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Safety profiling of hydroalcoholic extract of shuddha guggul (*commiphora wightii*) engler in swiss albino mice

Abstract

The acute or sub-acute toxicity of ethanol extract of guggle (*Commiphora wightii)* in Swiss albino mice was evaluated by oral administration of either a single dose of 1, 2, 3, 4, 5, and 6 g/kg or 100, 200, and 300 mg/kg body weight 80% guggul extract once daily for 90 days. Oral administration of 1-6 g/kg body weight ethanol guggul extract did not show any toxic effect up to a dose of 5 g/kg body weight in the acute toxicity study, whereas no morbidity and mortality were recorded after daily administration of 100-300 mg/kg body weight guggul extract up to 90 days. The administration of 100 to 300 mg/kg guggul extract for 90 days did not increase micronuclei frequency in the splenocytes, and also could not alter motility, viability, and DNA damage in the sperms. Similarly, RBC and WBC counts remained unaltered after 90 days of administration of 100-300 mg/kg guggul extract. The daily administration of various doses of guggul extract for 90 days did not alter serum creatinine and glucose levels and activities of creatinine kinase, aspartate aminotransferase, alanine aminotransferase, and cholesterol level. Ethanol guggul extract was safe up to 5 g/kg body weight in the acute toxicity study, and daily administration of 100-300 mg/kg guggul extract for 90 days did not induce toxicity in blood cells, splenocytes, sperms, liver, heart, and kidneys of mice, indicating the safety of guggul.

Keywords: *Commiphora wightii*, acute toxicity, chronic toxicity, micronuclei, comet assay, sperm dysfunction

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Introduction

The utilization of traditional medicine across various cultures has a rich historical background, stemming from the efforts of our ancestors to develop rudimentary remedies in response to natural calamities and ailments. The oldest system of healthcare, Ayurveda, uses herbs and natural products as medicine/drugs that reinforce their value in human healthcare as they have usually been found non-toxic.[1,2] The scientific evaluation of herbal and natural products could provide new inputs for the development of novel therapies for human healthcare.[3,4] Traditional, complementary, and alternative medicines provide healthcare to 80% of the world's population.[5,6] The application of nutraceuticals in healthcare has dramatically increased in developed countries as they are non-toxic and usually have no adverse side effects. This is indicated by the fact that the number of natural products and natural product-derived molecules rose to one-third of the new molecules approved by the FDA, USA.[7,8]

Plants secrete gums and resins as secondary metabolites, which are used as medicines for the treatment of different diseases in different systems of traditional medicine.[9] Oleoresin, gum guggul is an exudate from *Commiphora wightii*, a plant native to India and Pakistan, and contains several phytochemicals including guggultetrols, alkaloids, flavonoids, lignans, monoterpenoids, diterpenoids, sesquiterpenoids, and triterpenoids; steroids, aliphatic esters, ferulates, amino acids, and sugars. The guggul possesses Z- and E- E-isomers of guggulsterone and gugglusterols associated with it.[10–12] The oleo–gum–resin of *Commiphora wightii* Engler (Burseraceae) or guggul is also known as Mur or Myrrh in healthcare and possesses hypercholesterolemic, anti-inflammatory, antipyretic, antiseptic, antidiabetic, antidepressant, antiarthritic, cardioprotective, antiobesity, and antihistaminic activities.[11,13–15] Guggul has been reported to be active against hypercholesteremia in humans.[16,17] The ethanol extract of guggul was also active against fructose-induced insulin resistance and hypertriglyceridemia, abdominal pain, abscess, chest arthralgia, dysmenorrhea, heartache, gynecological benign tumors, postpartum stasis, rheumatic arthralgia, trauma, swelling, and wounds.[18,19] It has been reported to increase sperm count, motility, and abnormalities, and reduce glucose level in Wistar rats.[18,20] In folk, medicine guggul has been used to treat hypercholesteremia, dyspepsia, dysmenorrhea, endometritis, hypertension, impotence, bronchitis, malignant sores, caries, gingivitis, hysteria, ulcers, urinary complaints, obesity, intestinal worms, leucoderma, liver disorders, sinuses, edema, sudden paralytic seizures and inflammation.[15,21] In the present study, an attempt has been made to investigate the acute and sub-chronic (90 days) toxicity profiles of *Commiphora wightii* (guggul), in Swiss albino mice.

Material and methods

Chemicals

Ammonium oxalate, carboxymethyl cellulose (CMC), boric acid, phosphate buffered saline (PBS), NaOH (sodium hydroxide), NaCl (Sodium chloride), glacial acetic acid, petroleum ether, chloroform, methanol, absolute ethanol (HPLC grade), and dimethylsulphoxide (DMSO) were procured from Ranbaxy Laboratories Limited, Mumbai India, whereas agarose, low melting agarose (Cat No. A-4718), N-lauryl sarcosine, Triton-X 100, Trizma base, ethylenediaminetetraacetic acid (EDTA), concanavalin-A, fetal calf serum (FCS), cytochalasin-B, and RPMI-1640 medium, were supplied by Sigma-Aldrich Co. Ltd. (Bangalore, India). Acridine orange (BDH, England, Gurr Cat. No. 34001 9704640E), eosin Y, and ethidium bromide were requisitioned from BDH, England.

Collection of material

The purified form of dried shuddha guggul (oleo–gum–resin) of *Commiphora wightii* in solid form was purchased from M/s Guru Herbals, Udupi, Karnataka, India, and voucher specimen RBSGE04 is stored at the Department of Radiobiology, Kasturba Medical College, Manipal.

Preparation of the extract

The air-dried shuddha guggul (oleo-gum-resin), *Commiphora wightii,* (Arnot) Bhandari (Syn. *Balsamodendron mukul* Hook. Ex Stocks: *Commiphora mukul* Hook. (Ex Stocks) Engl. or *Commiphora roxburghii* (Ex Stocks) Engl. Family: Burseraceae was crushed into powder by a mortar and pestle before extraction. Briefly, five hundred grams of guggul powder was extracted with 2000 mL petroleum ether (60–80°C), followed by a repeated extraction in absolute methanol and subsequently in 80% ethanol in a Soxhlet apparatus. The ethanol extract was dried under reduced pressure in a rotatory evaporator (Buchi Rotavapour, Switzerland) and stored at minus 70°C until use. Henceforth, the shuddha guggul extract will be called CWE.

Animal care and handling

The care and handling of animals were carried out following the guidelines issued by INSA (Indian National Science Academy, New Delhi, India), World Health Organization, Geneva, Switzerland, and ARRIVE-2010 while conducting the experiments. Usually, six to eight-week-old male and female Swiss albino mice weighing 22 to 24 g were procured from an inbred colony maintained under the controlled conditions of 12 h of light and dark, 23±2°C temperature, and 50±5% humidity, respectively. The animals were given unrestricted acess to access to sterile food and water. Usually, five males and females were housed separately in an individual polypropylene cage consisting of sterile paddy husk (procured locally) during the experiments. All experiments have been approved by the Animal Ethical Committee of Manipal University, Manipal, India.

Preparation of the drug and mode of administration

The CWE was dissolved in 100 µl of ethanol and diluted further with 0.5% CMC in normal sterile physiological saline (SPS) as required. The animals were orally administered with CWE depending on the experimental protocol.

Acute Toxicity

The acute toxicity of CWE was ascertained according to OECD guidelines.[22,23] Briefly, 120 animals were allowed to fast by withdrawing food and water for 18 h, and were categorized into the following groups:

SPS: The animals of this group received 0.01mL/g body weight of sterile physiological saline orally.

CWE: The animals were orally administered a single dose of 0.5, 1, 3, 5, or 6 g/kg body weight CWE.

The animals were continuously monitored for the first 24 h and thereafter twice daily up to 14 days post-CWE treatment for signs of toxicity, morbidity, and mortality.[22,23]

Sub-chronic (90 day) toxicity -

The sub-chronic toxicity of CWE was evaluated following the OECD guidelines 2001 by randomizing an equal number of male and female mice in each group as described for acute toxicity, except that the animals were orally administered a single dose of 100, 200, or 300 mg/kg body weight CWE or SPS once daily consecutively for 90 days.[23,24]

The animals were monitored for all external general symptoms of toxicity, body weight changes, and mortality until the end of the 90th day. The animal weights were recorded before and after CWE treatment once a week for 90 days. The animals were euthanized on day 91, and the vital organs, including heart, lungs, liver, kidney, testes, and spleen, were removed. The seminal vesicles and *cauda epididymides* from males were excised and weighed using an analytical electronic weighing balance (Mettler Toledo GmbH, Switzerland). Viscera of the animals treated with CWE for 90 days were compared with those of the SPS group for the symptoms of toxicity, if any.

Hematological test

The blood was collected in heparinized vials from each mouse through the eye orbit under anesthesia. The erythrocytes (RBC) and leukocytes (WBC) were counted using a hemocytometer (American Optical Co., Southbridge, MA, USA) under a transmitted light microscope (Photomicroscope III, Carl Zeiss, Oberkochen, Germany), whereas hemoglobin was measured with the help of a hemoglobinometer (Contraves Digicell 3100H, Zurich, Switzerland). The hematocrit and platelet contents were estimated using an autoanalyzer.

Sperm dysfunction test

The sperm dysfunction in CWE-treated males for 90 days was determined using the sperm abnormality test, a parameter that allows a reliable assessment of germ cell mutagenicity and carcinogenicity.[25] The *cauda epididymides* and *vas deferens* from each animal were dissected out and transferred into individual centrifuge tubes containing 3 mL Krebs-Ringer's bicarbonate buffer. The sperm suspension was filtered through an 80 µm nylon mesh to remove tissue fragments and subsequently stained in 0.5 mL of 1% eosin-Y in a test tube. The contents were thoroughly mixed, and one drop of this suspension was placed onto a coded slide, spread, and screened for sperm abnormalities.[25]

Sperm comet assay

The effect of sub-chronic CWE treatment on sperm genotoxicity in male mice was assessed by a modified alkaline comet assay as described earlier.[26–28] Briefly, frosted slides were covered with 100 µL of 0.6% low-melting agarose prepared in PBS without Ca^{2+} and Mg^{2+} at 37°C, and the agarose was left for congealing on ice with a coverslip on it. Subsequently, the coverslips were removed. The sperm suspension was centrifuged for 5 min at 1,500 rpm. The pelleted sperms were resuspended in 80 µL of 1.2% low-melting agarose, layered onto the first layer, and allowed to solidify under a coverslip on ice. All the steps described above were carried out under diffused light to avoid additional DNA damage.

The slides embedded with sperms were placed into cold lysis buffer (pH 10) containing 2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris base (pH 10), 1% N-Lauryl sarcosine (sodium salt), 1% Triton X-100, 10% dimethylsulphoxide (added fresh) to solubilize proteins leaving DNA as nucleoids for 30 min in dark at 4°C. The slides were drained of lysis buffer and washed 3 times with buffer (90 mM Tris base, 90 mM Boric acid, and 2.5 mM Na_2EDTA, pH 8.3-8.4) before placing them into a horizontal gel electrophoresis apparatus filled with fresh electrophoresis buffer containing 300 mM NaOH, 1 mM Na_2EDTA, at pH 10 to a level of ~0.25 cm above the slides. The DNA unwinding of sperm was carried out by keeping the slides in the buffer for 20 min

and electrophoresing for 20 min at 1.25V/cm^2 and 300 mA under cold conditions. The slides were removed, drained off electrophoresis buffer, and flooded slowly with three changes of neutralization buffer (0.4M Trizma base, pH 7.5) for 5 min each.

The sperm DNA on each slide was stained with ethidium bromide and observed at 40X magnification in a fluorescence microscope as "comets" with a fluorescent head and a tail. The comet images were captured using an epifluorescence microscope (Olympus BX51, Olympus Microscopes, Tokyo, Japan) fitted with a 515-535 nm excitation filter, a 590 nm barrier filter, and a CCD camera (CoolSNAP-Procf Digital Color Camera Kit Ver 4.1, Media Cybergenetics, Silver Spring, Maryland, USA). Usually, 100 sperm were analyzed from each slide to give a representative result for the population of cells.[29] The ethidium bromide-stained comets were analyzed using Komet Software (Version 5.5, Kinetic Imaging Ltd, Bromborough, UK). The distance between the profile centers of gravity for DNA in the head and tail known as Olive tail moment or OTM[30] and percent tail DNA provide a good correlation of genotoxicity, the data for OTM and percent tail DNA were collected from three independent experiments, each containing quintuplicate measures and presented as Mean ± SEM (Standard error of the mean).

Splenocytes micronuclei assay

The animals administered with CWE for 90 days were killed by cervical dislocation. The animals were sterilized by thoroughly wiping with sterillium disinfectant (Bode Chemie, Hamburg, Germany), and their abdominal cavities were surgically opened using sterile scissors and forceps under aseptic conditions. The spleens of the animals were aseptically removed and twice washed in sterile PBS. The splenocytes were collected, and micronuclei were prepared.[31,32] Briefly, 40 h after the splenocyte culture, 5 µg/mL cytochalasin-B was added to each culture and allowed to grow for the next 32 h. The splenocytes were harvested 72 h after initiation of the cultures, subjected to 0.7% ammonium oxalate as the hypotonic treatment to retain the cell cytoplasm, and fixed in Carnoy's fixative (3:1 methanol: acetic acid). The cell suspension was centrifuged, resuspended in a small volume of fixative, and dropped onto precleaned coded slides to avoid the observer's bias. The splenocytes were stained with 0.025% acridine orange in Sorensen's buffer (pH 6.8). The slides were washed twice in Sorensen's buffer, mounted, and observed under a fluorescence microscope, equipped with a 450-490 nm BP filter set with excitation at 453 nm (Photomicroscope III, Carl Zeiss, Oberkochen, Germany), using a 40X Neofluar objective. A minimum of one thousand binucleate splenocytes (BNC) with well-preserved cytoplasm was scored from each culture, and the frequency of micronucleated binucleate cells (MNBNC) was determined. The micronuclei were identified and scored as described earlier.[31,32]

Biochemical studies

The blood from the eye orbit of each anesthetized animal receiving acute or sub-chronic CWE treatment was collected aseptically on days 30, 60, and 90 post-CWE treatment in coded nonheparinized tubes. The serum was separated and analyzed for aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), creatine kinase isoenzyme MB (CK-MB), cholesterol, urea, creatinine and glucose[33] using an autoanalyzer (Hitachi C211, Japan) according to the protocol given by the manufacturer (Ranbaxy Laboratories Limited, Mumbai, India).

Statistical analyses

The significance between the treatments was determined by Student's't' test, and a $p < 0.05$ was considered to be statistically significant. One-way ANOVA was used to measure significance among all treatments, and Tukey's post-hoc test was applied for multiple comparisons. The significance of treatment for micronuclei was tested using Fisher's exact test. GraphPad Prism 5 statistical software (GraphPad Software, San Diego, CA, USA) was used to analyze the statistical significance.

Results

The results of acute and sub-chronic toxicities after CWE treatment are shown in Table 1–8, and the data are expressed as mean± standard error of the mean (SEM).

Acute toxicity

The administration of varying doses of CWE, up to 5 g/kg body weight, did not result in any observable signs of toxicity or mortality, and hence it was considered as a no-observed-adverse-effect level (NOAEL) dose. However, locomotors activity declined marginally at a dose of 5g/kg CWE. When the dose of CWE was increased up to 6 g/kg body weight, one female exhibited anorexia, hypoactivity, increased salivation, and asthenia and succumbed to death after 3 days of CWE administration (Table 1). It was not feasible to test higher doses for acute toxicity determination due to the problems faced in dissolving a greater amount of the drug.

Table 1 Alteration in the survival of mice orally administered with different doses of *Commiphora wightii* hydroalcoholic extract for acute toxicity study

Commiphora wightii extract (mg/kg b. wt.)	Sex	No. of animals	No. of Deaths	Motility Latency	Toxic symptoms
0	Male	10	0	-	None
	Female	10	0	-	None
500	Male	10	0	-	None
	Female	10	0	-	None
1000	Male	10	0	-	None
	Female	10	0	-	None
3000	Male	10	0	-	None
	Female	10	0	-	None
5000	Male	10	0	-	Hypoactivity, piloerection
	Female	10	0	-	Hypoactivity, piloerection
6000	Male	10	0	-	Anorexia, hypoactivity, salivation
	Female	10	1	>45, <60	Anorexia, hypoactivity, salivation, asthenia

Citation: Jagetia GC. Safety profiling of hydroalcoholic extract of shuddha guggul (*commiphora wightii*) engler in swiss albino mice. *Int J Complement Alt Med.* 2026;19(1):73–81. DOI: 10.15406/ijcam.2026.19.00764

None = no toxic symptoms during the observation period; mortality latency = time to death (in days) after the drug was injected orally.

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

Mice in each dose group were carefully examined for any signs of toxicity (behavioral changes and mortality) for 14 days.

Sub-chronic toxicity

Oral administration of 100, 200, or 300 mg/kg CWE once daily consecutively for 90 days failed to elicit any visible signs of toxicity and mortality up to a dose of 300 mg/kg body weight CWE until the termination of the study, and hence this dose was considered as the safe dose.

Weight changes

The body weights of the animals were recorded every week before and after CWE treatment until the end of 90 days. CWE treatment caused a significant rise in body weights in the treated groups ($p<0.05$) when compared with the SPS group (Table 2).

Table 2 Body weight changes in mice orally administered chronically with different doses of *Commiphora wightii* hydroalcoholic extract daily for 90 days

***Commiphora wightii* extract**	**Mean body weight (g) ± Standard error of the mean**				
(mg/kg b. wt.)	**Sex**	**Day 0**	**Day 30**	**Day 60**	**Day 90**
0	Male	25.4 ± 1.3	27.4 ± 1.2	29.6 ± 1.6	31.9 ± 1.1[a]
	Female	24.7 ± 1.2	25.7 ± 1.3	27.7 ± 1.5	29.8 ± 0.9[a]
100	Male	24.9 ± 1.2	26.9 ± 1.2	30.5 ± 1.7	34.0 ± 1.2[b]
	Female	25.1 ± 1.1	26.1 ± 1.1	29.6 ± 1.4	33.2 ± 1.3[a]
200	Male	25.2 ± 1.1	27.2 ± 1.2	30.2 ± 1.2	34.6 ± 1.3[a]
	Female	25.0 ± 1.3	26.0 ± 1.1	29.5 ± 1.3	33.0 ± 1.1[a]
300	Male	25. 2 ± 1.0	28.2 ± 1.0	31.2 ± 1.5	34.0 ± 1.4[a]
	Female	24.3 ± 0.9	26.3 ± 1.2	29.3 ± 0.7	32.7 ± 1.0[a]

[a]$p<0.001$ when the values of each group is compared to the day 0 value.

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

N=10 for each group.

The condition of viscera and weight changes in vital organs, including heart, lungs, liver, kidney, testes, spleen, seminal vesicles, and *cauda epididymis*, were also recorded after 90 days of sub-chronic administration of various doses of CWE (Table 3). The sub-chronic CWE treatment did not alter the weight of vital organs significantly (Table 3) and the condition of viscera, when compared to the SPS group. The weight of testes and *cauda epididymides* did not show any alteration when compared to control in the CWE-treated animals, whereas the weights of seminal vesicles were significantly ($p<0.05$) higher (Table 3).

Table 3 Alteration in the organ weights of mice orally administered with different doses of *Commiphora wightii* hydroalcoholic extract for 90 days

***Commiphora wightii* extract**	***Weight (g)* ±Standard error of the mean**							
(mg/kg b. wt.)	***Heart***	***Lung***	***Liver***	***Kidney***	***Spleen***	***Testis***	***Seminal Vesicles***	***Cauda Epididymis***
0	0.50±0.02	0.80±0.07	6.01±0.13	1.59±0.04	0.51±0.07	0.65±0.02	0.56±0.02	0.24±0.01
100	0.52±0.03	0.85±0.09	6.55±0.22	1.61±0.06	0.66±0.09	0.68±0.01	0.83±0.04[a]	0.30±0.01
200	0.56±0.04	0.82±0.08	7.05±0.18	1.81±0.07	0.67±0.08	0.67±0.02	0.80±0.05[a]	0.29±0.02
300	0.55±0.03	0.86±0.09	6.56±0.22	1.70±0.05	0.66±0.07	0.69±0.02	0.76±0.03[a]	0.31±0.03

[a]$p<0.001$ when treatment groups are compared to control.

0 mg/kg body weight (b. wt.) corresponds to sterile physiological saline treated control group.

N=10 for each group.

Sperm dysfunction

The males receiving various doses of CWE once daily for 90 days did not reveal any alteration in the sperm motility, viability, and morphology when compared with the SPS group (Table 4).

Table 4 Changes in the Sperm viability and motility in mice administered with different doses of *Commiphora wightii* hydroalcoholic extract for 90 days

Commiphora wightii extract (mg/kg b. wt.)	Mean (%)±Standard error of the mean	
	Sperm viability	Sperm motility
0	75.0 ± 0.42	52.1 ± 0.38
100	75.2 ± 0.40	48.7 ± 0.36
200	76.5 ± 0.41	49.3 ± 0.39
300	78.1 ±0.43	50.05 ± 0.49

Note the non-significant changes.

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

N=10 for each group.

Sperm comet assay

The results of sperm DNA damage by alkaline comet assay in the sub-chronically CWE-treated mice are shown in Table 5. The single cell gel electrophoresis revealed that most of the DNA was confined into the sperm nucleus (head of the comet) in the animals receiving various doses of CWE once daily for 90 days, indicating that CWE treatment as such did not induce DNA damage as the OTM values were essentially similar to that of SPS group (Table 5).

Table 5 Alteration in the DNA damage in the sperms of mice administered with different doses of *Commiphora wightii* hydroalcoholic extract for 90 days by comet assay

Commiphora wightii extract (mg/kg b. wt.)	Mean (%) ± Standard error of the mean		
	Head DNA	Tail DNA	Olive Tail Moment
0	58.32 ± 3.40	5.90 ± 0.42	4.70 ± 0.38
100	67.54 ± 4.31	6.39 ± 0.44	5.67 ± 0.39
200	66.67 ± 4.38	6.02 ± 0.41	5.69 ± 0.39
300	64.01 ± 3.33	5.87 ±0.40	5.05 ± 0.40

Note the non-significant changes.

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

N=10 for each group.

Blood analysis

The administration of mice once daily with various doses of CWE for 90 days significantly increased the number of RBCs, blood platelets, and hemoglobin, but did not induce hemolysis when compared to the SPS-treated control group. Despite this increase in RBCs, blood platelets, and hemoglobin, the values were within the normal range (Table 6).

Table 6 Alteration in the hematological profile of mice administered with different doses of *Commiphora wightii* hydroalcoholic extract for 90 days

Commiphora wightii extract (mg/kg b. wt.)	Mean±Standard error of the mean				
	WBC (×103)	RBC (×106)	Hemoglobin (%)	Hematocrit (vol. %)	Platelets (x104 /µl)
0	5.5 ± 0.7	7.9 ± 0.2	12.2 ± 0.2	31.08 ± 0.35	62.38 ± 5.12
100	5.7 ± 0.9	8.8 ± 0.2	13.9 ± 0.2[b]	31.18 ± 0.89	71.43 ± 4.41
200	5.8 ± 0.7	9.2 ± 0.3[a]	14.4 ± 0.2[b]	30.98 ± 0.36	69.14 ± 4.39
300	5.9 ± 0.8	9.6 ± 0.3[a]	14.6 ± 0.3[b]	29.89 ± 0.52	89.43 ± 5.37b

[a]$p < 0.01$; [b]$p<0.001$ when treatment groups are compared to control.

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

N=10 for each group.

Splenocytes micronuclei

The results of the micronucleus assay in the splenocytes of mice administered with CWE once daily for 90 days are summarized in Table 7. The administration of 100, 200, or 300 mg/kg body weight CWE for 90 days led to a dose-dependent rise in the frequency of micronucleated binucleate cells (MNBNC); however, this rise in MNBNC was statistically nonsignificant in comparison to non-drug-treated controls (Table 7).

Table 7 Alteration in micronuclei formation in the cultured splenocytes of mice administered with different doses of *Commiphora wightii* hydroalcoholic extract daily for 90 days

Commiphora wightii extract (mg/kg b. wt.)	Micronuclei frequency (Mean ± Standard error of the mean)			
	One MN	Two MN	Multiple MN	Total MN
0	20.3 ± 1.23	0.99 ± 0.23	0.33 ± 0.16	21.62 ± 1.65
100	23.65 ± 1.45	1.56 ± 0.43	0.66 ± 0.25	25.87 ± 1.98
200	24.97 ± 1.76	2.65 ± 0.57	0.99 ± 0.32	28.61 ± 2.11
300	25.78 ± 1.98	2.98 ± 0.62	0.99 ± 0.35	29.75 ± 2.34

Note the non-significant changes.

0 mg/kg body weight (b. wt.) corresponds to sterile physiological saline treated control group.

N=10 for each group.

Citation: Jagetia GC. Safety profiling of hydroalcoholic extract of shuddha guggul (*commiphora wightii*) engler in swiss albino mice. *Int J Complement Alt Med.* 2026;19(1):73–81. DOI: 10.15406/ijcam.2026.19.00764

Biochemical estimation

The estimation of various biochemical parameters at 30, 60, or 90 days after oral administration of 100-300 mg/kg CWE once daily for consecutive 90 days did not significantly alter AST, ALT, and CK-MB activities. Similarly, serum creatinine, urea, and glucose levels also remained unchanged compared to the SPS group. In contrast, sub-chronic CWE treatment alleviated the cholesterol level non-significantly when compared to the SPS group, indicating that the sub-chronic administration of CWE was safe for the mouse heart, liver, and kidneys (Table 8).

Table 8 the biochemical changes in the serum of mice receiving various dosed of *Commiphora wightii* hydroalcoholic extract daily for 90 days

Commiphora wightii extract (mg/kg b.wt.)	Assay Time	Mean± Standard error of the mean					
		Glucose (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)	CK-MB(U/L)
0	Day 0	87.8 ± 5.0	60.1 ± 5.1	51.2 ± 3.2	139.2 ± 7.0	46.8 ± 6.0	127.55±16.24
	Day 30	87.5 ± 5.4	63.2 ± 3.2	52.2 ± 1.2	139.6 ± 5.4	50.1 ± 8.2	127.88±12.45
	Day 60	87.9 ± 3.1	61.0 ± 3.1	53.4 ± 1.6	139.1 ± 5.7	49.2 ± 7.7	128.02±16.34
	Day 90	90.8 ± 4.4	58.8 ± 4.3	53.7 ± 1.8	140.0 ± 7.3	44.7 ± 4.3	129.45±17.08
100	Day 0	90.7 ± 2.4	55.8 ± 3.8	53.4 ± 2.1	135.7 ± 3.9	52.0 ± 4.1	125.55±15.24
	Day 30	73.9 ± 2.8[a]	56.9 ± 3.7	52.0 ± 2.3	143.3 ± 8.7	50.7 ± 3.7	125.48±16.45
	Day 60	77.2 ± 3.3[a]	55.9 ± 4.4	50.6 ± 1.5	142.1 ± 8.9	45.1 ± 6.1	126.02±15.34
	Day 90	77.7 ± 6.2	63.2 ± 2.7	56.4 ± 2.4	139.4 ± 6.1	48.8 ± 3.5	128.45±17.08
200	Day 0	85.3 ± 3.	60.4 ± 3.8	50.1 ± 2.9	136.3 ± 3.9	50.2 ± 3.1	124.05±14.64
	Day 30	78.8 ± 6.5	53.0 ± 6.7	55.3 ± 1.7	134.2 ± 2.7	50.8 ± 2.8	123.40±15.45
	Day 60	77.8 ± 2.9	51.2 ± 4.7	53.0 ± 1.6	133.3 ± 4.3	46.5 ± 4.00	125.02±15.30
	Day 90	80.2 ± 3.7	62.2 ± 2.8	53.6 ± 1.6	138.1 ± 6.6	49.0 ± 2.8	127.45±17.08
300	Day 0	90.2 ± 3.5	62.4 ± 4.3	52.4 ± 1.6	140.6 ± 7.0	49.1 ± 3.8	124.76±18.85
	Day 30	71.6 ± 3.6[a]	50.3 ± 7.0	53.3 ± 1.6	139.9 ± 7.0	51.2 ± 2.6	122.44±14.45
	Day 60	78.5± 6.4	52.1± 6.5	50.3 ± 2.0	135.3 ± 5.7	48.7 ± 3.0	124.02±17.30
	Day 90	80.5 ± 3.9	61.9 ± 2.8	52.6 ± 1.3	131.4 ± 6.3	44.8 ± 4.1	124.45±18.08

[a] =p<0.05: Significance between each 100, 200, and 300 mg/kg *Commiphora wightii* extract and the control group;

0 mg/kg body weight (b. wt.) corresponds to the sterile physiological saline-treated control group.

N=10 for each group.

Discussion

The use of alternative and complementary medicine has risen tremendously in many developed countries in recent times.[34,35] Despite their widespread use, scientific evaluation of most of these medicines is lacking, and these medicines, though effective, are unable to pass through the stringent modern scientific tests applied to a single molecule. This is because of the complex nature of herbal medicines and natural products, which do not contain a single molecule but consist of several complex biomolecules.[3,36] The presence of a large number of chemical entities in herbs and natural products could be advantageous as they will employ multiple molecular pathways to eradicate the disease and also the presence of several molecules may make them less toxic or the toxic dose may be very high than the effective dose when compared to a single molecule-based drug/s.[1,2,37,38] In several cases of chronic diseases, where the modern medicinal system either failed or does not provide hope of cure, the traditional herbal medicines have been highly successful, which has reposed faith in the use of traditional herbal medicines, and more and more people are inclined to use them. The major constraints about herbal drugs have been their quality control and the absence of scientific data on their safety and toxicity profiles.[34,39,40] The scientific investigation of the safety and toxicity of herbal medicines is of paramount importance for their medical application. This will also substantiate the claim that herbal medicines are nontoxic, or the toxic doses are much higher than their effective doses. This has led us to investigate the acute and sub-chronic toxicity of guggul (*Commiphora wightii*), a natural product used in Ayurveda for the treatment of various ailments singly or in combination.

The results of the present study demonstrate that CWE did not exert any toxic effect in mice receiving 100, 200, and 300 mg/kg for 90 days or a single dose of 5000 mg/kg. Therefore, 5000 mg/kg CWE was considered as safe and a NOAEL dose.[41] Reports regarding the acute and sub-chronic toxic effects of *Commiphora wightii* in mice are scanty. However, *C. myrrha* resin orally administered at a dose of 1–5g/kg/d was found to be toxic, and the safe dose was found to be 0.25 g/kg/d.[42] The ethyl acetate extract of *Commiphora molmol* did not exert any toxic effect up to a dose of 3 g/kg in acute toxicity studies, whereas 100 mg/kg orally was non-toxic in sub-chronic toxicity studies.[43] The purified guggul did not trigger any toxic effect in mice administered with 1 to 3 g/kg body as a single dose and subacute toxicity at a dose of 300 mg/kg administered for consecutive 14 days.[44] Since the CWE has been previously shown to be pharmacologically active (dyslipidemic properties) when given by the oral route to mice at the minimum active dose of 3.6 mg/kg,[45] one may conclude that the active compound(s) present in the CWE exhibit a rather very low acute oral toxicity. The weights of vital organs did not change after administration of CWE for 90 days, and the condition of the viscera was normal and comparable to the control. The weights of testes and *cauda epididymides* were within the normal range; however, those of seminal vesicles were significantly higher (P<0.001) in the CWE

group than those of the SPS control group, which indicates the safety of CWE.

The hematopoietic system represents one of the most subtle parameters to measure the toxicity of drugs in humans and animals.[46] The present study revealed a significant rise in hemoglobin, RBC, and platelet counts except for total leukocytes that remained unchanged in 100-300 mg/kg CWE-treated animals when compared to SPS groups. Despite this increase, RBCs were within ($7X10^6$ to $11X10^6$) normal range.[47,48] The plant steroids may influence the secretion of androgen and erythropoietin, resulting in increased erythropoiesis.[49]

The DNA damage study by micronucleus assay did not reveal any alteration in the frequency of micronuclei in the splenocytes after sub-chronic administration of CWE. The amount of DNA damage impairs the expression of many genes, leading to cell death.[50] The evaluation of the adverse effect on sperm is of utmost importance as it affects the next generation or may cause male infertility.[51,52] The sperm dysfunction test showed no discernible alteration in the motility and viability of sperm after daily administration of guggul extract for 90 days. The sperm dysfunction and viability assays give precise information about toxic effects on male germ cells.[25,53] The drug-induced DNA damage detection in the sperms may serve as a good marker of fertility problems, and any sperm/s having very low DNA damage causes heritable changes in the next generation. The sperm DNA fragmentation and dysfunction can be easily detected by the alkaline comet assay.[54] The comet assay measures DNA damage in the cells subjected to an electric field; the increased DNA damage gives long comet tails.[29,55] The safety of 100-300 mg/kg CWE or 90 days on germ cells was indicated by sperm viability, motility, and the comet assay, which remained unaffected.[56]

The drug detoxification and biotransformation are carried out by the liver, and the liver toxicity can be studied by estimating AST and ALT levels. The higher amounts of these enzymes serve as a biomarker of toxic effects on the liver.[57] The raised level of ALT is the first indication of hepatic damage, and the rise in ALT is more specific to liver ailments. The AST is associated with diseases related to other organs like the heart, kidney, and muscles.[58,59] Treatment of mice with 100, 200, and 300 mg/kg CWE daily for consecutive 90 days did not change the serum levels of AST and ALT in mice when compared with the SPS group, indicating its safety up to a dose of 300 mg/kg. Likewise, an earlier study in mice has reported an insignificant decline in the AST and ALT levels in mice administered with 300 mg/kg purified guggul for 14 days.[44] Serum creatinine and urea levels are the important markers to assess nephrotoxicity, and any rise in creatinine levels is the only indication of functional damage to nephrons.[60,61] In the present study, serum creatinine and urea levels did not elevate significantly in mice receiving 100-300 mg/kg CWE, indicating its safety for the kidneys. Mice treated with 300 mg/kg purified guggul treatment for 14 days showed a non-significant decline in the serum creatinine and bilirubin levels.[44] Likewise, *C. molmol* also did not exert any kind of hepatotoxicity and nephrotoxicity in an earlier study.[43]

The treatment of mice with 100-300 mg/kg CWE reduced the cholesterol levels. Indeed, the antecedent cholesterol levels increase the risk of coronary artery disease.[62,63] The guggul has been reported to reduce total cholesterol levels in human serum significantly.[13] A marginal but non-significant reduction in the cholesterol level in mice receiving sub-chronic administration of 100, 200, and 300 mg/kg guggul may be due to the antagonistic action of guggul against farnesoid-X receptor, which plays a seminal role in the cholesterol and bile acid homeostasis.[64]

Conclusion

The present study reveals that a single oral administration of 80% CWE up to 5000 mg/kg body weight was non-toxic. The daily administration of 100-300 mg/kg body weight CWE for 90 days did not show any adverse signs of toxicity in the liver, kidney, heart, spleen and sperms as there was no significant alteration in the AST, ALT, creatinine, urea and DNA damage in cultured splenocytes (micronucleus assay) and sperms as assayed by and alkaline comet assay. The daily administration of 100-300 mg/kg CWE also did not adversely affect the hematological profile, indicating its safety. Our study demonstrates the safety of guggul administration in mice.

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Statement of conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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