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Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice

Amit D. Kadhare [a], Subhash L. Bodhankar [a,*], V. Mohan [b], Prasad A. Thakurdesai [b]

[a] Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Paud Road, Pune 411 038, India
[b] Indus Biotech Private Limited, 1, Rahul Residency, Off SalunkeVihar Road, Kondhwa, Pune 411 048, India

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ABSTRACT

The objective of the present work was to study acute and subacute (28-days repeated dose) oral toxicity effect of glycosides based standardized fenugreek seed extract (SFSE-G) *in vivo*. SFSE-G was prepared by resin-based chromatography and standardized to glycosides namely trigoneoside Ib (76%) and vicenin 1 (15%). The acute oral toxicity (AOT) and subacute toxicity studies were performed in Swiss albino mice (5 mice/sex/group) as per OECD 425 (up-and-down procedure) and OCED 407 guidelines respectively. Acute oral administration of 5000 mg/kg of SFSE-G showed 40% mortality with no mortality in lower dosages. The subacute oral administration of SFSE-G did not show observational or toxicological effects on the body or organ weights, food consumption, ophthalmic effects, locomotor activity, hematology, blood biochemistry, urinalysis, or histopathology at dose 250 mg/kg. However, SFSE-G (1000 mg/kg) showed mortality and minor alterations to body weight, relative liver weights, hematology and blood chemistry parameters related to treatment but it was within normal laboratory ranges. In conclusion, SFSE-G showed median lethal dose (LD_{50}) more than 4350 mg/kg and no-observed adverse effect levels (NOAEL) of 250 mg/kg for both sexes during AOT and sub-acute toxicity study, respectively.

1. Introduction

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. They often possess diversified phytoconstituents with unknown biological effects and can produce toxicity and drug interactions that harm human health (Mapanga and Musabayane, 2010; Palmer et al., 2003; Pittler and Ernst, 2003). Moreover, documentation on the safety profile of natural plant-based medicines is scarce and does not fulfill regulatory criteria of the category of drugs. Hence, the systematic studies of natural products and their phytoconstituents about efficacy and safety are required to utilize them as dietary supplements or botanical drugs.

Recently, phytoconstituents and/or secondary metabolites from plant source are being explored by many pharmaceutical industries to explore new drugs or dietary supplements. One such potential plant source is seeds from fenugreek (*Trigonella foenum graceum* family: *Fabaceae*). In Ayurveda, various parts of fenugreek plant have been widely used for the treatment of an array of diseases, including diabetes, high cholesterol, wounds, inflammation, indigestion, baldness and gastrointestinal ailments (Patil et al., 1997). In traditional Chinese medicine, fenugreek seed has been used as a tonic to treat weakness and edema of the legs (Murakami et al., 2000) whereas some studies have reported its use against male reproductive disorders (Basch et al., 2003).

An array of medicinal phytoconstituents is present in fenugreek seeds viz. mucilaginous fibers, proteins, alkaloids, flavonoids, free amino acids, saponins, glycosides (Snehlata and Payal, 2012; Ulbricht et al., 2008). One of the phytoconstituents that is present in abundance in fenugreek seeds is glycoside. These include a variety of furostanol (Kang et al., 2013; Yoshikawa et al., 1997) and flavonol (Han et al., 2001; Taylor et al., 2000) glycosides. Furostanol glycosides are known to be responsible for androgenic and anabolic (Aswar et al., 2010) and anti-inflammatory and anti-melanogenic (Kawabata et al., 2011) properties whereas flavonoid glycosides possess platelet aggregation inhibition (Pang et al., 2012) and anti-oxidant (Kenny et al., 2013) properties.

Organization for Economic Cooperation and Development (OECD) guidelines issued specific guidelines for preclinical acute and sub-acute toxicological evaluations (OECD, 2008). Therefore, toxicological evaluations of glycoside based fenugreek seed extract

* Corresponding author at: Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Paud Road, Pune 411 038, Maharashtra, India. Fax: +91 20 25439383.
E-mail address: sbodhindus@gmail.com (S.L. Bodhankar).

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in laboratory animals is needed before these are recommended to be safe for long-term human consumption.

Therefore, the present study was undertaken to prepare glycosides based standardized fenugreek seed extract (SFSE-G) and its toxicological evaluation in laboratory mice. The acute oral toxicity (AOT) and 28-days (sub-acute) oral toxicity of SFSE-G were evaluated using OECD Guidelines No. 425 (OECD, 1998b) and No. 407 (OECD, 1998a) respectively. In addition, the tissue distribution of SFSE-G in vital organ tissues after subacute exposure during the subacute oral toxicity study was studied.

2. Materials and methods

2.1. Animals

Swiss albino male and female mice (18–22 g, 6–8 weeks) were purchased from the National Institute of Biosciences, Pune (India) were used for the study. They were housed in cages at a temperature of 24 ± 1 °C and relative humidity 56–66%, with 12 h fluorescent light and 12 h dark cycle in an animal house facility. The mice had free access to water *ad libitum* throughout the study duration except during actual measurements. All experiments were carried out between 09:00 and 17:00 h. Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India approved the experimental protocol (CPCSEA/04/2014).

2.2. Preparation and characterization of SFSE-G

The SFSE-G was prepared from the hydroalcoholic extract of fenugreek seeds (Supplementary file-1).

2.3. Oral acute toxicity (AOT) of SFSE-G in mice

The acute oral toxicity study was performed according to the OECD Guideline 425 "Up and Down procedure" (UDP) (OECD, 1998b). There was six groups of Swiss albino mice (5 mice/sex/-group) viz. vehicle control (VC) (10 mL/kg, double distilled water), SFSE-G (55, 175, 550, 1750 and 5000 mg/kg, p.o.). Vehicle or SFSE-G was administered only once (on day 0) and observed until 14 days post treatment. At the end of the observation period of 14 days, all mice were euthanized under ether anesthesia, and macroscopical observations conducted on all organs and tissues. The various organs and tissues were excised and weighed after macroscopical observation.

2.4. Repeated dose 28-day oral toxicity (sub-acute) study of SFSE-G in mice

The subacute oral toxicity study was performed according to the OECD Guideline 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents" (OECD, 1998a). There were six groups of swiss albino mice (5 mice/sex/group) viz. VC (10 mL/kg, double distilled water), SFSE-G (250, 500, 1000 mg/kg), VC reversal and SFSE-G reversal (1000-R). A separate group of mice treated with SFSE-G (1000 mg/kg) were maintained for tissue drug distribution analysis. Vehicle or SFSE-G was administered daily for 28 days.

2.5. Blood collection and biochemical analysis

At the end of the experiment, the mice were euthanized under ether anesthesia. Blood was collected and organs (brain, kidneys, liver, lungs, heart, testes, ovaries, and spleen) were isolated weighted. Organ samples (kidneys, liver and lungs) used for analysis of tissue total proteins, superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (malondialdehyde content) (MDA) activities as per previously reported method (Kandhare et al., 2012c). Organ samples (brain, kidneys, liver, lungs, and small intestine) from two mice were stored at −20 °C until analysis of tissue drug distribution. Organ samples (kidneys, liver, lungs, heart, testes, ovaries, and stomach) from one mouse were either fixed in 10% formalin for histopathological examination.

2.6. Tissue drug distribution analysis

The weighed organs of mice were homogenized in phosphate buffer saline and centrifuged at 9000 rpm at 9 °C for 5 min. Supernatant was separated; 100 µl of acetic acid and 6 ml of distilled water was added. Then the sample was processed for solid phase extraction by passing through a C-18 cartridge column followed by washing with 6 ml distilled water. Then column was elute with 2 ml of methanol and elute was concentrated under nitrogen. The resultant residue was mixed with 1 ml of distilled water and analyzed by HPLC.

2.7. Statistical analysis

The data were represented as mean ± standard error of mean (SEM). Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). Data of body weight was analyzed using two-way ANOVA followed by Dunnett's test.

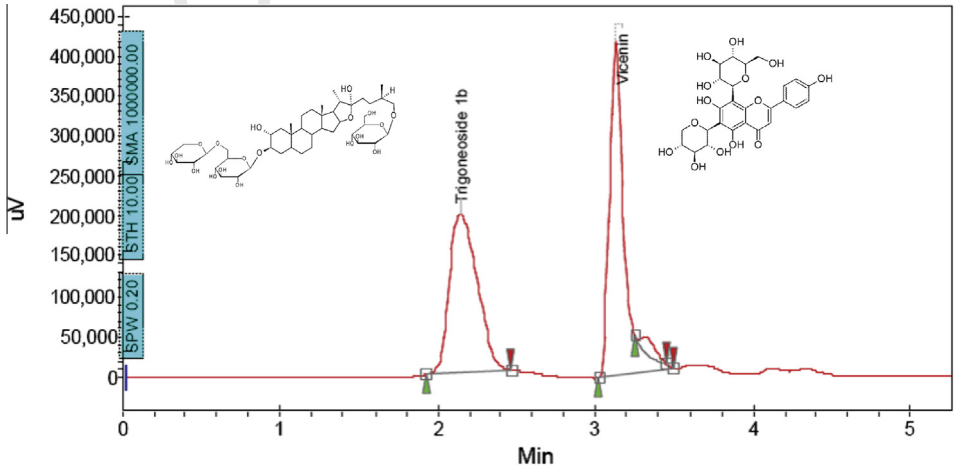

Fig. 1. HPLC chromatogram showing the composition of SFSE-G with structures of (A) trigoneoside Ib (RT = 2.3 min) and (B) vicenin 1 (RT = 3.2 min).

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