in rats treated with 200 and 600 mg/kg of ME of *O.europea* with values of 63.17 ±4.73 and 53.62± 4.91 nmol/ g tissue, respectively when compared with untreated group (80.79±3.27 nmol/ g tissue). The control group exhibited similar effect (41.48± 6.74 nmol/ g tissue) to that of rats receiving the standard drug; Glibil (51.31 ± 4.12 nmol/ g tissue). Also, the dose of 200 mg/kg and 600mg/kg of ME of *T.foenum-graecum* decreased lipid peroxidation (34.81 ±3.95 nmol/ g tissue ; 32.41±2.85 nmol/ g tissue). Similary, the 150mg/kg and 500mg/kg of ME of *E.globulus* decreased the MDA level compared to untreated diabetic rats (Figure 35).

Many reports suggested that polyphenol compounds present in the extract may either delay or inhibit the initiation step of lipid peroxidation by reacting with a lipid radical or by inhibiting their propagation step or by reacting with peroxyl or alkoxyl radicals (Antolovich *et al*., 2001 ; Pieme *et al*., 2014). Also, antioxidants in the extracts may further interfere with chain propagation reactions by forming peroxy-antioxidant stable compounds (Pieme *et al*., 2014). ROS damage macromolecules in cells and have been involved in several pathological processes. Antioxidant enzymes are capable of eliminating ROS and lipid peroxidation

products, therefore, they can protect cells and tissues from oxidative damage (Halliwell, 1997 ; Lee *et al*., 2003).

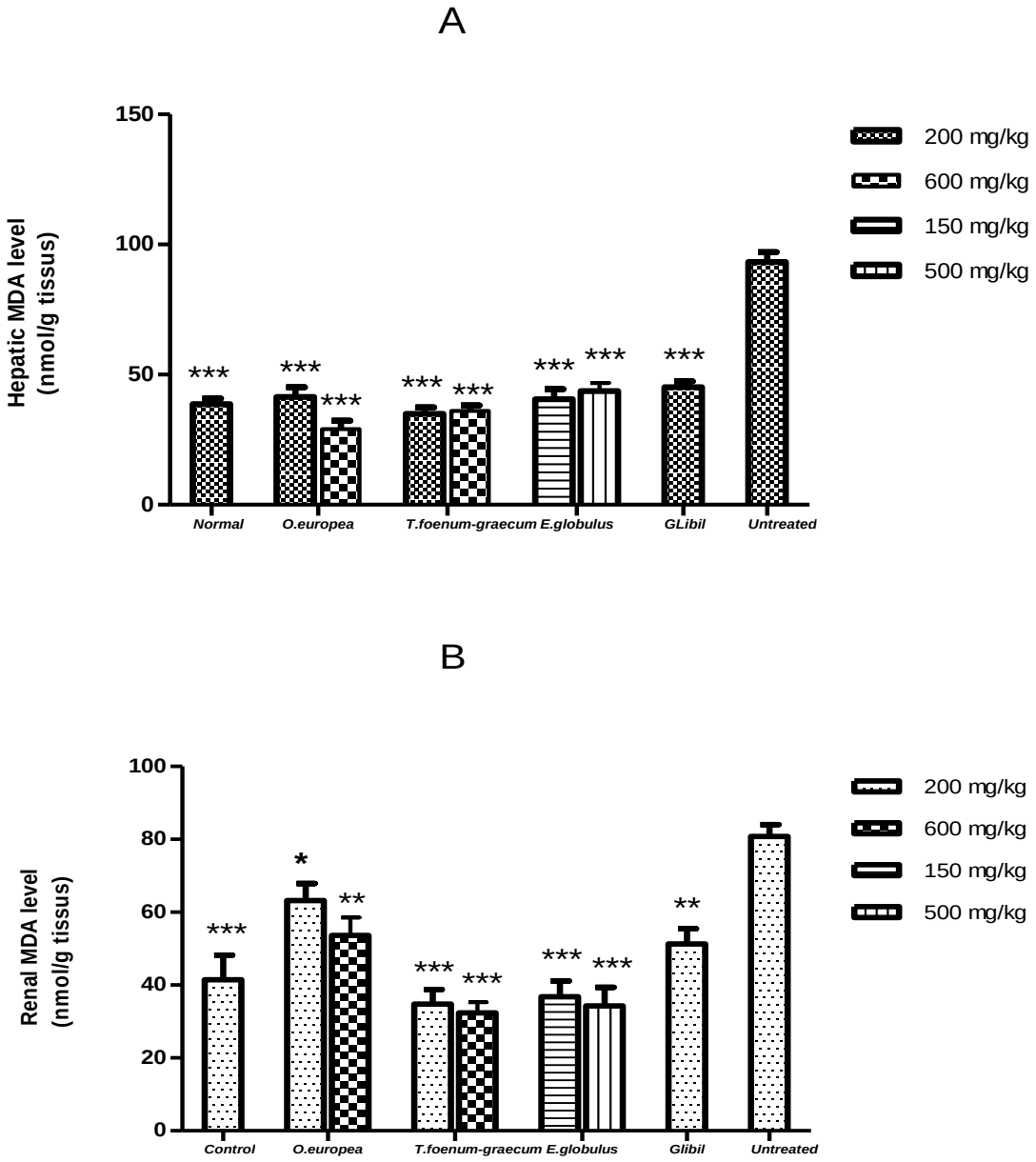


Fig.35 : Effect of methanolic extract of *O.europea*, *T.foenum-graecum*, *E.globulus* and Glibil on MDA level in liver (A) and kidney (B) of rats. Values are given as means ± SEM (n=6). (ns: no significant difference) compared to untreated group.

III.5.7.3. Effect of extracts on GSH level

GSH plays an important role in hepatic antioxidation and drug metabolism. The higher intracellular GSH content promotes reduced damage and better survival under oxidative stress (Dickinson *et al*., 2003). The physiological role of GSH is as an essential intracellular reducing agent for the maintenance of thiol groups on intracellular protein and antioxidant molecules. It was well established that GSH, the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation and hydroperoxide reduction. In addition, the GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. This system consists of GSH and an array of functionally related enzymes, of which GR is responsible for the regeneration of GSH, whereas GPx worked together with GSH in the decomposition of hydrogen peroxide or other organic hydroperoxides. Thus, the levels of GSH and activities of the GPx and GR were used to monitor the peroxidative balance (Mak *et al*., 1996) where the reduced GSH level could be a marker for diabetes (Couto *et al*., 2013).

The result of present study demonstrate that the treatment with plants extracts decreased the GSH level but this decrease was not significant ($p > 0.05$) in most groups where in liver, the untreated group showed a high decrease (58.61±3.18 µmol/g tissue) when compared to control group (87.51±2.86). While, groups treated with *O.europea, T.foenum-graecum and E.globulus* decreased slightly the level of GSH with values (71.78±2.36 µmol/g tissue ; 74.61±2.15 µmol/g tissue) ; (81.50±3.19 µmol/g tissue; 84.20±3.15 µmol/g tissue) ; (86.25±1.69 µmol/g tissue ; 72.46±2.30 µmol/g tissue) respectively. It was found that Glibil treated group had an effect on the level of GSH with value (76.12±3.38 µmol/g tissue).

In kidney, the GSH level was increased with values : (37.17±1.69 µmol/g tissue; 39.80±1.49 µmol/g tissue) ; (42.44±1.49 µmol/g tissue ; 42.47±1.10 µmol/g tissue) ; (41.12±0.62 µmol/g tissue ; 36.18±0.78 µmol/g tissue) for *O.europea, T.foenum-graecum and E.globulus* while,

Glibil group and control group had the values of (41±1.72 µmol/g tissue ; 43.56±1.86 µmol/g tissue, respectively) compared to untreated group (25.87±1.37 µmol/g tissue) (Figure 36).

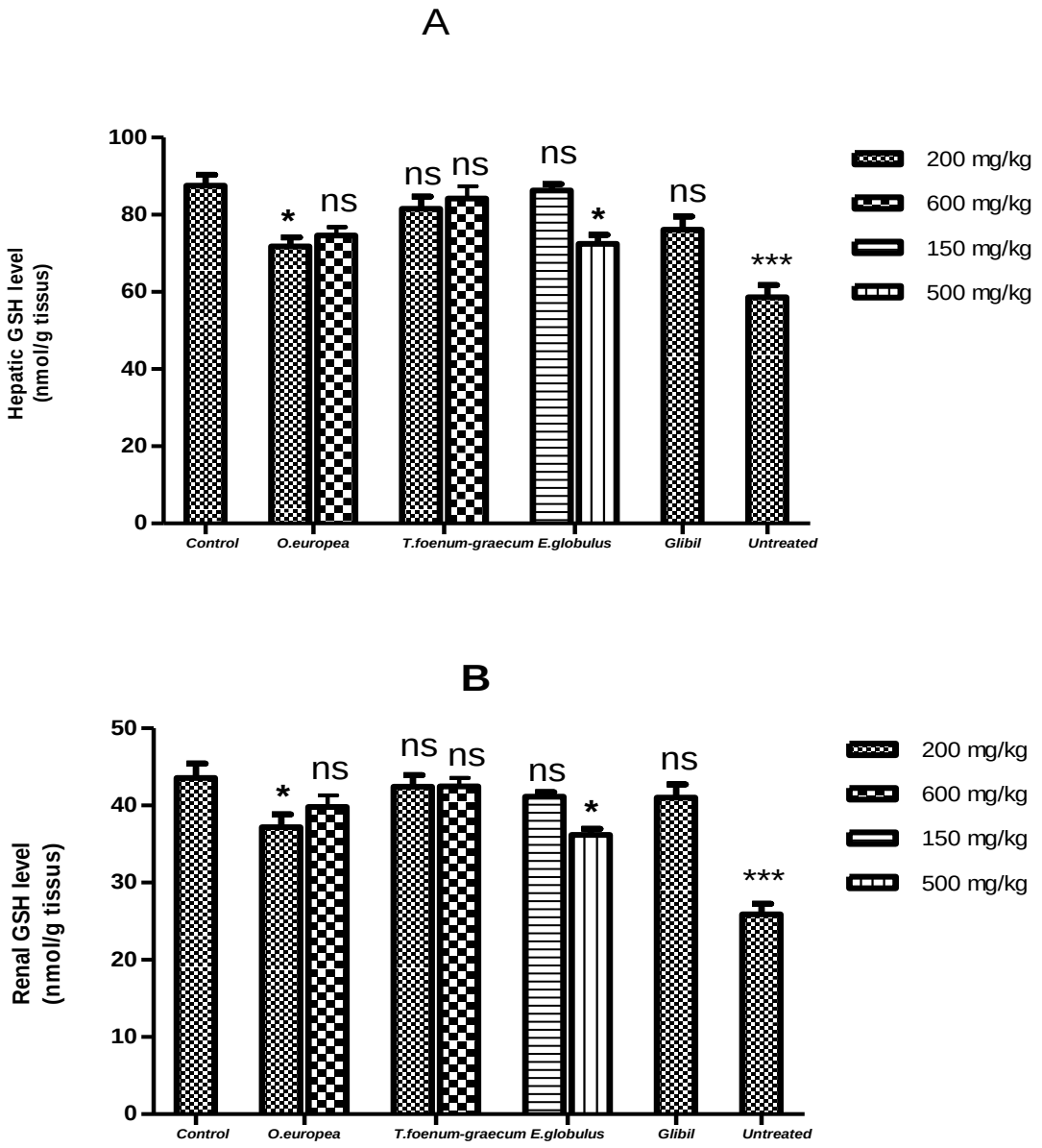


Fig.36 : Effect of methanolic extract of *O.europea, T.foenum-graecum and E.globulus* on reduced glutathione level in liver (A) and kidney(B) of rats. Values are given as means ± SEM (n=6). (ns: no significance difference) compared to control group.

Many studies affirm the decrease activities of Glutathione Reductase and Glutathione Peroxidase in type 2 Diabetes (Arthur, 2000 ; Colak *et al*., 2005). This decrease of GSH content

may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions including diabetes (Hussein, 2008).

Numerous studies have revealed lower antioxidant and enhanced peroxidative status in Type 2 diabetes mellitus because the STZ inactivates the glutathione and antioxidant system (Punitha *et al*., 2005; Pari and Suman,2010). SOD, CAT, GPx are enzymes that destroy peroxides and play a significant role in providing antioxidant defences to an organism (Punitha *et al*.,2005). CAT (Liedias *et al*., 1998) are involved in the elimination of H_2O_2 and SOD acts to dismutate superoxide radicals to H_2O_2 which is then acted upon by GPx (McCord *et al*.,1976). The function of all three enzymes are interconnected and lowering of their activities result in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats (Kaleem *et al*., 2006). So, the compromises in enzymatic antioxidant defense system and alterations in their activities have been implicated in the mechanisms of abnormal tissue function observed in diabetes (Martin *et al*., 2003).

Glutathione may modulate metal reduction, and the thiol portion is very reactive with several chemical compounds, mainly with alkylating agents such as STZ (Menegola *et al*., 1996; Nazirogˇlu and Cay, 2001). The GSH level in the groups treated were also lower than the control group. These findings are similar to those of other researchers studying GSH in relation to risk factors in diabetic subjects and animals (Damasceno *et al*., 2002; Menegola *et al*., 1996; Nazirogˇlu and Cay, 2001; Nazirogˇlu *et al*., 2004) but were highier than untreated group which may be due to the presence of phenolic compounds in the plants extracts which help to raise the antioxidant activities of such enzymes (Verma *et al*., 2009).

The flavonoid wich are in the studied plants also led to the reestablishment of antioxidant status and serum lipid profile as reported by Niture *et al* (2014) in STZ-treated diabetic rats fed a high-fat diet (HFD/STZ). Particularly, rutin can defend against and improve myocardial dysfunction, oxidative stress, apoptosis, and inflammation in the hearts of diabetic rats (Wang *et al*., 2015).

A recent report showed that rutin supplementation restored the reduced levels of glutathione (GSH) and decreased the level of thiobarbituric acid reactive substances (TBARS), which are formed as a byproduct of lipid peroxidation. Additionally, treatment with rutin in the diabetic retina showed anti-apoptotic activity by decreasing the intensity of caspase 3 and increasing the level of Bcl-2 (Ola *et al*., 2015).

III.5.7.4. Assessment of Total protein

The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This key function of liver makes it vulnerable to diseases in subjects with metabolic disorders, particularly diabetes (Levinthal and Tavill, 1999). Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into plasma indicates the sign of hepatic tissue damage (Ramaiah, 2007; Sreelatha *et al*., 2009).

As seen in Figure 37, the treatment with ME of *E.globulus* at the doses of 150 and 500 mg/kg did not change total protein level in liver (20.46±0.65 mg/ml ; 21.62±0.46) when compared to control group (21.60± 1.19 mg/ml). The total protein levels for *O.europea* and *T.foenum-graecum* at doses of 200 mg/kg and 600 mg/kg were found to be (11.70± 0.92 mg/ml, 13.79 ±0.46 mg/ml), (13 ± 0.99 mg/ml, 16.73 ±0.66 mg/ml), respectively. Glibil group had value of 11.05±0.48 while untreated group had the lower value with 7.22±0.35.

However in kidney, the administration of ME of *O.europea* had no effect (no significant difference, $p > 0.05$) on proteins level compared to control (13.41 ± 0.35 mg/ml) where untreated group showed low levels of proteins in kidney 7.25± 0.36 mg/ml. ME of *T.foenum-graecum* (600 mg/ml) caused a decrease in total proteins content (9.36 ± 0.83 mg/ml), whereas the highest content of 13.89 ± 0.94 mg/ml was noticed after treatment with for *E.globulus* (150 mg/kg) (Figure 37).

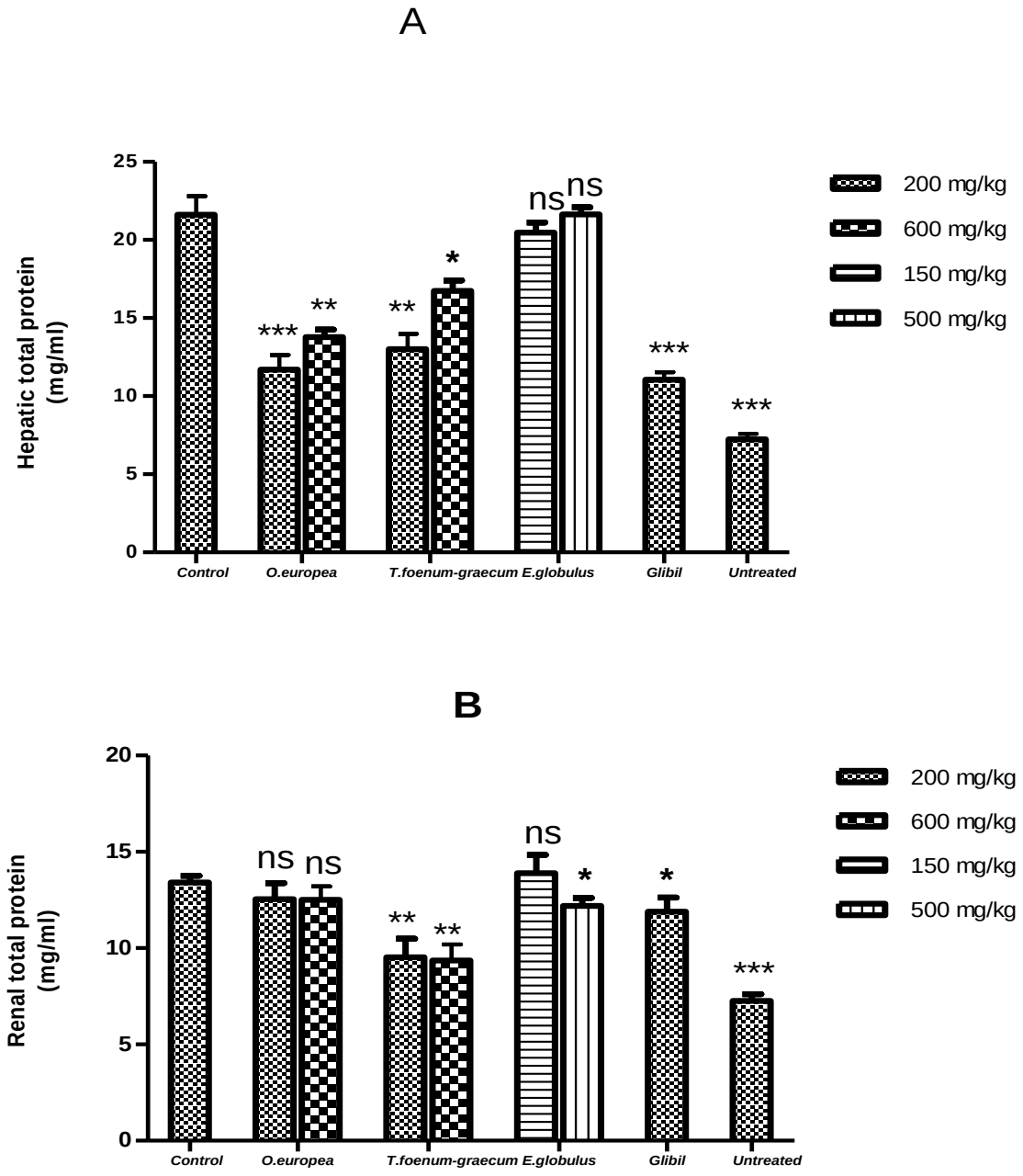


Fig.37 : Effect of methanolic extract of *O.europea, T.foenum-graecum and E.globulus* on total proteins level in liver (A) and kidney (B) of rats. Values are given as mean ± SEM (n=6). (ns: no significant difference) compared to control group.

The Kidney is an important physiological organ which when damaged may lead to alterations in metabolic activities. It is well known that increased reactive oxygen species (ROS) resulting from oxidative enzyme breakdown have been implicated in diabetes and its complications (Vats

et al., 2004). It has been reported that hyperglycemia leads to the generation of ROS in tissues from glucose autooxidation and protein glycosylation (Ajabnoor, 1990). The treatment of diabetic rats with the three plants extracts produced alteration in both renal and liver protein level compared with control animals, whereas, total proteins were very low in liver and kidney of untreated diabetic rats. The result showed that plants extract wer more effective than glibenclamide in restoring and protecting tissues (liver and kidney) towards normal. Whereas, untreated group showed a decrease in the level of proteins which may be due to the loss in adipose tissue and muscle which results from excessive fatty acid and tissue protein breakdown (Granner, 1996). This study suggested therefore, that extracts of the three studied plant extracts besides its ameliorative action could protect the kidneys and livers against impairment due to diabetes.

III.5.8. Determination of diuretic activity of plant extracts

Diuretics have a wide definition and commonly referred as excretion of water and Na^+ in larger volume. Whereas diuresis commonly known as a process to increase the formation of urine (Rang *et al*., 2007).

During 5h observation, treatment with ME of different extracts showed a significant dose-dependant diuretic activity in terms of cumulative urine excreted (Figure 38). The present study indicated that there is no significant increase at doses of MEE. But, the highest doses of extracts regulated the diuretic activity.

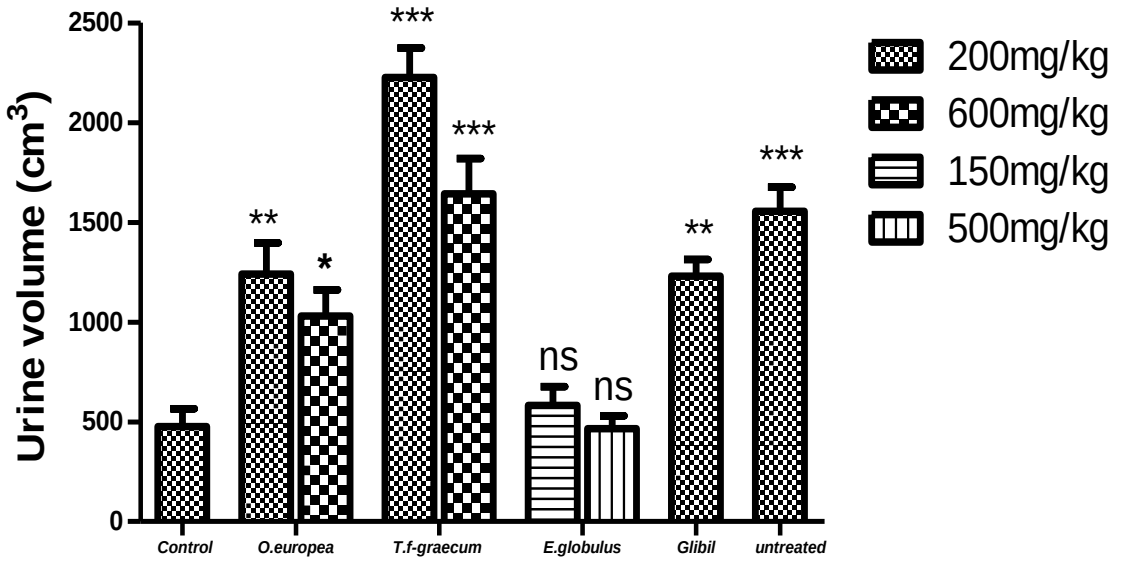


Fig.38 : Effects of ME of *O. europea*, *T. foenum-graecum* and *E. globulus* on urine output. Values are mean ± SEM, n=5.

Diuretics are synthetic drug which is used to increase the volume of urine excreted from body (Wright *et al*., 2007). Diuretics such as the high-ceiling/loop and thiazides diuretic are associated with several adverse effects, for example, ototoxicity, hyperuricemia, acute hypovolemia, potassium depletion, hypomagnesemia, hyponatremia, hypercalcemia, hyperglycemia, hyperlipidemia and hypersensitivity (Howland and Mycek, 2006). Insulin resistance and/or hyperinsulinemia are present in the majority of hypertensives, constitute a common pathophysiologic feature of obesity, glucose intolerance, and hypertension, possibly explaining their ubiquitous association, and may be linked to the increased peripheral vascular resistance of hypertension, which is putatively related to elevated intracellular sodium concentration (Modan *et al*., 1985). Hyperinsulinemia has been ascribed a pathogenetic role in obese hypertension via increased renal sodium retention. Abnormalities of cell membrane cation transport were demonstrated in obesity and hypertension (elevated intracellular sodium) (Trevisan *et al*., 1983) and in insulin-dependent diabetes (reduced intracellular and elevated

plasma potassium concentrations). These, as well as the obligatory linkage of cellular sodium efflux and potassium influx and the regulatory role of insulin in cell membrane cation transport, raise the possibility that such shifts in internal cation distribution are associated with insulin resistance and/or hyperinsulinemia (Modan *et al*., 1985).

Loop diuretics like furosemide (is a sulphonamyl derivative) increased the excretion of Na^+, K^+ and Cl^-. Nevertheless there are many ways for diuresis to occur. Likewise, herbs that contain of high sodium or potassium helps to promote diuresis by inhibit the reabsorption of renal tubular (Hook *et al*., 1993).

Wright *et al*. (2007) has wind up about 19 plants showing the potential diuretic effects.

ME extracts contain compounds that plays the same role of furosemide and thiazide. Sunitha Ch *et al* (2011) demonstrated diuretic action for plantsand diuretic activity higher than that of the standard furosemide. It may be due to the presence of flavonoids. However, the contribution of polyphenolic compounds to diuretic activity can not be ruled out (Sangita *et al*., 2009). The flavonoid glycosides are commonly hydrolyzed to their aglycones to produce effects *in vivo*. Deglycosylation by small intestinal epithelialcell β-glucosidasesis is a critical step in the absorption and metabolism of flavonoid glycosides (Walle *et al*., 2005). Flavonoid glycosides in general are absorbed as their aglycones after hydrolyzing along the digestive tract.

Conclusion and future considerations

Conclusion and future considerations

In this study the antioxidant, antidiabetic and diuretic activities of *Olea europea, Trigonilla foenum-graecum, Eucalyptus globulus* extracts are evaluated.

The human relationship with medicinal plant chemicals is therefore a primary one which can lead to problems such as toxication.

On one hand, Total phenolic are highly in EAE of *O.europea* and *Trigonella foenum-graecum* whereas it appeared in ME of *E.globulus*. However for flavonoids, EAE was the best for *O.europea* and *Trigonella foenum-graecum*, while, ChE had the maximum, this results are due to the different solvent and its degree of polarity also depending on several factors, including geographic distribution type, altitude, and habitat system : light quality and heat. These factors affect the biosynthesis of secondary metabolites and this alters antioxidant activity.

On the other hand, HPLC technique allowed to explore secondary metabolites in ME of plants and revealed the presence of: gallic acid, fumaric acid, gentisic acid, chlorogenic acid, catechin, 4-hydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, syringic acid, rutin, 4-hydroxybenzaldehyde, polidatine,ellagic acid, scutellarin, quercetin-3-β-D-glycoside, sinapic acid, naringin, diosmin, taxifolin, hesperidin, apegetrin, neohesperidin, myricetine, baicalin,p-caumaric acid, fistin,protocatechuic acid, morin, resveratrol, salicylic acid, quercetin, silibinin, cinnamic acid, apigenin, naringenin and kaempferol in *Oea europea* and gallic acid, fumaric acid, gentisic acid, 4-hydroxybenzoic acid, caffeic acid, rutin, 4-hydroxybenzaldehyde, polidatine, scutellarin, quercetin-3-β-D-glycoside, naringin, ferulic acid, diosmin, apegetrin, baicalin, p-coumaric acid, salicylic acid, apegenin and naringenin in *T.foenum-graecum* and gallic acid, fumaric acid, gentisic acid, chlorogenic acid, catechin, 4-hydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, syringic acid, rutin, 4-hydroxybenzaldehyde, polidatine, ellegic acid, scutellarin, quercetin-3-β-D-glycoside, sinapic acid, naringin, ferulic

acid, diosmin, taxifolin, hesperidin, apigetrin, neohesperidin,myricetine, baicalin, p-coumaric acid, fisetin, protocatechuic acid ethyl ester, morin, resveratrol, salicylic acid, quercetin, silibbinin,apegenin,naringenin, kaempferol, diosmetin, neochanin,eupatorin, wogonin and galangin in *E.globulus*.

In vitro, all extracts showed an antioxidant activity. EAE of *O.europea*, ChE of *Trigonella foenum-graecum* and EAE of *E.globulus* had the highest free radical scavenging activity in DPPH assay, whereas EAE, AqE, ME of *Trigonella foenum-graecum* and *E.globulus* exhibited the strongest antioxidant activity in ABTS assay. These results indicated that the extracts contain molecules with antiradical power.

However, ME of *O.europea* and *Trigonella foenum-graecum*, ChE of *E.globulus* showed greatest ability to catch the ferrous ion. ME and AqE are lower than EAE in phenolic contents, but they appeared to be a better chelator than EAE. This deponds on the nature of flavonoids present in the extract which have the capacity to stabilizing transition metal. While, EAE of *O.europea* and *Trigonella foenum-graecum*, ChE of *E.globulus* had powerful reducing power which is due probably to the phenolic acids and flavonoids in these extracts.

Furthermore, ChE of *O.europea* and EAE of *Trigonella foenum-graecum* and *E.globulus* possessed better antioxidant activity in β-carotene this due to their marked free radical scavenging activity.

ME of *O.europea* and EAE of *Trigonella foenum-graecum*, ME, ChE and AqE of *E.globulus* exhibited good antioxidant potential in FTC assay; whereas in TBA method, EAE of *O.europea*, *Trigonella foenum-graecum* and ME of *E.globulus* exhibited the best antioxidant activity.

All treatment with ME of different plant extracts showed a detectable amelioration of hematological parameters : increase of RBC, blood hemoglobin, PLT and they showed a decrease in total cholesterol, AST, Triglycerides, HDL, LDL, Albumin level.

The administration *per os* of ME of extracts to rats increased the plasma antioxidant capacity in DPPH assay and reducing power, high doses were stronger to raise the antioxidant capacity where, *E.golubus* was the best extracts, followed by *Trigonella foenum-graecum* and *O.europea*.

Histological observation of pancreas sections showed that the islet cells are destroyed while the acini were not effected. While, the liver and kidney have shown congestion and lymphocyte infiltration. Even other studies are needed to determine the mechanisms governing the diabetogenic effect of streptozocin.

In the present study, we noticed that plant extracts were very effective in controlling glucose levels than that observed with glibenclamide in streptozotocin induced diabetes in rats. ME of different plants decreased polyphagia and polydipsia and was associated with the increase in the relative weight of kidney and liver and a decrease in pancreas weight due to selective destruction of insulin-producing cells.

ME of different plants increased catalase activity, GSH level decreased MDA level or lipid peroxidation and this effects may be due to the phenolic contents in the plants extracts which help to raise the activity of such enzymes and increased total protein in liver and kidney.

ME extracts of plants showed a dose-dependent diuretic activity in terms of cumulative urine excreted due to its phenolic compounds especially flavonoids.

In future research, further studies are needed to enhance our findings ;

-Establishing the scientific basis of use of such plants in diabetes

-Studying the effects of some phytochemicals on glucose transport in the intestine

- Studying the effect of phytochemicals on glucose uptake by cells

-Studying the effect of plant extracts and plants derived phytochemicals on insulin secretion

-Isolating and purification of the active compounds and studying the effects of its antioxidant activity in reduction to diabetes

- Identifying each compound mechanism *in vivo*

-Studying the effects of plant extracts and plants derived molecules on the activity of some enzymes implicated in sugar metabolism such as α-glucosidase

ملخص

الهدف من هذه الدراسة هو تقييم النشاط المضاد للأكسدة والمضادة لداء السكري لمستخلصات *Olea europea*، *Trigonella foenum-graecum* و*Eucalyptus globulus* عن طريق إحداثه تجريبيا بحقن الإستربتوزوتوسين لجرعة 50 ملغ / كغ. في هذه الدراسة، أظهرت النتائج أن المستخلص الميثانولي (ME) والمستخلص الكلوروفورمي (ChE) لأوراق نبتة *E.globulus* يحتويان على نسبة عالية من عديدات الفينول والفلافونويدات بالترتيب. في حين، مستخلص خلات الإثيل (EAE) لأوراق نبتة *O.europea* وبذور نبتة *T.foenum-graecum* يحتوي على كمية معتبرة من عديدات الفينول والفلافونويدات. النتائج المتحصل عليها في HPLC أظهرت مركبات فينولية في ME. كذلك، مستخلص ME لنبتة *E.globulus* أظهر قوة إزاحة لجذر ABTS، ويمنع أكسدة حمض اللينوليك وثيوسيانات الحديد وكذا TBA.في حين EAE لنبتتي *O.europea*، *T.foenum-graecum* أبدى قوة تثبيطية لتقنية القدرة الإرجاعية، ABTS وTBA. بعد تحريض السكري، نشهد زيادة في حجم البول وإرتفاع السكر في الدم، وانخفاض في وزن الجسم للحيوانات غير المعالجة. أدت معالجة الجرذان بمستخلصي MEO وMET بجرعات 200 و600 ملغ / كغ ومستخلص MEE بجرعات 150و 500ملغ / كغ إلى زيادة في نشاط الكاتلا ز، مستوىGSH وانخفاض مستوى MDA في أنسجة الكبد والكلى، مستوى الكوليسترول والدهون الثلاثية المستويات في البلازما. وعلاوة على ذلك، تم تخفيض الأضرار النسيجية في البنكرياس والكبد والكلى. إذا فهذه النتائج أشارت إلى نشاط جيد لتخفيض نسبة السكرفي الدم ونشاط مضاد للأكسدة لمختلف النباتات الثلاثة المستعملة، مما قد يفسر استخدامها في الطب الشعبي في السيطرة على داء السكري ومنع مضاعفات هذا الداء من خلال تثبيط الجذور الحرة.

الكلمات المفاتيح: *Olea europea*، *Trigonella foenum-graecum*، *Eucalyptus globulus*، مضادات الأكسدة، مضاد السكري، عديدات الفينول

Abstract

The objective of this study is to evaluate the antioxidant and antidiabetic activity of *Olea europea*, *Trigonella foenum-graecum* and *Eucalyptus globulus* extracts by Streptozotocin- induced diabetes by intravenous injection of 50 mg/kg of Streptozotocin. In this study, the results showed that methanolic extract (ME) and chloroformic extract (ChE) of *Eucalyptus globulus* leaves contained high polyphenolics and flavonoids contents, respectively, whereas ethyl acetate extract (EAE) of *Olea europea* leaves and *Trigonelle foenum-graecum* seeds contain the most important quantity in polyphenols and flavonoids. The obtained results from HPLC technique allowed the detection of phenolic compounds in methanolic extracts. In addition, ME of *E.globulus* showed a strong ABTS radical scavenging activity, and inhibited the linoleic acid oxidation in ferric thiocyanate method and TBA. While, the EAE of *O.europea* and *T.foenum-graecum* exhibited a good activity in reducing power, ABTS and TBA assays. After Induction of diabetes, the volume of urine and glucose increased and the body weight decreased in the untreated animals. The administration of MEO and MET at doses of 200 and 600 mg/kg and MEE at doses of 150 and 500mg/kg increased catalase activity, GSH level and decreased lipid peroxidation in the tissues of liver and kidneys, serum total cholesterol and triglycerides levels. Furthermore, histological damages in pancreas, kidney and liver tissues were reduced. These results indicated a good hypoglycemic and antioxidant activity of the three studied plants, could explain their use in folk medicine in the control of diabetes and preventing diabetic complications by scavenging free radicals.

Key words : *Olea europea L*, *Trigonella foenum-graecum*, *Eucalyptus globulus*, antidiabetic, antioxidant, polyphenols

Résumé

L'objectif de cette étude est d'évaluer l'activité antioxydante et antidiabétique des extraits des *Olea europea*, *Trigonella foenum-graecum* et *Eucalyptus globulus* en induissant expérimentalement le diabète par l'injection intraveineuse de 50 mg / kg de streptozotocine. Dans cette étude, les résultats ont montré que l'extrait méthanolique (EM) et l'extrait chloroformique (ECh) des fleurs *Eucalyptus globulus* ont contenu des teneurs élevées en polyphénols et flavonoïdes, respectivement, tandis que l'extrait ethyl acetate (EEA) des fleurs *Olea europea* et les graines *Trigonelle foenum-graecum* étaient les meilleurs en polyphénols et flavonoïdes. Les resultats obtenus par la méthode HPLC permettent de détecter des composés phénoliques dans les éxtraits méthanoliques. En outre, EM d'*E.globulus* a montré une forte activité de piégeage des radicaux ABTS, et a inhibé l'oxydation de l'acide linoléique par le test de thiocyanate ferrique et TBA. Alors que, EEA d'*O.europea* et *T.foenum-graecum* montraient une bonne activité dans les tests pouvoir réducteur, ABTS et TBA. Après l'induction du diabète, le volume d'urine et de glucose ont augmenté et le poids corporel a diminué chez les animaux non traités. L'administration de EMO et EMT aux doses de 200 et 600 mg / kg et EME à des doses de 150 et 500mg / kg a entrainé d'augmentater l'activité de catalase, le niveau de GSH et une diminution de la peroxydation lipidique dans les tissus du foie et des reins, le taux de cholestérol total et triglycérides dans le serum. En outre, les dommages histologiques des tissus de pancréas, de rein et de foie ont été réduits. Ces résultats indiquent une bonne activité hypoglycémique et antioxydante des trois plantes étudiées, peuvent ainsi expliquer leurs utilisations dans la médecine traditionnelle dans la lutte contre le diabète et la prévention des complications du diabète en piégeant les radicaux libres.

Mots clés: *Olea europea L*, *Trigonella foenum-graecum*, *Eucalyptus globulus*, antidiabétiques, antioxydant, les polyphénols