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Protective effects of *Trigonella foenum-graecum* crude extract over damage induced by Sreptozotocin diabetes rats

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ABSTRACT

The seeds of *Trigonella foenum-graecum* (fenugreek) are used for treatment of diabetes mellitus in traditional medicine. This paper examines the protection effects of fenugreek from the damage induced by streptozotocin diabetes rats. Tannins content of *T. foenum-graecum* was also estimated *in vitro*. Normoglycemic male Wistar rats, weighing 170-250 g, were selected and randomly divided into five groups (n= 6): normal control, diabetic + TFGE (200mg/kg), diabetic+ TFGE (600mg/kg), diabetic + Glibil (3mg/kg), untreated group. Diabetes was induced after a single intraperitoneal injection of streptozotocin (50 mg/kg body weight) and Fenugreek was given every day via orogastric tube for 18 days. At the end of experiment, rats were sacarificed. Organ weight was estimated of all groups. *Trigonella foenum-graecum* administration significantly improved the polydipsia, polyphagia, and it also compensated weight loss of diabetic rats ($P<0.05$, $P < 0.01$). Moreover, fenugreek had a significant concentration of tannins (806.22 ±0.036 µg TAE/gE). The results revealed that fenugreek improves the damage in diabetic rats that in some ways validates the traditional use of this plant in treatment of diabetes.

Keywords: Antidiabetic activity, Protective effect, Streptozotocin, Tannins, *Trigonella foenum-graecum*

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INTRODUCTION

Diabetes mellitus is a complex disease characterized by hyperglycemia and associated with insulin insufficiency or insensitivity of target organs to insulin. Hyperglycemia in diabetes produces the classical symptoms of polyuria, polydipsia, polyphagia and diabetes complications [1] which occur due to the abnormalities in carbohydrate, fat, and protein metabolism [2].

Streptozotocin (STZ) had toxic effects on islet beta cells that is why it widely used to induce insulin-dependent diabetes mellitus in experimental animals [3, 4]. STZ produced severe lesions in pancreas, liver, kidney of progressively increased with time after treatment from seven to forty two days [5]. The acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis were considered as biological actions of STZ [6].

Medicines from plant sources have played a vital role in the healthcare of the population. The crude extracts of varieties of plants have been used in clinical practice from long time. Because of diversity in phytoconstituents with unknown biological effect[7, 8, 9].

Trigonella foenum-graecum L.(Fenugreek) is an annual plant of the *Fabaceae* family. It has long been cultivated in the Mediterranean area, in India and in North Africa [10]. Fenugreek is an annual grass with round stem, smooth, upright, from 20 to 50 cm with characteristic odor. Principal components of Fenugereek are mucilage, saponins, diosgenin, flavonoids, protids, prolamin, essential oil, nicotinic acid, mineral elements:iron, phosphorus, calcium., vitamins, A, B1, C, alkaloids (trigonelline…), choline… [11].

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 [11]. Therefore, this study was conducted to assess the tannins content and to evaluate the anti-diabetic effect of *Trigonella foenum-graecum L* crude extract in normal and diabetic rats and their role to improve the damage over diabetes (polyuria, polyphagia and organs weight).

MATHERIALS AND METHODS

Reagents and standards

Methanol (MeOH), Tannic acid, Streptozotocin. The products used were purchased from Merck and Sigma.

Plant samples

Seeds of *Trigonella feonum-gracum* (Fenugrec, elhalba) were collected from Setif, Algeria. The plant was authentified by Pr. Laouer Hocine (University FerhatAbbass, Setif-1, Algeria). The plant samples were air dried in shadow and powdered.

Extraction of plant phenols

Extractions of *Trigonella feonum-gracum* seeds polyphenols was carried out according to [12] with slight modification. 1 liter of 85% methanol was added to 100g of powdered plant material. After 3days, the filtration of the residue obtained was re-extracted with 1 liter of 50 % methanol for 24 h. Under reduced pressure (at 40 C°) using a rotary evaporator, the first and the second extractions were concentrated to obtain methanolic extract of *Trigonella feonum-gracum* (TFGE). Then, the extract were stored at -20 °C until use

Animals

Male adult albino rats weighing 170–280 g obtained from Pasteur Institute (Algiers, Algeria) were maintained in an air-conditioned animal room (25 ± 2°C), with 12/12h light/dark cycle and given free access to water and food for 7days, prior to experiments.

In vitro: Determination of tannins content

Tannins content was estimated by the method of [13]. Briefly a volume of plant extract was diluted to give a concentration of total polyphenols approximately 500 µg/ml and mixed with an equal volume of haemolysed sheep blood (Absorbance equal to 1.6), after 10 minutes this solution was centrifuged for 20 minutes and the absorbance of the supernatant was measured at 576 nm and the effectiveness of the precipitation of the solutions tested is expressed as µg tannic acid equivalent/ g extract.

Experimental induction of diabetes

Anti-diabetic activity of *T. feonum-gracum* extracts was evaluated by Streptozotocin induced rats according to the method of [14] with slight modifications. By single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/kg) to overnight fasted rats, diabetes was induced. Rats with blood glucose levels ≥250 mg/dl were considered diabetic and were used for the study.

Experimental designs

Male Wistar rats weighing 170–280 g were divided into five equal groups (n = 6).

Group1: (Control), received distilled water

groups 2,3: treated with TFGE extract (200,600mg/kg respectively)

group 4: received glibenclamide or Glibil used as a standard oral hypoglycemic agent (3mg/kg),

group 5: untreated group did not receive any thing

For 18 days, the treatment was given every day via orogastric tube. After sacrificed of animals, all organs were removed. The organs, the food and water intake were also measured to determine food, water intake and the change in the weight of organs and were statistically analyzed.

Statistical analysis

Data were performed using Graph Pad Prism (version 5.01 for Windows) and were analyzed by One-way analysis of ANOVA followed by Tukey's test. Whereas *in vitro* experiments were calculated as mean ± SD (n=3). The P< 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Tannins content

Based on the absorbance value of the plant extract solution reacting with haemolysed sheep blood and compared with the absorbance values of standard solutions of Tannic acid, the relative contents of tannins in TFGE was estimated as 806,22 ±0,036 µg TAE/gE.

Water and food intake

The present study was designed to observe the effects of streptozotocin (STZ)-induced diabetes and studying the association between the increase of polydipsia, polyphagia and diabetes.

Table 1 shows significant differences in water and food intake in the different experimental groups. The consumptions of water and food in normal rats were 496.6±5.77ml/day; 198.55±5.95g/day, respectively. Whereas, the water and food intake values in untreated rats was 1070 ± 14.14 ml/day and 225.95 ± 21.45g/day, respectively. TFGE doses (200mg/kg and 600mg/kg) administration compensated the reduced polydipsia (605.3±7.07ml/day; 535±7.07ml/day respectively) and hyperphagia (216.25±11.75g/day; 198.6±5.9g/day respectively). Glibil group showed a significant reduce of polydipsia and hyperphagia (P < 0.001; P< 0.01).

Table 1: Effects of TFGE administered on water intake and food intake of normal and diabetic rats

	Control	TFGE (200mg/kg)	TFGE (600mg/kg)	Glibil (3mg/kg)	Untreated
Water intake (ml/day)	496.6±5.77	605.3±7.07***	535±7.07*	715±7.17***	1070±14.14***
Food intake (g/day)	198.55±5.95	216.25±11.75***	198.6±5.9ns	207.85±3.35**	225.95±21.45***

The values present the mean ± SEM for six rats in each group (ns: no significant difference, *p < 0.05, ** p < 0.01, *** p < 0.001) compared to control group.

In the present study, streptozotocin was used in a dose of 50 mg/kg body weight intraperitoneal injection for producing hyperglycaemia in accordance with [15] used the same dose of STZ but intravenously in rats. While [16] used high dose of STZ (90 mg/kg body weight intraperitoneally in rats). The selection of higher dose was not tolerated and survive for rats and should adopted the lower dose. Traditional medicine use the food tannins because of it's good

preventive on human health. Also, some tannins are considered as antioxidants. The most properties of tannins are cardio-protective, anti-inflammatory, anti-carcinogenic and antimutagenic, and others. They act as free radical scavengers or activate antioxidant enzymes. Also, in diabetic rats, tannins have been considered as anti-hyperglycemic agents [17]. The phenolic compounds could reduce glycemia by reduction in the absorption of nutrients (food intake) [18].

Organs weight

The results showed that organs weight changed slightly compared to control group (Table2).

Heart and lungs weights of untreated or diabetic rats (0.65±0.03g; 1.23±0.05g respectively) showed a significant decrease (P <0.05) as compared to normal control rats (0.88±0.16g; 1.68±0.27g respectively). Treatment with two doses extracts produced no significant change of the testicles (2.66±0.6g; 2.51±0.28g respectively) and stomach (1.53±0.015g; 1.41±0.11g respectively) whereas, the values of untreated group was being (2.43±0.05g; 1.40±0.10g) respectively. Concerning pancreas the present data showed a higher significant decrease (P <0.001) in untreated group when compared with control group (Table 2). So, all the treatment with two doses extract showed a detectable elevation of all organs weight in diabetic rats.

Glibil treated group were reported to have measurable effect on organs weight (Heart, lungs, stomach, spleen, testicles), Whereas, Untreated group remained the lower values.

Table 2: Organs weight changes in the treated and untreated animals (g)

	Control	TFGE (200mg/kg)	TFGE (600mg/kg)	Glibil (3mg/kg)	Untreated
Heart	0.88±0.16	0.66±0.08*	0.63±0.07*	0.67±0.11*	0.65±0.03*
Liver	5.17±0.94	4.01±1.29*	4.09±1.06*	4.33±0.45**	4.15±0.7**
Testicles	3.14±0.22	2.66±0.6ns	2.51±0.28ns	2.9±0.37ns	2.43±0.05ns
Kidneys	1.80±0.06	1.66±0.13ns	1.26±0.08***	1.53±0.02ns	1.5±0.14***
Spleen	1.02±0.10	0.95±0.14 ns	0.74±0.11*	0.6±0.01**	0.61±0.07***
Stomach	1.55±0.19	1.53±0.015ns	1.41±0.11ns	1.43±0.13ns	1.40±0.10 ns
Pancreas	0.25±0.03	0.20±0.02ns	0.15±0.02*	0.14±0.02**	0.12±0.02***

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

A notable significant decrease of liver in untreated group (P< 0.001) when compared to control one (Table 2). The recorded values of diabetic rats showed a high decrease in kidneys weight (0.85±0.14 g) as compared with the normal control rats (1.80±0.06 g). An increase (hypertrophy) in the weight of liver in proportion to the body weight was observed in untreated group were compared with control group despite the fact that the mean weight of all the animals in treated group with TFGE, Glibil and untreated groups decreased (Table 3).

Table 3: Comparison between organ weight in the treated and untreated animals in grams to weight of animal in kilograms of rats (X ± S.E.M.).

	Control	TFGE (200mg/kg)	TFGE (600mg/kg)	Glibil (3mg/kg)	Untreated
Body weight	257.14±13.16	209.48±12	212±14.06	226.87±8.12	202.40±2.68
Liver	19.67g/kg	19.14 g/kg	19.26g/kg	19.08g/kg	20.50g/kg
Kidneys	7 g/kg	7.92 g/kg	6.12g/kg	6.74g/kg	7.41g/kg
Pancreas	0.97g/kg	0.95g/kg	0.70g/kg	0.61g/kg	0.59g/kg

The selective destruction and disappearance of insulin-producing cells (β cells) attributed to the decrease in the weight of pancreas [19, 20]. Alkylation of DNA, produced hyperglycaemia and necrotic lesions was caused by injurious effects of STZ leads to loss of organs weight .The results of the present study are in agreement with the findings of [5, 21, 22]. Also, [23] concluded that the reduction in body weight was associated with increase (hypertrophy) in the relative weight of kidney and liver whereas the pancreas weight was unaffected.

CONCLUSION

The inclusion of the different two doses of TFGE (200mg/kg, 600mg/kg) resulted in increased weight near to normal level. Also, TFGE showed a considerable effect on the water and food intake. The ameliorating effects of *Trigonella foenum-graecum* could be related to its tannins constituents. In conclusion, using Fenugreek seeds exhibits preventive effects against diabetes which, in turn, validates the traditional use of the plant in the treatment of diabetes mellitus. Further comprehensive chemical and pharmacological investigations with isolated active principles of the plant may shed more light on the use of fenugreek for protection in diabetes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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