https://doi.org/10.33472/AFJBS.6.1.2024.193-203

African Journal of Biological Sciences

Acute And Subacute Toxicity Profiles Of Gandhaga Thailam In Wistar Albino Rats

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Article History
Volume 6, Issue 1, 2024
Received:10 December 2023
Accepted: 9 January 2024
doi:10.33472/AFJBS.6.1.2024.193-203

ABSRTACT:
Gandhaga Thailam (Medicated oil) is an unique Siddha medicine having Gandhagam (sulphur) , Vediuppu (potassium nitrate), Manosilai (arsenic disulfide), Navacharam (ammonium chloride), and Veeram (mercuric per chloride)as its ingredients. The special feature about the oil is that it can be used an internal medicine. In siddha system this oil is mainly used to treat many skin diseases. Since Gandhaga thailam is a totally mineral based medicine documenting the safety profile of the drug becomes need of the hour. In this study Acute and Sub acute toxicity studies were done for the Gandhaga thailam in Wistar albino rats of both sexes. In acute toxicity study 2000mg of Gandhaga thailam was administered orally as a single dose and the mortality and behavioral changes were monitored for 14 days. No mortality and behavioral changes were observed. Then a repeated oral toxicity study for 28 days was done with 3 doses namely 100mg, 200mg and 400 mg of Gandhaga thailam. Hematological, Biochemical and Histological parameters were observed after 28 days of treatment. No significant abnormality could be observed in any of the mentioned parameters except SGPT which is slightly increased ($p < 0.001$) compared with the control group. Since there is no positive correlation with any of the other observed parameters and the histological appearance of the liver appears to be normal, It could be claimed that our drug Gandhaga thailam did not produce any toxicity in acute and subacute toxicity studies and it is safe to use upto 400mg .

Key Words: Gandhaga thailam , Toxicity ,OECD, Siddha, SGPT, Thailam

BACKGROUND

Nestled within the Siddha medical system are 32 kinds of external and 32 types of internal drugs that are widely applicable for treating a wide range of illnesses. The many external drugs that are currently in use could serve as evidence of the adoration for the Siddha system. Among them is our test drug, Gandhaga Thailam (Medicated oil), which has its own potent medicinal properties confirmed by its constituents: Gandhagam (sulphur) , Vediuppu (potassium nitrate), Manosilai (arsenic disulfide), Navacharam (ammonium chloride), and Veeram (mercuric per chloride). These ingredients are triturated with cow's butter using the Sudar Thailam Procedure to yield Thailam.

Thus, Gandhaga Sudar Thailam is another name for the preparation. The fact that it can be administered as internal medicine helps to facilitate its effect as well.

Dermatologists have been using sulfur, a biologically active ingredient, for millennia (1,2). In the realm of biomedicine, it possesses antibacterial, antifungal, antiviral, and keratolytic properties in addition to its antitumor action (3). In siddha system, Numerous dermatological conditions, including dandruff, acne, and scabies, are treated with sulfur(4). In the Siddha system, potassium nitrate, also known as Vediuppu, is a common diuretic medication used to treat compromised renal functioning (5). Arsenic disulfide is an anti-cancer drug that efficiently decreases cell viability by inducing apoptosis (6).

While every component of Gandhaga Thailam has several significant therapeutic benefits, there isn't enough scientific evidence to support each of these claims. Rather of acknowledging the medicinal value of heavy metals, there is a persistent notion that they are toxic. The Siddha system is unusual in that it makes use of different heavy metals in every way to treat deadly ailments easily and efficiently. Not all metals are utilized in their raw state for any purpose. They undergo appropriate purification to change their metallic form, improve their bioavailability, and eliminate. When taken correctly, along with the recommended adjuvant, and within the allotted time frame, these medications based on minerals and metals have no negative side effects. Gandhaga Thailam is utilized in the Siddha system and is useful for treating skin conditions as well as reestablishing cutaneous homeostasis. Moreover, Gandhaga thailam is a medicinal oil that is made differently from other oils using a process known as Sudar thailam. The combination of all the aforementioned minerals and cow's ghee is burned over direct flames till droplets, known as sudar thailam, are formed.

Due to increased knowledge of the risks and detrimental effects of chemicals on human health as well as the possibility of chemical residues in animals raised for human food, the use of alternative medicines is becoming more and more popular in today's world. Thus, this work aims to use histopathological, hematological, and biochemical analysis to examine the acute and subacute oral toxicity profiles of Gandhaga thailam in Wistar albino rats.

Materials and Methods:

The preparation of Gandhaga thailam :

Purified Gandhagam (Sulphur) – 35 gram
Purified Vediuppu (Potassium Nitrate) – 35 gram
Purified Manosilai (Arsenic di Sulphide – 35 gram
Purified Navacharam (Ammonium chloride) – 2.5 gram
Purified Veeram (Mercuric Per Chloride) – 2.5 gram

Each ingredient was purified and powdered separately. Then they are triturated with Cow's butter (140 grams). The Gandhaga thailam is prepared by a special preparatory procedure namely "Sudar Thailam Procedure (7).

Experimental animals:

Six-week-old, healthy, and weighing between 100-150 grams for both sexes of Wistar albino rats were obtained. Before the test drug was given, the rodents were given seven days to acclimatize to the controlled laboratory environment. A standard laboratory animal diet was given to the rats along with unlimited water. The Institutional Animal Ethics Committee (IAEC) gave its approval for the research.

Acute Toxicity study (8):

Acute toxicity test was performed as per (OECD) guidelines 423. Female Wistar albino rats were used for the experiments. Animals were split into two groups at random(n=3). The test material was taken orally in a single dosage. The animals received their dose after a three-hour fast.

The oral method was used to provide a single dosage of 2000 mg/kg body weight of Gandhaga thailam. A standard diet was given to the control group. Food for the rats was stopped for two hours following the drug administration. For a full day, the treatment groups were monitored at 2-hour intervals.

For fourteen days, animals were monitored for behavioral changes following the guidelines. At the end of the 14th day, all animals were sacrificed.

Sub-acute toxicity study (9):

Sub-acute toxicity test was performed as per (OECD) guidelines 407. Experiments were performed using Wistar albino rats of both sexes. The animals were divided into 4 groups (n=10). Group I was considered as control receiving a normal diet, whereas Group II, III, and IV were administered a dosage of 100, 200, and 400 mg/kg of Gandhaga Thailam respectively. The animals were subjected to bi-daily clinical sign observations, while their body weight and food intake were documented every week. Hematological parameters were analyzed using an Erba blood analyzer (H360). Biochemical parameters were analyzed through auto auto-analyzer.

Gross pathology and Histopathological examination:

The animals were subsequently subjected to humane euthanasia, using ketamine xylazine. The internal organs were examined during necropsy in order to detect any obvious abnormal changes. Every experimental animal's heart, liver, kidney, adrenal gland, brain, lungs, pancreas, spleen, ovary, and testis were weighed. After that, the organs were processed histo pathologically and placed in 10% buffered formalin. The specimens were sectioned at a thickness of 5 µm, embedded in paraffin wax, trimmed, and stained with hematoxylin and eosin.

Statistical analysis :

For data analysis, GraphPad Prism (version 9.5) programme was used. The data for each group were presented as mean ± SD followed by One-way Analysis of Variance (ANOVA) Dunnett's test was used in the study for comparison and assessment.

Results:

Acute Toxicity study:

Following drug administration, systematic observations and records were made in accordance with the guidelines. Gandhaga thailam at a dosage of 2000 mg showed no signs of mortality. During the acute toxicity study, no notable behavioural alterations were seen as shown in table 1.

Table1: Acute toxicity study: General appearance and Behavioural assessments for treated and control groups.

Observations	control	Gandhaga thailam (2000 mg/kg bw)
Alertness	Alert	Alert
Visual placement	Active	Active
Stereotypy	Absent	Absent
Grooming	Unaffected	Unaffected
Restlessness	Absent	Absent
Irritability	Absent	Absent

Fearfulness	Absent	Absent
Spontaneous activityand reactivity	Normal	Normal
Touch response	Present	Present
Pain response	Absent	Absent
Tremors	Absent	Absent
Convulsions	Absent	Absent
Body posture	No effect	No effect
Limb tone	Normal	Normal
Body tone	Normal	Normal
Pinna	Present	Present
Corneal	Present	Present
Pupil size	No effect	No effect
Salivation	No effect	No effect
Body temperature	No change	No change
Heart rate	Normal	Normal
Skin color	No change	No change
Respiratory rate	Normal	Normal

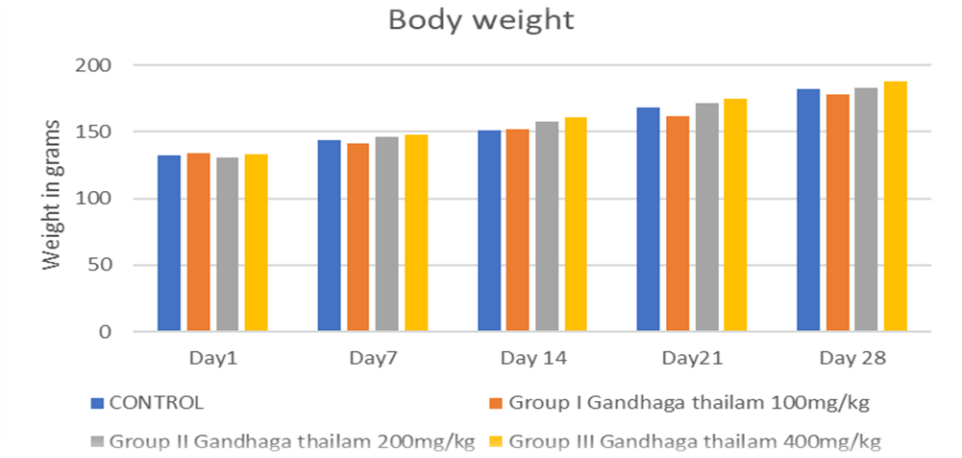
Sub-acute Toxicity study:

Table 2: Mortality status after 28 days of oral administration of Gandhaga thailam:

GROUPS		Dead %	Survival %
I	Control	0	100
II	100mg/kg	0	100
III	200mg/kg	0	100
IV	400mg/kg	0	100

No mortality was observed throughout 28 days of study

Graph 1: Body weight changes during the periods of the 28 days treatment of Gandhaga thailam



There was no statistically significant change in body weight as compared to the control group.

Table 3 :Effect of Gandhaga thailam on Haematological parameters after 28 days of treatment

Parameters	Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400 mg/kg
Hb (g/dl)	13.31 ± 0.59	12.84 ± 0.43	13.34 ±0.54	13.12 ± 0.61
RBC (x 106 cells/µL)	7.18 ± 0.28	6.91 ± 0.56	6.94 ± 0.61	6.9 ± 0.42
WBC (x 103 cells/µL)	4.08 ± 0.42	4.05 ± 0.45	4.30 ± 0.36	4.25 ± 0.29

Neutrophils (%)	47.42 ± 2.71	45.03 ± 1.28	47.65 ± 3.78	46.20 ± 0.91
Lymphocytes (%)	40.13 ± 1.16	38.79 ± 2.22	39.20 ± 1.10	39.25 ± 1.68
Eosinophils (%)	4.10 ± 0.20	4.05 ± 0.11	3.88 ± 0.21	4.02 ± 0.18
HCT(%)	37.99 ± 0.89	37.63 ± 1.66	37.08 ± 0.64	35.35 ± 4.43
MCV (fL)	60.57 ± 1.52	58.66 ± 3.26	62.69 ± 5.30	62.52 ± 5.01
MCH(pg)	17.12 ± 0.59	17.04 ± 0.76	16.51 ± 0.61	16.89 ± 0.94
MCHC(g/dl)	29.04 ± 0.25	28.97 ± 0.66	28.42 ± 0.85	29.22 ± 0.51
PLT(x 103 cells/µL)	6.98 ± 0.54	7.25 ± 0.28	7.11 ± 0.39	7.26 ± 0.29

Each value represents the mean ± SD.

From the table it was evident that Gandhaga thailam did not produce any significant deviation in the hematological parameters after 28 days of treatment.

Table: 4 Effect of Gandhaga thailam on Biochemical parameters after 28 days treatment

Parameters	Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400 mg/kg
SGOT (IU/L)	32.66 ± 3.38	34.2 ± 3.39	35.9 ± 6.4	34.47 ± 3.46
SGPT (IU/L)	228.67 ± 6.15	228.88 ± 5.93	244.1 ± 8.04**	254.4 ± 9.22***
Albumin (g/dl)	2.78 ± 0.18	2.93 ± 0.48	3.25 ± 0.54	2.97 ± 0.46
ALP (IU/L)	228.86 ± 9.21	229.18 ± 5.76	241.6 ± 7.53	241.8 ± 20.48
Urea (mg/dl)	27.5 ± 4.03	25.5 ± 3.21	25 ± 1.94	26.4 ± 2.41
Creatinine (mg/dl)	0.36 ± 0.04	0.35 ± 0.05	0.37 ± 0.04	0.37 ± 0.07
Total cholesterol (mg/dl)	108.83 ± 11.8	109.77 ± 8.31	111.17 ± 5.33	100.02 ± 4.65
TGL (mg/dl)	90.33 ± 3.44	92.2 ± 4.28	90.55 ± 3.78	90.52 ± 5.44
Glucose (mg/dl)	98.66 ± 5.31	93.2 ± 12.3	94.7 ± 9.84	94.7 ± 9.38
Total protein (g/dl)	5.36 ± 0.30	5.29 ± 0.37	5.45 ± 0.47	5.24 ± 0.35
HDL (mg/dl)	35.17 ± 2.99	33.9 ± 3.66	35.6 ± 2.51	35.6 ± 3.86
LDL (mg/dl)	81 ± 2.61	79.8 ± 3.85	78 ± 3.36	78.3 ± 5.12
TC: HDL	1.34 ± 0.17	2.38 ± 1.08	2.33 ± 0.93	2.08 ± 0.74
LDL: HDL	2.32 ± 0.25	2.37 ± 0.22	2.20 ± 0.19	2.29 ± 0.24

Each value represents the mean ± SD.

** significant differences at $p < 0.01$,*** at $p < 0.001$ compared with the control group

From the table, it was evident that Gandhaga thailam did not produce any significant deviation in the Biochemical parameters except SGPT, which seems slightly raised in animals administered with 400mg of Gandhaga thailam group after 28 days of treatment.

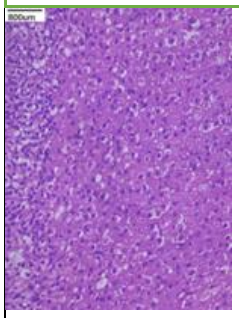
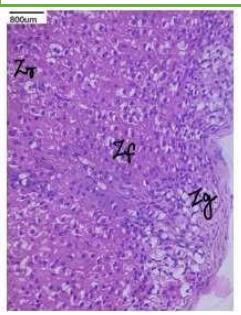
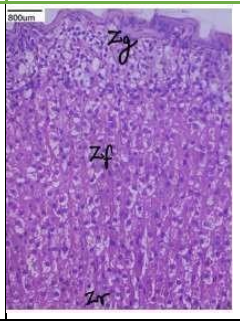
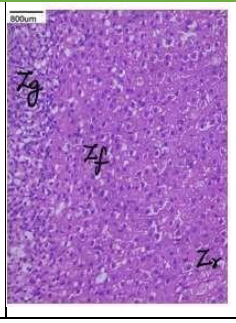
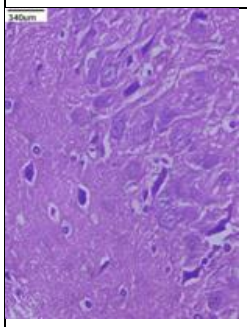
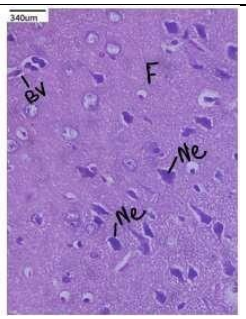
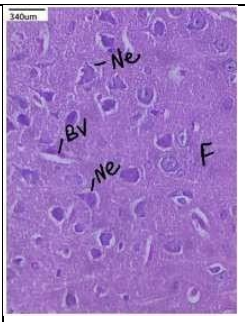
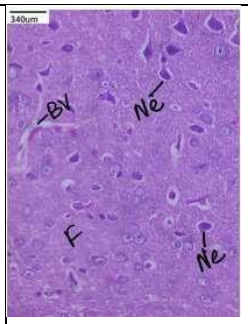
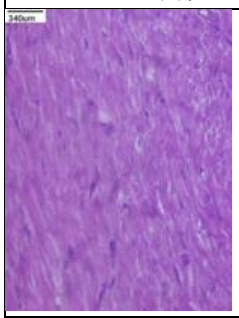
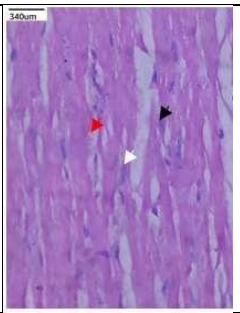
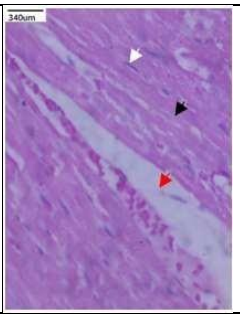
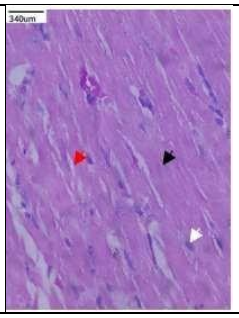
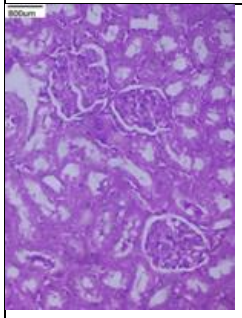
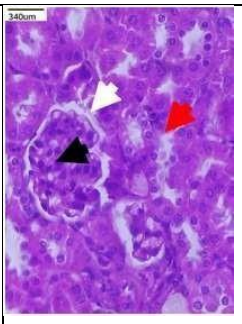
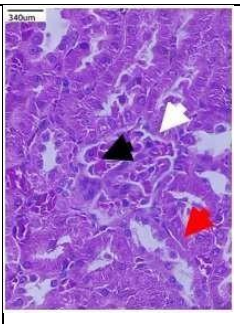
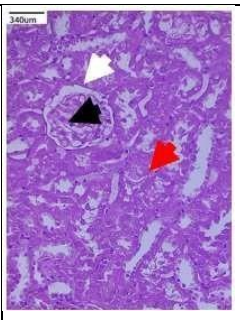
Table:5 Effect of Gandhaga thailam on Relative organ weights after 28 days of treatment

Relative Oragans	Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400 mg/kg
Adrenal	0.05 ± 0.002	0.04 ± 0.002	0.04 ± 0.001	0.04 ± 0.001
Brain	1.29 ± 0.06	1.06 ± 0.05	1.07 ± 0.03	1.16 ± 0.02
Heart	0.67 ± 0.03	0.56 ± 0.03	0.56 ± 0.01	0.61 ± 0.01
Kidney	1.29 ± 0.06	1.07 ± 0.06	1.07 ± 0.03	1.17 ± 0.02
Liver	4.58 ± 0.21	3.77 ± 0.20	3.79 ± 0.11	4.12 ± 0.07
Lung	1.01 ± 0.04	0.83 ± 0.04	0.83 ± 0.02	0.91 ± 0.01
Spleen	0.37 ± 0.01	0.31 ± 0.01	0.31 ± 0.009	0.34 ± 0.005
Testis	0.05 ± 0.002	0.05 ± 0.00	0.05 ± 0.001	0.05 ± 0.002
Ovary	0.06 ± 0.002	0.050 ± 0.002	0.05 ± 0.001	0.05 ± 0.001

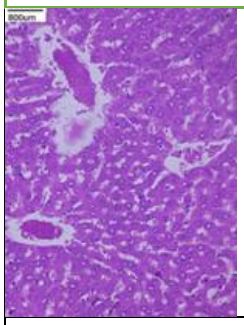
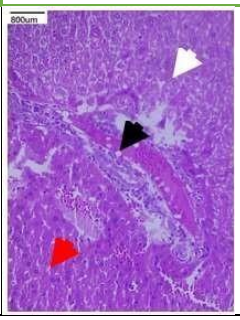
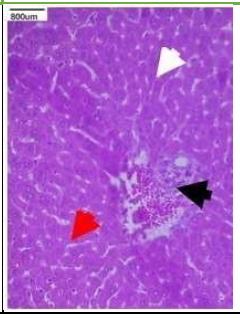
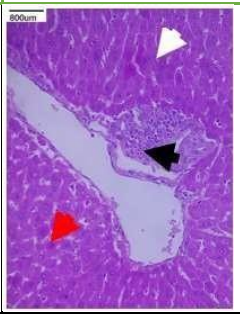
Each value represents the mean ± SD . Organ weights are represented in grams.

From the table, it was evident that Gandhaga thailam did not produce any significant deviation in the relative organ weights after 28 days of treatment

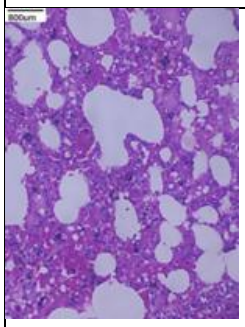
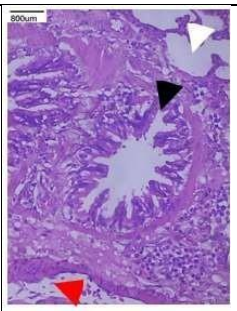
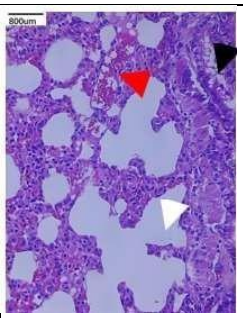
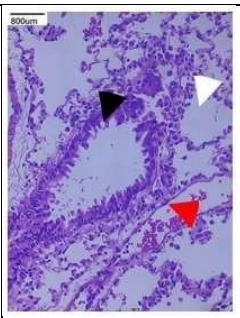
Fig.1: Histopathology of organs – Sub-acute toxicity study of Gandhaga Thailam:

Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400mg/kg
			
H&E section of Adrenal with 10 x magnification: showed normal zonal layers [zg– zona glomerulosa, zf– zona fasciculata, zr – zonareticularis]			
			
H&E section of Brain with 10 x magnification: showed normal neurons (Ne), neurofibrils(F), and normal blood vessels (BV).			
			
H&E section of Heart with 10 x magnification: showed normal cardiomyocytes (black arrow) with intact myonuclei (white arrow) and normal intermysial connective tissue spaces (red arrow).			
			

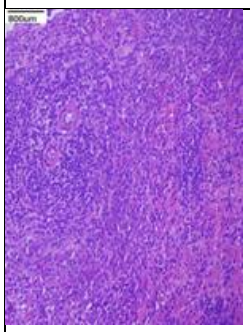
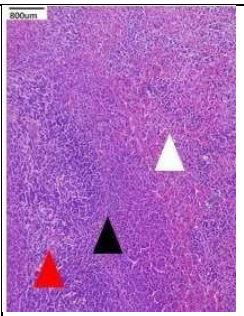
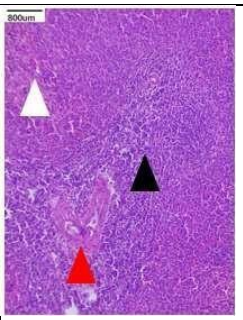
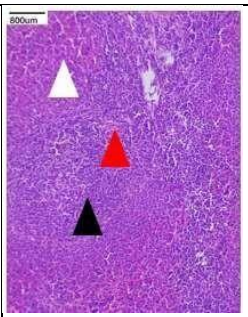
H&E section of the kidney with 10 x magnification: showed normal glomerular architecture (black arrow) with intact bowman's capsule (white arrow) and tubules (red arrow).

Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400mg/kg
			

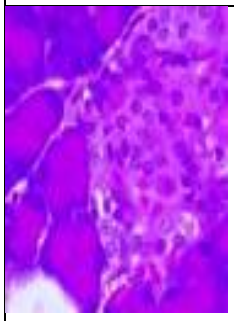
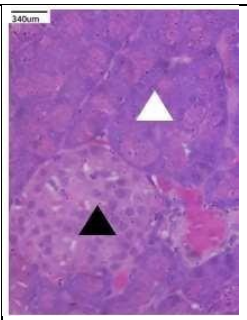
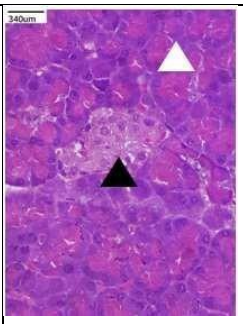
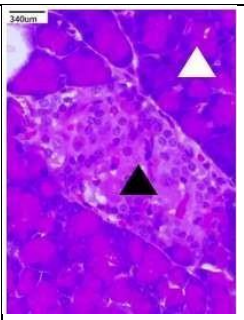
H&E section of Liver with 10 x magnification: showed normal hepatocytes (white arrow), normal portal triad (black arrow) and normal sinusoids(red arrow).

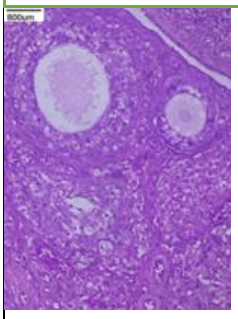
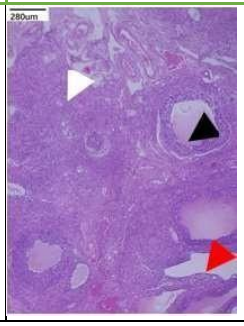
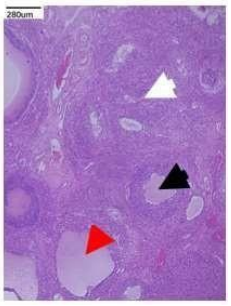
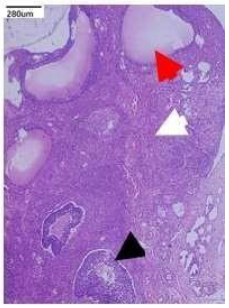
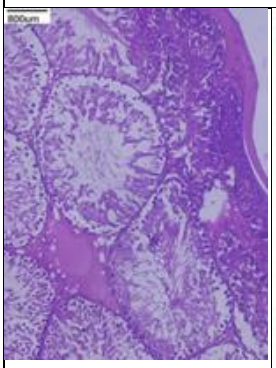
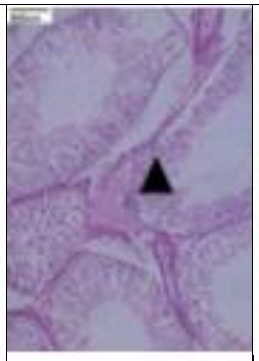
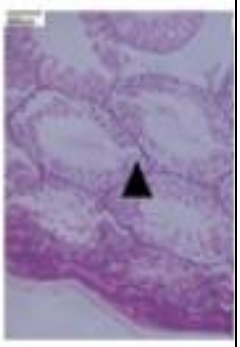
H&E section of Lung with 10 x magnification: showed normal alveolar structures (white arrow), bronchioles (black arrow), and normal blood vessels (red arrow).

H&E section of Spleen with 10 x magnification: showed normal white pulp (black arrow), red pulp (white arrow), and normalcentral arteriole (red arrow).

H&E section of Pancreas with 10 x magnification: showed normal endocrine islet of Langerhans (black arrow) and normal exocrine glands (white arrow).

Control	Gandhaga thailam 100mg/kg	Gandhaga thailam 200mg/kg	Gandhaga thailam 400mg/kg
			
H&E section of Ovary with 10 x magnification: showed normal primary follicle (black arrow) and secondary follicles (red arrow) and normal ovarian stroma (white arrow).			
			
H&E section of Testis with 10 x magnification: showed normal seminiferous tubules (black arrow), spermatogonia (white arrow), and normal spermatids (red arrow).			

Discussion:

Gandhaga thailam is a popular treatment for a variety of skin disorders, with an emphasis on inflammatory ones. The medication Gandhaga thailam is based on minerals. While Siddha literature mentions the extensive pharmacological properties of Gandhaga thailam, there is insufficient evidence regarding its toxicity profile in humans and animals. It was clear that long-term exposure to metals and minerals would impact a variety of biological processes in human organs at the molecular, cellular, and intercellular levels, potentially leading to morphological changes (10). Therefore, it becomes crucial to record the toxicity profile of a Siddha medication that is entirely mineral-based and pharmacologically active.

The animals were given a single oral dosage of 2000 mg/kg body weight of Gandhaga thailam, and the results were compared with the control group. All of the animals' behavior and mortality did not significantly alter (Table 1). Thus, Gandhaga thailam may be safe at doses up to 2000 mg/kg of body weight.

Thus, for 28 days, a subacute toxicity study using oral dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg of Ganthaga thailam was conducted. Changes in body weight are the primary indicator of systemic toxicity in drugs (11). There were no noticeable differences in food intake or weight gain in our study (Graph 1).

In repeated oral toxicity study, organ weight may be a helpful indicator of treatment-related modifications, with or without accompanying histological evaluation (12). After being exposed to

toxic compounds, the weight of internal organs usually decreases, which is also an indication of toxicity (13,14). After the 28-day treatment period, no appreciable changes in the relative weight of all the harvested important organs were seen when compared to the control (Table 5). It is possible to conclude that Gandhaga thailam has no harmful effects on the harvested organs because the weight of the target organs for toxicity was not appreciably changed.

The changes in the blood parameters in animals have been considered a useful indicator for predicting animal toxicity. (15) The administration of 3 different doses of Gandhaga thailam caused no significant change in WBC, RBC, HCT, MCH, MCV, MCHC, and PLT when compared with the control group (Table 3).

Clinical biochemistry investigations were performed to assess potential dose-dependent changes in the hepatic, renal, and lipid profiles of treated rats relative to control rats to identify potential pathological abnormalities (16). During the 28-day treatment period, Gandhaga thailam did not exhibit any significant alterations in the majority of the biochemical parameters. However, significant alterations were noted in SGPT in rats given 200mg and 400 mg of Gandhaga thailam (Table 4). Moreover, there was no positive correlation between high SGPT and SGOT. Serum bilirubin and ALP levels were similarly inversely correlated with SGPT at the same time. Furthermore, there were no obvious degenerative alterations noticed in the liver upon histological analysis.

When drugs or bioactive compounds are absorbed in the intestines and metabolized into other compounds, the liver is the primary target organ. These compounds may or may not be hepatotoxic to animals(17). Numerous investigations have established that there is no direct correlation between liver injury and increased serum levels of hepatic enzymes and transaminases namely , SGPT and SGOT(18). However, cellular permeability and inflammation are brought on by increased SGPT levels (19). Thus, it is possible that certain minerals, which have the potential to be toxic to the liver at increasing doses and induce liver damage, are responsible for the increase in SGPT following the intake of Gandhaga thailam.

While some biochemical alterations were noted during the course of the 28-day treatment, histopathological analysis of the target organs revealed normal architecture (Fig 1), indicating that the 28-day administration of Gandhaga thailam did not result in any deleterious changes or morphological disturbances.

It is said that different ideas indicated in Siddha literature for processing and purifying metallic and mineral medicines with traditional herbal juices aid in changing the molecular structure and lessening their poisonous character (20). It was therefore clear from the data that Gandhaga thailam did not cause any appreciable impairment in either the acute or subacute toxicity studies.

Conclusion:

The findings from this investigation provide valuable data on the Acute and subacute Toxicity profile of Gandhaga thailam in Wistar albino rats , In acute toxicity study no mortality had been identified at the dose level of 2000 mg/kg. From the results of Sub acute toxicity study it is evident that Gandhaga thailam exhibited no toxic effects on the body and relative organ weights, hematological, biochemical, and histopathological parameters of rats upto 400 mg/kg.

Thus Gandhaga thailam is safe up to a dose level of 400 mg/kg.

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