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Evaluation of acute and sub-acute toxicity assessment of marine diatom *Thalassiosira weissflogii*

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ABSTRACT

With positive effects on human health, several bioactive compounds from marine microalgae have been demonstrated for their diverse biological activities. As new technologies are emerging for the study of bioactive compounds from natural sources, determination of their toxicity profile becomes inevitable. Since diatoms are rich in minerals and produce secondary metabolites, diatoms were reported as potent anti-cancer agents. It has been proved that diatoms can be used as potentially effective carrier in treating cancer diseases especially in targeted drug delivery treatments. Various *in vivo* studies have shown that there are no significant symptoms of tissue damage in animal models, thus suggesting the aptness of a diatoms extract of *Thalassiosira weissflogii* in cancer treatments. The diatoms methanolic extract showed hopeful paths for further preclinical studies in discovering their pharmacological potential. In future, these marine diatoms which are rich in cytotoxic compounds against cancer cells will be connected for the discovery of new lead compounds. The diatoms of *T. weissflogii* could be further assessed for their efficiency against cancer models to attain targeted delivery and harmless treatment.

1. INTRODUCTION

Since ancient times, natural remedies have been practiced for the treatment of many diseases all over the world. Resistance of micro-organisms to commonly used antibiotics has enhanced morbidity and mortality and triggered the search of the new drug, especially from marine diatoms such as *Thalassiosira weissflogii* [1]. During the search of new drug, toxicological investigation is an important step because this investigation is done to determine the applicability of any novel compound and to assure the health benefits. It follows the observation of the usage boundaries such as toxicokinects, dosage restrictions detailed signs of toxicity and mortality, and ways of administration [2].

The toxicological modesties were the aim of the present investigation. The investigation also aimed at potential effects of these toxicological modesties on behavioral and biochemical alterations in experimental animals and to offer guidelines to select the security dosage for humans [3].

Nearly 60% of the drugs used have their origin from natural resources and nowadays, marine origin drugs have become more drugs [4]. Historically, the Asiatic medicines, especially algae were used for the screening of various biological entities even during the second half of the 20th century. Among the algal sources, microalgae found to produce a potential class of microbial metabolites with significant antibacterial, antifungal, and anticancer properties [5,6]. Microalgae are very small algal creatures where they convert the solar energy to biomass using photosynthetic components. Most of the microalgae possess therapeutic properties which are majorly associated with their biological constituents such as, amino acids, pigments, vitamins, and lipids etc. [7]. Several novel compounds with major medicinal values have been isolated and tested for various applications with positive effects [8].

Thalassiosira weissflogii belongs to the species of centric diatoms, which are unicellular microalgae. It is found in many parts of the

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world and they belongs to marine and inland water environments. The species can produce various secondary metabolites such as pigments, phenolics and tocopherols that contain anti-microbial and anti-inflammatory properties [9]. Hence, the present study aimed at the extract of *T. weissflogii* which are tested for their sub acute toxicity against sexually mature female Swiss albino mice for their biocompatibility nature.

2. MATERIALS AND METHODS

2.1. Collection and Production of Microalgae

Thalassiosira weissflogii were collected from coastal regions of Marakanam, Tamil Nadu. The microalgae collected was further cultivated in medium composed of seawater collected from the microalgae culture pond and was enriched by adding growth *f*/2 medium prepared in the laboratory. Using filtered natural seawater and with the required ingredients as prescribed, the *f*/2 medium was prepared and it was then autoclaved [10]. The medium was cooled and the microalgal cultures were grown under continuous light at 20ºC for 5 days in *f*/2 medium [11] and regularly monitored for their presence of growth till it reaches stationary phase. Then matured grown cultures were harvested by centrifugation at 10,000 rpm for 3 minutes. The pellets obtained were shade dried. After drying the pellet was made into a coarse powder using mechanical grinder and stored in an airtight container till further use.

2.2. Extract Preparation of *Thalassiosira Weissflogii*

The attained algal biomass was partially dehydrated by subjecting it to centrifugation at 2,500 rpm/minutes for 10 minutes. Then about 25 g of this partially dehydrated algal biomass was subjected to extraction using Soxhlet apparatus for a time period of 30 minutes. 150 ml of organic solvent, i.e., methanol was used for extraction [12].

2.3. Preparation of Toxicity Studies

Female Swiss albino mice weighing 20–30 g which are sexually mature were obtained from TANUVAS, Madhavaram, Chennai for toxicity studies. They were maintained under conventional laboratory housing and nourishing conditions. The animals were marked to permit individual identification by random selection and they were caged for 7 days before dosing. The principles of laboratory animal care were followed carefully to get the approval of the Institutional Animal Ethical Committee for the usage of the animals and the study design was approved by the IAEC No: XLVIII/03/CLBMCP/2018.

2.4. Acute Toxicity Studies as per OECD 423 Guidelines

The animals were grouped according to the dosage levels, six animals were used in each group. The animals were deprived of only food for overnight before administration of *T. weissflogii,* the weight of each animal also noted. The animal was given the dose level of 400, 800, and 1,600 mg/kg body weight and the dose was administered orally. A control group was noted by administering only an equal volume of vehicle (normal saline). After administration, the feeding was again withdrawn for a period of 3 to 4 hours.

Systematically and continuously the observations were recorded as per the guidelines. The changes such as skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements were visually observed. Finally, the quantity of stayers was noted after 24 hours and these animals were then monitored further for 14 days and observations were made daily. The toxicological effect was evaluated based on the biochemical analysis includes AST, ALT, ALP, LDH, Lipopolysaccharides, and catalase activity [13].

2.5. Sub-Acute Toxicity Studies as per OECD 407 Guidelines

The healthy adult male and female mice were grouped into three in order to determine the subacute toxicity. The group I serve as a control, and groups II and III were administered orally with methanolic extract of *T. weissflogii* by giving the doses of 500 and 1,500 mg/kg body weight per day respectively. Their weight was measured weekly for four weeks, i.e., the observations were made for 28 days. On the 28th day, a cardiac puncture was made while the mice were anaesthetized and its blood was collected from each animal.

Two groups of test tubes were used for the collection of blood samples i.e., with and without anticoagulant. Automated Hematology Analyzer was used to determine the hematological parameters such as WBCs, RBCs, HGB, HCT, MCH, MCHC, and platelets using the samples collected with anticoagulant. The blood chemistry such as glucose, urea, creatinine, total protein, ALT, and AST was analyzed to study renal and hepatic functions using Automated Clinical Chemistry Analyzer from the blood samples that are collected without anticoagulant.

After the collection of blood, the mice were killed by cervical dislocation. For histopathological study, the liver was dissected out surgically and fixed in 10% buffered formalin. After fixation, the tissue samples were dehydrated, washed, and enclosed in paraffin blocks. Then, by rotary microtome, thin tissue sections of 5 µm were obtained which was stained with hematoxylin-eosin and they were analyzed microscopically for pathological examinations and photomicrographs were recorded.

3. RESULTS AND DISCUSSION

During toxicity studies conducted in mice with different dosage forms such as 400, 800, and 1,600 mg/kg body weight, no mortality was observed for the study period of 14 days. The results are as follows:

The body weight of test animals with a dose range of 400 mg/kg body weight was normal and whereas it was decreased in 800 mg/kg and 1,600 mg/kg of doses as shown in Table 1. After the dosage, the behavioral observation of test animals showed the assessment of postures, salivation, limb paralysis, skin color, defecation, locomotion, and urination were found to be normal in 400 mg/kg but they were found to be abnormal in 800 and 1,600 m. The other parameters such as body tone, pile erection, sensitivity response, and muscle grips were found to be normal in all dose ranges.

Based on Table 2, alertness, grooming, and touch response of the test animals were found to be present in control and 400 mg/kg

Table 1: Behavioral signs of acute toxicity studies.

SL	Observation	Group 400	Group 800	Group 1600
1.	Body weight	Normal	Naturally decreased	Normally decreased
2.	Assessments of posture	Normal	Normal	Normal
3.	Signs of Convulsion Limb paralysis	Normal	Absence of sign (−)	Absence of sign (−)
4.	Body tone	Normal	Normal	Normal
5.	Lacrimation	Normal	Absence	Absence
6.	Salivation	Normal	Absence	Absence
7.	Change in skin color	No significant color change	Significant color change	Significant color change
8.	Piloerection	Normal	Normal	Normal
9.	Defecation	Normal	Abnormal	Abnormal
10.	Sensitivity response	Normal	Normal	Normal
11.	Locomotion	Normal	Abnormal	Abnormal
12.	Muscle gripness	Normal	Normal	Normal
13.	Rearing	Mild	Mild	Mild
14.	Urination	Normal	Abnormal	Abnormal

Table 2: Changes found during acute toxicity studies.

No	dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	400 mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	800 mg	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-
4.	1,600 mg	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch response 7. Decreased motor activity 8. Tremors 9. Convulsions 10. Muscle spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality (+ Present, − Absent).

groups, and they were negative for 800 mg and 1,600 mg dosages. Aggressiveness, pile erection, gripping, motor activity, and respiration were found to be normal in all dose forms, whereas, convulsions and writhing were present in 800 mg and 1,600 mg groups.

Water intake and food consumption were continuously monitored in test animals for 14 days and presented in Figures 1 and 2. Even though there are no significant changes [*p* value (*p*)* = Non significant] in the water and food intake among the test animals, the intake of water and food varies with groups. The intake of water increases from day 1 to 14 in control group and is less dose group the water intake decreases in the 7th day and increases in the 14th day. Among the mid dose and high dose experimental groups, the water intake gradually decreases from day 1 (Fig. 1). The intake of food increases from day 1 to 14 among control and less dose groups, whereas the intake of food gradually decreases in the mid and high dose experimental groups (Fig. 2).

Biochemical parameters in blood of test animals were analyzed and found to increase with the increase in dosage forms. All the parameters were found higher in dosage groups than in control group animals (Fig. 3).

Based on Table 3, the body weight of mice in different dosage forms was observed at different time intervals. Even though there is slight increase in body weight among the rat groups, there is no significant changes during the study period of 28 days.

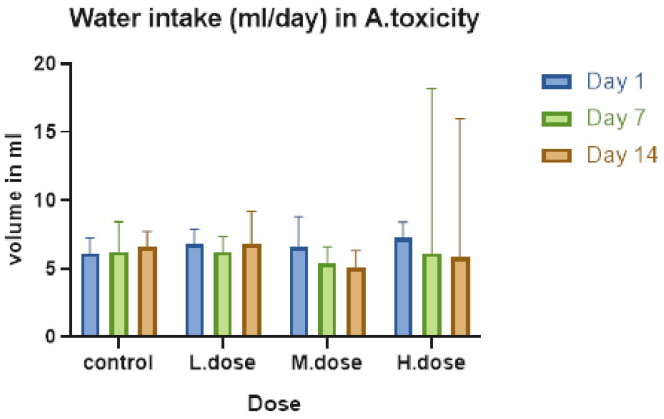

Figure 1: Water intake (ml/day) of Swiss albino mice group treated with *T. weissflogli*.

After 28 days, blood which was collected from each of the test animals were observed for the hematological parameters such as hemoglobin, WBC, platelets, neutrophil, monocyte counts in all the three groups. Even though no significant changes were observed in the test groups, increasing changes were noted in the percentage of neutrophils, eosinophil, platelets, and total RBC of group with high dose than other groups (Table 4).

In order to check the function of liver, the level of glucose, triglycerides, cholesterol, and ALP were observed at the end of the

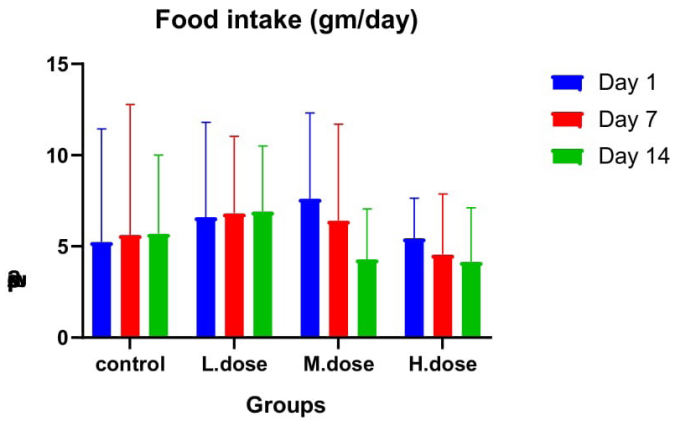

Figure 2: Food intake (g/day) of Swiss albino mice group exposed to *T. weissflogli.*

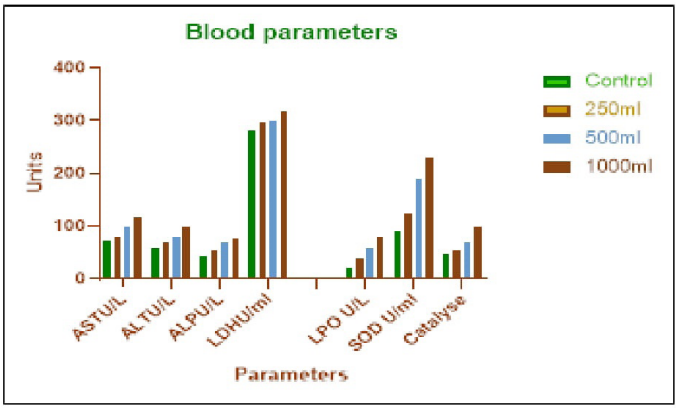

Figure 3: Biochemical parameters in blood.

experimental period (Fig. 4). It was observed that when the dosage of *T. weissflogii* increases, the values of these parameters in the blood decreases. It was noted that the values of all the parameters were decreased in the treated groups than control except the triglycerides (mg/dl). In the high dose group, the level of triglycerides increases than the control group (Table 5). Biochemical parameters were analyzed after 28 days of oral administration of *T. weissflogii*. The results were observed similar to the liver function test. The values of the BUN, LDH, creatinine, total protein, and albumin were lower than the control groups (Table 7).

After 28 days of oral administration of *T. weissflogii*, the experimental animals were sacrificed, the organs were dissected out and the organ weights were measured. The histological section of the kidney from control group mice showed normal cortex, medulla, and normal glomeruli compared to that of high dose *T. weissflogii* treated mice that show abnormal interstitial sections. In the control group, the lobular structure was normal in the histopathological section of the liver. High dosage (1,500 mg/wt) of *T. weissflogii* administered mice showed abnormal architectural lobes, central veins, and sinusoids. Histological section of spleen

Table 3. Body weight of wistar albino rat groups exposed to *Thalassiosira weissflogii.*

	DAYS		
Dose	**1**	**14**	**28**
Control	280.2 ± 10.03	281.2 ± 10.24	281.6 ± 24.61
Low dose	260.2 ± 40.20	260.5 ± 30.14	261.8 ± 52.40
High dose	248 ± 01.10	248 ± 10.30	249 ± 04.32
p value (*p*)*	NS	NS	NS

NS, Not significant, **($p > 0.01$).
*($p > 0.05$), $n = 10$ values are mean ± S.D (one way analysis of variance followed by Dunnett's test).

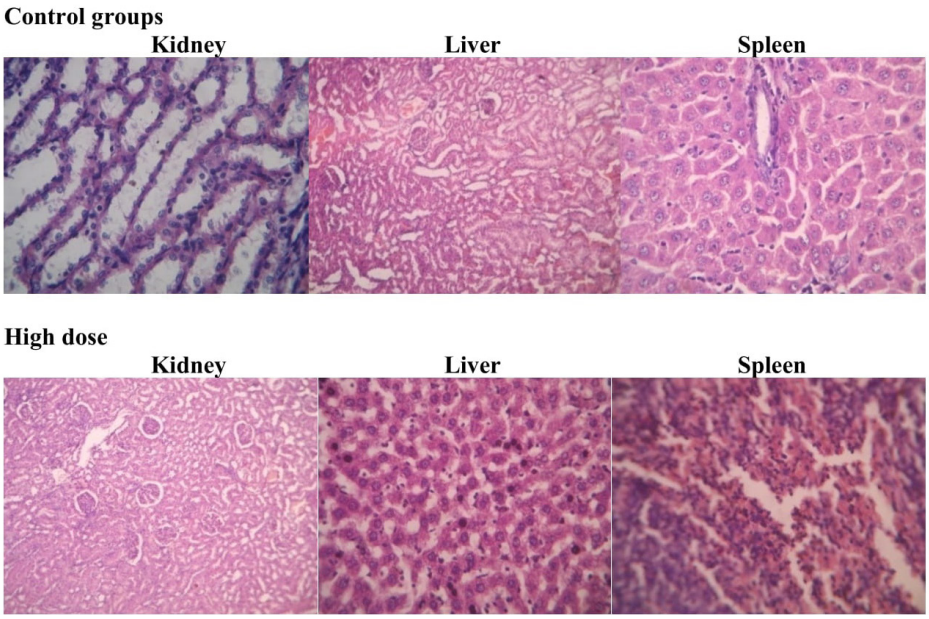

Figure 4: Histopath ological report of experiment animals after 28 days of oral administration of *T. weissflogli.*

Table 4: Hematological parameters of Swiss albino mice group treated with *Thalassiosira weissflogii*.

Category	Control	Low dose	High dose	*p* value (*p*)*
Hemoglobin (g/dl)	10.2 ± 0.24	10.10 ± 0.36	12.28 ± 0.26	N.S
Total WBC (×10³l)	14.52 ± 0.05	14.42 ± 0.13	14.40 ± 6.16	N.S
Neutrophils (%)	26.15 ± 0.01	26.11 ± 0.22	28.20 ± 2.30	N.S
lymphocyte (%)	79.10 ± 1.06	79.23 ± 1.02	79.26 ± 4.46	N.S
Monocyte (%)	0.9 ± 0.03	0.9 ± 0.05	0.9 ± 0.07	N.S
Eosinophil (%)	3.2 ± 0.04	3.2 ± 0.06	3.53 ± 0.02	N.S
Platelets cells 10³/µl	604.16 ± 2.66	604.10 ± 4.26	608.06 ± 4.54	N.S
Total RBC 10⁶/µl	8.49 ± 0.01	8.09 ± 0.50	8.64 ± 0.32	N.S
PCV%	37.65 ± 0.6	37.30 ± 1.32	37.66 ± 2.24	N.S
MCHC g/d	38.4 ± 1.42	38.06 ± 0.47	38.30 ± 2.34	N.S
MCV fl (µm³)	54.04 ± 4.60	54.06 ± 3.43	54.34 ± 2.14	N.S

Table 5: Liver function tests of Swiss albino mice group treated with *Thalassiosira weissflogii*.

Group	Treatment	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	SGPT/ALT (U/l)	ALP(U/l)
I	Control	123.64 ± 10.24	66.24 ± 7.74	66.61 ± 4.45	89.64 ± 6.57	215.08 ± 10.48
II	Low dose	118.44 ± 09.24	76.14 ± 6.22	62.10 ± 6.46	66.11 ± 3.39	230.40 ± 11.68
III	High dose	108.22 ± 2.11	69.14 ± 09.32	57.14 ± 2.43	72.11 ± 1.24	212.44 ± 10.94

Table 6: Biochemical parameters of experimental animals after 28 days of oral administration of *Thalassiosira weissflogii*.

Group	Treatment	BUN (mg/dl)	LDH (U/mg)	T. Protein (g/dl)	Albumin (g/dl)	Creatinine (mg/dl)
I	Control	21.10 ± 0.20	280.40 ± 18.19	7.14 ± 0.12	3.33 ± 0.19	0.6 ± 0.02
II	Low dose	18.16 ± 0.90	240.10 ± 42.03	5.84 ± 0.24	2.85 ± 0.11	0.2 ± 0.04
III	High dose	16.14 ± 1.22	221.18 ± 44.15	5.36 ± 0.42	2.70 ± 0.04	0.3 ± 0.04

shows pulp congestion in both control and *T. weissflogii* treated mice. Slight variations also observed in penciler artery.

Thalassiosira weissflogii belongs to diatoms that are imperious organism which accounts for global primary production. They can uptake nitrogen and assimilation very rapidly when colossal nutrients were present. These organisms have been at the bottom of the food chain. It is also important for its secondary metabolites and its bioactive compounds which can be used for pharmacological and medicinal purposes. These organisms were rich in proteins, unsaturated fatty acids, carbohydrates, minerals, and many micro and macronutrients. They have the potential benefits that include antioxidant, antitumor, immune stimulant, antibacterial, and angiotensin-converting enzyme inhibitor-I inhibition activity.

Most of the primary organs like, kidney, liver, heart, lungs, and spleen, were found to be unnatural by metabolic reaction triggered by tested toxicant. Liver was vulnerable to damage due to variety of chemicals which affect the metabolic process [14,15]. Bogne *et al*. [16] studied the toxicity effects of microalgae (methanolic extract of *H. floribunda* and *Neocarya macrophylla* seed cake, respectively) in Wistar rats and reported that there is a change in body weight which was considered as low toxicity effect. In contrast, there are reports [17] on the weight of the organs such as, heart, kidney, and spleen of the test rats, which were found to be comparable with those of control rats in both the acute and sub-chronic toxicity studies. The observations of the present study displayed that in body weight of mice in both control and high dose administered groups there is no significant change. Similarly, the biochemical test showed that is no significant changes were seen in values of ALP, ALT, and AST of *H. floribunda* fed Wistar rats, these results were well correlated with this study.

The weight of the organ is a major factor of physiological and pathological status in the tested animals. The weight of comparative organ is fundamental to ascertain whether the organ was showing to the injury or not during the treatment. Kafaie *et al*. [18] investigated the acute and sub-chronic toxicities of *Nannochloropsis oculata* in rats. They reported that *N. oculata* biomass supplemented rats did not showed any biologically changes in both kidney and liver either in the male or female rats which concludes that feeding the rats with *N. oculata* biomass of equal or less than the body weight results in no acute or chronic adverse effects in the rats. Similarly in the study of [19] there is no histopathological changes were observed in rat liver, testes, and kidneys when treated with the herbal extract of *Eurycoma longifolia*. In this present study for *T. weissflogii* showed some pathological changes in liver, kidney, and spleen. While in sub-acute toxicity study of *E. longifolia* [19] showed no significant changes in hematological and biochemical tests, similarly *T. weissflogii* also showed no changes in both tests of sub-acute toxicity study. In another report, acute and sub acute toxicity effect of three different microalgae *Isocrysis galbana*

(MG2), *Tetraselmis gracilis* (MG6), and *Chromulina friebergensis* (MG10) were tested against albino rats and the LD50 values were found to be 2,000 mg/kg for MG2 and for the microalgal extract of MG6 and for MG10 it was above 3,500 mg/kg. They also studied the histopathological effect of algal extract and found to have toxic effects on various organs such as liver, spleen, kidney, and heart [20].

Hosseinzadeh *et al*. [21] from Iran studied the acute and sub-acute toxic effects of safranal in rats and mice and found that, there is a significant decrease in RBC, platelets, and hemoglobin counts were observed in the sub-acute toxicity study. They decreased the level of cholesterol, triglycerides, and ALP and increases the LDH and serum urea. In contrast, the *T. weissflogii*, hematological and biochemical tests did not show any significant changes. In acute toxicity study, the pathological examination of safranal showed a toxic effect on liver spleen and heart, similarly, the *T. weissflogii* showed some changes in histopathological examination of liver, kidney, and spleen.

4. CONCLUSION

The extracts of *T. weissflogii* was processed and tested for acute and sub-acute toxicity in Swiss albino mice. In conclusion, the present result of the study clearly showed that the extracts of *T. weissflogii* under the conditions studied no mortality was found when different dosage was tested. The behavioral observation of test animals after dosage were found to be normal; however various biochemical parameters in blood were found to be increased with the increase in dosage forms when compared with control group animals. Interestingly, the body weight of tested animals at different dosage forms at different time intervals found to have no significant changes for the entire study period of 28 days. The hematological parameters also were noted that there are no significant changes were observed in control when treated with high dose and low dose groups. The histopathological studies after 28 days showed that dosage plays an important role, and mid dose showed no abnormal changes in the organs. Thus, extracts of *T. weissflogii* found to be potential candidate against cancer thus can be used as the defensive agent against hepatocellular carcinoma.

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6. CONFLICTS OF INTEREST

No conflicts of interest among authors.

7. AUTHOR CONTRIBUTIONS

AA collected data and prepared the basic manuscript under the guidance of authors MS and VA who planned, corrected and structured the manuscript.

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