Oral toxicity evaluation of gokshuradi guggulu, an ayurvedic formulation

Manish M. Wanjari, Yadu Nandan Deya, Mahendra Yadav, Deepti Sharma, Bhavana Srivastava, Shrirang B. Jamdagni, Sudesh N. Gaidhani and Sharad Pawar

ABSTRACT
Gokshuradi guggulu is an important classical polyherbal formulation used in Ayurvedic system of medicine for the treatment of various chronic diseases like kidney stones and diabetes. However, no scientific attempts were made to evaluate its oral toxicity. Hence, the present study evaluated the acute and 28 days repeated dose sub-acute oral toxicities of gokshuradi guggulu in rats. Gokshuradi guggulu was tested for its compliance using physicochemical and analytical parameters as per standards prescribed in Ayurvedic Pharmacopeia of India. In acute oral toxicity study, Wistar rats were orally administered a single dose of gokshuradi guggulu (2700 mg/kg) and clinical signs and mortality or moribund stage were observed for 14 days along with weekly body weight. On day 15, the rats were euthanized and the gross morphology was carried out during necropsy. In sub-acute (repeated dose) oral toxicity study, the rats were orally administered gokshuradi guggulu (270, 1350 and 2700 mg/kg) once daily up to 28 days. Clinical signs and mortality or moribund stage, weekly body weight, weekly feed and water consumptions, biochemical and hematological investigations, urine analysis, and major organ weights and histopathology were carried out. In acute and sub-acute toxicity studies, gokshuradi guggulu administration did not show any alteration in parameters or any adverse effect as compared to vehicle treated group. There was no mortality or moribund state observed in any group in both studies. Administration of gokshuradi guggulu in acute and 28 days repeated doses did not exhibit any toxicity or adverse effect at the doses used and NOAEL was found to be 2700 mg/kg.

ARTICLE HISTORY
Received 1 May 2020
Revised 3 December 2020
Accepted 5 December 2020

KEYWORDS
Ayurveda; gokshura; Tribulus terrestris; acute toxicity; sub-acute toxicity

1. Introduction
Ayurvedic medicine is the part of Indian traditional system of medicine widely practiced in India since ancient times. Many ayurvedic drugs are used in clinical practice for the treatment of various ailments without any safety or toxicity studies due to deep-rooted belief that they are safe. However, some ayurvedic formulations and herbal medicines were reported to exhibit adverse effects (Chaudhary et al. 2010, Chan 2003). The non-availability of safety/toxicity data of these medicines restricts their global popularity and acceptability. Hence, it is required to scientifically document the safety and toxicity of Ayurvedic drugs and polyherbal formulations.

Gokshuradi guggulu is an important ayurvedic compound polyherbal formulation mentioned in Sharangdhar samhita, an ayurvedic classical compendium. It is an official drug in Ayurvedic Formulary of India (Anon 2003) and Ayurvedic Pharmacopeia of India (Anon 2008B). It is classified as nitratal category of drug. It contains 9 plants depicted in Table S1 (Supplementary material) among which Tribulus terrestris (Gokshura) and Conmiphora mukul (guggulu) are the chief ingredients. Gokshuradi guggulu (orally, 2–3 g daily in divided doses) is prescribed in Ayurveda for treatment of urinary tract problems like urinary disorders (prameha), dysuria (mutraghata), urinary obstruction (mutraghata), calculus (ashmari), and other like excessive vaginal discharges (pradara), gout (vatarakta), vata roga and vitiation of semen (shukraasosa). Incidentally, an Iranian male patient using the Tribulus terrestris extract for prevention of kidney stone formation has been reported to develop nephrotoxicity, hepatotoxicity and neurotoxicity (Talasaz et al. 2010). In another study, the steroidal saponins like Protodioscin from hydro alcoholic extract of Tribulus terrestris also demonstrated nephrotoxicity in streptozotocin-diabetic rats and exhibited its accumulation in kidney tissues (Gandhi et al. 2013). Further, there are reports of hepatorenal syndrome and neurotoxicity and poisonings in goats and sheep due to Tribulus terrestris while grazing (Aslani et al. 2003, Aslani et al. 2004, Jacob and Peet 1987, Bourke 1987, Bourke 1983). These reports raise concerns about the toxic effects resulting from long term use of ayurvedic/herbal formulations containing Tribulus terrestris. These reports, thus, encouraged us to investigate the toxicity profile...
of gokshuradi guggulu in experimental animals which has not been conducted so far. Hence, the present study evaluated the acute and 28 days repeated dose sub-acute oral toxicity of gokshuradi guggulu in rats.

2. Materials and methods

2.1. Drugs and chemicals

Gokshuradi guggulu (Batch No. AGU 13) was procured from M/s Indian Medicines Pharmaceutical Corporation Ltd. (IMPCL), Mohan, Distt. Almora, Uttarakhand, India. Clinical chemistry kits (M/s Erba Mannheim, Germany and Proton Biologicals, Bangalore) and hematological reagents (Sysmex Corporation, Japan) were procured from M/s Transasia Biomedicals Pvt. Ltd, Mumbai, India. All other common chemicals and reagents were of analytical grade.

2.2. Quality compliance evaluation of test drug – gokshuradi guggulu

Before proceeding the toxicity evaluation, the test drug – gokshuradi guggulu was first standardized for its compliance as per pharmacopeial standards (Anon 2008b) by evaluating its physico-chemical tests like heavy metals (lead, arsenic, mercury and cadmium) content, microbial contamination (total viable aerobic count, total enterobacteriaceae and total fungal count), test for specific pathogens (Escherichia coli, Salmonella spp., Staphylococcus aureus and Pseudomonas aeruginosa), test for aflatoxins (B1, B2, G1, G2) and pesticide residue (organochlorine pesticides, organophosphorus pesticide and pyrethropids) analysis (Khandelwal 2006).

Thin layer chromatography analysis of gokshuradi guggulu was carried out as per the method described in Ayurvedic Pharmacopeia of India (Anon 2008b).

2.3. LC–Ms/MS analysis

LC–ESI–MS/MS analysis was carried out by using a UHD Accurate-Mass 6538 Q-TOF LCMS system (Agilent Technologies) with a Infinity Lab Poroshell 120SB-C18 analytical column (3.0 x 100 mm, 2.7 µm). Q-TOF system is state of the art platform for MRM analysis with less than 5 ppm error yielding very high mass resolution. The mobile phase was comprised of solvent A (H₂O: Acetonitrile: Formic acid; 90:9:0.1), solvent B (Acetonitrile: H₂O: Formic acid; 90:9:0.1) run in a gradient mode (5% to 97%). The injection volume was 20 µl and the column temperature was maintained at 40°C. Parameters for analysis were set using positive ion mode with spectra acquired over a mass range from m/z 100 to 1700 for MS and from m/z 50 to 1700 for MS/SM, data was acquired at 2 GHz extended dynamic range with narrow isolation width. The MS/MS data was analyzed using quantitative analysis software (Version B.10.0 Agilent Technologies, USA), the compounds were indentified using commercially available licensed METLIN metabolite PCDL library. The accuracy for confirmation of the compounds was established on the basis of their error less than 5 ppm and MS/MS fragment matching.

2.4. Animals

Healthy Wistar rats 68 (equal number of males and females) were procured from National Laboratory Animal Center, Central Drug Research Institute, Lucknow, Uttar Pradesh, India. Animals were acclimatized for 7 days before initiation of the study and maintained at Central Animal Facility of the Institute under standard housing conditions of temperature 25 ± 2°C, relative humidity 55 ± 5% and light and dark cycles of 12 h. Animals were housed in polypropylene cages with lids and rice husk bedding. Animals were provided with standard pellet diet (Ashirwad Brand, Chandigarh) during study period. The purified filtered water was provided ad libitum using plastic nozzle bottles. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee (IAEC) for conducting the experiments (Proposal No. NRIASHRD-GWL/IAEC/2014/02).

2.5. Dose selection

As the biochemical and functional systems vary in different species altering the pharmacokinetics of drugs, the scaling of dose based on the body weight (mg/kg) alone is not the right approach. Therefore, extrapolation of dose from animals to humans needs consideration of body surface area (BSA), pharmacokinetics, and physiological time to increase clinical trial safety (Nair and Jacob, 2016). Even the United States Food and Drug Administration (USFDA) guidance document also suggests to use BSA for calculating animal equivalent dose from Human dose based on correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m²). In the present study, therapeutic dose of test drug for rats (test species) was calculated from body surface area calculation suggested by Paget and Barnes (1964) and some previous studies (Gokarn et al. 2017, Timbadiya et al. 2015, Joshi et al. 2016) on similar type of ayurvedic formulations.

The human dose of gokshuradi guggulu is 2–3 g in divided doses.

Taking human dose is 3 g (maximum) in a day, animal equivalent dose is calculated as follows.

\[
\begin{align*}
(3000 \text{ mg}) \times (a) & \times \text{ conversion factor (b) } 0.018 = (c) \\
\text{per } 200 \text{ g of rat } & \\
3000 \text{ mg } \times 0.018 \text{ (b) } & = 54 \text{ mg (c)/200 g of rat} \\
54 \times 1000/200 & = 270 \text{ mg/kg}
\end{align*}
\]

Hence, the calculated therapeutic dose (TD) is 270 mg/kg. Based on this, ten times TD was selected 270 × 10 = 2700 mg/kg as high dose (HD) as the maximum feasible dose based on available human therapeutic dose. In acute toxicity study, high dose (10TD) (2700 mg/kg) was used and in sub-acute toxicity study high dose (10TD) (2700 mg/kg), mid dose (10TD) (1350 mg/kg) and low dose (TD) (270 mg/kg) were used.
2.6. Acute oral toxicity study

The study was performed as per the protocol mentioned in guideline prescribed by Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi based on Organization for Economic Cooperation and Development (OECD) guideline 423 (Anon 2001) and Schedule Y of the Drugs and Cosmetic Act, 1940. For acute oral toxicity, total 20 rats of 10–12 weeks age were selected and randomly divided into 2 groups. The animals were randomized before grouping and randomization schedule was kept in the record. Each group consisted of 10 animals (5 males and 5 females). Females were non-pregnant and nulliparous. Group I (vehicle control group) received suspension of 4% w/v gum acacia in distilled water while group II (test group) received 
gokshuradi guggulu (2700 mg/kg, orally once). Animals were housed individually and numbered as per their groups. The cage side observations were made for clinical signs and mortality after dosing, at 5–10 min, 30–45 min, 1 (±10 min), 2 (±10 min), 4 (±10 min), 6 (±10 min) and 24 (±2 min) h post dosing followed by once daily throughout 14 days which included changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Body weights of each animal were recorded at the start of study and thereafter at weekly intervals. All the animals were euthanized by CO2 asphyxia on 15th day of the study and subjected to a detailed postmortem examination. Liver, kidneys, spleen, heart, thymus and brain were collected and weighed. The relative organ weights of each animal were calculated by the following formula.

\[
\text{Relative body weight} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100
\]

2.7. Sub-acute oral toxicity study

The study was performed as per the protocol mentioned in guideline prescribed by Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi based on Organization for Economic Cooperation and Development (OECD) guideline 423 (Anon 2001) and Schedule Y of the Drugs and Cosmetic Act, 1940. For sub-acute (28 days repeated dose) oral toxicity study, total 48 rats (24 Males + 24 Females) of 6–7 weeks age were selected and randomly distributed to 4 groups (Group I, II, III and IV). The animals were randomized before grouping and randomization schedule was kept in the record. Each group consisted of 12 animals (6 males and 6 females). Females were non-pregnant and nulliparous. Group I (vehicle control group) received suspension of 4% w/v gum acacia in distilled water while groups II, III and IV (test groups) received 
gokshuradi guggulu in TD (270 mg/kg), 5 times of TD (1350 mg/kg) and 10 times of TD (2700 mg/kg), respectively. Animals were housed individually and numbered as per their groups. The vehicle and 
gokshuradi guggulu were administered orally once to individual animals of control and test groups, respectively in a dose volume of 1.0 ml/100g of body weight. The following observations were made during the course of study up to 28 days.

2.7.1. Clinical signs and mortality

Rats were examined for clinical signs and mortality after dosing, at 5–10 min, 30–45 min, 1 (±10 min), 2 (±10 min), 4 (±10 min), 6 (±10 min) and 24 (±2 min) h post dosing followed by once daily up to 28 days in sub-acute toxicity study. Cage side observations included changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. All animals were observed for morbidity and mortality daily.

2.7.2. Body weights

Body weights of each animal were recorded at the start of study and thereafter at weekly intervals.

2.7.3. Feed and water consumption

In sub-acute toxicity study, the weekly feed and water consumptions of rats were recorded by measuring the difference between feed/water offered and feed/water left over on subsequent weeks. The weekly feed consumed per cage was calculated and presented in terms of feed/water consumed by each rat.

2.7.4. Biochemistry and pathology

In sub-acute toxicity study, blood collection was carried out on 29th day at the end of the study to assess the changes in hematological and biochemical parameters of animals. The blood was collected from overnight fasted rats through retro-orbital plexus (Parasuraman et al., 2010) under light ether anesthesia. For hematology, blood was collected in K3 EDTA (potassium Ethylene Di-amine Tetra Acetate) vacutainers whereas for biochemical analysis centrifuge tubes containing heparin anti-coagulant were used. The plasma was separated and processed for biochemical analysis.

The hematological parameters were evaluated using fully automated veterinary Hematology Analyzer (Sysmex XT-2000iV) which included hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count and percent, platelet count, and other related corpuscular parameters.

Clinical biochemistry determinations on blood were carried out using semi-automated clinical chemistry analyzer – Macrolab 300 (Vital Scientific). The determinations of glutamic-oxaloacetate transaminase (GOT), glutamic-pyruvate transaminase (GPT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), fasting glucose, triglycerides, cholesterol, urea, creatinine, albumin, total bilirubin, total protein, calcium, phosphorus, chloride, sodium, potassium and magnesium were made.

Animals were shifted to metabolic cages (Orchid Scientific and Innovative India Pvt. Ltd., Nasik, India) and for urine collection for 14–16 h during week 4 (Day 21). Feed was withheld during the phase of urine collection in metabolic cages. The urine volume was measured using calibrated centrifuge tubes manually. Color and visual appearance of the clarity were noted manually. Specific gravity, glucose, bilirubin, ketones, occult blood, pH, protein, urobilinogen, nitrite and
leukocytes of urine were estimated by Uridip 10 A (Erba Mannheim, Germany). The visual observations of the strips were performed.

2.7.5. Necropsy, organ collection and histopathology
Animals were euthanized by CO₂ asphyxia on 29th day of the study and subjected to a detailed postmortem examination. Brain, heart, lungs, liver, kidneys, adrenals, pancrease, spleen, thymus, gastrointestinal tract, ovaries/testes, skin and sciatic nerve were collected and weighed, wherever necessary. The relative organ weights of important organs of each animal were calculated. All organs except testes were fixed in 10% neutral buffered formalin while testes were first fixed in modified Davidson’s fluid and subsequently transferred to 10% neutral buffered formalin on the next day. All the organs were processed for histopathology by Study Pathologist at Regional Ayurveda Institute for Fundamental Research, Pune.

2.8. Statistical analysis
The statistical analysis was performed using Graph pad prism, version 5.0. All the values are expressed as the Mean ± SEM. The data of weekly body weight in acute toxicity study were analyzed by Student’s ‘t’ test. In 28 days repeated dose sub-acute toxicity study, the data of hematological parameters, biochemical parameters and organ weights were analyzed by one-way ANOVA followed by Tukey’s multiple comparison post hoc test while the data of weekly body weight, feed and water consumption were analyzed by two-way ANOVA followed by Bonferroni post hoc test. A value of \( p < 0.05 \) was considered statistically significant.

3. Results
3.1. Quality compliance evaluation of gokshuradi guggulu
The results of the all the physico-chemical parameters are presented in Table S2. The parameters were found within prescribed pharmacopeial limits (Anon 2008b). There was no detection of any heavy/toxic metals (Pb, As, Hg and Cd), aflatoxins (B1, B2, G1 and G2) and pesticide residues observed in gokshuradi guggulu. The total bacterial and fungal count in gokshuradi guggulu powder was 1650 and 10 cfu/g, respectively while enterobacteriaceae were found absent. All the results were within the standard limits prescribed in ASU guidelines (Lohar 2011).

3.2. Metabolite profiling of gokshuradi guggulu
The LC-MS chromatogram of methanolic extract gokshuradi guggulu is represented in Figure 1. Results from LC-MS analysis revealed the identification of 11 phytoconstituents in extract after integrating with the libraries. The details of the compounds are mentioned in Table 1.

3.3. Clinical signs and mortality
The cage side observation showed no signs of alterations in any of the parameters of rats upon acute and 28 days repeated dose administration of gokshuradi guggulu compared to vehicle control group. No mortality or moribund stage was observed throughout the study period.

3.4. Effect on body weights
In acute toxicity study, there was no significant change noted in body weights of male and female rats (Table S3) after single dose administration of gokshuradi guggulu compared to vehicle control rats. In sub-acute toxicity study there was no significant change noted in weekly body weights of male and female rats after 28 days repeated administration of gokshuradi guggulu (Table S4) compared to vehicle control rats.

3.5. Effect on feed and water consumptions
Gokshuradi guggulu administration for 28 days did not show any significant change in feed consumption of male and female (Table 2) rats compared to vehicle control rats. The

![Figure 1. A base peak LC-MS chromatogram of gokshuradi guggulu (100 µg, 20 µg of 5 mg/ml solution).](image)
Table 1. LC-MS-MS analysis of Gokshuradi guggulu.

| S. No. | Name of the compound | METLIN | Retention time | m/z   | Charge state | Mass    | Chemical formula | Abundance | Error PPM (<5ppm) | Structure |
|-------|----------------------|--------|----------------|-------|--------------|---------|------------------|-----------|------------------|-----------|
| 1.    | Calabricoside B      | 50684  | 1.562          | 453.1226 | [M + 2H]2+ | 904.2304 | C41 H44 O23     | NA        | 3.4              |           |
| 2.    | Dillen tin 5-glucoside-7-glucuronide | 50826  | 1.686          | 357.0677 | [M + 2H]2+ | 668.1577 | C29 H32 O18     | 11325     | -1.8             |           |
| 3.    | Quercetin 3-(2''-caffeylsambubioside)-7-glucoside | 50572  | 3.173          | 483.1025 | [M + 2Na]2+ | 920.2252 | C41 H44 O24     | 3818      | 3.2              |           |
| 4.    | Kaempferol 3-apioside-7-rhamnosyl-(1->6)-(2''-caffeoylglactoside) | 50384  | 3.226          | 467.1049 | [M + 2Na]2+ | 888.2319 | C41 H44 O22     | 7126      | -0.55            |           |
| 5.    | Licorice saponin A3  | 93142  | 3.331          | 515.217  | [M + 2Na]2+ | 984.4556 | C48 H72 O21     | 674       | -1.05            |           |
| 6.    | Ampeloside Bf1      | 87998  | 4.04           | 580.2613 | [M + 2H]2+ | 1114.544 | C51 H86 O26     | 610       | 3.06             |           |
| 7.    | Ampeloside Bf2      | 87997  | 3.552          | 477.2535 | [M + 2H]2+ | 952.4915 | C45 H76 O21     | 2065      | 3.76             |           |
treatment showed significant increase in water consumption at dose of 2700 mg/kg in 4th week and decrease in water consumption at doses 1350 and 2700 mg/kg in 2nd and 3rd weeks, respectively in male rats (Table 2). In female rats, the decrease in water consumption was observed in 1st week (Table 2) at the dose 270 and 1350 mg/kg of gokshuradi guggulu.

3.6. Effect on hematological and biochemical parameters

Gokshuradi guggulu treatment for 28 days did not show significant change in any of the hematological and biochemical parameters in male and female rats compared to vehicle control rats (Table S5 and S6, respectively).

3.7. Effect on urine parameters

The analysis urine of male and female rats showed no noticeable change in any of the urinary parameter due to gokshuradi guggulu treatment compared to vehicle control rats (Table S7).

3.8. Effect on gross morphological changes during necropsy

During necropsy, no gross morphological changes were observed in the organs of all test group animals compared to organs of vehicle control rats in both acute and sub-acute toxicity studies.

3.9. Effect on weights and histopathology of organs

In acute toxicity study, there was no significant change observed in relative organ weights of male and female rats (Table S8) after single dose administration of gokshuradi guggulu compared to vehicle control rats. In sub-acute toxicity study, gokshuradi guggulu administration for 28 days did not show significant change in relative organ weights of male and female rats compared to the vehicle control group (Table S9).

Histopathology evaluation of various organs/tissues of high dose group revealed normal tissue architecture without any evidence of tissue damage as compared to vehicle control animals (Figure 2).

4. Discussion

Traditional medicines are used by different groups of population in the communities for the treatment of various ailments without proper dosage monitoring with a belief that they have negligible side effects. Some ayurvedic medicines are used for prolonged time period against the disease like diabetes, arthritis, etc. However, these medicines may lead to toxic manifestation after their chronic uses (Eran et al. 2016). Some of them are reported while most of the times they remain neglected or unattended. So, proper scientific documentation of toxicity studies of the traditional medicines is

| No. | Name of the compound                  | METLIN Retention time | m/z | Charge state | Mass | Chemical formula | Abundance | Error PPM (<5ppm) | Structure |
|-----|--------------------------------------|-----------------------|-----|--------------|------|------------------|-----------|-------------------|-----------|
| 8   | Agavasaponin C                       | 12.754                | 351.2164 | [M+H]^+      | 350.2087 | C20 H30 O5       | 1325      | 2.35              |           |
| 9   | Methyl acetoxy-6-gingerol            | 15.5928              | 358.2317 | [M+H]^+      | 364.2504 | C21 H32 O5       | 1018      | 2.35              |           |
| 10  | Acetoxy-8-gingerol                   | 17.9624              | 351.2164 | [M+H]^+      | 364.2504 | C21 H32 O5       | 1018      | 2.35              |           |
| 11  | 18:0-Glc-Sitosterol                  | 19.1277              | 439.3834 | [M+2Na]^2+   | 842.6991 | C53 H94 O7       | 2999      | 0.96              |           |

Table 1. Continued.
required for estimation of their safe and effective doses of administration and the possible toxicological signs. Gokshuradi guggulu is used in Ayurvedic clinical practice for various diseases like urolithiasi and diabetes. The toxic effects are also reported for its main ingredient, Gokshura (*Tribulus terrestris*). Hence, the present study assessed the acute and sub-acute oral toxicity of gokshuradi guggulu in rats.

Acute oral toxicity study at the high dose of gokshuradi guggulu (2700 mg/kg) revealed that there was no toxicity of any nature, mortality or moribund stage noted during the observation period. The clinical signs were normal and no gross morphological changes were noted in the organs of test group rats as compared to vehicle control rats during necropsy. This indicates that maximum tolerated dose of gokshuradi guggulu was 2700 mg/kg and thus, no observed adverse effect level (NOAEL) of gokshuradi guggulu is more than 2700 mg/kg with approximate LD$_{50}$ more than 2500 mg/kg.

The repeated dose sub-acute toxicity provides information regarding dosage regimen, target organ toxicity, and identification of observable adverse effect that may affect the average life span of experimental animals (Porwal *et al.* 2017). The present study indicated that repeated oral dose administration of gokshuradi guggulu for 28 days did not cause any mortality or moribund stage during the observation period. The clinical signs were normal and there was no significant effect of gokshuradi guggulu on neurological behavior. The body weight gained by the animals and their feed intake were not significantly affected compared to vehicle treated rats. The water consumption changes observed were not consistent in all groups when compared to vehicle control animals. Thus, change was not considered to be a treatment related effect. This indicates that gokshuradi guggulu did not adversely affect the basic metabolic processes of the experimental animals, which was also confirmed from the insignificant changes in glucose, cholesterol and triglycerides levels and the histology of liver.

Clinical biochemistry and hematological parameters play an important role in determining the health condition whose alterations indicate diseased condition or adverse effects. Blood is a connective tissue which transports the xenobiotics throughout the body. Exposure to toxic compounds may cause damage to blood cells which leads to dysregulation of the normal body function (Adeneye *et al.* 2006). Gokshuradi guggulu administration for 28 days did not cause any significant damage. Hemoglobin is generally broken down to bilirubin and its elevated concentration in blood reflects hemolysis and liver damage. Gokshuradi guggulu did not cause any significant change in haematological parameters suggesting no adverse influence on the haemopoietic system of body. Hemoglobin is generally broken down to bilirubin and its elevated concentration in blood reflects hemolysis and liver damage. Gokshuradi guggulu did not cause any significant change in levels of bilirubin or liver architecture indicating its safety in hepatic system. White blood cells are the first line of cellular defense that respond to infectious agents and inflammation. Neutrophils, lymphocytes, and monocytes reflect the absence of inflammation or immune system activation. The white blood cell counts did not show any significant change in the treated rats, which are known to be the source of inflammation or immune system activation in all groups, when compared to vehicle control rats. Hence, the present study assessed the acute and sub-acute oral toxicity of gokshuradi guggulu in rats. The toxic effects are also reported for its main ingredient, Gokshura (*Tribulus terrestris*). Hence, the present study assessed the acute and sub-acute oral toxicity of gokshuradi guggulu in rats. The toxic effects are also reported for its main ingredient, Gokshura (*Tribulus terrestris*). Hence, the present study assessed the acute and sub-acute oral toxicity of gokshuradi guggulu in rats.

| Parameters                          | Vehicle Control | Test (Gokshuradi guggulu) |
|------------------------------------|----------------|--------------------------|
|                                   |                | 270 | 1350 | 2700 |
|                                   |                | Males | Female |
|                                   |                | Week | Vehicle Control | Test (Gokshuradi guggulu) | Vehicle Control | Test (Gokshuradi guggulu) |
| Weekly Feed Intake (g)            |                | 1st  | 187.66 ± 4.69  | 180.66 ± 3.41  | 183.33 ± 4.31  |
|                                   |                | 2nd  | 190.66 ± 3.90  | 191.66 ± 0.86  | 174.00 ± 3.83  |
|                                   |                | 3rd  | 177.66 ± 2.14  | 182.50 ± 1.38  | 164.33 ± 7.10  |
|                                   |                | 4th  | 159.83 ± 0.69  | 171.66 ± 4.80  | 152.00 ± 4.38  |
|                                   |                | 1st  | 269.83 ± 2.12  | 278.83 ± 6.18  | 243.83 ± 1.73  |
|                                   |                | 2nd  | 281.83 ± 10.13 | 278.50 ± 1.11  | 234.50 ± 6.33  |
|                                   |                | 3rd  | 272.16 ± 5.46  | 277.66 ± 3.07  | 250.33 ± 7.20  |
|                                   |                | 4th  | 246.83 ± 5.67  | 263.16 ± 5.01  | 223.90 ± 10.22 |
| Weekly Water Intake (ml)          |                | 1st  | 269.83 ± 2.12  | 278.83 ± 6.18  | 243.83 ± 1.73  |
|                                   |                | 2nd  | 281.83 ± 10.13 | 278.50 ± 1.11  | 234.50 ± 6.33  |
|                                   |                | 3rd  | 272.16 ± 5.46  | 277.66 ± 3.07  | 250.33 ± 7.20  |
|                                   |                | 4th  | 246.83 ± 5.67  | 263.16 ± 5.01  | 223.90 ± 10.22 |

Values are mean ± SEM, (n = 6). Doses are expressed in mg/kg. $p < 0.05$, $\alpha p < 0.01$, $\beta p < 0.001$ compared to vehicle control.
platelets. In the current study, repeated doses of gokshuradi guggulu did not cause any type of nephrotoxicity in rats as indicated by the normal plasma urea and creatinine levels as compared to vehicle control rats. This is further evident from the unaffected urine volume, pH, kidney weights and histology. Adrenal gland is a very important endocrine gland which releases aldosterone, epinephrine and norepinephrine, which are essential for normal physiological functions. The organ weights and histology of heart, adrenal and thymus did not show significant changes in test group animals as compared to vehicle control rats. These findings suggest that gokshuradi guggulu did not produce any adverse effect on cardiovascular and urinary systems. The gastrointestinal system did not show any toxicity as seen by the normal clinical signs and no alterations in the histological structures of stomach and intestine in both sexes of rats.

In the present study, repeated dose of gokshuradi guggulu did not show any significant difference in the organ weight, necropsy and histology of testes and epididymus of male rats as well as ovary and uterus of female rats. It suggests that the treatment did not show any toxicity in reproductive system of animals.

Gross and histopathology evaluations of various organs/tissues from males and females of vehicle control and high dose group revealed that repeated dose administration of gokshuradi guggulu for 28 consecutive days in Wistar rats did not show any lesion or histopathological alterations related to the treatment/doses of test drugs compared to the vehicle control group.

The results of the present study are in contrast to earlier reports of nephrotoxicity observed due to treatment with Tribulus terrestris (Talasaz et al. 2010) or its saponin fraction (Gandhi et al. 2013). The possible reason is that gokshuradi guggulu is a compound formulation containing various other ingredients including Tribulus terrestris. Hence, the amount of drug or its constituent is present in very small quantity to elicit any toxic response as compared to single drug/isolated constituents used in above studies. Further, other ingredients might be taking care of metabolism and elimination of toxic influences, if any, of Tribulus terrestris. It is pertinent to mention here that ayurvedic medicines are formulated in view of holistic approach of treatment and contains ingredients based on the pathophysiology of the disease and body constitution (prakriti) of the diseased individual (Kshirsagar and...
Anon, 2008b. The Ayurvedic Pharmacopoeia of India, Part II (Formulations), Vol 2, 1st ed. New Delhi, India: Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy, 112–114.
Aslani, M.R., et al., 2003. Experimental Tribulus terrestris poisoning in sheep: clinical, laboratory and pathological findings. Veterinary Research Communications, 27 (1), 53–62.
Aslani, M.R., et al., 2004. Experimental Tribulus terrestris poisoning in goats. Small Ruminant Research, 51 (3), 261–267.
Bourke, C.A., 1983. Hepatopathy in sheep associated with Tribulus terrestris. Australian Veterinary Journal, 60 (6), 189.
Bourke, C.A., 1987. A novel nigrostriatal dopaminergic disorder in sheep affected by Tribulus terrestris staggers. Research in Veterinary Science, 43 (3), 347–350.
Chan, K., 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere, 52 (9), 1361–1371.
Chaudhary, A., Singla, S.K., and Tandon, C., 2010. In vitro evaluation of Terminalia arjuna on calcium phosphate and calcium oxide crystalization. Indian Journal of Pharmaceutical Sciences, 72 (3), 340–345.
Eran, B.A., et al., 2017. Toxicological studies of rassadindra, an ayurvedic formulation. Indian Journal of Pharmaceutical Sciences, 79 (4), 633–640.
Ilanchezhan, R., Roshy Joseph, C., and Acharya, R., 2011. Concept of shodhana (purification/processing) and its impact on certain poisonous herbal drugs. Journal of Ayurveda, 5 (4), 69–76.
Jacob, R.H. and Peet, R.L., 1987. Poisoning of sheep and goats by Tribulis terrestris (caltrop). Australian Veterinary Journal, 64 (9), 288–289.
Joshi, A.J., et al., 2016. Evaluation of immunomodulatory activity of Balachatur bhadra Churna – an Ayurvedic formulation. Indian Journal of Natural Products and Resources, 7 (4), 293–300.
Khandelwal, K. R., 2006. Practical pharmacognosy. 15th ed. Pune, India: Nirlal Prakashan, 149–156.
Khuriasgar, M. and Magno, A. C., 2011. Ayurveda – a quick reference handbook. Twin Lakes, WI: Lotus Press.
Lohar, D. R., 2011. Protocol for testing of ayurvedic, siddha and unani medicines. Pharmacopeial Laboratory for Indian Medicine, AYUSH. Ministry of Health and Family Welfare, Ghaziabad, India: Government of India.
Maurya, S.K., et al., 2015. Sodhana: an ayurvedic process for detoxification and modification of therapeutic activities of poisonous medicinal plants. Ancient Science of Life, 34 (4), 188–197.
Nair, A. B., and Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy, 7 (2), 27–31.
Paget, G. E. and Barnes, J. M., 1964. Toxicity tests. In: D.R. Laurence, A.L. Bacharach, eds. Evaluation of drug activities: Pharmacometries. Vol. 1, 1st ed. London, UK: Academic Press, 135–166.
Parasuraman, S., Raveendran, R., and Kesavan, R., 2010. Blood sample collection in small laboratory animals. Journal of Pharmacology & Phamacotherapeutics, 1 (2), 87–93.
Porwal, M., Khan, N.A., and Maheshwari, K.K., 2017. Evaluation of acute and sub-acute oral toxicity induced by ethanolic extract of Mardensia tenacissima leaves in experimental rats. Scientia Pharmaceutica, 85 (3), 29.
Talasaz, A.H., et al., 2010. Tribulus terrestris-induced severe nephrotoxicity in a young healthy male. Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association – European Renal Association, 25 (11), 3792–3793.
Timbadiya, M.J., et al., 2015. Experimental evaluation of antipyretic and analgesic activities of Amalakya Gana: an ayurvedic formulation. Ayu, 36 (2), 220–224.