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Safety Evaluation of Methanolic Extract of Fenugreek Seeds on Hepatic, Renal and Cardiac Parameters: An Animal Experiment

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ABSTRACT

Introduction: Fenugreek is one of the plants with great therapeutic significance. It contains numerous bioactive compounds. Knowing the safety of crude extracts on various body organs is a must.

Aims & Objectives: To assess the safety of methanol extract (MEFS) of fenugreek seeds on various hepatic, renal and cardiac parameters.

Place and Duration of Study: The study was conducted in the department of Pharmacology, University of Karachi for a period of 2 months from 1-2-2023 till 30-3-2023.

Materials & Methods: Safety evaluation of MEFS was conducted on 28 white rabbits of both genders weighing 1-1.5 kg after getting ethical approval. Animals were apportioned into 4 groups by simple random sampling. Each group consisted of 7 animals. Group I was categorized as control and given distilled water 1m1/kg per oral route O.D. Remaining 3 groups were test groups. They were fed with fenugreek extract using 3 different doses (50, 100 and 200 mg/kg body weight) in the same amount given to Group I per oral route O.D for 45 days. Blood sample from marginal vein of ears of rabbits were taken on 46th day and tested on Humalyzer using standard reagents. Data was analyzed using SPSS version 24, via one-way ANOVA and post hoc tukeys HSD test.

Results: After 45 days of feeding the extract, insignificant decrease in aspartate transaminase (80.12±14.5), alanine transaminase (67.32±15.5), γGT (8.46±0.14), total bilirubin (0.82±0.11), direct bilirubin (0.14±0.02), total protein (07.21± 1.11), urea (37.23±0.54), creatinine (0.78±0.15), lactate dehydrogenase (356.23±16.11),creatinine phosphokinase (404.33± 76.45) and blood electrolytes (Na 2.76± 0.21, K 3.55±0.43 and Ca 3.81±0.26) was observed in all test groups when compared to control group.

Conclusion: The study indicates higher safety of fenugreek seeds extract on liver, kidney and heart as observed from effects on various hepatic, renal and cardiac parameters. Isolation of active compounds and testing on large sample size are suggested for future studies.

Keywords: Fenugreek seeds, Methanol extract, Liver enzymes, Renal parameters, Cardiac enzymes, Rabbits

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) an ancient medicinal herb pertains to the family Fabaceae is well mentioned in literature.[1] It is approximately 30-60 cm in height and produced yearly. [2] The straight hairy plant bears three layered, green, raggedy leaves with long stalks.[3,4] Comprehensive nomenclature of plant is revealed in Figure 1. Pods are structured as knife-edge, having bent beak tip, filled with seeds. Seeds are brown in color, hard and small. They are compressed and have a diverse diamond shape (Figure 2). They have a peculiar smell and bitter taste[5]. With a rich history spanning centuries, fenugreek seeds have been a valued remedy in traditional medicine worldwide.[6,7] It is inherent to western Asian countries. The worldwide farming is distinctive of its alteration to changing weather conditions. It gives specific essence and tint to the food due to various aromatic compounds present in it.[8] The whole plant is rich in carbohydrates, protein, lipid, and fibers. It also encloses many essential minerals, vitamins and bioactive compounds such as steroidal saponins, alkaloids, and polyphenols.[9] Therapeutic significance of Fenugreek is comprehensively quoted in Ayurveda, Greek and Latin books. Leaves are used to treat indigestion and flatulence.[10] Chinese consumed the seeds for the cure of edema and

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weakness.[11] In India, seeds were used for milk stimulation and as a medicine for stomach ache and hair fall.[12,13] Leave and seeds extract were also used for the therapy of oral blisters and throat congestion. It also reduced muscular and arthritic pain in gout. Greek people consumed this to stimulate labor pain. Fenugreek has also been used as a remedy for flu, influenza, nasal congestion, and constipation. Various scientifically proven activities of plant have been found in literature such as anticancer, anti-diabetic, neuroprotective and antioxidant.[14,15,16] The current research was particularly intended to examine the safety of methanol extract prepared from fenugreek seeds on various hepatic, renal and cardiac parameters in rabbits.

Figure: 1 Fenugreek Plant

Figure: 2 Fenugreek Seeds

MATERIALS AND METHODS

This experimental study (randomized control animal trial) was performed on 28 healthy white albino rabbits of either gender in the weight range of 1-1.5 kg after obtaining ethical approval from IRB of Karachi University (Ref:09/23/IRB/KIMS dated 14-1-23). Rabbits were sited at the animal house of Pharmacology department under temperature (22±2 ^{0}C) and humidity (50-60%) in an alternative 12-h light/dark cycle. Animals were given standardized diet and water on regular basis. Rules given by the National institute of Health (NIH) were followed for animal's conduct and experiments. Health of animals were monitored by carefully observing appetite, activity level, urine output, feces, skin and hair condition, any signs of lethargy or respiratory issues and color of conjunctiva on daily basis. Body weight measurements were done weekly. Sample size was calculated by online sample size calculator (Confidence level: 95%, margin of error:5%, population proportion 50%, population size: 30). Simple random sampling was used to divide the animals into four groups containing equal number of animals. Group I was labelled as control and provided with distilled water 1m/kg per oral route O.D. Group II, III and IV were labelled as test groups and fed with methanol extract of Fenugreek in 03 different doses (50,100 and 200 mg/kg) in the same quantity given to control group per oral route once a day. Extract was administered to test groups for 45 days.

Fenugreek seeds were procured from reliable herbal shop in Karachi and recognized by Dr. Mohtasheem, Chairman, Pharmacology department. University of Karachi (UOK). Voucher specimen no: (FGS-01-14/16) was issued and deposited in the same department.

Methanolic extract of fenugreek seeds was prepared based on cold extraction method. Seeds were cleansed from any dirt and dissolved in 85% 1500 ml methanol for 15 days in sealed containers. Jars were shaken frequently on every alternate day. Solvent was filtered through What-Mann No.1 filter paper, evaporated using rotary evaporator at 45 ^{0}C under reduced pressure and freeze dried at-30 ^{0}C. The acquired crude extract was conserved in petri dishes at -20 ^{0}C. Blood samples were collected on day 46 from marginal veins of the rabbit ears in test tubes. Centrifugation of blood was performed on 14 K Humax centrifuge machine (Human Germany) at 2000 RPM for 15 minutes. Serum obtained were kept at -20 ^{0}C temperature. A number of hepatic (liver enzymes including aspartate aminotransferase, alanine transaminase, γ glutaryl transferase, total and direct bilirubin), renal (total protein, urea and creatinine) and cardiac tests (lactate dehydrogenase and creatine phosphokinase) and serum electrolytes assessments were performed on Humalyzer (Human Germany) using standard reagents.

Statistical Analysis: All the results acquired were analyzed on SPSS version 23. Obtained values are shown as mean and standard error of mean (SEM). Data was analyzed using one-way ANOVA and post

hoc Tukey's HSD test. Shapiro Wilk test was used to check the normality of data. Results showing P 0.05 were considered as statistically significant and p<0.001 as highly significant.

RESULTS

Table 1 describes the outcome of MEFS on various hepatic markers AST, ALT, GT, total bilirubin and direct bilirubin after 45 days of regular supply. It is obvious that Groups II, III and IV given the extract at 50, 100 and 200 mg/kg body weight respectively demonstrated statistically insignificant reduction in AST 81.11 ± 10.43, 78.51 ± 62.13, 80.12± 14.5 IU/L respectively as compared to group I (control) 83.18 ± 25.25 IU/L. Similarly, all the Groups II, III, and IV revealed insignificant lowering in ALT enzymes 67.34±15.15, 62.13±20.1, 67.32±15.5 IU/L respectively as compared to control group I (control) 71.82 ±10.43 IU/L. GT levels were also decreased insignificantly in group II, III and IV 11.43±0.65,10.31±1.34, 8.46±0.14 IU/L respectively as compared to group I (control) 12.21±2.11 IU/L. Total and direct bilirubin also decreased insignificantly. Total bilirubin in groups II, III and IV were 0.84±0.15, 0.81±0.13, 0.82±0.11 mg/dl respectively when compared to Group I (control) 0.86±0.23 mg/dl. Direct bilirubin in group II, III and IV were 0.12±0.05, 0.12±0.07, 0.14±0.02 mg/dl respectively when compared to group I (control) 0.14±0.06 mg/dl.

Table 2 presents the outcome of MEFS on renal tests i.e. total protein, urea and creatinine after 45 days. It is apparent that there were insignificant effects on renal values of group II, III and IV in comparison to control group. Total protein in groups II, III and IV were 07.22± 0.11, 08.86 ± 1.21, 07.21± 1.11g/dl respectively when compared to group I (control) 07.31± 2.14 g/dl. Urea levels in groups II, III, IV were 38.12±1.13, 36.13±0.76, 37.23±0.54 mg/dl respectively when compared to group I (control) 38.56±4.23 mg/dl. Creatinine levels in group II, III and IV were 0.75±0.43, 0.73±0.05, 0.78±0.15mg/dl respectively when compared to group I (control) 0.75±0.21mg/dl.

Table 3 has shown the action of MEFS on two important cardiac enzymes including CPK and LDH after 45 days of feeding. Effect on cardiac enzyme CPK in group II, III and IV was insignificant 403.44± 43.37, 404.37± 56.32, 404.33± 76.45 IU/L respectively in comparison to group I (control) 411.32 ± 82.46. Effect on cardiac enzyme LDH in group II, III and IV was insignificant 356.32±25.03,363.28±18.15, 356.23±16.11 IU/L respectively in comparison to group I (control) 357.23 ±85.21.

Table 1: Effect of Fenugreek seeds extract on hepatic function tests

Dosage & Group	Aspartate Amino Transferase (AST) IU/L	Alanine Amino Transferase (ALT) IU/L	γ Glutaryl Transferase (γGT) IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
1mg/Kg DW Group I	83.18 ± 25.25	71.82 ±10.43	12.21±2.11	0.86±0.23	0.14±0.06
50 mg/Kg Group II	81.11 ± 10.43	67.34±15.15	11.43±0.65	0.84±0.15	0.12±0.05
100mg/Kg Group III	78.51 ± 62.13	62.13±20.1	10.31±1.34	0.81±0.13	0.12±0.07
200 mg/Kg Group IV	80.12± 14.5	67.32±15.5	8.46±0.14	0.82±0.11	0.14±0.02

Table 2: Effect of Fenugreek seeds extract on renal tests

Dosage & Group	Total Proteins (gm/dl)	Urea (mg/dl)	Creatinine (mg/dl)
1mg/Kg DW Group I	07.31± 2.14	38.56±4.23	0.75±0.21
50 mg/Kg Group II	07.22± 0.11	38.12±1.13	0.75±0.43
100mg/Kg Group III	08.86 ± 1.21	36.13±0.76	0.73±0.05

Table 3: Effect of Fenugreek seeds extract on cardiac tests

Dosage & Group	CPK (IU/L)	LDH (IU/L)
1mg/Kg DW Group I	411.32 ± 82.46	357.23 ±85.21
50 mg/Kg Group II	403.44± 43.37	356.32±25.03
100mg/Kg Group III	404.37± 56.32	363.28±18.15
200 mg/Kg Group IV	404.33± 76.45	356.23±16.11

Electrolytes: Table 4 has shown the outcome of MEFS on various electrolytes after 45 days of feeding. Effect on sodium, calcium, and potassium in all the test animals was revealed to be insignificant in comparison to the control.

Table 4: Effect of Fenugreek seeds extract on Serum Electrolytes

Dosage & Group	Na (ug/ml)	K (ug/ml)	Ca (ug/ml)
1mg/Kg DW Group I	3.23±0.21	2.65 ±0.11	2.32±0.11
50 mg/Kg Group II	2.31± 0.11	2.43±0.25	3.61±0.81
100mg/Kg Group III	2.97± 0.52	3.22±2.1	4.18±2.2
200 mg/Kg Group IV	2.76± 0.21	3.55±0.43	3.81±0.26

DISCUSSION

Plants are utilized as a source of medication virtually in all regions. Approximately 40% of the entire drug consumption is ascribed to herbal products in China.[17] In Japan, Herbal preparations are more popular than other medicines.[18] Similar to other underdeveloped countries, cure with herbal medicines is well accepted in Pakistan.[19] In Pakistan, people commonly use plant derived medicines particularly in rural areas due to ease of availability, low cost and less side effects. [20] Pharmacological effects of several plants are researched in detail.[21] Safety and efficacy of these products is not yet authenticated. Evidence regarding active constituents, toxic and adverse effects of these preparations is still lacking. Knowing the harmful effects and implementation of regulations for proper labeling of products are measures which can improve the quality and efficacy of herbal preparations for medicinal use.[22] Essential tests related to specific organs are usually performed to investigate the safety of new drugs, extracts, or preparations. Organs release various enzymes in blood in case of harm or injury caused by some new drug or extract. Calculating the level of leaked enzymes helps to diagnose harm to that tissue. ALT and AST enzymes are indicators of liver injury and can evaluate the degree of harm to liver.[23] ALT enzymes increase in serum whenever there is increased membrane permeability. Present study revealed insignificant decrease in liver enzymes AST and ALT in comparison to control. Also, there was insignificant effect on γGT, total bilirubin and direct bilirubin levels in contrast to control after 45 days of regular feeding to them indicating the safe use of extract for liver. Levels of total protein, urea and creatinine indicate kidney function.[24] If their values are elevated, this shows reduced kidney function. The present study highlighted insignificant effect of extract on all these markers at all 3 doses in comparison to control group suggestive of safe for kidney. Effect on cardiac enzymes after 45 days of feeding of extract was also found to be insignificant in all test groups in comparison to control. Electrolytes perform an essential role in various body functions such as governing body fluid acid base equilibrium, neuronal transmission and contraction of muscles. Electrolyte disproportion due to kidney disease, dehydration state, or emesis can cause various complications [25]. There was insignificant effect of MEFS on blood sodium, potassium and calcium levels in all the test groups after 45 days.

CONCLUSION

The study indicates higher safety of Fenugreek seeds extract on liver, kidney and heart as observed from the effects on various hepatic, renal and cardiac parameters. Moreover, no signs and symptoms of gross toxicity were noted during the entire study period. Isolation of active compounds and testing on large sample sizes are suggested for future studies.

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