



Research Article

ACUTE TOXICITY OF *TRIGONELLA FOENUM-GRÆCUM* (FENUGREEK) SEEDS AQUEOUS EXTRACT ON LIVER IN MALE MICE, HISTOPATHOLOGICAL STUDY

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Article Received on: 09/03/17 Approved for publication: 18/04/17

DOI: 10.7897/2230-8407.080443

ABSTRACT

Acute toxic effect of fenugreek on liver histology has not been fully explored. Hence the objective of this study is to investigate the acute toxicity of fenugreek seeds aqueous extract (FSA) on liver histology. Twelve male Swiss albino mice, were randomly divided into control (C), and three treatment (T₁, T₂, and T₃) groups (n = 3 each). T₁, T₂, and T₃ were given 3g, 6g, and 9g/kg body weight FSA respectively. Intragastric divided doses of FSA were given as per OECD guidelines 425. Continuous observation of signs of acute toxicity and survival set up. The mice were euthanized on day 14 and the liver was dissected and processed for histopathological examination. 3, 6, and 9g/kg body weight FSA doses failed to induce any of signs of acute toxicity. Histopathological examination revealed that, all FSA administered doses showed mild portal inflammation, mild mononuclear cell infiltration in hepatic parenchyma, in addition to mild bile stasis induced only in mice received 9g/kg of FSA. Administered doses of FSA showed mild liver histopathological inflammatory changes.

Key words: Acute toxicity, FSA, OECD.

INTRODUCTION

Different pharmaceutical dosage forms of fenugreek are available these days as an herbal drug for medical uses in the treatment of: bronchitis, abscess, diabetes, hypercholesterolemia, and as a protective drug for liver against lipid accumulation¹, and for kidney against diabetic nephropathy². Due to its documented historical and traditional use as a spice and medicinal herb in various parts of the world, fenugreek has been granted "generally recognized as safe" (GRAS) status by the U.S. food and drug Administration (FDA)-SP/ESO, GRAS - 182.10, 182.20³. Significant clinical harmful adverse effects (in human), due to consumption of fenugreek as a food or as medicinal supplement have not been reported⁴. But data of safety of different fenugreek extracted forms, and the acute & chronic toxicity doses still not anticipating the increasing medical use of fenugreek. Various pharmacologically active compounds with different concentrations have been isolated from fenugreek seeds such as: Alkaloids, flavonoids, tannin like phenolic compounds, polyphenols, steroids, saponins, free amino acids, unusual amino acid 4-hydroxyisoleucine, lipids, phospholipids, mucilaginous fibers, vitamins, and minerals⁵, some functions of these active compounds are known, but many still unknown. Even though no fenugreek adverse effects on human has been reported to date, testing of fenugreek toxicity effect on liver histology in animal models is the first step to open the window for future clinical trials to investigate safety of fenugreek for applied medical uses.

Fenugreek seeds aqueous extract (FSA) has been tested for many therapeutic uses, therefore toxicological histopathological evaluation in laboratory animals is needed before FSA is recommended to be safe for human usage, hence this study aimed to investigate the FSA acute toxicity on liver histology in male mice model.

MATERIALS AND METHODS

Animals

Twelve Swiss albino male mice were purchased from Sapphire enterprise (Selangor -Malaysia). Their ages were 6 to 7 weeks, their weights were 25 to 26 grams. The mice were housed in animal cages under standard experimental conditions of temperature of 24 °C, with relative humidity 60 ± 5%, 12-hour dark / light cycle, and they had free access to water ad libitum. The mice were acclimatized for one week to the new environment before starting the experiment. The experiment carried out for 14 days, and conducted according to OECD guide lines 425 (2008). The experimental protocol was approved by the International Islamic University Malaysia Institutional Animal Care and Use Committee (IACUC-IIUM) IIUM (No. of IACUC Approval: IIUM / IACUC Approval /2016 / (11) (68).

Preparation of fenugreek seeds aqueous extract (FSA)

One kilogram of fenugreek seeds was purchased from local market in Yemen (Rada'a market). The fenugreek seeds were identified and verified by Botanist at Biodiversity Unit, institute of Bioscience University Putra Malaysia in cooperation with Herbarium department Kuliyah of Pharmacy IIUM, voucher specimen identification was deposited at the Herbarium, Kuliyah of Pharmacy under voucher specimen No.: PIUM 0226-2. Using a modified method of traditional medicinal practitioners, FSA extract was prepared⁶. The prepared fenugreek seed powder was put in distilled water in a ratio of 1 gram of powder in 20 ml of distilled water, followed by stirring for 24 hours using magnetic stirrer at room temperature. After that, the aqueous extract was transferred into 50 ml falcon tubes and centrifuged at 10000 rpm for 5 minutes using centrifuge machine (Hettich UNIVERSAL 320R, Germany), the yield of the extract was then frozen at -80 °C in (Haier ULT FREEZER, China). Then the extract was put in the freeze dried machine for 7 days (Freeze Alpha 1-2LDplus CHRIST, Germany). Then FSA powder form, was stored in -20 °C freezer (Haier freezer, China) until use.

Fenugreek seeds aqueous extract doses and administration

Based on: Evaluated maximum tolerated dose of fenugreek (MTD) (9.77 g/kg)⁷, LD₅₀ of fenugreek leaves aqueous extract (10 g/kg)⁸, previously tested maximum safe dose of fenugreek (3g/kg)¹, and OECD guidelines 425⁹ for testing chemicals, the following doses of fenugreek seed aqueous extract was selected for acute toxicity study: 3g/kg, 6g/kg, and 9g/kg. There were three treatment groups of Swiss albino male mice (3 per group), and one control group. FSA was administered once in divided doses in the first day of the experiment, with continuous observation for 14 days' post treatment. At the end of the experiment (14 days) all mice were euthanatized and liver was dissected for histopathological processing after macroscopic examination.

Histopathological processing

The dissected livers were grossly examined for: surface smoothness, colour change, and any abnormal growth, and then immediately was fixed in 10% neutral buffered formalin for histological examination. The livers were processed using automated tissue processor (Leica TP 1020). The tissues were embedded into paraffin blocks (Leica EG1160). The tissues were sectioned at 4 µm thickness and stained with hematoxylin and eosin (H&E) stain according to the histopathology laboratory work procedure and safety guidelines of IIUM Pathology Department using Haematoxylin and Eosin staining¹⁰.

Table 1: Acute toxicity effects of fenugreek seeds aqueous extract on liver histopathology

Group	Histopathological change			
	Portal inflammation	Mononuclear cell infiltrate	Steatosis	Bile stasis
Control	Absent	Absent	Absent	Absent
T1 (3g/kg)	Mild (+1)	Mild (+1)	Absent	Absent
T2 (6g/kg)	Mild (+1)	Mild (+1)	Absent	Absent
T3 (9g/kg)	Mild (+1)	Mild (+1)	Absent	Mild (+1)

Number of animals per group = 3. T1: Treatment group 1 received 3g/kg Fenugreek Seeds Aqueous extract, T2: Treatment group 2 received 6g/kg Fenugreek Seeds Aqueous extract, T3: Treatment group 3 received 9g/kg Fenugreek Seeds Aqueous extract. Mild (+1) portal inflammation = Portal inflammation can be identified but is limited. Mild (+1) mononuclear cell infiltrate = Very limited mononuclear cell infiltration.

Mild (+1) bile stasis = Limited bile stasis.

RESULTS

Acute toxicity effect study of FSA- on liver histology

Table 1; summarizes the histopathological findings in the liver of each of the experimental groups, all FSA administered doses showed mild portal inflammation, mild mononuclear cell infiltration in hepatic parenchyma, in addition to mild bile stasis induced only in mice received 9g/kg of FSA. Group T1 (received FSA dose 3g/kg body weight) and group T2 (received FSA dose 6g/kg body weight) when compared to the control (Figure 1.A and Figure 1.B), showed mild portal inflammation, with minimal hepatic parenchymal mononuclear cell infiltration as shown in Figures (1.C to 1.F). Group T3 (received FSA 9g/kg body weight) when compared to the control, showed mild portal inflammation, mild liver parenchymal mononuclear cell infiltration, with mild bile stasis, however no steatosis was observed (Figure 1.G and Figure 1.H).

DISCUSSION

The current study showed evidence of mild hepatic inflammation in the form of portal inflammation and mononuclear cell infiltration, in all of the treatment groups, in a dose dependent pattern. In addition to the previous mentioned findings, the

treatment group which received dose of 9g/kg fenugreek extract showed very mild bile stasis. The biochemical results of our preceding study; correlate with the mild histopathological finding for the evidence of mild toxic effect of high doses of fenugreek seeds aqueous extract on the liver. Kandhare et al., (2015) found a significant biochemical and histopathological liver changes on repeated doses for 28 days (subacute study) with oral administration of 1000mg/kg body weight glycoside based fenugreek extract¹¹. Another earlier study of acute toxicity in which 2g/kg fenugreek powder were used showed no biochemical or histopathological abnormality in the liver¹². The results of the present study showed possible mild toxic effect on the liver by doses of 3gm, 6gm, and 9gm/kg body weight of FSA extract in male mice which could be due to induction of hepatitis, or might be due to altered gene expression induced cell injury¹³.

CONCLUSION

In the present study, administration of fenugreek seeds aqueous extract at doses of 3gm, 6gm, and 9gm/kg body weight resulted in mild portal inflammation, mononuclear cell infiltration in liver parenchyma, and mild bile stasis in male Swiss albino mice.

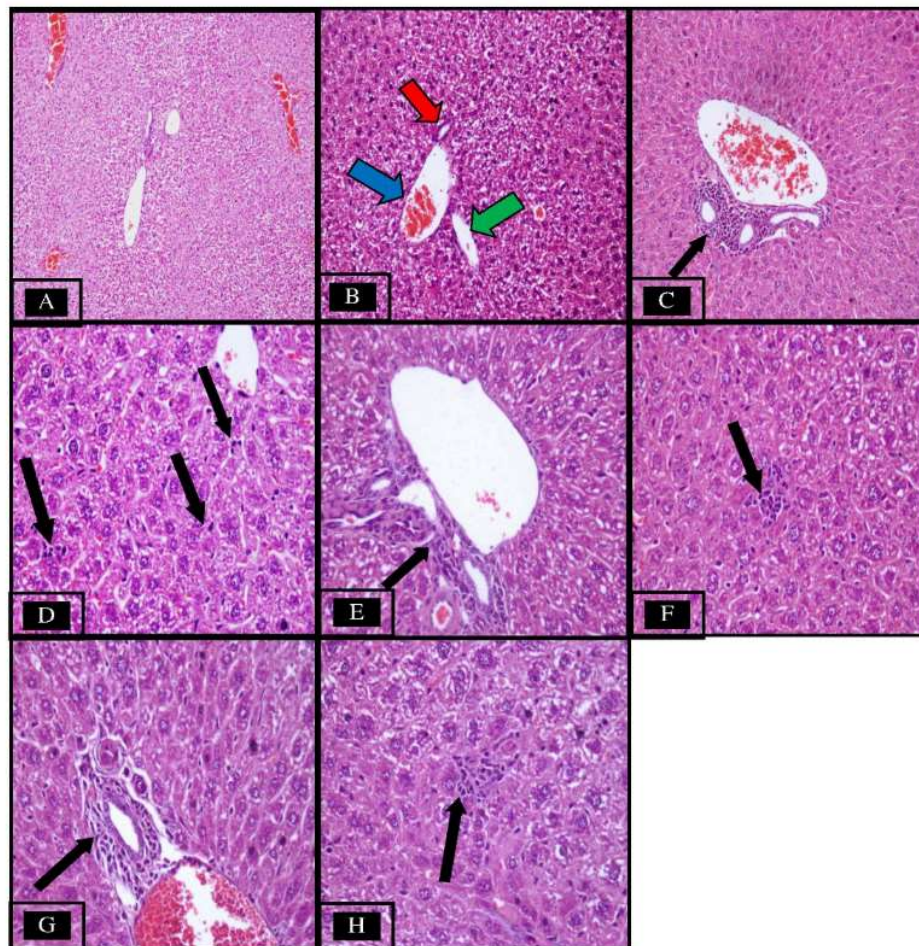


Figure 1: Photomicrograph of liver histology by Hematoxylin and Eosin stain from all the groups. (A) Liver histology from the control group (100x magnification). (B) Liver histology from control group (200x magnification) shows portal triad (red arrow - hepatic artery, blue arrow - portal vein, green arrow -bile duct). (C) Liver histology from treatment group T₁ (200x magnification) shows mild portal inflammation (arrow). (D) Liver histology from treatment group T₁ (400x magnification) shows mild mononuclear cell infiltrate in hepatic parenchyma (arrows). (E) Liver histology from treatment group T₂ (400x magnification) shows mild portal inflammation (arrow). (F) Liver histology from treatment group T₂ (400x magnification) shows mild mononuclear cell infiltrate in hepatic parenchyma (arrow). (G) Liver histology from treatment group T₃ (400x magnification) shows mild portal inflammation (arrow). (H) Liver histology from treatment group T₃ (400x magnification) shows mild mononuclear cell infiltration in liver parenchyma (arrow).

ACKNOWLEDGEMENT

Authors would like to thank the research management center (RMC International Islamic University Malaysia- Kuantan) for their funding of this research under the research initiative grant scheme (RIGS-15-083-0083), and thanks extends also to Lab workers of pharmaco-physiology, and histopathology labs of International Islamic University Malaysia.

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Cite this article as:

Hamad Abdulsalam Hamad Alfarisi et al. Acute toxicity of *Trigonella foenum-graecum* (fenugreek) seeds aqueous extract on liver in male mice, histopathological study. *Int. Res. J. Pharm.* 2017;8(4):24-27 <http://dx.doi.org/10.7897/2230-8407.080443>

Source of support: RMC International Islamic University Malaysia- Kuantan, Conflict of interest: None Declared

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