International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

PRENATAL DEVELOPMENTAL TOXICITY EVALUATION OF LOW MOLECULAR WEIGHT GALACTOMANNANS BASED STANDARDIZED FENUGREEK SEED EXTRACT DURING ORGANOGENESIS PERIOD OF PREGNANCY IN RATS

PALLAVI DESHPANDE[a], VISHWARAMAN MOHAN[a], MUKUL PORE[b], SHAILESH GUMASTE[b], PRASAD THAKURDESAI[a,*]

[a]Indus Biotech Private Limited, Kondhwa, Pune, Maharashtra, 411048, India, [b]Intox Pvt. Ltd., Pune, Maharashtra, 412115, India
Email: prasad@indusbiotech.com

Received: 01 Feb 2016 Revised and Accepted: 30 Mar 2016

ABSTRACT

Objective: To evaluate the prenatal developmental toxicity of low molecular weight galactomannans based standardized fenugreek seed extract (LMWGAL-TF).

Methods: Rats received oral administration of LMWGAL-TF (250, 500 and 1000 mg/kg) during the period of gestation from day 5 (implantation day)–19 (1 d before expected day of parturition) post conception. Maternal, embryo, and fetal toxicity parameters were evaluated.

Results: LMWGAL-TF exposure did not produce maternal (clinical observations, body weight gain, food intake) and embryo–fetal toxicity. Occasional skeletal and visceral malformations, unrelated to the treatments, were seen in both LMWGAL-TF-treated and vehicle control (VC) groups.

Conclusion: Oral exposure of LMWGAL-TF during the prenatal period did not induce significant maternal and embryo–fetal toxicity up to a dose of 1000 mg/kg in rats. The dose of 1000 mg/kg was considered as NOAEL for LMWGAL-TF.

Keywords: Developmental toxicity, Low molecular weight galactomannans, Standardized fenugreek seed extract, OECD Test No. 414, Reproductive system, Rat.

INTRODUCTION

The increased use of botanicals or food supplements by consumers and industry has raised concerns among scientific and regulatory communities. Medicinal supplements from plants possess functional properties or health benefits. The sources of food supplement may vary from staple food plants to herbals used in folk medicine and comprise the whole plant, extracts thereof or purified components [1, 2]. The crude extracts of varieties of plants have been used in clinical practices from a long time [3] and often possess diversified phytoconstituents with unknown biological effects and can produce toxicity and drug interactions that harm to human health [4, 5]. There is a need for safety reports for a botanical extract of crude as well as purified fractions for their validated activities.

Trigonella foenum-gracum (TF), known as fenugreek, is one of the most promising medicinal herbs, known from ancient times. Fenugreek seeds have known for many applications towards female health. In ancient Rome, fenugreek seeds were used to aid labor and delivery [6]. Fenugreek seeds or powder used by traditional Ayurvedic physicians to enhance milk production to breastfeeding mothers [6-8]. These benefits on female health have been attributed to unique dietary fiber components of fenugreek seeds [9]. The endosperm of the fenugreek seed is a rich source of fiber (20 %) and gum (32.4 %) [10-12], which include galactomannans (GAL). Fenugreek GAL is a group of oligosaccharides (OS) composed of mannose as the backbone with galactose as side groups in the ratio of 1:1.

Fenugreek seeds contain both soluble and insoluble types of fibers [13]. The soluble dietary fiber content of fenugreek seeds is known to be responsible for health benefits such as effective restoration of both glucose and fat metabolism in DM [6, 14, 15]. The soluble dietary fiber fraction of fenugreek seeds of various compositions reported to have antidiabetic [16, 17], hypolipidemic [18, 19] and a food stabilizer properties [12].

Soluble fiber fraction of fenugreek is rich in low molecular weight galactomannans (LMWGAL) containing oligosaccharides (OS) such as raffinose (three monosaccharide units) and stachyose (five monosaccharide units). LMWGAL from fenugreek seeds demonstrated anti-hyperglycemic [15] and anabolic potential in male rats [20] and against diet-induced obesity in C57/BL6 mice [21]. Furthermore, the clinical benefit of LMWGAL based standardized fenugreek seed extract on strength and body composition in resistance-trained males has been reported [22].

A crude form of fenugreek seed powder or extract was found to be safe in many prior studies [23, 24]. Oral exposure of LMWGAL-TF is reported to be safe during acute and subchronic toxicity studies in male and female rats [25] and during 8-weeks of exposure to resistance-trained male subjects [22]. However, the safety or toxicity information of LMWGAL-TF in females during the gestational period of pregnancy is not yet known. Looking at potential applications of LMWGAL-TF for the area of female health, the study involving prenatal exposure on reproduction related parameters in pregnant mothers and fetuses is necessary. Therefore, we undertook the present study to evaluate the effects of LMWGAL-TF on pregnant female rats and their fetuses during oral exposure to female rats during the period of gestation, beginning from the implantation day as per OECD 414 guidelines, an internationally accepted procedure.

MATERIALS AND METHODS

Animals

Adult and young nulliparous and non-pregnant healthy female Wistar rats (of 14-15 w) and adult, proven fertile male rats were inbred at the lab of INTOX Private Limited, Pune, India. Animals were housed in solid polypropylene cages with stainless steel grill tops and bedding of clean and sterilized paddy husk for at least eight days before mating. 'Nutrilab' brand extruded rodent pelleted feed (M/s Provimi Animal Nutrition India Pvt. Ltd., Bangalore) and filtered water were provided *ad libitum*. During the entire study,

animals were kept at the following controlled conditions: temperature of 22±2 °C, 50±20% of relative humidity, and a light/dark cycle of 12 h. Two females cohabited overnight with a male and examined for the presence of sperm in their vagina. The day that sperm was detected was defined as gestational day 0. After mating is confirmed, on '0' day, pregnant rats were housed individually and randomly assigned to the vehicle control (VC) and treatment groups. The study was conducted at INTOX Private Limited (Pune, India), a GLP Certified laboratory and with prior approval from Institutional Animal Ethics Committee (IAEC) in accordance with guidelines set by Committee Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

The test compound, LMWGAL-TF, was supplied by Indus Biotech Private Limited (Pune, India) after preparation and characterization (HPLC and LC-MS) as per reported procedure [15]. LMWGAL-TF is standardized fenugreek seed extract (96.21 % water soluble low molecular weight galactomannans). LMWGAL-TF is available as Torabolic® as marketed dietary supplement. The LMWGAL-TF is water soluble powder. The fresh solution was prepared daily as 1% w/v in distilled water (vehicle) and administered once daily by oral route to rats in a volume of 5 ml/kg body weight. Dosing formulations were prepared appropriate concentrations as per study design to meet dosage level requirements.

Methods

The study was conducted in accordance with OECD Guidelines (Test No. 414: Prenatal Development Toxicity Study) [26]. Dose-range finding study was conducted before the main study.

Dose-range finding study

Female rats were cohabited with male rats (1 male: 2 females) overnight and examined for the presence of spermatozoa in vaginal smear next day morning. The day on which the sperms were found in the vagina was considered gestational day 0 (GD 0). Four groups of seven female rats each ('0' day pregnant) were administered with either vehicle or LMWGAL-TF (250 mg/kg, 500 mg/kg or 1000 mg/kg) from GD5 to GD19 and checked for systemic toxicity, body weight, and food consumption. All dams were sacrificed on GD20 and subjected to necropsy examination for ovaries and uterine contents. Fetal abnormalities were assessed by external examination. The highest dose level of 1000 mg/kg body weight did not result in any remarkable maternal or fetal toxicity in the exposed rats. Based on the findings, doses 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight were selected for the main study.

Main study

One hundred and twenty female rats ('0' day pregnant) were selected and randomized into 4 groups (n=30 in each group). The day on which the sperms were found in the vagina was considered GD0. The rats were gavaged daily from GD5 to GD19. Each group of rats was treated either with Vehicle (G1: VC) or LMWGAL-TF (G2:250 mg/kg, G3: 500 mg/kg or G4: 1000 mg/kg). Cage-side observations of females were conducted at least once daily. Food consumption was measured at 3 d intervals starting on GD5. Maternal body weights were recorded on GD 0, 5, 8, 11, 14, 17 and prior to their terminal sacrifice on day 20. All animals were sacrificed on GD 20 by CO_2 asphyxiation. The uterus from each female was examined for the number and placement of uterine implantation sites, number of live and dead fetuses, number of early and late resorptions and any abnormalities of the uterus or embryonic sac. Dams were examined for the number of corpora lutea of ovaries. Sex and the body weight of each fetus were determined. Pre-implantation and post-implantation loss was calculated as follows: pre-implantations loss = [(no. of corpora lutea – no. of implantations)/no. of corpora lutea] × 100 and post-implantation loss = [(no. of dead implants)/no. of total implantations] ×100 [27]. Uteri which had no visible implantation sites were stained with ammonium sulfide (10%) to detect very early resorptions [28]. All live fetuses were individually weighed, sexed, and examined for external anomalies in a uniform order (from head to tail) for external malformations. Live fetuses were euthanized by using diethyl ether vapors. One-half of the fetuses from each litter was selected in a random manner and subjected to visceral examination by Wilson's Technique for assessment of soft tissue development [29]. The remaining half number of the fetuses were sacrificed, eviscerated and processed for skeletal examination using Alizarin Red S staining method [30].

Data analysis

The data was presented as mean±standard deviation (SD). The litter was used as the basis for the analysis of fetal variables. Gestational body weights, corrected body weight, body weight gain and food consumption of pregnant rats, litter weight (total, male and female), average pup weight, uterus weight (absolute and relative), pre-implantation loss (%), post-implantation loss (%), dead and live fetuses (%), were analyzed by one-way analysis of variance, followed by Dunnett's test. Number of the male and female pup, sex ratio, the number of corpora lutea, the number of implantation, the number of live and dead fetuses, and the number of early and late resorptions and incidences of fetal visceral and skeletal malformations were evaluated by using the Kruskal–Wallis test, followed by the Mann–Whitney test. Numbers of pregnant and non-pregnant females, the number of live and dead females were analyzed by using Fisher's test. Treated groups were compared to VC. The results of the statistical analysis were assessed at 5% level of significance ($p<0.05$).

RESULTS

Maternal toxicity

No mortality was observed in any of treatment groups during the study period. No evident clinical signs were observed in any dam during the study. The data related to maternal finding is presented in table 1. No abortion was observed in any of the dams during the study period. There was no significant difference in body weight gain of pregnant rats between LMWGAL-TF exposed groups and the VC group throughout the experimental period. There were no differences between the treatment groups and VC for the maternal weight and food consumption. No significant difference was found in the pregnancy rate of treatment groups of 250 (86.6%), 500 (96.6%) and 1000 (96.6%) mg/kg dose groups as compared to VC (86.6%).

Table 1: LMWGAL-TF. Maternal findings

Parameters	G1	LMWGAL-TF treatment		
		G2	G3	G4
	VC	250 mg/kg	500 mg/kg	1000 mg/kg
Pregnancy data				
Initial animals per group	30	30	30	30
Confirmed pregnancy at necropsy	26	26	29	29
Pregnancy rate (%)	86.66	86.66	96.66	96.66
Maternal data				
Body weight (g) GD5	214.69±12.76	212±12.47	215.69±12.51	211.55±11.19
Final body weight (g)GD20	283.54±26.44	274.5±27.58	279.07±23.71	276.62±17.35
Body weight change (g) during GD5 to GD20	68.85±16.00	62.5±19.77	63.38±19.76	65.07±12.56
Corrected Body weight (g)	237.17±12.70	232.06±14.87	232.32±18.31	229.08±12.97

Values are expressed as mean±SD. n = 30 female rats per group were randomized. VC-Vehicle control, GD-Gestational Day.

Table 2: LMWGAL-TF. Embryo–fetal toxicity related to reproductive findings

Parameters	G1	LMWGAL-TF treatment		
		G2	G3	G4
	VC	250 mg/kg	500 mg/kg	1000 mg/kg
Gravid uterus weight (g)	46.37±18.89	42.44±16.56	46.75±12.81	47.54±12.71
Corpora lutea (No.)	10.85±1.91	9.73±2.16	9.93±1.87	10.14±1.36
Implantations per female (No.)	9.12±3.00	8.46±3.11	8.72±2.19	9.00±1.63
Early resorptions (No.)	0.50±1.14	0.54±0.71	0.48±1.02	0.52±1.09
Late resorptions (No.)	0.04±0.20	0	0	0
Pre-implantation loss (%)[a]	16.6±21.38	15.53±23.19	12.03±18.89	11.21±14.84
Post-implantation loss (%)[b]	8.78±18.46	8.79±19.76	6.82±18.86	6.95±15.09
Litter size (No.)	8.58±3.57	8.24±2.82	8.54±2.12	8.48±2.23
Live fetuses (No.)	8.58±3.57	8.24±2.82	8.54±2.12	8.48±2.23
Dead fetuses (No.)	0	0	0	0
Live male fetuses (No.)	5.08±2.17	4.21±1.74	4.63±1.69	4.83±1.87
Live female fetuses (No.)	3.88±2.07	4.20±1.94	4.38±1.83	3.66±1.65
Male/female sex ratio (No.)	1.57±1.12	1.26±1.30	1.50±1.42	1.81±1.45
Average fetal weight (g)	3.30±0.43	3.45±0.33	3.59±0.30	3.82±0.87*
Average male fetal weight (g)	3.47±0.32	3.60±0.31	3.69±0.31*	3.77±0.29*
Average female fetal weight (g)	3.20±0.46	3.33±0.37	3.46±0.33*	3.53±0.28*

n = 30 female rats per group were randomized. Values are expressed as mean±SD, [a][(Number of Corpora Lutea-Number of implantations)/Number of Corpora Lutea] × 100, [b][(Number of dead implants)/Total number of implantations] × 100, *P<0,05 v/s. VC group. VC-Vehicle control.

Table 3: LMWGAL-TF. Fetal malformations and variations

Parameters	G1	LMWGAL-TF treatment		
		G2	G3	G4
	VC	250 mg/kg	500 mg/kg	1000 mg/kg
Total no. fetuses (litters) examined[a]				
External	223 (26)	206 (26)	239 (29)	246 (29)
Visceral	106 (25)	97 (24)	113 (28)	116 (29)
Skeletal	117 (26)	109 (25)	126 (28)	130 (29)
External Malformation	0	0	0	0
Visceral variations				
Brain: Hydrocephalus of lateral ventricles (slight)	0	1 (1)	1(1)	0
Kidney: Hypoplasia (both)	0	0	1(1)	0
Eye: Retinal folding (left)	0	0	1(1)	0
Eye: Retinal folding (both)	0	0	1(1)	0
Skeletal variations				
Skull ossification	2 (2)	3(3)	2 (2)	15 (7)
Sternebra Variations				
Incomplete/ Poor ossification	17 (7)	13(9)	19 (10)	14 (10)
Un-ossified	12 (9)	16 (7)	10 (8)	4 (4)
Spilt, rudimentary, dumbbell shaped, asymmetrical, misshapen, hyperplasia, extra	29 (22)	31 (18)	28 (18)	26 (16)
Rib Variations				
Rudimentary (14th-right/left/both)	22 (13)	37 (20)	34 (19)	34 (22)
Extra/Accessory (right/left/both)	2 (2)	6 (6)	7 (7)	6 (6)
Undulated, nodulated, short, asymmetrical, wavy, absent	6 (6)	8 (5)	1 (1)	3 (3)
Vertebrae variations				
Cervical: No/Poor ossification	3 (3)	0	0	0
Sacral: No/Poor ossification	1 (1)	0	0	0
Lumbar: Incomplete/ Poor ossification	0	2 (2)	0	0
Thoracic *vertebra centra*: Dumbbell shaped, spilt, asymmetrical, misshapen	7 (7)	14 (3)	15 (7)	14 (11)
Sacral: Fused	0	0	1 (1)	0
Limb Variations				
Fore limb: Fingers misshapen	0	0	0	1(1)

Fetuses from pregnant female rats per group were examined. [a] The incidence of the individual defect is presented as a number of fetuses (numbers of litters). VC-Vehicle control

Reproductive findings related to embryo–fetal toxicity

The data of embryo–fetal toxicity related to reproductive findings are presented in table 2. The gravid uteri of females sacrificed on GD20 did not reveal any remarkable alterations indicative of adverse effects of LMWGAL-TF. There was no significant difference in a number of corpora lutea, live fetuses, and implantation sites, early and late resorption, pre-and post-implantation loss in LMWGAL-TF treated groups as compared to VC group.

The fetal weights of male and female fetuses in LMWGAL-TF (500 and 1000 mg/kg) were significantly ($P<0.05$) more than that of VC groups.

Fetal malformations and variations

Each fetus was exposed to examine for any abnormal findings with respect to length, cranium, eyes, palate, limbs, tail, genitals, sex along with skeletal and visceral abnormalities of exposed fetuses and data is presented in table 3. No statistical difference was observed in the frequency of external variations and malformations in LMWGAL-TF treated groups as compared to VC group. In the visceral examination, hydrocephalous of lateral ventricles of the brain was observed in one fetus from LMWGAL-TF (250 mg/kg) group and one fetus from LMWGAL-TF (500 mg/kg) group. Hypoplasia of kidneys was observed in one fetus from LMWGAL-TF (500 mg/kg) group. Retinal folding was observed in one fetus from LMWGAL-TF (500 mg/kg) group. These incidences of fetal soft tissue or skeletal abnormalities are normal variants or minor anomalies and considered to be incidental and not treatment related.

These findings are commonly seen in 20 d. old rat fetuses. As per skull, the anomaly is concerned; variation from the ossification patterns was seen like unossified, scrambled, poorly and incompletely ossified skulls. Such variations were observed in two fetuses from the VC group three from LMWGAL-TF (250 mg/kg) group, two fetuses from LMWGAL-TF (500 mg/kg) group and 15 fetuses from LMWGAL-TF (1000 mg/kg) group. These were classified as normal variants. Poor and incomplete ossifications of sternebrae were also seen in 17 fetuses from VC, 13 from LMWGAL-TF (250 mg/kg) group, 19 from LMWGAL-TF (500 mg/kg) group and 14 from LMWGAL-TF (1000 mg/kg) group. Unossified sternebrae were also encountered in all dose groups like 12 fetuses in VC, 16 in LMWGAL-TF (250 mg/kg) group, 10 in LMWGAL-TF (500 mg/kg) and 4 in LMWGAL-TF (1000 mg/kg). These were all classified as normal variants. Poor, incomplete and unossified cervical, lumbar, sacral and caudal vertebrae were observed in VC and LMWGAL-TF (250 mg/kg) group. Minor anomalies like split, rudimentary, dumbbell shaped, asymmetrical, dumbbell shaped, extra, misshapen and agenesis of sternebrae were seen in all dose groups. These anomalies were observed in 29 fetuses from VC, 31 fetuses LMWGAL-TF (250 mg/kg) group, 28 from LMWGAL-TF (500 mg/kg) group and 26 fetuses from LMWGAL-TF (1000 mg/kg) dose group. Minor anomalies in ribs like rudimentary, wavy, extra, accessory, asymmetrical, nodulated, undulated ribs were observed in 30 fetuses from VC, 51 fetuses from LMWGAL-TF, 42 fetuses from LMWGAL-TF (500 mg/kg) and 43 fetuses from LMWGAL-TF (1000 mg/kg) group. Dumbbell/asymmetrical, dumbbell shaped, split, asymmetrical, misshapen and fused vertebrae (thoracic and sacral) were observed in 7 fetuses from VC, 14 fetuses from LMWGAL-TF (250 mg/kg), 16 from LMWGAL-TF (500 mg/kg) and 14 fetuses from LMWGAL-TF (1000 mg/kg) group. One fetus from LMWGAL-TF (1000 mg/kg) group was observed with misshapen forelimb fingers.

In general, the skeletal examination did not show any major abnormalities which could have caused any functional damage to these fetuses, if allowed to grow in the normal course. The abnormalities could be considered as the variations which occur during development but are repaired and normalized during the normal course of development.

DISCUSSION

The prenatal developmental toxicity study provides general information concerning effects of exposure to the developing organism during the gestational period. Developmental toxicity studies cover any detrimental effect produced by exposures to developing organisms during embryonic stages of development. Such lesions can be either irreversible or reversible. Teratological studies use the investigations on maternal, embryonic and fetal toxicity.

The present study, for the first time, evaluated the LMWGAL based standardized extract of fenugreek seeds, LMWGAL-TF, for prenatal developmental toxicity during the gestational period (prenatal exposure, GD 5 through GD 19) on maternal and fetus development. The LMWGAL-TF was studied at 3 dose levels, namely 250, 500 and 1000 mg/kg.

In the present study, LMWGAL-TF was found to be safe on prenatal exposure with the no-observed-adverse-effect-level (NOAEL) of 1000 mg/kg/day. In the past, there have been inconsistent reports on reproductive toxicity on crude extract of fenugreek seeds on prenatal exposure in rabbit [31], mice [32-34] and rats [31, 35] with one case study in human [36]. On the other hand, fenugreek seed extract was reported to be safe during pregnancy period [37, 38]. However, these reports utilized whole and crude extract with unknown composition and not standardized to any marker compounds. Furthermore, the toxicity of crude extract may not be directly correlated to purified or standardized extracts. For example, a well-known sweetener, steviol glycosides (SGs) of *Stevia rebaudiana* leaves, did not show reproductive toxicity in rats [39] whereas crude stevia leaf extracts showed renal and cardiovascular adverse effects with reduced fertility in male and female rats [40-44].

Toxic constituents of some plants may contribute to birth defects [45]. The prenatal exposure to LMWGAL-TF during gestational days (GD5 through GD19), did not cause any toxic birth defects effects in terms of weight (body, gravid uterine), food intake, hematology, or biochemical parameters. Pregnancy rate is the proportion of mated pairs that have produced at least one pregnancy within a fixed period where pregnancy is determined by the earliest available evidence that fertilization has occurred. There was no maternal mortality observed during the gestational period in pregnant rats. Maternal parameters related to pregnancy rate are maternal deaths during pregnancy, females with the viable fetus (%) and females with resorption (%). The numbers of utereal implantations in the left and right arms of the uterus were counted in LMWGAL-TF treated all dams. A number of resorptions and implantation affect litter size per dam. There was no significant change in a total number of implantation in terms of pre and post implantation loss after LMWGAL-TF exposure. LMWGAL-TF treated groups did not show any dose dependent early and late resorption in any dam. These findings suggest normal fertilization process with LMWGAL-TF exposure in the gestational period in rats.

LMWGAL-TF treated dams did not show adverse effects in terms of a number of fetuses and sex ratio of fetuses. The mean live fetal weights of male and female fetuses from LMWGAL-TF (500 and 1000 mg/kg) treated group were significantly higher as compared to VC group and is considered as incidental and not considered to be biologically significant since this significant change is not comparable with other fetal data observed. The incidence of normal fetuses observed in this study was 100% in control group and in all treatment groups. There were no any external abnormalities observed in the study. There were some visceral and skeletal abnormalities found in control as well as LMWGAL-TF treated groups. In general, there were no major abnormalities which could have caused any functional damage to the fetuses, if allowed to grow in the normal course.

Thus, at NOAEL of LMWGAL-TF for prenatal oral exposure during the gestational period was found to be greater than or equal to 1000 mg/kg/day. Human equivalent dose (HED) can be derived from NOAEL by using USFDA guidance for Industry [46]. Considering NOAEL of 1000 mg/kg in pregnant female rats, HED is almost 9.7 g considering the human weight of 60 kg and this HED is much higher than efficacy doses that are reported in male volunteers [22].

CONCLUSION

In conclusion, LMWGAL-TF did not produce overt signs maternal toxicity, or signs of embryo–fetal toxicity and found well-tolerated

during the gestation period. Therefore, a dose of 1000 mg/kg can be considered as the NOAEL of LMWGAL-TF for prenatal oral exposure in rats. This safety information during a gestational period in pregnant female rats will form an important basis for the clinical development of LMWGAL-TF as a safe dietary supplement or botanical agent for female specific applications.

ACKNOWLEDGEMENT

This study was performed at the Intox Private Limited, Pune, India.

CONFLICT OF INTERESTS

The study is supported by Indus Biotech Private Limited, Pune, India.

REFERENCES

1. Schilter B, Andersson C, Anton R, Constable A, Kleiner J, O'Brien J, *et al.* Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. Food Chem Toxicol 2003;41:1625-49.
2. Walker R. Criteria for risk assessment of botanical food supplements. Toxicol Lett 2004;149(1-3):187-95.
3. Banerjee R, Verma AK, Das AK, Rajkumar V, Shewalkar AA, Narkhede HP. Antioxidant effects of broccoli powder extract in goat meat nuggets. Meat Sci 2012;91:179-84.
4. Palmer ME, Haller C, McKinney PE, Klein-Schwartz W, Tschirgi A, Smolinske SC, *et al.* Adverse events associated with dietary supplements: an observational study. Lancet 2003;361(9352):101-6.
5. Pittler M, Ernst E. Systematic review: hepatotoxic events associated with herbal medicinal products. Aliment Pharmacol Ther 2003;18:451-71.
6. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. Altern Med Rev 2003;8:20-7.
7. Patil SP, Niphadkar PV, Bapat MM. Allergy to fenugreek (*Trigonella foenum graecum*). Ann Allergy Asthma Immunol 1997;78:297-300.
8. Schaffir J, Czapla C. Survey of lactation instructors on folk traditions in breastfeeding. Breastfeed Med 2012;7:230-3.
9. Madar Z, Stark AH. New legume sources as therapeutic agents. Br J Nutr 2002;88 Suppl 3:S287-92.
10. Sharma R. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. Nutr Res 1986;6:1353-64.
11. Sharma R, Raghuram T, Rao NS. Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. Eur J Clin Nutr 1990;44:301-6.
12. Meghwal M, Goswami T. A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J Food Process Technol 2012:3:181.
13. Madar Z, Shomer I. Polysaccharide composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats. J Agric Food Chem 1990;38:1535-9.
14. Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ. *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol Cell Biochem 2001;224(1-2):45-51.
15. Kamble H, Kandhare A, Bodhankar S, Mohan V, Thakurdesai PA. Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. Biomed Aging Pathol 2013;3:145-51.
16. Hannan JM, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, *et al.* Soluble dietary fiber fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. Br J Nutr 2007;97:514-21.
17. Al-Habori M, Raman A, Lawrence MJ, Skett P. *In vitro* effect of fenugreek extracts on intestinal sodium-dependent glucose uptake and hepatic glycogen phosphorylase A. Int J Exp Diabesity Res 2001;2:91-9.
18. Boban PT, Nambisan B, Sudhakaran PR. Hypolipidaemic effect of chemically different mucilages in rats: a comparative study. Br J Nutr 2006;96:1021-9.
19. Venkatesan N, Devaraj SN, Devaraj H. A fibre cocktail of fenugreek, guar gum, and wheat bran reduces oxidative modification of LDL induced by an atherogenic diet in rats. Mol Cell Biochem 2007;294(1-2):145-53.
20. Aswar U, Mohan V, Bhaskaran S, Bodhankar SL. Study of galactomannan on androgenic and anabolic activity in male rats. Pharmacologyonline 2008;2:56-65.
21. Kandhare A, Bodhankar SL, Mohan V, Thakurdesai PA. Prophylactic efficacy and possible mechanisms of oligosaccharides based standardized fenugreek seed extract on high-fat-diet-induced insulin resistance in C57BL/6 mice. J Appl Pharm Sci 2015;5:35-45.
22. Poole C, Bushey B, Foster C, Campbell B, Willoughby D, Kreider R, *et al.* The effects of a commercially available botanical supplement on strength, body composition, power output, and hormonal profiles in resistance-trained males. J Int Soc Sports Nutr 2010;7:34.
23. Narasimhamurthy K, Viswanatha S, Ramesh B. Acute and subchronic toxicity assessment of debitterized fenugreek powder in the mouse and rat. Food Chem Toxicol 1999;37:831-8.
24. Muraki E, Hayashi Y, Chiba H, Tsunoda N, Kasono K. Dose-dependent effects, safety and tolerability of fenugreek in diet-induced metabolic disorders in rats. Lipids Health Dis 2011;10:240.
25. Bodhankar SL, Kandhare A, Mohan V, Thakudesai PA. Toxicological evaluations of the low molecular weight galactomannans from fenugreek seeds [OP-9]. XXXIII Annual Conference of Society of Toxicology (STOX). Mathura, India; 2013.
26. OECD. Test No. 414: Prenatal Development Toxicity Study. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris: OECD Publishing; 2001.
27. Burdan F, Szumiło J, Dudka J, Klepacz R, Błaszczak M, Solecki M, *et al.* Morphological studies in modern teratological investigations. Folia Morphol (Praha) 2005;64:1-8.
28. Salewski E. Färbemethode zum makroskopischen nachweis von Implantationsstellen am Uterus der ratte. Naunyn-Schmiedeberg's Arch Pharmacol 1964;247:367.
29. Monie IW, Kho KG, Morgan J. Dissection procedures for rat foetuses permitting alizarin red staining of skeleton and histological study of viscera. 1965. p. 163-73.
30. Taylor P. Practical Teratology. London: Academic Press; 1986.
31. Kassem A, Al-Aghbari A, Molham AH, Al-Mamary M. Evaluation of the potential antifertility effect of fenugreek seeds in male and female rabbits. Contraception 2006;73:301-6.
32. Khalki L, M'hamed SB, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from *Trigonella foenum-graecum* (L.) in mice. J Ethnopharmacol 2010;131:321-5.
33. Mital N, Gopaldas T. Effect of fenugreek (*Trigonella foenum graecum*) seed based diets on the birth outcome in albino rats. Nutr Rep Int 1986;33:363-9.
34. Khalki L, Bennis M, Sokar Z, Ba-M'hamed S. The neurobehavioral developmental effects of fenugreek seeds on prenatally exposed mice. J Ethnopharmacol 2012;139:672-7.
35. Kamal R, Yadav R, Sharma J. Efficacy of the steroidal fraction of fenugreek seed extract on fertility of male albino rats. Phytother Res 1993;7:134-8.
36. Skalli S. Malformations associées à la prise de fenugrec au cours de la grossesse. Bulletin d'informations de pharmacovigilance; 2006. p. 3.
37. Mittal N, Gopaldas T. Effect of fenugreek *(Trigonella foenum graceum*) seeds based on the birth outcome in albino rats. Nutr Reproduction Int 1986;33:362-9.
38. Mittal N, Gopaldas T. Effect of fenugreek (*Trigonella foenum-graecum*) seed based diets on the lactational performance in albino rats. Nutr Report Int 1986;33:477-84.
39. Curry LL, Roberts A, Brown N. Rebaudioside a: two-generation reproductive toxicity study in rats. Food Chem Toxicol 2008;46:S21-S30.
40. Planas GM, Kuć J. Contraceptive properties of *Stevia rebaudiana*. Science 1968;162:1007.
41. Melis MS. Effect of crude extract of *Stevia rebaudiana* on renal water and electrolytes excretion. Phytomedicine 1999;6:247-50.

42. Melis MS. Effects of chronic administration of *Stevia rebaudiana* on fertility in rats. J Ethnopharmacol 1999;67:157-61.
43. Melis MS. Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: renal effects. J Ethnopharmacol 1995;47:129-34.
44. Melis MS. A crude extract of *Stevia rebaudiana* increases the renal plasma flow of normal and hypertensive rats. Braz J Med Biol Res 1996;29:669-75.
45. Taloubi L, Rhouda H, Belahcen A, Smires N, Thimou A, Mdaghri AA. An overview of plants causing teratogenicity: Fenugreek (*Trigonella foenum graecum*). Int J Pharm Sci Res 2013;4:516.
46. Center for Drug Evaluation and Research. Guidance for Industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. US Department of Health and Human Services, Food and Drug Administration; 2005.