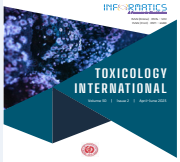
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Toxicity Studies of *Trayodashang guggulu*, A Classical Ayurvedic Formulation in Experimental Animals

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Abstract

The present study was undertaken to evaluate the Maximum Tolerated Dose and No Observed Adverse Effect Level of *Trayodashang guggulu* (TG), an Ayurvedic classical formulation, in Wistar rats. TG was administered orally to Wistar rats in as single dose (2000 mg/kg body weight) in an acute toxicity study. Ninety days repeated dose oral toxicity (subchronic) study was carried out by using three dose levels (250, 500 and 1000 mg/kg body weight) administered orally daily for 90 consecutive days and derived from 28 days dose range-finding study. In acute and subchronic toxicity studies, animals were observed for general clinical signs, mortality, weekly body weight changes, weekly feed intake, weekly water intake, blood biochemical investigation, haematological parameters, and gross pathological and histological investigations. In an acute toxicity study, the dose level of 2000 mg/kg of TG was found to be safe when given at a single dose. In the dose range-finding study and subchronic toxicity study TG was found to be safe at all tested dose levels. No significant changes in food and water consumption, haematological and blood biochemical parameters were noticed at any dose level in both studies. No major changes were noticed during histopathological evaluation in ninety days repeated dose oral toxicity study. The study concluded that the Maximum Tolerated Dose of TG was found at 2000 mg/kg body weight and the No Observed Adverse Effect Level was found at 1000 mg/kg in Wistar rats.

Keywords: Acute Toxicity, Dose Range Finding Study, Sub-chronic Toxicity Study, Toxicity Study, *Trayodashang guggulu*

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1. Introduction

Guggul, an oleo-gum resin, is an exudate of plants from the Family Burseraceae. *Trayodashang guggulu* (TG) is an Ayurvedic formulation consisting of 13 ingredients of herbal origin. The ingredients are 1 part each of *Acacia arabica* (Lam.) Willd, the root of *Withania somnifera* (L.), the fruit of *Juniperus communis* L., the stem of *Tinospora cordifolia* (Willd.) Miers, root tuber of *Asparagus racemosus* Willd., fruit of *Tribulus terrestris* L., root of *Ipomoea petaloidea* Choisy, *Pluchea lanceolate* (DC.) Oliv. and Hiern, the fruit of *Anethum sowa* Roxb. ex Fleming, rhizome of *Hedychium spicatum* Sm., the fruit of *Trachyspermum ammi* (L.) Sprague and rhizome of *Zingiber officinale* var. rubrum Theilade, 12 parts of *Commiphora wightii* (Arn.) Bhandari, is one part of clarified butter from cows milk (Goghrta). The use of TG in the treatment of joint diseases is mentioned in The Ayurvedic Formulary of India[1]. *Scientific studies on the ingredients of* TG *reported having beneficial effects in arthritis and other joint diseases.* Clinical studies showed that TG is effective in treating patients with cervical spondylosis[2]. In modern medicine, different classes of allopathic drugs viz. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), corticosteroids, hyaluronic acid, Disease-Modifying Anti-Rheumatoid Drugs (DMARDs), and opioid derivatives are being used for the treatment of arthritis[3], however, the limitations of these allopathic drugs are their adverse effects on the patient. Herbs are a potential source of medicine and much scientific literature is available regarding the therapeutic efficacy of different herbs against arthritis. In the recent past, many scientists claimed that users of Ayurvedic drugs may be at risk of heavy metal toxicity, and hence, quantification of heavy metals in Ayurvedic drugs as well as *in-vivo* comparative toxicity screening was emphasized[4,5]. Hence, the present study was undertaken to evaluate the safety/toxicity of the formulation through acute and 90-day oral repeated dose sub-chronic toxicity study in Wistar rats to find out the target toxicity organ and to establish No Observed Adverse Effect Level (NOAEL).

2. Materials and Methods

2.1 Trial Drug Preparation

TG is an Ayurvedic formulation consisting of thirteen ingredients of herbal origin prepared in clarified butter from cow's milk. The Trial drug TG (Batch No. 001, MFG. Date: April 2018) prepared and standardized as per the protocol of Ayurvedic Pharmacopoeia of India was obtained from the Pharmacy division, Central Ayurveda Research Institute for Drug Development, CCRAS, Ministry of AYUSH, Government of India, Kolkata[1].

2.2 Toxicity Studies

A Dose Range-Finding (DRF) study and ninety days repeated dose oral toxicity study were performed on Wistar rats of both sexes. While an acute toxicity study was carried out only in female Wistar rats.

Approval from the Institutional Animal Ethics Committee (IAEC), Central Ayurveda Research Institute for Drug Development, Kolkata was taken before the experimental work (IAEC approval no. for acute toxicity study- 14/P/S/IAEC/2018, dated 23.06.2018; IAEC approval no. for DRF and ninety days toxicity study- 15/P/S/IAEC/2018, dated 23.06.2018).

2.3 Experimental Animals

Adult healthy Wistar rats maintained at Animal House Facility (Registration No. 694/GO/RBi/S/02/CPCSEA) were used for acute, DRF study and 90 days repeated dose oral toxicity studies. The body weight of the experimental rats ranged from 150 g to 200 g. The female animals selected were nulliparous and non-pregnant. The animal studies were carried out as per the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA). Temperature and relative humidity were maintained at 23±2^{0}C and 50-60%, respectively and illumination was controlled to give approximately a sequence of 12 hours of light and 12 hours of dark. Animals were acclimatized for seven days after randomization to the environmental conditions of the animal house before initiating the experiment. Randomization was done in such a way that the means of groups are the same and body weight variation is ±20% of the mean body weight. A pelleted rodent diet obtained from the National Institute of Nutrition, Hyderabad, was provided along with purified water using plastic nozzle bottles *ad libitum* during the study period.

2.4 In-life Phase and Pathology

2.4.1 Acute Toxicity Study

Acute toxicity study was carried out as per OECD guideline no. 423[6–8]. TG was administered at the single dose of 2000 mg/kg body weight, orally to three female Wistar

rats of the treated group. Three female rats of the vehicle Control group received purified water at the dose of 20 ml/kg body weight. The animals were observed for clinical signs, symptoms and mortality at 5 m, 30 m, 1 h, 2 h, 4 h, 6 h, 24 h and thereafter daily up to 14 days post-treatment. The animals were observed for changes in the skin, fur, eyes, mucus membrane, and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Weekly body weight, feed and water consumptions were recorded. Faecal consistency across the groups during the entire study period was also observed. At the termination of the study, the animals were euthanized for organ necropsy.

2.4.2 Ninety Days Repeated Dose Oral (Sub-Chronic) Toxicity Study

Ninety days repeated dose oral (sub-chronic) toxicity study was carried out as per OECD guideline no. 408[9]. Doses for the ninety days repeated dose toxicity study were evaluated from 28 days DRF study in Wistar rats. For ninety days repeated dose toxicity study, adult Wistar rats of both sexes were selected based on body weight and randomly distributed into six groups namely, vehicle control, high Dose (1000 mg/kg), mid-dose (500 mg/kg), low dose (250 mg/kg), satellite control and satellite treated (1000 mg/kg) group. The animals of vehicle control group and satellite control group received only oral purified water at the dose of 20 ml/kg body weight. The trial drug was administered orally daily for 90 consecutive days during the ninety days repeated dose toxicity study. TG was suspended in the purified water; the suspension was subjected to thorough mixing, with the help of a vortex mixer. The preparations were freshly prepared every day during the study period. The animals were observed for clinical signs, symptoms and mortality daily throughout the study period. Weekly body weight, feed and water consumptions were recorded till the termination of the study. Haematological and blood biochemical investigations were carried out on the 30th, 60th, and 90th day of the study, and in case of satellite groups on the 118th day. Animals of vehicle control, high dose, mid dose and low dose (250 mg/kg) group were sacrificed by using deep CO_2 euthanasia on the 91st day, while animals of satellite control and satellite high hose group were sacrificed on the 119th day. All animals in the study were subjected to a full, detailed gross necropsy. Weights of all major organs of all animals were recorded soon after dissection to avoid drying and were preserved in 10% neutral buffer formalin for histopathology.

Histopathology was carried out on the preserved organs and tissues of all animals in the control and high-dose groups only.

2.5 Statistical Analysis

The data was analyzed statistically by using one-way ANOVA. Dunnett's post hoc test was applied to compare the dose groups over the control arm. Student's '*t*'-test was done for the satellite control group and satellite high dose group data. All the statistical tests were done at a 5% level of significance.

3. Results

3.1 Trial Drug

Physicochemical analysis of TG i.e. loss on drying, total ash value, acid insoluble ash value, water soluble ash value and alcohol soluble extractive value showed 8.50, 9.1, 2.9, 45.1 and 18.6%, respectively. The trial drug was also found to be free from heavy metals (lead, mercury, arsenic, cadmium), and pesticide residues and have below detection limits of mycotoxins (aflatoxin B1, B2, G1, G2 and other harmful microorganisms (*Staphylococcus aureus, E. coli, P. aeruginosa* etc.).

3.2 Acute Toxicity Study

TG at the single dose of 2000 mg/kg body weight produced neither deaths nor treatment-related signs of toxicity in any of the animals during the study. There was no significant effect on weekly body weight, feed and water consumption in the acute toxicity study. Further, no significant gross pathological lesion of internal organs (liver, lungs, kidney, heart, spleen, stomach, gastrointestinal tract) was observed at terminal sacrifice on the 14th day.

3.3 Ninety Days Repeated Dose Oral (Sub-Chronic) Toxicity Study

In ninety day repeated dose (sub-chronic) toxicity study, no significant adverse effect on behavioural patterns, clinical signs, body weight, feed consumption and water consumption was observed in the animals when treated with TG at a dose level of 1000 mg/kg, 500 mg/kg and 250 mg/kg body weight by oral route for ninety consecutive days. There was no significant adverse effect on body weight, haematological and blood biochemical param-

eters in the treated rats of all study groups throughout the study period (Tables 1 and 2). No gross pathological changes were observed in any of the study groups on terminal sacrifice on the 90th day and on the 118th day in the satellite group. In histopathology, no treatment-related histopathological abnormal features have been noticed in the tissue sections of major internal organs across the dose groups (Figure 1).

Table 1. Weekly absolute body weight (g) of Wistar rats in ninety days repeated dose toxicity study

Week	Vehicle Control	High Dose (1000mg/kg)	Mid Dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between groups)	Satellite Control	Satellite High Dose (1000mg/kg)	p-Value (Between groups)
Male rats								
Initial	173.1±28.58	173.9±28.03^{ns}	175.2±26.28^{ns}	173.1±24.98^{ns}	1.00	165.8±6.64	165.7±19.81^{ns}	0.98
1st	197.5±32.79	195.3±25.95^{ns}	188.9±26.66^{ns}	189.0±26.06^{ns}	0.86	181.5±10.93	176.5±18.61^{ns}	0.58
2nd	214.1±35.94	210.0±28.99^{ns}	210.7±25.06^{ns}	205.4±29.5^{ns}	0.93	197.5±9.83	188.3±20.10^{ns}	0.34
3rd	233.6±39.14	227.5±31.41^{ns}	228.9±24.98^{ns}	223.9±26.93^{ns}	0.91	214.7±9.18	201.8±18.66^{ns}	0.16
4th	236.9±35.99	241.3±27.53^{ns}	246.0±25.63^{ns}	240.1±27.06^{ns}	0.92	228.3±8.16	219.7±17.63^{ns}	0.30
5th	252.8±34.35	248.4±31.01^{ns}	254.0±22.87^{ns}	248.3±22.89^{ns}	0.95	241.0±8.25	235.8±14.63^{ns}	0.47
6th	266.5±35.16	261.0±32.41^{ns}	266.3±23.8^{ns}	262.1 ± 23.61^{ns}	0.96	253.0±12.65	245.8±15.30^{ns}	0.39
7th	279.5±33.48	276.0±33.77^{ns}	277.3±30.31^{ns}	275.8±23.07^{ns}	1.0	265.0±13.78	264.0±14.75^{ns}	0.91
8th	281.2±33.83	274.7±33.88^{ns}	286.7±29.7^{ns}	286.2 ±20.49^{ns}	0.82	279.0±16.57	282.0±15.65^{ns}	0.76
9th	300.9±31.30	287.6±35.0^{ns}	294.6±29.55^{ns}	285.4±13.44^{ns}	0.67	297.7±14.02	301.8±13.08^{ns}	0.62
10th	310.5±29.75	292.2±36.0^{ns}	306.9±30.11^{ns}	293.9±12.0^{ns}	0.46	310.5±17.59	317.0±10.37^{ns}	0.48
11th	320.4±28.84	303.4±34.79^{ns}	318.3±32.83^{ns}	304.2±10.54^{ns}	0.48	310.7±17.52	312.4±7.27^{ns}	0.84
12th	308.5±43.66	305.8±32.35^{ns}	319.3±32.02^{ns}	309.0±15.21^{ns}	0.85	308.2±16.25	310.2±7.08^{ns}	0.80
13th	314.4±31.73	298.3±41.06^{ns}	311.0±37.74^{ns}	313.6±16.1^{ns}	0.71	314.2±15.63	320.2±6.98^{ns}	0.45
14th	–	–	–	–	–	324.5±21.99	329.6±6.18^{ns}	0.63
15th	–	–	–	–	–	334.0±23.66	340.8±8.40^{ns}	0.55
16th	–	–	–	–	–	338.3±26.77	349.4±10.76^{ns}	0.41
17th	–	–	–	–	–	344.7±29.09	359.8±14.27^{ns}	0.31
Female rats								
Initial	145.4±15.01	144.4±8.09^{ns}	147.0±12.95^{ns}	155.1±13.44^{ns}	0.23	143.2±15.38	143.5±10.69^{ns}	0.96
1st	151.9±15.53	153.4±9.34^{ns}	155.8±15.85^{ns}	160.5±12.76^{ns}	0.52	148.8±16.25	148.5±10.09^{ns}	0.96
2nd	157.9±17.36	162.0±9.60^{ns}	165.9±15.40^{ns}	167.8±10.40^{ns}	0.38	153.7±15.74	154.2±9.70^{ns}	0.94
3rd	164.5±16.11	170.8±10.33^{ns}	173.2±16.74^{ns}	173.4±10.81^{ns}	0.44	159.3±15.72	160.2±8.90^{ns}	0.91
4th	166.7±16.74	174.2±11.19^{ns}	177.4±15.95^{ns}	176.6±10.38^{ns}	0.30	164.5±14.75	165.8±9.17^{ns}	0.85
5th	172.4±18.36	177.8±13.13^{ns}	181.7±16.03^{ns}	179.4±9.95^{ns}	0.54	169.3±15.23	170.8±8.68^{ns}	0.83
6th	178.1±20.21	181.4±11.82^{ns}	185.6±16.07^{ns}	183.5±9.95^{ns}	0.71	174.7±16.18	175.8±8.15^{ns}	0.88
7th	180.6±19.80	185.1±11.96^{ns}	188.7±16.12^{ns}	186.0±10.27^{ns}	0.68	179.5±15.86	179.7±8.40^{ns}	0.98
8th	183.5±20.17	189.7±12.61^{ns}	188.9±18.86^{ns}	188.3±10.83^{ns}	0.80	180.7±16.92	180.8±7.67^{ns}	0.98
9th	185.0±20.42	191.1±13.46^{ns}	192.0±18.68^{ns}	190.9±10.21^{ns}	0.76	182.0±15.99	181.3±8.14^{ns}	0.99
10th	186.4±20.52	193.3±13.37^{ns}	194.5±18.08^{ns}	193.5±10.36^{ns}	0.66	181.5±16.59	181.6±8.84^{ns}	0.99
11th	188.4±20.39	195.8±12.97^{ns}	194.7±16.18^{ns}	196.3±10.68^{ns}	0.66	181.5±16.71	182.8±9.20^{ns}	0.88
12th	193.5±20.28	198.6±13.37^{ns}	199.6±18.56^{ns}	200.9±12.17^{ns}	0.78	181.7±17.33	182.2±10.62^{ns}	0.95
13th	196.8±20.57	201.6±14.62^{ns}	203.1±19.07^{ns}	203.5±11.94^{ns}	0.82	181.2±17.16	182.2±10.80^{ns}	0.91
14th	–	–	–	–	–	181.8±17.35	181.8±11.37^{ns}	0.99
15th	–	–	–	–	–	181.8±16.77^{a}	182.6±11.63^{ns}	0.93
16th	–	–	–	–	–	182.7±16.82	183.6±11.01^{ns}	0.92
17th	–	–	–	–	–	184.2±16.23	185.8±8.78^{ns}	0.84

Values sharing common superscript do not differ significantly in one way ANOVA ($p>0.05$)
^{ns}No Significant difference compared with the satellite control group in Student's 't' test.

Table 2. Blood biochemical and haematological parameters of male & female Wistar rats in ninety days repeated dose toxicity study

Parameters	Control	High Dose (1000mg/kg)	Mid dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between groups)	Control	High Dose (1000mg/kg)	Mid dose (500mg/kg)	Low Dose (250mg/kg)	p-Value (Between groups)
	Male rats					Female rats				
Albumin (gm/dl)	4.06± 0.09	3.99± 0.19^{ns}	4.01± 0.17^{ns}	3.87± 0.15^{ns}	0.12	4.25 ± 0.12	3.95 ± 0.53^{ns}	4.21 ± 0.27^{ns}	3.89 ± 0.37^{ns}	0.08
SGOT (U/L)	130.3± 13.85	120.9± 19.77^{ns}	98.64± 6.46*	123.5± 17.07^{ns}	0.02	125.6± 22.63	111.8 ± 44.61^{ns}	99.59 ± 17.39^{ns}	144.3 ± 71.45^{ns}	0.18
SGPT (U/L)	76.03± 15.87	67.37± 5.03^{ns}	64.26± 12.04^{ns}	77.49± 13.62^{ns}	0.10	53.54 ± 12.70	44.48 ± 9.11^{ns}	48.75 ± 10.70^{ns}	68.44 ± 11.69*	<0.01
Bilirubin (mg/dl)	0.15± 0.04	0.12± 0.06^{ns}	0.09± 0.04^{ns}	0.11± 0.07^{ns}	0.14	0.09 ± 0.04	0.23± 0.21*	0.12 ± 0.09^{ns}	0.07 ± 0.06^{ns}	0.04
Alkaline phosphatase (U/L)	248.4± 87.59	244.3± 63.39^{ns}	254.1± 45.44^{ns}	217.3± 48.30^{ns}	0.64	141.2 ± 42.52	172.3 ± 115.1^{ns}	132.4 ± 27.05^{ns}	143.5± 28.29^{ns}	0.58
Creatinine (mg/dl)	0.53± 0.02	0.54± 0.02^{ns}	0.52± 0.03^{ns}	0.54± 0.06^{ns}	0.84	0.58± 0.03	0.54± 0.06^{ns}	0.52± 0.09^{ns}	0.53± 0.07^{ns}	0.26
BUN (mg/dl)	15.90± 1.15	15.15± 1.50^{ns}	15.18± 0.87^{ns}	17.37± 2.01^{ns}	0.01	16.60± 1.32	16.33± 2.12^{ns}	16.31± 1.29^{ns}	16.15± 2.06^{ns}	0.95
Glucose (mg/dl)	116.5± 6.56	126.0± 11.13^{ns}	136.7± 6.01*	115.0± 9.24^{ns}	<0.0001	123.5± 25.23	111.8± 18.45^{ns}	119.2± 14.35^{ns}	118.5± 14.40^{ns}	0.61
Haemoglobin (gm/dl)	14.03± 0.47	13.88± 0.46^{ns}	14.37± 0.57^{ns}	13.77± 0.43^{ns}	0.09	13.21± 1.05	13.99± 0.49^{ns}	13.84± 0.95^{ns}	13.73± 0.80^{ns}	0.23
RBC (10e6/mm3)	7.92± 0.42	7.88± 0.27^{ns}	8.07± 0.30^{ns}	7.78± 0.36^{ns}	0.43	7.14± 0.59	7.51± 0.21^{ns}	7.47± 0.54^{ns}	7.26± 0.38^{ns}	0.27
WBC (10e3/mm3)	11.05± 1.68	11.41± 1.87^{ns}	13.33± 2.89^{ns}	14.69± 3.31*	0.02	6.48± 1.98	7.63± 2.82^{ns}	6.29± 2.75^{ns}	7.93± 1.62^{ns}	0.35
PCV (%)	46.16± 1.91	45.51± 1.58^{ns}	46.76± 2.02^{ns}	45.47± 1.3^{ns}	0.40	43.28± 3.68	45.99± 2.37^{ns}	45.59± 3.28^{ns}	45.16± 2.33^{ns}	0.22
Platelet Count (Lakhs/ cumm)	7.34± 0.98	7.85± 1.06^{ns}	8.20± 1.12^{ns}	7.84± 0.88^{ns}	0.44	7.26± 0.50	8.11± 1.17^{ns}	7.57± 1.13^{ns}	7.30± 1.00^{ns}	0.24
ESR (1Hr) mm	12.88± 2.35	12.78± 1.64^{ns}	11.29± 2.14^{ns}	12.33± 2.50^{ns}	0.49	14.20± 2.94	12.11± 2.80^{ns}	12.33± 3.00^{ns}	13.00± 3.53^{ns}	0.45
Neutrophil (%)	20.63± 3.58	21.89± 6.39^{ns}	22.71± 8.26^{ns}	26.67± 9.47^{ns}	0.36	13.90± 3.31	19.67± 8.24^{ns}	16.89± 4.67^{ns}	20.78± 7.36^{ns}	0.08
Lymphocyte (%)	77.13± 3.72	75.67± 6.24^{ns}	74.86± 8.55^{ns}	70.67± 9.33^{ns}	0.30	83.90± 3.41	78.00± 8.38^{ns}	80.67± 4.52^{ns}	77.00± 7.50^{ns}	0.09
Monocyte (%)	1.0	1.0^{ns}	1.0^{ns}	1.0^{ns}	-	1.0	1.0^{ns}	1.0^{ns}	1.0^{ns}	-
Eosinophil (%)	1.25± 0.46	1.44± 0.53^{ns}	1.43± 0.53^{ns}	1.67± 0.50^{ns}	0.4	1.20± 0.42	1.33± 0.50^{ns}	1.44± 0.53^{ns}	1.22± 0.44^{ns}	0.67
Basophil (%)	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	-	0.0± 0.0	0.0± 0.0	0.0± 0.0	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.

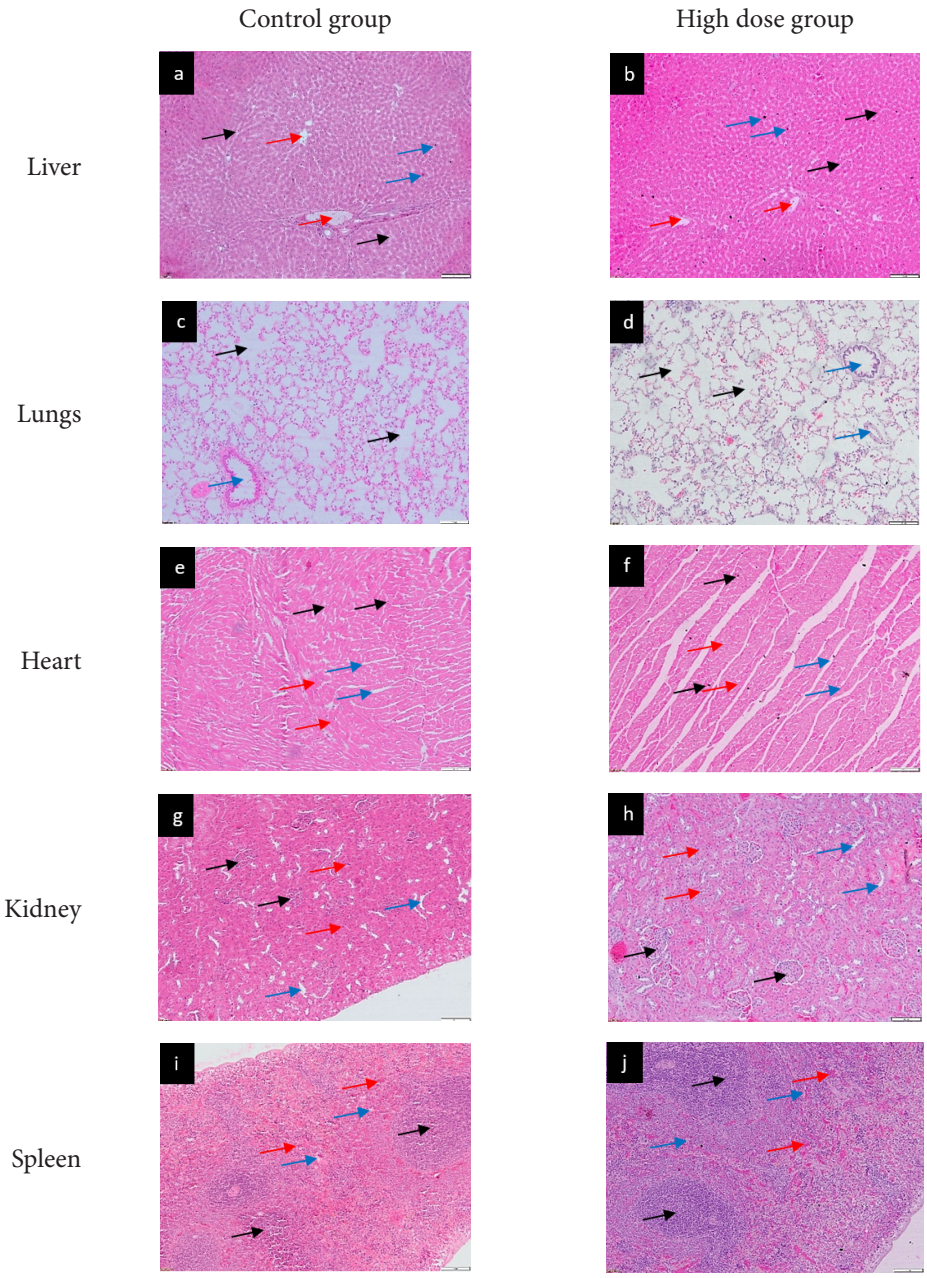


Figure 1. Histology (H&E, 100x) of the liver (**a, b**), lungs (**c, d**), heart (**e, f**), kidney (**g, h**), and spleen (**i, j**) of control and high dose group. Section of the liver (a-control group and b-high dose group) showed normal hepatocytes (blue arrow), hepatic sinusoid (black arrow), portal vein (red arrow) revealed, and normal architecture with granulated cytoplasm. Section of lungs (c-control group & d-high dose group) normal alveoli (black arrow) and bronchioles (blue arrow). Section of the heart (e-control group and f-high dose group) showed a normal central nucleus (black arrow), branching fibre (blue arrow) and perinuclear halo (red arrow). Section of the kidney (g-control group and h-high dose group) showed normal distal tubules (blue arrow), proximal tubules (black arrow), and size of glomeruli (red arrow) with normal tubules. Section of the spleen (i-control group and j-high dose group) showed normal germinal centre (black arrow), red pulp (red arrow), and white pulp (blue arrow).

4. Discussions

Herbs are a potential source of medicine and many scientific literature are available regarding therapeutic efficacy of different herbs against arthritis. *Aloe barbadensis* Mill., *Boswellia*, *Zingiber officinale* Ros., *Curcuma longa* L., *Glycyrrhiza glabra, Strychnos potatorum* (Linn.), *Urtica pilulifera* L., *Cinnamomum zeylanicum* L., *Polygonum viviparum* L., and many other medicinal herbs have shown to be effective against rheumatoid arthritis[10]. Guggulu (*Commiphora wightii* (Arn.) Bhandari), the main ingredients of TG, reported to possess therapeutic benefits against inflammation and pain in joints, bones and muscles. Guggulu contains an active phytoconstituent known as guggulsterone which suppress DNA binding of NF-kappa B induced by inflammatory markers Tumor Necrosis Factor and Interleukin[11]. It is reported that purified guggul, an oleo gum resin has no toxic effect up to 3 g/kg body weight oral dose in acute toxicity study and 300 mg/kg/day in 14 days sub-acute toxicity studies in mice[12]. In the present study, TG when administered at a single dose of 2000 mg/kg body weight, orally to Wistar rats no mortality, signs or symptoms of toxicity study was observed. Swarna guggulu was found to be safe on 90 days repeated dose sub-chronic oral toxicity study in Wistar rats and the No Observed Adverse Effect Level was found to be 500 mg/kg body weight[13].

In the present study, no significant adverse effect on behavioural patterns, clinical signs, body weight, feed consumption, water consumption, or haematological and biochemical parameters were observed in all the surviving animals when treated with TG at a dose level 1000 mg/kg, 500 mg/kg and 250 mg/kg body weight by oral route for ninety consecutive days.

Liver is the major organ for metabolism including drugs and it is a site of cholesterol disposal or degradation and synthesis. In the same perspective, it controls glucose synthesis and generates free glucose from hepatic glycogen stores and also controls the different hepatic enzymes[14]. Since no significant changes were observed in serum SGOP, SGPT and bilirubin levels in this study, it suggests that TG had no effect on the hepatic enzyme level in rats. Further, drugs did not show toxicity in liver affects the transaminases, (Aspartate Aminotransferase) AST, and (Alanine Amino Transferase) ALT which are well-known enzymes used as good indicators of liver function and biomarkers predicting possible toxicity[15]. In our study, both AST and ALT did not show any treatment related increase even at the high dose compared to the control group. In addition, AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum[15]. Thus, no significant increases observed in ALT and AST activities strongly suggest that the chronic administration of TG did not alter the hepatocytes and consequently the metabolism in the rats. Further, TG did not show any histological changes in the liver, lungs, kidney, heart, and spleen indicating no toxicity sign on different internal organs.

No gross pathological changes were observed in any of the study group on terminal sacrifice on the 90th day and on the 118th day in the satellite dose group. Histopathological evaluation of tissues of the vehicle control group and high-dose group showed no dose-dependent histopathological changes. Thus, from the result of the present study, it can be concluded that the Maximum Tolerated Dose of TG is 2000 mg/kg body weight in Wistar rats and the No Observed Adverse Effect Level (NOAEL) is established as 1000 mg/kg in Wistar rats.

5. Conclusion

This study provides valuable data on the acute and 90 day oral toxicities profile of *Trayodashang Guggulu* (a classical Ayurvedic formulation for a number of ailments) that could be very useful in its future clinical study. TG seemed to be nontoxic as was observed following acute and consecutive 90 days oral administrations. Further, teratogenic, mutagenic and carcinogenic studies with this formulation are needed to complete the safety profile of this plant.

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