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Safety Evaluation of Vyosadi Guggulu, an Ayurvedic Formulation through Acute and Subchronic Toxicity Studies in Wistar Rats

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Abstract

The objective of this study is to assess the safety profiles of Ayurvedic formulation Vyosadi guggulu (VG) in Wistar rats. Acute, subacute and subchronic toxicity studies were conducted in accordance with the guidelines of Organization for Economic Cooperation and Development (OECD) and the Committee for Control and Supervision of Experiments on Animals (CCSEA). Approvals from the Institutional Animal Ethics Committee (IAEC), Central Ayurveda Research Institute, Kolkata were obtained prior to carry out the animal experimental studies. Standardized trial drug VG supplied by Indian Medicine Pharmaceutical Corporation Limited (IMPCL), Uttarakhand. A single dose of VG at 2000mg/kg body weight was given to Wistar rats through oral gavage in acute toxicity study. Subchronic oral toxicity study was conducted out at three doses 250, 500 and 1000 mg/kg body weight and administered orally daily for 90 successive days. The doses were derived from a 28 days repeated dose oral toxicity study. Animals in the acute toxicity study were monitored for gross pathological examination, weekly feed and intake, mortality, weekly body weight changes, and general clinical symptoms. Animals were examined for mortality, weekly changes in body weight, general clinical signs, weekly feed and water intake, clinical biochemical investigations, hematological parameter analysis, examination for gross pathological changes, organ weight measurement, and histopathological investigations in the 90-day toxicity study. In acute toxicity study, the single dose of 2000mg/kg of VG was found to be safe. In 28 days and 90 days repeated dose oral toxicity studies all tested doses of VG were proven to be safe. In both studies, there was no significant adverse effect in food and water consumptions, hematological and clinical biochemistry parameters at any dose. No treatment related abnormal features were noticed

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during histopathological evaluation in 90 days oral toxicity study. The no observed adverse effect level from subchronic toxicity was found to be 1000mg/kg in Wistar rats.

Major findings: The present study showed that Vyosadi guggulu is safe at single oral dose of 2000mg/kg in Wistar rats. In the subchronic toxicity studies all tested doses of VG were proven to be safe and the No Observed Adverse Effect Level was found to be 1000mg/kg body weight in Wistar rats.

Keywords: Acute Toxicity, Ayurvedic Formulation, Subchronic Toxicity, Vyosadi guggulu, Wistar Rats

1. Introduction

Guggulu is the gum resin obtained from the plant *Commiphora wightii* (Arn.) Bhandari from family *Burseraceae*. Formulations containing Guggulu has been used to cure arthritis, inflammation, obesity, bone fracture, gout, rheumatism, dyslipidaemia etc. from long time back[1]. Vyosadi Guggulu (VG) is an Ayurvedic formulation containing one part each of Shunthi, Marica, Pippali, Agni (Citraka), Musta, Haritaki, Bibhitaki, Amalaki, Vidanga and nine parts of Guggulu-suddha. The use of Vyosadi Guggulu for the treatment of hyperlipidemia (Medoroga) is cited in the Ayurvedic classical text[2].

The primary ingredients of Ayurvedic formulations are herbs. Herbs possess the potential to be a valuable source of medicine, and a wealth of studies has been done on the effects of different herbs on a range of illnesses, including cancer, diabetes, arthritis, and dyslipidemia. Shunthi (*Zingiber officinale* Rosc.), one of the key ingredients of VG shown to have hypoglycaemic, hypocholesterolaemic and hypolipidaemic activities[3]. Marica (*Piper nigrum* L.) was found to reduce the risk of atherosclerosis markedly by virtue of its hypolipidemic and antiatherogenic effects[4]. Pippali (*Piper longum* L.) showed significant antidiabetic and antihyperlipidemic activity *in vivo*[5]. Citraka (*Plumbago zeylanica* L.) has antimicrobial, anti-inflammatory, antidiabetic, antioxidant, hepa-toprotective and antihyperlipidemic activities[6,7]. Musta (*Cyperus rotundus* L.) has different pharmacological activities such as hypolipidemic, analgesic, antipyretic, anticonvulsant, antidiarrhoeal properties[8]. Vidanga (*Embelia ribes* Burm. F.) exhibits pharmacological properties such as antibacterial, antioxidant, anticancer, wound healing, antihyperglycemic, anti-inflammatory, antidepressant, antiobesity and antihyperlipidemic activities[9].

The need to perform preclinical toxicity studies of Ayurvedic formulations in experimental animals has increased due to safety concerns. A clear understanding about safety or toxicity profile of Vyosadi Guggulu is not established yet though various studies reported the safety profiles of its individual ingredients, therefore, the present study was carried out with the objective to establish the target organ toxicity and the No Observed Adverse Effect Level (NOAEL) to evaluate the formulation's safety and toxicity.

2. Materials and Methods

2.1 Trial Drug

The trial drug VG (Batch No. AYU-05, Mfg. Lic No. A and U 1012/83, Mfg. Date: July, 2020, Exp. Date June, 2025) was supplied by Indian Medicine Pharmaceutical Corporation Limited (IMPCL), Mohan, Almora, Uttarakhand and certificate of analysis of VG was obtained from IMPCL.

2.2 Animal Welfare

The Institutional Animal Ethics Committee (IAEC), Central Ayurveda Research Institute, Kolkata granted permission to undertake the experimental works with the approval numbers 29/P/S/CARIDD/IAEC/2018 and 38/P/S/CARIDD/IAEC/2018, dated 25.08.2020.

2.3 Experimental Animals

Twenty-eight days and subchronic toxicity studies were carried out on both male and female Wistar rats. While only female Wistar rats were used in acute toxicity study. Adult disease free Wistar rats of 150g to 200g weight kept and maintained at the Animal House Facility of Central Ayurveda Research Institute, Kolkata, were used for the oral toxicity studies. The animal studies were conducted out in accordance of the guidelines of CCSEA. Relative humidity and temperature were kept at 50-60 % and 23±2 ^{0}C, respectively and the lightings were adjusted to provide 12 hours of light and 12 hours of darkness. Before starting the experiment, the animals were acclimated to the environment of the animal house. The randomization process was carried out to ensure that difference of body weight is ±20% of the mean and that means of groups are identical. *Ad libitum* quantity of standard rodent pelleted

diet and purified water was provided to the animals during the study.

2.4 Animal Experimental Study

2.4.1 Acute Toxicity Study

Acute toxicity study was conducted in accordance with Organization for Economic Cooperation and Development guideline no. 423[10]. Three female Wistar rats in the treated group received VG at a dose of 2000mg/kg body weight through oral gavage while, purified water (20ml/kg body weight) was given to the animals of vehicle control group. Following treatments, the experimental rats were monitored at the intervals of 5m, 30m, 1h, 2h, 4h, 6h, 24h and thereafter daily upto 14 days. Changes in the skin, fur, eyes, mucus membrane and behavior patterns of the animals were noted. Somatomotor activity, circulatory system, respiratory system, autonomic and central nervous systems were measured by cage side observations. Respiratory pattern, respiratory rate, convulsions, tremors, salivation, lacrimation, faecal excretions and salivations of the animals were all monitored closely. All the animals were observed twice daily for systematically recording morbidity and mortality with individual records being maintained for each animal. Body weight, water and feed consumptions were recorded on weekly intervals, the animals were euthanized for gross pathological examinations of the internal organs at the termination of the study.

2.4.2 Subchronic Toxicity Study

Subchronic toxicity study was conducted in accordance with OECD guideline no. 408[11]. Doses for the ninety days subchronic toxicity study were assessed from a 28 days repeated dose oral toxicity study in Wistar rats at the three dose levels of 1000mg/kg, 500mg/kg, 100mg/kg body weight carried out as per OECD guideline no. 407[12]. The No Observed Adverse Effect Level (NOAEL) of VG was established as 1000mg/kg body weight in Wistar rats in the 28 days repeated dose oral toxicity study. Henceforth, three dose levels of 1000mg/kg, 500mg/kg and 250mg/kg body weight were selected for the subchronic toxicity study. Adult Wistar rats of both sexes were chosen for ninety days subchronic toxicity study. The animals were assigned to 6 groups namely control, high dose (1000mg/kg), mid dose (500mg/kg), low dose (250mg/kg), satellite control and satellite treated (1000mg/kg) group, after being chosen based on their body weights. VG was orally administered daily for 90 consecutive days. Only purified water (20ml/kg body weight) was administered to the animals of both control and satellite control groups. Throughout the study period the animals were monitored daily for general clinical observations. Measurements of body weight, water and feed consumptions were taken throughout the study at weekly interval. Clinical biochemistry and haematological analysis were conducted on day 30th, 60th, 90th day of the experiment, and on day 30th, 60th, 90th and 118th for satellite groups. Except for animals of satellite groups, which were sacrificed on day 119th, all animals were sacrificed on 91st day and an extensive gross necropsy was performed. Before drying out, the weights of each animal's major organs were noted as soon as they were dissected, and they were then stored in 10% neutral buffer formalin for histopathology. Only the preserved organs and tissues from the animals in the control and high dose groups were subjected to histopathology.

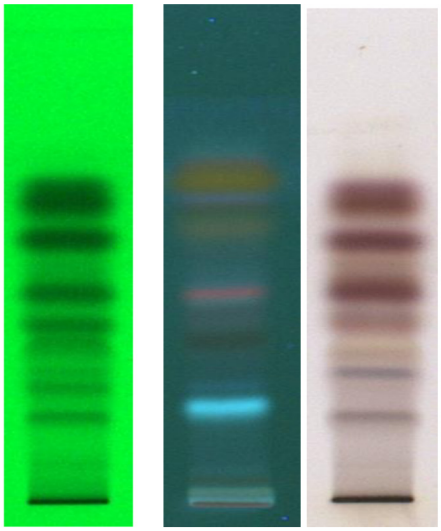
2.5 Statistical Analysis

One-way ANOVA taking dose group, as a factor was used to analyze normally distributed data, whereas, not normally distributed data was analyzed by Kruskal-Wallis H test. Dunetts (2-sided)/Mann-Whitney U test, wherever appropriate was used to compare the dose group over the control arm. Both satellite groups were subjected to student's *'t'* test. All the values were expressed as mean ± standard deviation (Mean±SD) and the statistical analysis were assessed at $p<0.05$.

3. Results

3.1 Trial Drug

Loss on drying, total ash value, acid insoluble ash value, water soluble extractive value and alcohol soluble extractive value of VG were found as 9.82, 10.28, 1.81, 24.15 and 22.70 %, respectively. The trial drug has below detection limits of heavy metals (lead, mercury, arsenic, cadmium), pesticide residues and mycotoxins (aflatoxin B1, B2, G1, G2). VG was also found to be free from harmful microorganisms *viz. Staphylococcus aureus, Salmonella*, *Escherichia coli* and *Pseudomonas aeruginosa*. High Performance Thin Layer Chromatography (HPTLC) profile of the trial drug VG showed R_f values: At 254nm: 0.09, 0.18, 0.27, 0.34, 0.43, 0.48, 0.54, 0.58, 0.63 and 0.67, at 366nm: 0.06, 0.18, 0.26, 0.32, 0.34, 0.41, 0.47, 0.49, 0.52, 0.56, 0.62 and 0.67 and at white light after derivatisation: 0.15, 0.19, 0.24, 0.31, 0.39, 0.46, 0.51, 0.58, 0.63 (Figure 1).



(254nm) (366 nm)(White light)

Figure 1. High Performance Thin Layer Chromatography (HPTLC) profile *Vyoshadi guggulu*.

R_f values:

At 254 nm: 0.09, 0.18, 0.27, 0.34, 0.43, 0.48, 0.54, 0.58, 0.63 and 0.67

At 366 nm: 0.06, 0.18, 0.26, 0.32, 0.34, 0.41, 0.47, 0.49, 0.52, 0.56, 0.62 and 0.67

At white light after derivatisation: 0.15, 0.19, 0.24, 0.31, 0.39, 0.46, 0.51, 0.58, 0.63.

3.2 Acute Toxicity Study

No animal had treatment-related toxicity or mortality during the study when given VG at a dose of 2000 mg/kg body weight. In the acute toxicity investigation, there was no significant change in weekly body weight, feed, or water intake. Furthermore, at the terminal sacrifice, none of the major inner organs namely liver, lungs, kidney, heart, spleen, stomach, and gastrointestinal system, showed gross pathological abnormality.

3.3 Subchronic Toxicity Study

No significant effect was observed on body weight, feed and water consumption, clinical signs, haematological and clinical biochemical parameters in the 28 days repeated dose oral toxicity study of VG at all three dose levels 1000mg/kg, 500mg/kg, 100mg/kg body weight in Wistar rats. In gross necropsy, no gross lesions of major internal organs were observed and there was no dose related effect on absolute and relative organ weights of the experimental animals. Therefore, the No Observed Adverse Effect Level (NOAEL) of VG was established as 1000mg/kg body weight in Wistar rats in the 28 days repeated dose

Table 1. Weekly absolute body weight (g) of Wistar rats in subchronic toxicity study

Week	Vehicle Control	High Dose (1000mg/kg)	Mid Dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between groups)
Male Rats					
Initial	188.40±11.57	188.40±9.92^{ns}	188.30±8.73^{ns}	187.70±7.93^{ns}	0.998
1st	198.0±9.60	198.30±9.27^{ns}	198.40±8.00^{ns}	197.20±7.72^{ns}	0.989
2nd	208.10±9.67	210.0±8.86^{ns}	209.10±7.62^{ns}	206.70±8.17^{ns}	0.847
3rd	218.90±7.65	220.0±8.86^{ns}	219.0±8.85^{ns}	216.60±8.26^{ns}	0.831
4th	229.30±7.90	228.80±7.96^{ns}	227.50±8.84^{ns}	226.40±7.84^{ns}	0.855
5th	240.30±6.96	239.30±8.0^{ns}	238.40±8.61^{ns}	237.60±7.79^{ns}	0.883
6th	249.70±6.23	249.80±8.48^{ns}	249.0±9.15^{ns}	247.50±7.42^{ns}	0.909
7th	259.50±7.70	260.90±8.53^{ns}	258.70±8.95^{ns}	257.0±7.07^{ns}	0.751
8th	268.40±6.68^{ns}	271.50±8.48^{ns}	269.70±8.39^{ns}	266.10±8.02^{ns}	0.491
9th	279.10±7.40	280.60±8.40^{ns}	281.10±9.55^{ns}	277.70±6.96^{ns}	0.784
10th	292.60±6.48	295.20±8.54^{ns}	292.50±11.19^{ns}	289.70±6.78^{ns}	0.556
11th	305.70±7.78	307.90±9.66^{ns}	303.20±12.18^{ns}	300.80±7.64^{ns}	0.263
12th	319.40±7.30	321.30±7.51^{ns}	315.50±12.53^{ns}	309.40±7.57*	0.014
13th	329.50±5.60	332.80±8.16^{ns}	326.40 ±12.65^{ns}	318.50 ±7.53*	0.004
Female Rats					
Initial	165.50±5.68	166.10±5.04^{ns}	166.40±5.50^{ns}	166.40±6.50^{ns}	0.983
1st	177.2±6.05	176.9±5.13^{ns}	176.9±4.86^{ns}	175.6±7.73^{ns}	0.936

Table 1. Continued...

Week	Vehicle Control	High Dose (1000mg/kg)	Mid Dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between groups)
2nd	187.9±5.84	188.9± 6.77ns	189.0±3.74ns	186.0±5.27ns	0.442
3rd	196.4±11.63	199.8± 7.19ns	200.1± 4.81ns	196.5± 7.76ns	0.719
4th	207.6± 12.16	209.4± 6.78ns	209.4± 6.73ns	206.7± 7.40ns	0.716
5th	220.6± 9.40	220.4± 7.30ns	222.3± 5.63ns	219.1± 7.76ns	0.829
6th	231.5± 7.94	230.7± 8.74ns	233.2± 7.23ns	229.6± 8.94ns	0.798
7th	241.8± 9.04	241.5± 8.74ns	245.1± 6.02ns	240.5± 8.38ns	0.618
8th	251.7±8.55	251.6±8.34ns	253.1±6.55ns	251.1± 8.66ns	0.952
9th	261.6± 8.12	262.5±7.41ns	264.8± 7.27ns	261.3± 8.83	0.782
10th	274.0± 10.31	274.3±7.88ns	277.3± 6.12ns	278.2± 9.85ns	0.626
11th	286.0± 8.39	285.77±6.90ns	290.0± 5.35ns	290.2± 10.55ns	0.234
12th	300.4±4.78	297.88±7.55ns	303.22±4.38ns	301.0±9.50ns	0.438
13th	312.3±4.54	310.0±8.80ns	314.55±5.38	310.55±8.47ns	0.472

Mean with the same superscript do not differ significantly in one way ANOVA (p>0.05).

Table 2. Weekly absolute body weight (g) of male Wistar rats of satellite groups in subchronic toxicity study

Week	Satellite Control	Satellite High Dose (1000mg/kg)	p-Value (Between groups)
Male Rats			
Initial	188.0±3.34	188.16±4.07ns	0.937
1st	198.33±2.73	198.17±3.31ns	0.926
2nd	211.17±4.91	208.67±2.94ns	0.31
3rd	221.0±4.89	218.67±1.96ns	0.171
4th	230.83±4.91	226.5±3.50ns	0.085
5th	241.17±4.16	239.83±4.70ns	0.615
6th	252.50±4.59	250.83± 4.49ns	0.539
7th	261.83±4.44	260.33±4.45ns	0.572
8th	271.33±5.78	269.83±2.56ns	0.369
9th	281.17±4.79	280.50±3.27ns	0.370
10th	290.0±4.33	294.0±5.25ns	0.181
11th	282.83±4.08	308.17±5.11ns	0.02
12th	306.50±5.24	317.83±7.08*	0.01
13th	314.67±4.08	327.0±6.35*	0.003
14th	321.33±5.27	335.83±7.35*	0.003
15th	327.33±4.32	344.50±8.19*	0.001
16th	334.17±4.30	354.5±8.19*	<0.001
17th	342.17±5.15	364.83±8.27*	<0.001

Table 2. Continued...

Week	Satellite Control	Satellite High Dose (1000mg/kg)	p-Value (Between groups)
Female Rats			
Initial	165.66±2.80	166.0±2.68ns	0.838
1st	176.50±2.07	176.17±2.92ns	0.825
2nd	187.50± 2.07	187.16± 3.81ns	0.855
3rd	199.33±1.03	197.66±3.88ns	0.351
4th	210.50± 1.22	209.5± 2.58ns	0.412
5th	222.50± 2.42	221.5± 3.08ns	0.546
6th	232.50± 2.42	231.33±3.77ns	0.539
7th	243.33± 2.87	242.0± 4.77ns	0.571
8th	255.33± 3.83	254.5± 6.09ns	0.783
9th	267.83± 2.40	266.5± 6.95ns	0.672
10th	274.50± 2.58	278.5± 8.01ns	0.289
11th	282.66± 2.33	292.16± 7.57ns	0.015
12th	289.5±1.64	304.33± 7.11*	<0.001
13th	298.0±3.03	316.67±8.52*	<0.001
14th	307.66±3.38	328.5±10.09*	0.001
15th	313.0±3.79	337.16±10.45*	<0.001
16th	318.83±2.99	346.16±10.08*	<0.001
17th	325.33±4.96	355.33±10.25*	<0.001

nsNo Significant difference in Student's 't' test compared with the control group.

Table 3. Clinical biochemistry and haematological parameters of male Wistar rats in subchronic toxicity study at 90th day

Parameters	Vehicle Control	High Dose (1000mg/kg)	Mid Dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between Groups)
Albumin (gm/dl)	2.89±0.33	3.09±0.12^{ns}	2.91±0.34^{ns}	3.11±0.18^{ns}	0.138
SGOT (U/L)	102.45±12.84	91.46±18.81^{ns}	97.60±23.01^{ns}	94.99±10.82^{ns}	0.563
SGPT (U/L)	39.60±9.24	45.18±5.43^{ns}	44.01±6.06^{ns}	39.44±7.63^{ns}	0.205
Bilirubin (mg/dl)	0.26±0.15	0.29±0.21^{ns}	0.41±0.37^{ns}	0.44±0.21^{ns}	0.304
Alkaline phosphatase (U/L)	114.23±41.75	122.27±36.26^{ns}	179.31±31.50*	166.92±31.02*	<0.001
Triglyceride (mg/dl)	43.90±11.10	54.43±12.66^{ns}	71.28±24.18*	59.66±17.86^{ns}	0.021
Creatinine (mg/dl)	0.57±0.03	0.33±0.19*	0.31±0.18*	0.51±0.28^{ns}	0.001
Urea (mg/dl)	39.86±2.96	38.09±3.27^{ns}	38.11±2.60^{ns}	40.64±3.77^{ns}	0.214
BUN (mg/dl)	18.62±1.38	17.79±1.52^{ns}	17.81±1.21^{ns}	18.99±1.76^{ns}	0.214
Glucose (mg/dl)	119.15±15.05	127.17±10.46^{ns}	127.31±17.99^{ns}	109.15±16.52^{ns}	0.057
Haemoglobin (gm/dl)	13.92± 3.80	14.42± 2.17^{ns}	15.0±0.50^{ns}	14.91±0.40^{ns}	0.692
RBC (10e6/mm^3)	7.43±1.98	7.02±1.64^{ns}	7.61±0.35^{ns}	7.76±0.36^{ns}	0.619
WBC (10e3/mm^3)	5.91±2.21	7.24±2.63^{ns}	7.99±1.46^{ns}	6.94±1.73^{ns}	0.201
PCV (%)	40.61±11.13	38.62±9.09^{ns}	42.27±1.17^{ns}	43.14±1.67^{ns}	0.817
MCV (femtolitres)	54.40± 1.51	54.96± 2.1^{ns}	55.57± 1.34^{ns}	55.62± 1.24^{ns}	0.308
Neutrophil (%)	24.0±2.98	22.70±4.19^{ns}	23.70±3.16^{ns}	25.0±3.26^{ns}	0.635
Lymphocyte (%)	67.70±3.94	68.90±3.38^{ns}	66.50±3.13^{ns}	65.70±3.19^{ns}	0.192
Monocyte (%)	5.0±1.82	5.0±1.69^{ns}	6.70±0.94*	6.10±1.52^{ns}	0.042
Eosinophil (%)	3.30±1.15	3.40±1.26^{ns}	3.10±0.87^{ns}	3.20±0.91^{ns}	0.865
Basophil (%)	0	0	0	0	-

Values are expressed as Mean ± SD, N=10 per group, p>0.05, *P<0.05 significant difference compared to control group according to one way ANOVA in Dunnet's Post Hoc test.

oral toxicity study. In ninety days repeated dose toxicity study, the animals treated with VG at the oral dose levels of 1000mg/kg, 500mg/kg and 250mg/kg body weight for consecutive 90 days showed no marked negative effect on clinical signs, feed consumption and water consumption. No dose dependent significant changes in body weights of the treated animals were observed except significant decrease in body weight of male animals at 12th and 13th week at 250 mg/kg dose. Significant increase in body weights of both male and female rats of satellite high dose group (1000mg/kg) were observed from 11th to 17th weeks as compared to satellite control group. Significant increase in serum alkaline phosphatase is observed in male animals from low dose and mid dose group as compared to vehicle control group. Significant decrease in serum creatinine of male rats from mid dose and high dose groups and female animals from mid dose and low dose groups was observed as compared to vehicle control group. Blood glucose of female animals from low dose group was significantly decreased as compared to vehicle control. Other than these, the rats in all the study groups did not exhibit any significant changes in hematological and clinical biochemical parameters throughout the study period (Table 1-4). On terminal sacrifice, none of the experimental groups showed any significant gross pathological alterations. Neither the high dose group nor the vehicle control group showed any treatment-related abnormalities in histopathology of the main internal organs (Figure 2).

4. Discussions

The primary ingredients of the VG, Guggulu (*Commiphora wightii* (Arn.) Bhandari) is reported to have health benefit against arthritis, obesity, gout, paraplegia *etc*. Guggulsterone, an active phytoconstituent present in Guggulu is responsible for its therapeutic effect[1]. Other active ingredients of VG namely *Zingiber officinale* Rosc,

Table 4. Clinical biochemistry and haematological parameters of female Wistar rats in subchronic toxicity study

Parameters	Vehicle Control	High Dose (1000mg/kg)	Mid dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between Groups)
Albumin (gm/dl)	3.36± 0.22	3.37± 0.32^{ns}	3.11± 0.15^{ns}	3.18±0 .33^{ns}	0.108
SGOT (U/L)	109.37±26.09	101.87±17.95^{ns}	101.02±13.14^{ns}	107.07±10.61^{ns}	0.711
SGPT (U/L)	32.68± 13.90	40.10± 10.58^{ns}	36.78± 7.96^{ns}	45.98± 19.38^{na}	0.210
Bilirubin (mg/dl)	0.24±0.25	0.20±0.14^{ns}	0.19±0.21^{ns}	0.36±0.18^{ns}	0.275
Alkaline phosphatase (U/L)	114.47±43.74	97.3567±22.10^{ns}	128.50±46.09^{ns}	102.23±21.60^{ns}	0.267
Triglyceride (mg/dl)	38.29±18.08	37.07±3.44^{ns}	39.44±3.31^{ns}	23.90±13.24^{ns}	0.028
Creatinine (mg/dl)	0.44±0.07	0.47±0.17^{ns}	0.22±0.11*	0.60±0.12*	<0.001
Urea (mg/dl)	40.70±5.24	37.07±3.44^{ns}	39.44±3.31^{ns}	38.88±4.80^{ns}	0.344
BUN (mg/dl)	19.02±2.44	15.59±5.68^{ns}	18.43±1.54^{ns}	16.35±6.12^{ns}	0.284
Glucose (mg/dl)	128.50±20.57	138.82±12.35^{ns}	112.65±12.80^{ns}	109.69±17.57*	0.002
Haemoglobin (gm/dl)	14.05±1.27	13.66±1.67^{ns}	14.54±0.68^{ns}	14.48±0.42^{ns}	0.390
RBC (10e6/mm^3)	7.07±0.69	6.65±0.94^{ns}	7.30±0.46^{ns}	7.39±0.23^{ns}	0.093
WBC (10e3/mm^3)	5.49±1.10	3.81±0.79*	4.66±1.0^{ns}	5.74±1.49^{ns}	0.003
PCV (%)	41.76±3.91	39.31±5.68^{ns}	42.62±2.35^{ns}	43.67±1.27^{ns}	0.054
MCV (femtolitres)	55.19±12.30	59.05±1.06^{ns}	58.43±2.23^{ns}	59.10±1.63^{ns}	0.979
Platelet Count (10^3/ µL)	697.8±139.01	727.11±84.26^{ns}	730.66±69.71^{ns}	714.22±124.90^{ns}	0.924
Neutrophil (%)	22.30±3.52	25.33±3.04^{ns}	24.11±3.14^{ns}	22.55±2.55^{ns}	0.142
Lymphocyte (%)	67.30±4.37^{ns}	64.11±2.75^{ns}	66.66±3.80^{ns}	66.22±3.45^{ns}	0.412
Monocyte (%)	6.80±0.91	7.22±1.09^{ns}	5.77±1.64^{ns}	7.1±1.05^{ns}	0.061
Eosinophil (%)	3.60±0.96	3.33±1.22^{ns}	3.44±1.58^{ns}	4.11±0.78^{ns}	0.518
Basophil (%)	0	0	0	0	-

Values are expressed as Mean ± SD, N=10 per group, p>0.05, *P<0.05 significant difference compared to control group according to one way ANOVA in Dunnet's Post Hoc test.

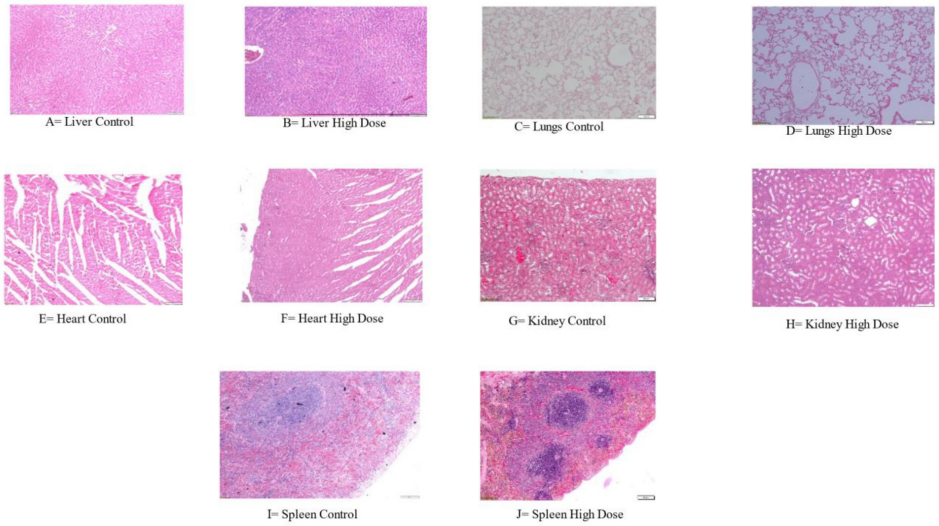


Figure 2. Histology (H and E, 10x X 10x) of liver **(A, B)**, lungs **(C, D)**, heart **(E, F)**, kidney **(G, H)**, spleen **(I, J)** of control group and high dose group. Lungs, liver, heart, kidney and spleen tissue sections from control and high dose groups showed normal architecture and cells.

Piper nigrum L., *Piper longum* L., *Plumbago zeylanica* L. *Cyperus rotundus* L., *Embelia ribes* Burm. F., *Terminalia chebula* Rezt. and *Emblica officinalis* have various pharmacological activities due to presence of different types of bio-active phyto-constituents as evident from many published scientific literatures. Bio-active compounds such as gingerols, shogaols, paradol and zingerone present in *Zingiber officinale* possesses potent antioxidant and anti-inflammatory properties. The antioxidant mechanism of *Zingiber officinale* is linked to activation of Nrf 2 signaling pathway[13]. Phytochemical piperine in *Piper nigrum* and *Piper longum* shown to inhibit the expression of inflammatory mediators like tumor necrosis factor-α, inducible NO synthase, and LPS induced COX-2 in macrophages and suppresses release of IL-1β in LPS-primed bone marrow-derived macrophages[14]. Pyrogallol, an active compound present in *Emblica officinalis* shown to inhibit proliferation of human lung cancer cells by inducing G2/M phase cell cycle arrest and apoptosis[15]. Purified guggulu showed no adverse effect upto an oral dose of 3g/kg body weight and at 300mg/kg/day in acute toxicity study and 14 days sub-acute toxicity study, respectively, in mice[16]. In a clinical trial, VG was found to be safe at the dose of 1.5g twice daily for 12 weeks when used in combination with Haritaki Churna in obesity patients[17]. Gokshuradi guggulu was found to be safe upto 2700mg/kg body weight in acute and sub-acute oral toxicity study in rats[18]. Earlier studies reported that Shunthi (*Zingiber officinale* Rosc.), one of the active ingredients of VG, is safe at the dose of 2000mg/kg body weight in Wistar rat[19]. Pippali (*Piper longum* L.), another ingredient of VG, showed no mortality and behavioural changes when administrated at the maximum dose up 3750mg/kg body weight to Wistar rats[20]. Haritaki (*Terminalia chebula* Rezt.) shown to be safe orally in both acute (5000mg/kg body weight) and chronic toxicity (300, 600 and 1200 mg/kg body weight) studies in rats[21]. Hydro-alcoholic extract of Amlaki (*Emblica officinalis*) administered at the oral doses of 250, 500 and 1000 mg/kg body weight upto 3 weeks did not show any untoward effect in rats[22]. However, no safety or toxicity studies of VG has been reported in animal models. In the current study, Wistar rats were given VG orally at a single dose of 2000mg/kg body weight; no mortality or toxicity-related signs or symptoms were noted. Trayodashang guggulu, an Ayurvedic formulation containing guggulu showed similar results in acute oral toxicity study in rats[23].

When VG was administered orally to the animals for a consecutive 90 days, in the current study, at the three different doses, no drug-related significant adverse effect was observed on general behavioral, clinical symptoms, feed and water intake, body weight, haematological, or clinical biochemical parameters. The variations in the body weight and some blood parameters at only one specific time point observed in these male and female rats in the study is not be considered test drug related effect, but rather a result of chance on biological variation. Whatever changes observed in the parameters of the study are within the normal ranges[24,25]. On terminal sacrifice, none of the experimental groups showed any significant gross pathological alterations. Histopathology of the major internal organs did not show treatment-related abnormalities in the high dose and vehicle control groups. The present study demonstrated that VG is safe at a single oral dose of 2000mg/kg in Wistar rats. From the ninety days repeated dose oral toxicity study the No Observed Adverse Effect Level (NOAEL) is established as 1000mg/kg in Wistar rats.

5. Conclusion

The present study delivers important insights about the oral toxicity profiles of Vyosadi Guggulu which could be very useful in formulating dosage recommendations for clinical trials as Vyosadi Guggulu appears to be safe as demonstrated from its acute and consecutive 90 days of oral administrations. However, understanding the exact mechanisms behind Vyosadi Guggulu's safety and efficacy will require further research on its pharmacokinetics and pharmacodynamics behaviours. Applications of *in vitro* models and *in silico* tools such as molecular docking, molecular dynamic simulations, systems biology approaches etc can be valuable tools for determining the mechanism of safety and efficacy of Vyosadi Guggulu.

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