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Acute And Sub Acute Toxicity Of Methanolic Extract Of Sphaeranthus Amaranthoides

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ABSTRACT

Sphaeranthus amaranthoides (SA) is one of the most popular medicinal plants used in Siddha system. It is used to treat blood disorders, helminthiasis, eczema, vomiting, abdominal discomfort, and also used to increase the semen consistency. It also prevents the destruction and aging of the viscera, muscles, nerves, bones, bone marrow, blood cells etc. To date there is no documented evidence corroborating its safety. This study thus aimed to evaluate the toxicity profile of the methanolic extract of Sphaeranthus amaranthoides. Acute and Subacute toxicity changes after oral administration of methanolic extract of sphaeranthus amaranthoides (SAME) were reported in Male adult Wistar albino rats. The results demonstrate that, a single dose and short term oral intake of methanolic extract of Sphaeranthus amaranthoides caused no toxicity up to a dose of 2000 mg/kg b.w. Thus, prolonged uses of Sphaeranthus amaranthoides orally at lower doses and mid doses were proved to be very safe.

KEYWORDS: Acute, Toxicity, Lethal dose, Sphaeranthus amaranthoides, Herb, Methanolic extract

INTRODUCTION

Plants are used traditionally for the treatment of various diseases. Therefore it is necessary to rule out the toxic effects of a particular plant. In addition toxic profile of a plant helps in enhancing the efficacy of that particular plant. Almost any substance can be harmful at some doses; similarly can be without harmful effect at lower doses. Between these two limits there is a range of possible effects, from subtle long-term chronic toxicity to immediate lethality [18]. The large array of toxic chemicals produced by plant based products, usually referred to as secondary plant compounds, is often said to have evolved as defense mechanisms against herbivorous animals, particularly insects and mammals. Many chemicals that have been shown to be toxic are constituents of plants that form part of the human diet [18].

Sphaeranthus amaranthoides Burm belongs to the family Asteraceae is a rejuvenator herb of Siddha system. Sphaeranthus amaranthoides has been used as an ingredient in certain Siddha polyherbal formulation possessing antioxidant property. The entire herb possesses therapeutic value, even though leaves has more value. The plants possess astringent and mild hot taste and hot potency. These tastes got biotransformation into hot taste after absorption. The plant has aromatic, astringent, stomachic, antispasmodic, emmenagogue and diuretic properties (12).

The phytochemical analysis of the plant showed that it contains steroids, triterpenoids, phenolic compound, flavonoids, tannins, and glycosides. The leaves of Sphaeranthus amaranthoides has have been reported for their antioxidant, antimutagenic, antimicrobial, and phytochemical activities

[19]. Sphaeranthus indicus is a closely resembling plant of the same family on which many research works has been done. This article deals with the acute and subacute toxicity effects of methanolic extract of spahaeranthus amaranthoides on adult wistar albino rats.

EXPERIMENTAL SECTION

Plant Materials/Extract Preparation

The plant Sphaeranthus amaranthoides was sourced in September 2018, from the Agricultural lands of different parts of Tiruvarur and Salem district, Tamilnadu, India. The prime works like washing, drying were done. The plant materials were identified and authenticated by DR. P. Murugan M.Sc., PhD, Department of Medicinal Botany, Srisairam Siddha Medical College & Research Centre. The collected plant material was free from disease and contamination of other plants was strictly avoided. About 2.5 kg of air-dried, powdered leaves of Sphaeranthus amaranthoides (SA) were defatted with petroleum ether (60–80 °C) to remove fat, latex and non-polar compounds of high molecular weights. The defatted plant residues were extracted by maceration in methanol for 24 h, with intermittent stirring at 45 °C, to obtain the methanol extract (SAME). The solvent was regularly changed until colour disappears. The collected extract was filtered through Whatman filter paper (No. 1). Finally the filtrate was concentrated in vacuum using rotary evaporator and the concentrated extract was dried using a freeze dryer followed by incubation in an oven (45 °C).

2.2. Oral Acute Toxicity Study: Experimental Design

The acute oral toxicity study was sanctioned to be conducted in compliance with OECD guideline 423, which stipulate the use of only three animals (OECD 423, [(2)]. Three of the test animals were fasted overnight (~12 h) and weighed. Test doses of Sphaeranthus amaranthoides methanolic extract (SAME) were calculated in relation to the body weight of every fasted animal; and administered via oral gavage at 2000 mg/kg (Fig 1)[(1)]. The animals were regularly and individually observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days [(3)]. Finally, on the 15th day, weights of the animals were measured, and gross physical examinations were carried out. After sacrificing the rats, gross pathological observation was carried out on vital organs.

2.3. Oral Sub-Acute Toxicity Study (28 Days)

2.3.1. Procedure

The study was conducted in compliance with OECD guidelines No. 407. The experimental animals were divided into two groups of 5 rats each (160–240 g b.w.) in separate cages. The groups were treated daily with two doses of SAME (250 and 500 mg/kg b.w.) respectively for 28 days. All extracts were administered via oral gavage.

Observation

Clinical signs were observed at least twice a day during the 28-day treatment period. Body weights were measured once a week. On the 29th day, the animals were fasted overnight and blood samples collected from orbital sinus. Vital body organs were dissected, cleansed of adhering tissues and rinsed in normal saline before their weights were measured. The kidneys and livers were immediately stored in 10% paraffin for histology. Paraffin sections were made and stained with hematoxylin and eosin for a thorough histopathological study [(5)]. Hematological analysis of the blood samples was performed using an automatic hematology analyzer. The parameters which were evaluated included: red blood cells (RBC) count; hemoglobin (Hb); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); platelets (PLT); leukocytes (WBC) count; and neutrophils, eosinophils, basophils, lymphocytes and monocytes counts. For biochemical analysis purposes, the blood samples were centrifuged at 3000 rpm for 15 min. Diagnostic kits were used to evaluate these parameters, which included the serum levels of total proteins (TP), bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphate (ALP), creatinine, and albumin (ALB) . Histopathological examination was also conducted on the liver, Kidney and gonads of the treated control groups. In this study, the values obtained for the control group were considered as the reference values and statistical analysis was conducted against the control group.

Ethical Consideration

The study was conducted after having approval from the Institutional Ethical and Scientific committee, Sathyabama Institute of Science and Technology, Tamilnadu, India. Animals used in this study were not subjected to any unnecessary painful and terrifying situations [15]. To keep the pain and suffering minimal during any surgical intervention all animals were given Xylazine anesthetic and the procedure was carried out by a well-trained person. The animals were protected from pathogens and placed in appropriate environment. The numbers of animals were reduced to the minimum possible that allows investigators achieving the scientific objectives of the study.

Histopathological Studies

The liver, brain, and kidney sections taken randomly for tissue processing were fixed in 10% neutral buffered formalin overnight at room temperature. After fixation, the tissue sections were washed with water to remove excess fixatives for about six hours and dehydrated with increased concentration of alcohol of 70% for two hours, 90% for two hours, absolute alcohol-I, II for one and half hours, and III overnight. The dehydrated tissues were cleared in two changes of xylene-for one and half hours and two and half hours. The tissues were then infiltrated with three changes of paraffin wax-for one and half hours, two and half hours, and overnight. Finally, the tissues were embedded in paraffin wax in square metal plates forming tissue blocks, by each tissue block was labeled and stored at room temperature till sectioned.

The tissue blocks were sectioned in ribbons at a thickness of 5 μm. The ribbons of the section were collected and put onto the surface of a warm water bath. The floating ribbons over the surface of

warm water were mounted onto precleaned slides spread with egg albumin. The slides containing paraffin wax were arranged within the slide holder and placed in an oven with temperature of 40°c for about 20 minutes so as to fix the tissue to the slides and allowed to cool at room temperature for 30 minutes and stained regressively with routine Harris haematoxylin for 6 minutes and then eosin for 17-20 second (H and E).

The tissue sections were washed with tap water for five minutes and stained regressively with Harris haematoxylin for 6 minutes and then washed under running tape water for five minutes again. The slides were immersed in acidic alcohol for differentiation and controlling over stained haematoxylin for 1 second and then put in bluing solution (sodium bicarbonate) until they became blue. After bluing, the slides were counter stained with eosin for 17-20 seconds and then washed in tap water for two minutes. The sections were dehydrated with increasing alcohol concentration of 50%, 70%, 95%, absolute I and II for two minutes each. The dehydrated sections were cleared with xylene I and xylene II for three minutes each and permanently mounted on microscopic slides using DPX and cover slips and then observed under light microscope for the investigations of any histological change, thereby the histology of the treated groups was compared with histology of the control group. After examination, photomicrographs of selected samples of liver and kidney section from both the treated and control rats were taken under a magnification of x10 and x40 objective using automated built-in digital photo camera.

RESULTS AND DISCUSSION

Acute oral Toxicity Study of SAME (14 days)

Commonly Acute toxicity study assesses the adverse effects that occur within a short time following the administration of a single dose of a test drug. The present acute toxicity study did not show any toxicity signs and symptoms at 2000mg/kg. No morbidity or mortality was observed in the treated animals at this maximum dose during acute toxicity study. No sign of toxicity was observed in the wellness parameters during the 14-day observation period. As a result, the LD_{50} of the SAME extract could be greater than 2000mg/kg body weight. The methanolic extract of Sphaeranthus amaranthoides (SAME) may, therefore, be considered relatively safe on acute exposure.

Body weight change is an important index for assessment of toxicity [9]. In the present study, there was a gradual normal increase in the mean body weight of the treated groups like control group. In the present study, there was a regular normal increase in the mean body weight of the treated groups like control group. At the end of Acute toxicity study the mean body weight for the control rats was 204 g. The mean body weight for rats treated with 2000 mg/kg 207 g, respectively. However, the weight gain difference between control and treatment groups was statistically insignificant

PARAMETERS	DOSES	MEAN ±SE
DAY 1	Control	180.83±2.19
	2000mg/kg	180.5±2.33

DAY7	Control	191± 3.61
	2000mg/kg	195±3.94
DAY14	Control	204±2.33
	2000mg/kg	207±3.41

Table 1: Comparison of the effect of SAME on body weight of treated and control rats during acute toxicity study.

Liver and kidneys of rats are used by many researchers to assess the safety or toxicity of herbal drugs or plant materials [10]. In the present acute toxicity study, gross pathological examination of the liver and kidneys of treatment groups did not show any major visual difference in size, shape, color, and texture compared with control group. In addition, there was no significant difference in the absolute weight of liver and kidneys of treated rats compared to control group. Our study agrees with the acute toxicity study done by Thanigavelan et al (12), which revealed that sphaeranthusamaranthoides was safe of acute exposure.

Sub-Acute (28 days) Oral Toxicity studyof SAME

Subacute toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rodents. This test provides information on target organs and on the potential of the test chemical to accumulate in the organism and then is used as the basis for the determination of the no observed effect level (11)

Effect of Oral Administration of Sphaeranthusamaranthoides Methanolic Extract (SAME) on General Behavior

In the sub-acute toxicity study, ratswere administered with 250 mg/kg b.w. and 500 mg/kg b.wof ofmethanolic extract of Sphaeranthusamaranthoides did not exhibit symptoms of toxicity. There was no morbidity or mortality during the study period. There was no behavioural variations in comparison with control rats.

Effect of Oral Administration of Sphaeranthusamaranthoides Methanolic Extract (SAME) on Body and Organs Weights

The rats showed increase in mean body weight in comparison with their initial body weights in a non – significant fashion. The initial mean body weight of control group was 189.681±4.9 g, and final mean body weight was 216.884±4.915 g. The initial mean body weight of rats treated with the dose of 200 mg/kg was 188.522±4.874 g, and final mean body weight was 217.5108±3.666 g. The initial mean body weight of rats treated with the dose of 400 mg/kg was 187.136±3.844 g, and the final mean body weight was 217.952±4.331 g.

The gross pathological examination of the liver and kidneys of the treated rats showed no change in color, shape, size, and texture compared to the control group. Gross observation of the liver and kidneys of the treated rats showed no significant changes compared with the control group and no

significant difference was observed in the mean absolute organs weight between control and treated groups. The mean absolute weights of the liver were 7.07±0.1734g (at 200mg/kg) and 7.01±0.2277g (at 400mg/kg), compared with the control 6.61±0.7282g . Similarly, the mean absolute weights of the kidneys of rats were in the control and extract treated groups was not significantly different.

	DOSES	BODY WEIGHT (Mean ±SE)
I WEEK	CONTROL	189.681±4.9
	HD	187.136±3.844
	LD	188.522±4.874
II WEEK	CONTROL	198.585±5.703
	HD	198.538±4.874
	LD	197.146±3.844
IIIWEEK	CONTROL	208.136±5.469
	HD	208.719±4.871
	LD	206.36±3.354
IV WEEK	CONTROL	216.884±4.915
	HD	217.952±4.331
	LD	217.5108±3.666

Table 2: Comparison of the effect of SAME on body weight of treated and control rats during Subacute toxicity study.

	CONTROL	HIGH DOSE	LOW DOSE
Brain	1.87±0.0260	1.74±0.0313	1.89±0.0420
Kidney	1.41±0.0192	1.53±0.0598	1.19±0.0655
Liver	6.61±0.7282	7.01±0.2277	7.07±0.1734

Table 3: Comparison of the effect of SAME on organ weight of treated and control rats during Subacute toxicity study.

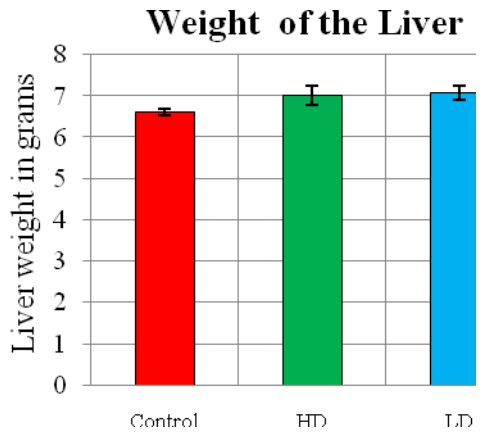


Figure 1: Bar graph of mean weight of liver in rats treated with (LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.

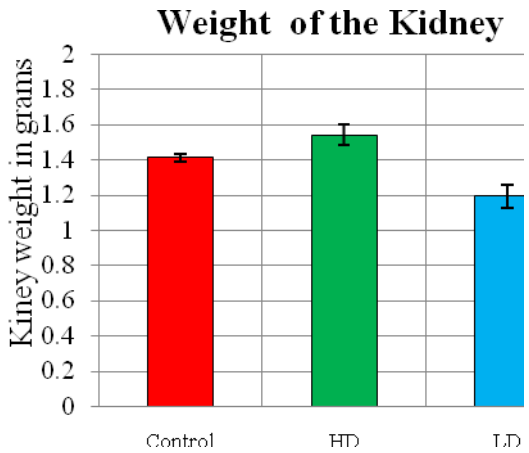


Figure 2: Bar graph of mean weight of kidney in rats treated with (LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.

Effect of Oral Administration of SphaeranthusamarnthoidesMethanolic Extract (SAME) on Plasma Hematological Parameters

Assessment of hematological parameters can be used to determine the extent of harmful effect of foreign compounds including plant materials on blood (14) Hematological parameters of the rats were examined as shown in Table 4. No significant changes were observed in plasma. Hematological parameters in animals treated with SAME compare with the control, with the statistical significance Hb(%) , RBC ($\times 10^6/mm^3$), PCV(%), MCV(fl), MCH (pg),MCHC (g/L), WBC (mm^3), platelets($\times 10^3/mm^{3)}$ ESR(mm) .

All the haematological parameters except a few are same for the animals administered with 400 mg /kg BW ,whereas all the haematological parameters are same as control for the animals administered with 250 mg /kg BW. The erythrocytes were slightly elevated when compared to control, but not in a significant in 500 mg /kg b.w. Haemoglobin values for control and low dose animals did not show any significant difference. This indicates that the 250mg of extract may not possess toxic substance that can cause anemia or other abnormalities. This may be well documented when given subchronically.

PARAMETERS	CONTROL	HD	LD
RBC	6.92±0.092	6.91±0.071	6.87±0.096
HB	0.44±0.07	0.37±0.062	0.46±0.076
WBC	11023.67±108	10938.67±149.97	11370.17±125.47

Platelets	1215.66±6.49	1286.66±14.34	1263.5±7.97
ESR	2	2	2
PCV	43.97±0.15	46.97±0.32	44.71±0.144
MCV	54.78±0.17	56.45±0.421	55.43±0.2678
MCH	18.36±0.05	13.44±0.048	18.87±0.06
MCHC	30.65±0.07	30.94±0.075	31.03±0.090

RBC-Red blood cell count, Hb-Hemoglobin ,WBC- White blood cell count,ESR- erythrocyte sedimentation rate, PCV- packed cell volume,MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin , MCHC - Mean corpuscular hemoglobin concentration Platelets

Table 4: Comparison of the effect of SAME on plasma haematological parameters of treated and control rats during subacute toxicity study.

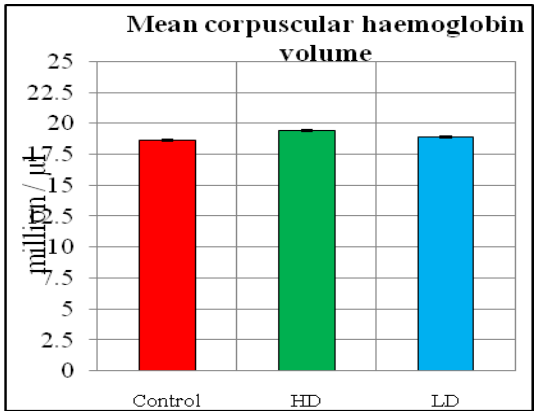


Figure 3: Bar graph of mean Corpuscular haemoglobin volume in rats treated with (LD) 250mg/kg and (HD) 500mg/kg of SAME as compared to the control group during subacute toxicity study.

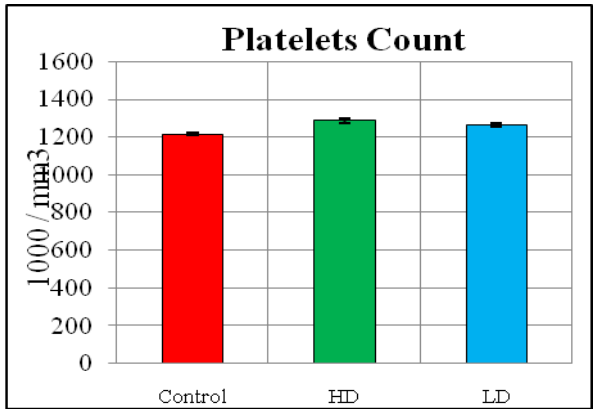


Figure 4: Bar graph of mean platelet count in rats treated with (LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.

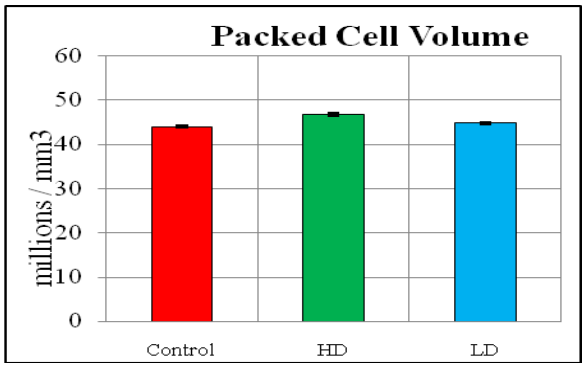


Figure 5: Bar graph of mean packed cell volume in rats treated with (LD)250 mg/kg and (HD)500 mg /kg

Effect of Oral Administration of SphaeranthusamaranthoidesMethanolic Extract (SAME) on Serum Electrolytes Levels

	CONTROL	HD	LD
Sodium	136±0.346	135.76±0.348	135.5±0.341
Potassium	4.61±0.055	4.38±0.116	4.56±0.136
Calcium	8.4±0.08	8.38±0.071	8.25±0.072

Table 5: Comparison of the effect of SAME on mean serum electrolytes levels of treated and control rats during Subacute toxicity study.

Electrolytes (ions) play an important role in the body. They regulate the osmotic pressure in cells and help to maintain the function of muscle and nerves. If electrolyte levels vary, cell and organ functions will decline, which might lead to dangerous conditions. The electrolyte levels of the treated animals were insignificantly variable than control. The slight change in potassium levels may be physiological and should be correlated with other biochemical parameters.

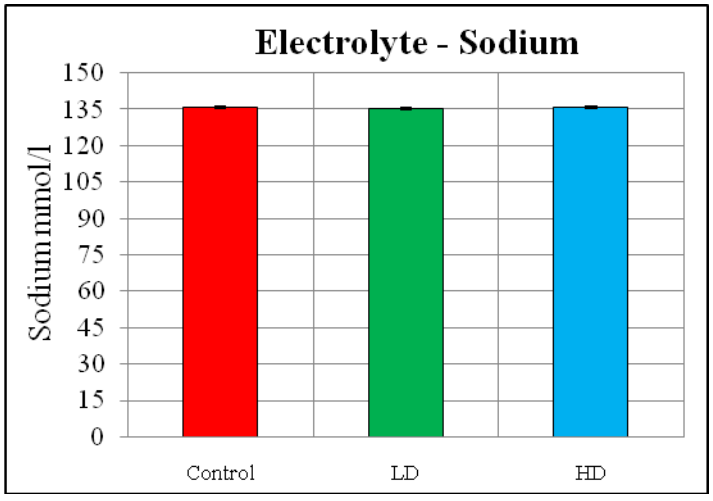


Figure 6: Bar graph of mean sodium level in rats treated with (LD)250 mg/kg and (HD) mg /kg of SAME as compared to the control group during subacute toxicity study during subacute toxicity study.

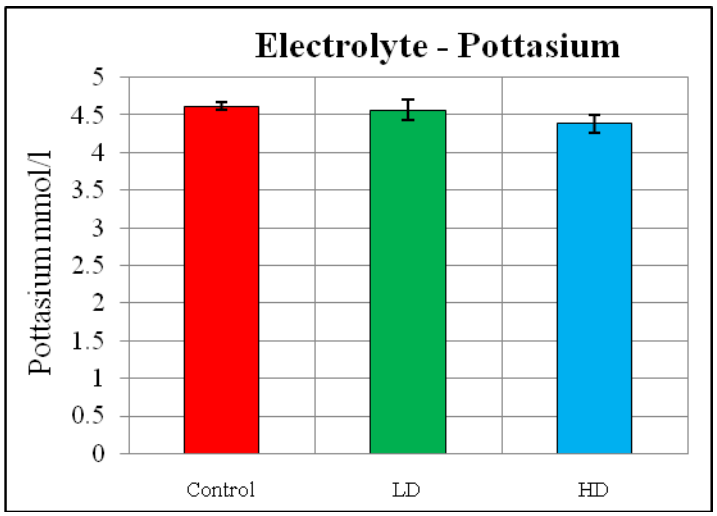


Figure 7: Bar graph of mean potassium level in rats treated with(LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.

Sodium, potassium, chloride, calcium and Total protein concentrations in the treated groups were not significantly different from those in the control group.

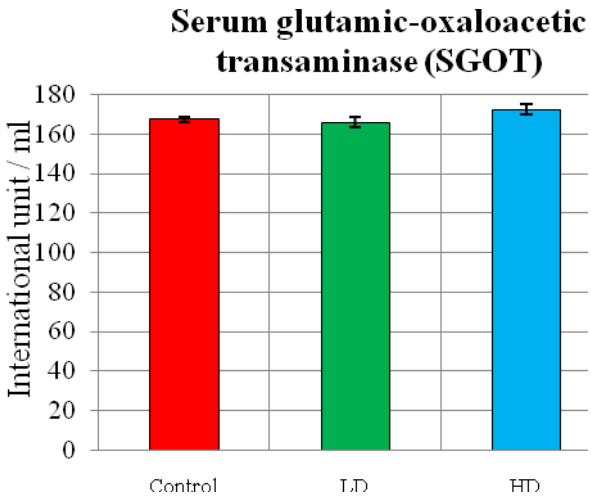
Effect of Oral Administration of *Sphaeranthus amaranthoides (SAME)* Extracts on Serum Biochemical Parameters

In toxicological evaluation, biochemical parameters have significant roles as a marker because of their response to clinical signs and symptoms produced by toxicants. Evaluation of hepatic and renal function is of prime importance to assess the toxic properties of extracts and drugs .The albumin is the one of the most important plasma protein, maintain the osmotic pressure of blood and prevent the escape of fluids from blood to tissue. Almost all the Biochemical parameters of the treated animals did not show significant change when compared to the control group. GOT is more important than SGPT . The levels of markers were slightly elevated in those animals treated with 500mg / kg bw.but the changes were insignificant. These changes might be physiological.

Table 6: Comparison of the effect of SAME on mean serum Biochemical parameters of treated and control rats during Subacute toxicity study.

PARAMETERS	CONTROL	HD	LD
Bilirubin Total (mg/dl	0.26±0.008	0.009±0.009	0.008±0.008
Bilirubin Direct(mg/dl	0.1±2.53372581020338E-18	0.1±2.53372581020338E-18	0.1±2.53372581020338E-18
Bilirubin Indirect	0.16±0.16	0.15±	
SGOT	167.55±1.343	172.3	
SGPT	58.23±3.148	58±1.	
Alkaline phosphatase	541.33±11.885	544.5	
Total protein	8.3±0.014	8.41±	
Albumin	4.03±0.047	3.96±0.032	4.05±0.027
Globulin	4.3±0.014	4.43±0.020	4.36±0.008

Figure 8: Bar graph of mean SGOTlevel in rats treated with(LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.



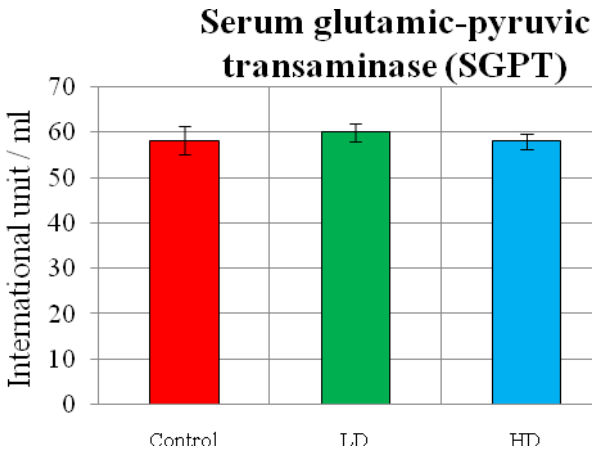


Figure 9: Bar graph of mean SGPT level in rats treated with(LD)250 mg/kg and (HD) 500 mg /kg of SAME as compared to the control group during subacute toxicity study.

3.2.7. Histopathological Examination

Histopathological studies of the liver sections in the control group (a) showed normal appearance of central vein (CV) and hepatic sinusoids (S) lined by endothelial cells with normal radiating

hepatocytes. There was also normal appearance of the portal triad including hepatic portal vein, interlobular bile duct, and branches of hepatic artery. Rats treated with High dose 500 mg/ kg bw (b) of SAME and with Low dose 250mg / kg bw of SAME also showed normal appearance of the central veins (CV) and hepatic sinusoids lined with endothelial cells with normal radiating hepatocytes. Histological evaluation showed no specific change in the hepatic lobules in the treated rats as compared with the control. The result was also accompanied by the no adverse effects of the extract in any of the biochemical markers (such as SGOT and SGPT), which showed statistically insignificant changes compared with control group

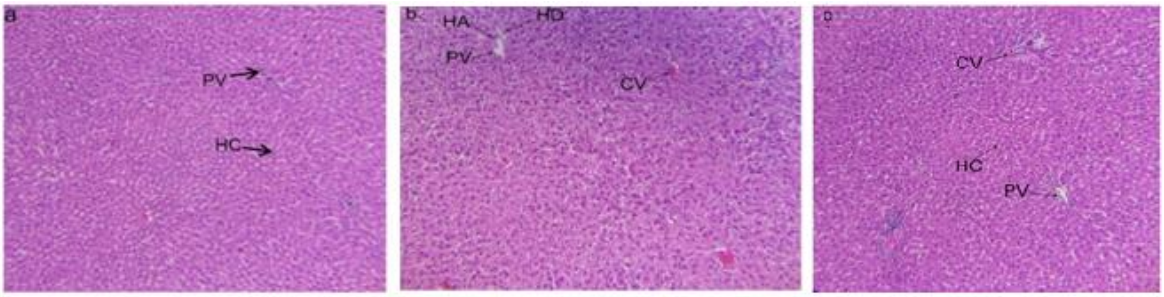


Figure : 10 Photomicrographs of the Liver sections. (H & E 10x). (a) Control rats , (b) High dose and (c) Low dose . CV= Central vein, PV= Portal vein, HC = Hepatocytes, HA= Hepatic artery, HD= Hepatic duct

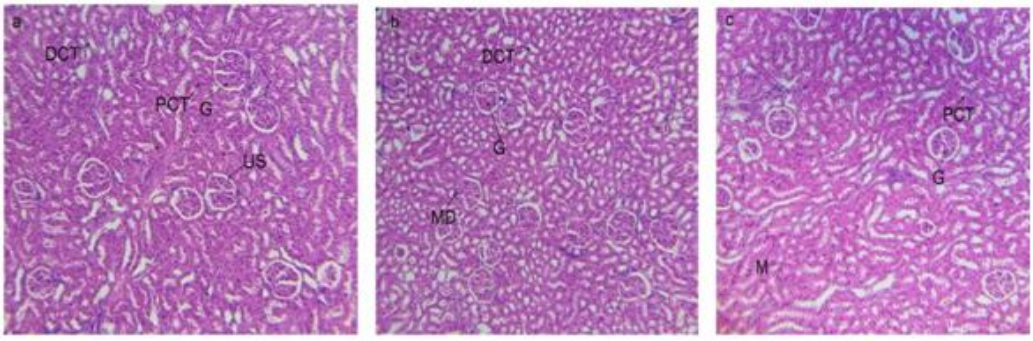


Figure 11 Photomicrographs of the kidney sections. (H & E 10x). (a) Control rats, (b) High dose (c) Low dose PCT= proximal convoluted tubule, DCT= distal convoluted tubule, MD= macula densa, G= glomerulus, US= urinary space, M = Medullary region

5. Conclusion

The acute toxicity study of the SAME did not produce adverse effects on the behavior and gross pathology of the rats at treated doses. Therefore, the oral LD_{50} of the methanolic extract of the Sphaeranthus amaranthoides was greater than 2000mg/kg. Meanwhile, subacute toxicity study of the SAME did not adversely affect the body weight and hematological and biochemical parameters of tested doses. There were no signs of toxicity observed in the kidney and liver sections of treated rats. However, well designed sub chronic and chronic toxicity studies should be carried out in order to set the clear picture of the safety of the plant part before develop in Sphaeranthus amaranthoides based health product.

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