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Original Research Article

Acute and Subacute Toxicity Study of *Trigonella foenum-graecum.L* Seed Extract in Wistar Rat

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ABSTRACT

Fenugreek *(Trigonella-foenum-graecum.L*) seeds are commonly utilized in cuisine as spices, in the traditional treatment and in the prevention of various troubles. This study attempts to assess the acute and subacute toxicity of its extract in *wistar rats*. In the acute toxicity, single oral administration of four doses (0,05 g, 0,3 g, 2 g and 5 g /kg.bw) to *wistar rats* was tested and to test subacute toxicity, quotidian oral administration of fenugreek seed extract at dosages of 1, 2 and 3 g/kg.bw was used for 28 days. The results of acute toxicity did not show any deaths and no indication of intoxication was observed in the rats for 14 days following receipt of the various doses of the extract. The subacute toxicity results demonstrated that repeated administration of the extract led to a significant weight gain ($p<0.05$) in treated *rats* in comparison to control *rats*. Treatment of *rats* with fenugreek seed extract did not cause any signs of intoxication and did not affect the hematological parameters of the *rats* compared to control rats ($p>0.05$). Gross examination revealed that the appearance of vital organs was not altered and histological examination revealed steatosis and an increase in the nuclei of hepatocytes in *rats* given a repeated dose of 3 g /kg of fenugreek seed extract. Fenugreek seeds extract is generally tolerated by *rats* even at high doses but more care should be taken at chronic use.

Keywords: acute toxicity, extract, subacute toxicity, *Trigonella-foenum-graecum.L*, *wistar rat*

Introduction

Trigonella-foenum-graecum L. is an annual living plant that belongs to Fabaceae family that grows on many continents including Asia, Europe, and Africa., where it is used in the preparation of different recipes and in traditional medicine.[1] The presence of a diverse array of phytochemicals in fenugreek contributes to its health-promoting and disease prevention properties. In fact, its seeds contain flavonoids,[2] Saponins,[3] proteins, polyphenols, fibers (soluble and insoluble fibers),[4] coumarin compounds and alkaloids (trigonelline, gentianine, carpaine)[5] and lipids.[6]

Trigonella-foenum-graecum.L seeds are frequently utilized in traditional medicine and its biological activities have been confirmed in several studies[7]. Previous study showed that they possess a set of health benefits and pharmacological properties including anti-diabetic activity,[8] anti-cancer effect,[9] antimicrobial effect,[10] anti-inflammatory and hypocholesterolemic activities,[11] anabolic activity,[12] antioxidant effect.[13] However, its toxicological effects increase considerably.[14] The objective of the current study was to investigate the acute and subacute toxicity of oral treatment of fenugreek seed extract (FSE) in *wistar rats*.

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Materials and Methods

Preparation of plant material

Seeds recently harvested were obtained in Ksar Elkebir city (North-western Morocco) (Figure 1) in November 2021 and identified by Professor L. Zidane (Faculty of Sciences, Ibn Tofail University. Morocco) and a voucher specimen (N1352021) was placed at Biotechnology and Valorization of Natural Resources Laboratory.

To prepare the plant extract, the dried seeds of fenugreek were crushed to a powder. 40 g of fenugreek seed powder underwent extraction cycles with methanol (99%) using a Soxhlet at 30℃ for sex hours. The resulting solution was subsequently concentrated employing a rotavapor (25℃). Afterward, the generated pasty product was dried at 30℃ in an oven until the dry extract was obtained. The extraction yield was 8.2%.

Yield (%) = (The quantity obtained (g)/the initial quantity (g)) × 100

Animals used in the study

Wistar rats were acquired from the Science Faculty's animal facility at Ibn Tofail University in Morocco (Ethical approval Number 1274/2/21). *Rats* were separated into groups based on their body weight and kept at a regular temperature of 22-26 °C and a dark/ light cycle of 12 hours. Animals were acclimated for 5 days and having unlimited food and water availability before the trials began, and all required precautions were followed to reduce pain and animal distress.

Acute toxicity

The oral acute toxicity was carried out according to the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals [15]. To evaluate the toxicity of the extract and estimate its Lethal Dose 50 (LD 50), Four groups of six *rats* were formed. Animals were fasted deprived of food the night preceding the experiment and they were given only water. Different dose levels of the fenugreek seeds extract were administered to rats through gastric gavage; the group1 received 50 mg/kg, the group (2) 300 mg/kg, the

group (3) 2000 mg/kg, and the group (4) 5000 mg/kg body weight of FSE. After receiving their doses, treated *rats* were monitored for 6 hours and thereafter for 14 days [15] to look for indicators of intoxications such as mortality, behavioral changes, respiratory and digestive system alterations, diarrhea, lethargy, coma, tremors, and convulsions.

Subacute toxicity

To investigate the subacute toxicity of fenugreek seeds, 4 groups each containing 6 *wistar rats* were formed; Group (1) used as group control receiving only distilled water and a standard diet, groups 2,3 and 4 received a daily dose of 1g /kg, 2g /kg and 3g /kg.bw respectively of the extract for 28 days. Each group composed of 6 *rats* weighed between 117.1 and 128.3g at the beginning of the study.

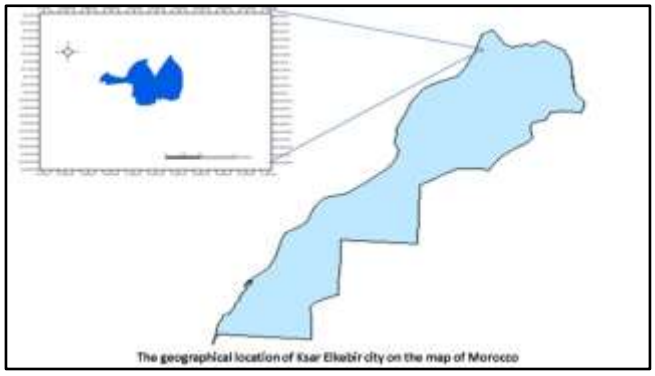

Figure 1: the source region of the fenugreek seeds used in this study

Clinical signs and body weight of rats

The *rats* were fasted for 2 hours before receiving their plant doses, and they were monitored for 4 hours afterward to detect symptoms of toxicity. The progression of *rat*'s body weight was measured each week during all the study period.

Hematological analyses, gross and histopathological examination of vital organs

After four weeks of daily treatment, the *rats* were euthanized under ether anesthesia and Blood samples were taken in tubes for hematological analyses. Then, *rats* were sacrificed in order to obtain their kidneys and livers for gross examination. After that, the organs were preserved in paraformaldehyde until histological analysis using the usual technique of hematoxylin and eosin staining was performed.[16]

Statistical analyses

In all groups, means and standard deviations (MSD) were determined for measurement data. by means of t-Student test and One-Way Analysis of Variance (ANOVA). The statistical significance of the experimental and control groups was compared and statistical significance was determined as a P-value less than 5%.

Results and Discussion

The treatment of the *rats* by only one dose gavage of 50, 300, 2000, and 5000 mg /kg.bw of FSE did not cause any deaths, and all animals lived normally for the period of the experiment. No clinical symptoms of toxicity were reported directly or throughout the post-treatment period even with the highest possible dosage of FSE of 5000 mg/kg.bw. These findings indicated that FSE is not toxic and its LD50 is higher than 5g/kg.[15] Our results are in agreement with earlier studies.[12,17] Another study showed that fenugreek seeds extract show media lethal dose (LD50) higher than 4350 mg/kg.[18] Contrariwise, another previous study have find that *rats* treated with a saponins extracted form fenugreek showed behavioral changes and mortality.[19]

Observations of the *rats*' behavior after taken their doses during the 28 days of treatment did not show any remarkable sign of intoxication except the death of one *rat* in group (1) on the eighth day of the study; This case was not a dose-related effect, and it was not observed in other animals given higher doses. Our results are supported by report where *rats* fed with 1,5 and 10% debitterized fenugreek powder for three months showed no evidence of signs of toxicity or mortality.[20] These findings agrees also with a previous study which showed no alterations were identified in animals after the eye examination following *rat*'s treatment with consecutive doses of 250, 500 and 1000 mg/kg of fenugreek seed extract.[17] However, our findings are not entirely in accordance with the results of a earlier study which have find some clinical signs of toxicity sch as for-limb inflammation, alopecia, inflammation and inflammatory in *rats* following a chronic treatment of 1000 mg/kg of fenugreek extract.[21] Further, another study investigation showed a high percentage of mortality in *mice* treated with 1000 mg/kg of glycosides based standardized fenugreek seeds.[18]

All *rats* of the experiment recorded a positive body weight gain. Indeed, *rats* treated with the extract proved a remarkable elevation ($p<0,05$) in their body weight when compared with the control group. However, the difference between the three groups given various doses of the extract is not statistically significant ($p>0,05$) (Table 1). These outcomes are consistent with an earlier study.[21] In contrary, in a previous study, male *mice* fed with 1000 mg/kg.bw of glycosides derived from standardized fenugreek seeds extract lost weight.[18]

Table 1: The Evolution of body weights of rats (W: week)

Groups	W 0	W 1	W2	W 3	W 4
Control	122.10 ± 21	129.15 ± 16	135.18 ± 16	143.17 ± 16	150.21 ± 16
1000mg/kg	118.16 ± 10	130.10 ± 16	141.17 ± 13	153.20 ± 18	164.08 ± 14
2000mg/kg	123.05 ± 12	134.09 ± 19	145.13 ± 20	158.17 ± 18	169.10 ± 14
3000mg/kg	126.10 ± 13	137.90 ± 19	149.50 ± 16	152.09 ± 09	166.78 ± 18

Table 2: Hematological indicators of different study groups (G)

Indicators	G 1	G2	G 3	G 4
Red cells (106 / mm3)	6.37 ± 1.26	6.42 ± 2.23	6.41 ± 3.43	6.39 ± 1.23
Hemoglobin(g/dl)	11.42 ± 1.24	11.74 ±4.36	11.23 ±3.18	11.35 ± 2.32
Hematocrit (%)	32.63 ± 1.32	34.13 ±4.22	33.89 ±3.24	33.23 ± 2.41
Mean corpuscular volume	53.34 ± 3.26	52.72 ±1.14	53.93 ±2.23	52.55 ± 1.28
mean blood count hemoglobin	17.18 ± 1.35	17.43 ±1.24	18.28 ±1.18	17.58 ± 3.42
Lymphocytes (%)	71.54	73.84	72.62	71.07
Platelets (thousand/ mm3)	319.18 ± 12.73	318.45±6.3	321.7 ±7.13	318.44±5.49

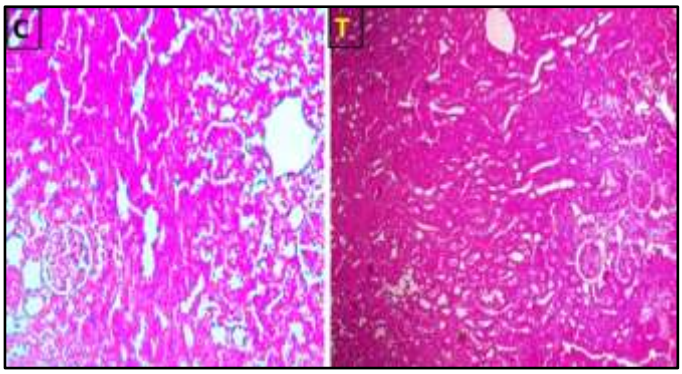

Figure 2: A normal kidneys in control group (C) and in group treated with 3 g/kg.bw (T

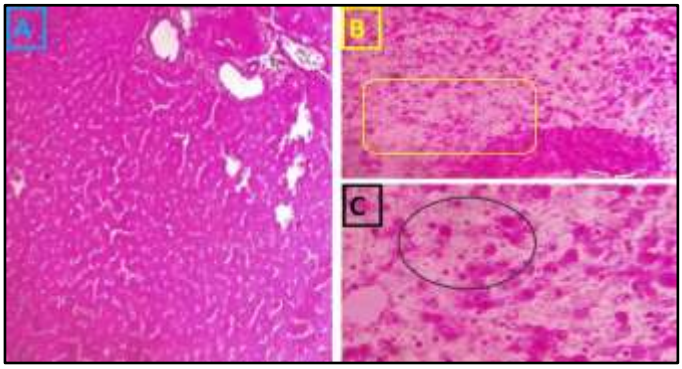

Figure 3: histological sections showing a normal liver in group control (A) and Steatosis (B) and an increase in the size of cell nuclei of rats given a dose of 3 g / kg of fenugreek seed extract.

Oral administration of 1000, 2000 and 3000 mg/kg.bw of FSE to *rats* during four weeks did not caused significant changes in hematological parameters and hematological indicators were within normal margin in comparison with the group control (Table 2). These findings are consistent with earlier researches, which has proven that that repeated administration of extracts from fenugreek seeds does not cause any variations in the hematological parameters of the animals studied.[17,21] In the contrary, an earlier study have shown some changes in hematological parameters of mice after oral administration of repeated dose of glycosides based standardized fenugreek seeds.[18]

Histological sections taken after treatment of the *rats* revealed no changes in the structure of the rat kidneys compared to the control group (Figure 2). However, histological examination of the *rat*'s livers showed steatosis (Figure 3.B) and the increase of the size of the nuclei of the hepatic cells (Figure 3.C) in *rats* given a dose of 3g/kg.bw of the FSE in comparison with a normal liver (Figure 3.A). A previous study reported renal damage and inflammatory cells in kidney and vascular congestion of the hepatocytes in the liver of *rats* administered repeated dose of 1000 mg/kg of standardized fenugreek seed extract based on glycosides.[18] On the other hand, other studies didn't report any remarkable changes in the structure of vital organs of animals treated with fenugreek seeds extracts.[17,20]

Previous studies showed that fenugreek seeds extracts improve the immune status,[22] ameliorate healthy sperm, cardiovascular health, mental alertness and overall performance in human subjects,[23] enhance symptoms of testosterone deficiency syndrome,[24] and protect cellular inflammation and metabolic alternations.[25] An earlier study found that fenugreek glycoside supplementation has beneficial effects on endurance during training,[26] Another recent study found that fenugreek seeds stimulate breast milk production in postnatal women as well as helping children in gaining weight during their first week of life.[27]

The pharmacological properties as well as the toxicological effects attributed to medicinal plants are due to their chemical composition.[28,29] Fenugreek exposure, either short or long duration, can have a variety of impacts including detrimental impact on reproductive performance and probable teratogenic consequences in fetuses,[30] antifertility activity,[31] spermatotoxic effect.[21] At high doses, fenugreek causes teratogenicity, fetotoxicity, reproductive changes and abnormal sperm shapes associated with DNA damage.[32] An earlier report have shown Trigonelline may have unfavorable effect on the skeletal system.[33] Moreover, fenugreek seeds aqueous extract impairs sensorimotor and coordination function.[34] Among its side effects, fenugreek seeds extract causes hypothyroidal effect.[35] In humans, chronic administration of fenugreek seeds to diabetic patients caused transient diarrhea and excess of flatulence,[36] dyspepsia and mild abdominal distension.[37] It was demonstrated that fenugreek extract may cause a slight stomach discomfort.[38] Another study has found that hepatotoxicity was detected in certain subjects treated with coumarin.[39]

Conclusion

The results of the acute toxicity study demonstrated that even on a scale of 5000 mg/kg.bw the extract of fenugreek seeds did not cause mortality in *wistar rats*. Equally, the findings of the subacute toxicity study showed that repeated oral gavage of a high dose during 28 days did not affect the behavior and hematological parameters of *rats*. However, consumption of fenugreek seeds caused a statistically significant elevation in the body weights of *rats* compared to control rats and tissue-scale changes have been observed such as increased hepatocytes nuclei size and steatosis in the group treated with a dose of 3g/kg for 4 weeks. Further, more investigations are needed to determine the safety of fenugreek seeds in the medium and long term, as well as in humans.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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