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AQUEOUS ETHANOLIC EXTRACT OF *ACALYPHA INFERNO* LEAVES IS SAFE IN ANIMALS

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Keywords:

Acalypha inferno, Medicinal plants, Acute toxicity, Subchronic toxicity, Biochemical parameters

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ABSTRACT: *Acalypha inferno*(AI) is a well-known ornamental plant, presently with no known documentation concerning its therapeutic use and safety profile. However, other plants of the same genus, Acalypha, have been reported to have various uses ranging from food to medicine with their related adverse effects. This study therefore focused on the acute and subchronic toxicity effects of aqueous ethanolic leaf extract of *Acalypha inferno* leaves (AIE) in animals. The acute toxicity study was performed using the fixed dose method. In the subchronic assessment, both male and female rats were separately administered with AIE at doses of 100 mg/kg, 250 mg/kg and 500 mg/kg twice daily for 28 days. Change in body weight, relative organ weight, haematological parameters and biochemical parameters were recorded and analysed. In the acute toxicity study, no deleterious effect was observed up to 5000 mg/kg, hence the $LD_{50} \geq 5000$ mg/kg. In the subchronic toxicity study, significant increase in weight was observed at all doses in male rats whilst a significant decrease was observed only at 500 mg/kg in female rats. A significant increase in weight of uterus was also observed at 100 mg/kg and 250 mg/kg. Decreased levels of ALT, AST, creatinine, WBC and P-LCR were observed, whereas increasing levels of FBG, platelet count and total bilirubin were observed in male rats at high doses. AIE produced no significant adverse effects and could therefore be considered safe with controlled use.

INTRODUCTION: Medicinal plants are generally plants with therapeutic and pharmaceutical properties. Over the years, they have gained great popularity not just amongst the folklores, but also in modern medicines where they are used in primary healthcare. A number of conventional drugs produced today contain vast variety of pharmacologically active components found in these plants. These phytochemicals are a totality of secondary metabolites produced and accumulated by plants. They include terpenoids, glycosides, flavonoids, alkaloids, saponins, sterols and volatile oils [1]. With these diverse ingredients present, medicinal plants do not customarily aim at a particular ailment, but as a source of holistic array of chemicals focusing on the entire health of an individual. For this reason, fewer side effects are reported as compared to conventional drugs and hence they are thought to be safe and as effective as conventional drugs.

However, some allelochemicals, pathogens, agro-chemical residues, phytotoxins, fungal toxins and heavy metals present, pose several health threats when consumed [2]. This therefore raises a concern of safety, hence the need for toxicity studies. The plant of study was the *Acalypha inferno* (family Euphorbiaceae), commonly known as the Flame Copper leaf, which originated from the South Pacific Island [3].

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Presently, *Acalypha inferno* has no known documentation on uses, phytochemistry or adverse effects, but is known to be a potent phytoremediator of zinc from contaminated soils [4]. Nevertheless, several species of the genus, Acalypha are known for various ethnopharmacological uses. In Tanzania, leaf decoction of *Acalypha fruticosa*is used in treating epilepsy and various fungal infections [5]. In southern Nigeria, the leaves of *Acalypha godseffiana*, known to be rich in saponins, flavonoids, carotenoids and Vitamin C, areeaten as vegetables to manage hypertension and diabetes [6, 7].

FIG. 1: *ACALYPHA INFERNO* PLANT [12]

A study by Onocha *et al*., [8] also revealed *Acalypha hispida*as a potent scavenger of free radicals minimizing oxidative damage in living cells. In an acute toxicity study of *Acalypha fruticosa*, no toxic symptoms were observed in rabbits upon the administration of methanol extract of the aerial parts of the plant, at a single dose level of 5 g/kg [9].

Nonetheless, a study by Iniaghe *et al*., [10] revealed a decline in the Mean Corpuscular Volume (MCV) in wister albino rats treated with aqueous extract of *Acalypha wilkesiana* leaves. This is the potential cause of microcytic red blood cell formation which could interfere with iron uptake. *Acalypha hispida*, *Acalypha wilkesiana* and *Acalypha pendula* have all been assigned toxicity classes of 2 and 4, inferring they could cause minor ailments such as diarrhoea on ingestion and induce skin irritation respectively [11].

Current studies on AI in our laboratory has demonstrated the 50 % hydroethanolic extract to be rich in triterpenoids, sterols, alkaloids, coumarins, flavonoids, saponins, glycosides and hydrolysable tannins; high free radical scavenging activity and total phenols and significant antibacterial activity against some clinical bacterial isolates. The presence of iron and zinc were also demonstrated in both raw and crude extracts. The current study reported the acute can subchronic toxicity of the hydroethanolic extract in animals.

MATERIALS AND METHODS:

Plant Preparation and Extraction: The leaves of *Acalypha inferno* were collected from the forecourts of Biochemistry Annex in October, 2016 before 9:00 am, washed, shade dried and milled. The plant material was authenticated at the Department of Pharmacognosy, KNUST-Kumasi by Dr. George Sam (Taxonomist) and a voucher specimen deposited at the herbarium for reference. The 50 % hydro-ethanolic extract was prepared by dissolving 100 g of the sample into 1000 ml of 50% hydroethanol (50:50 v/v ethanol: water).

The mixture was left on a mechanical shaker for 24 hours at room temperature. The mixture was filtered and concentrated using a Heidoph Rotary Evaporator (Germany) at 60°C under reduced pressure. Plant extracts were freeze dried to obtain the *Acalypha inferno* aqueous-ethanolic leaf extract (AIE). This was re-dissolved in normal saline and respective doses prepared for the study. All extractions were performed in the laboratories of the Department of Biochemistry and Biotechnology, KNUST-Kumasi.

Animals: Swiss albino mice of either sex or wistar albino rats of both sexes were obtained from the animal facility of the University of Ghana School of Medical Sciences, Accra-Ghana. The animals were kept in aluminum cages bedded with wood shavings at the animal holding facility of the Department of Biochemistry and Biotechnology, KNUST.

They were allowed to adapt to the laboratory conditions for 7 days. Standard laboratory conditions of 24 ºC - 26 ºC temperature and relative humidity of 40% - 70% were observed. Animals had free access to food and water within this period and throughout the experiment. They were marked exclusively on their tails using permanent markers for easy identification. All animal experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA)

and Guide for Care and Use of Laboratory Animals [13]. Animals were handled humanely throughout the duration of the experiment.

Acute Oral Toxicity Studies: Swiss albino mice of either sex were used for the acute oral toxicity studies. The mice were put in 5 groups of 5 animals each. Treatment began after an overnight fast with doses selected based on the fixed dose method [14]. The control group, I, received 0.3 ml normal saline, whereas the treated groups, II, III, IV and V, received 100 mg/kg b.wt., 1000 mg/kg b.wt., 2500 mg/kg b.wt. and 5000 mg/kg b.wt. respectively. Doses were administered orally with the aid of a feeding needle. After treatment, mice were observed for signs of toxicity and mortality within the first critical 4 hours, then daily for 7 days. The Oral Median Lethal Dose (LD_{50}) was calculated as the geometric mean of the dose that caused 0% and 100% mortality in the Swiss albino mice. This was used to guide the selection of three doses (100 mg/kg, 250 mg/kg and 500 mg/kg) for subchronic toxicity studies.

Subchronic Toxicity Studies: The subchronic toxicity studies was carried using methods described earlier [15] using 16 male and 16 female Wistar albino rats which were put in 4 groups of 4 rats each. The control group, I, received 1 ml /kg b.wt normal saline whereas the treated groups, II, III and IV received 100 mg/kg b.wt., 250 mg/kg b.wt. and 500 mg/kg b.wt. AIE respectively after an over night fast. The animals were treated with AIE twice daily for 28 days and observed daily for signs of toxicity.

Effect of AIE on Body Weight of Animals: Rats in each group were weighed on the first day (D0) and thereafter, at the end of every four days (D4, D8 …D28) using a mass balance. The percent change in body weight was calculated using the formula:

$$Percent\ Change\ in\ Body\ Weight = \frac{Weightn - Weight_0}{Weight_0}\ X\ 100\%$$

Where
$Weight_n$ is the weight on Day 4, D8 … D28 and $Weight_o$ is the weight on the first day (D0).

Effect of AIE on some Haematological Parameters of animals: After 28 days of treatment, the animals were sacrificed by ether anesthesia after an overnight fast. Incisions were quickly made in the cervical regions of sacrificed animals using sterile blade and blood collected into EDTA tubes for haematology analysis using Sysmex Haematology System. The parameters; Haemoglobin (HGB), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet count, Lymphocytes, Neutrophils, Haematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Plateletcrit, Platelet Distribution Width (PDW) and Platelet Larger Cell Ratio (P-LCR) were determined.

Effect of AIE on some Biochemical Parameters of Animals: Part of blood samples were collected into gel activated tubes, left to clot and centrifuged for 10 minutes at 3500 rpm. The serum obtained was analysed for the levels of alanine amino transferase (ALT), aspartate amino transferase (AST), total bilirubin, total cholesterol (TC), high density lipoproteins (HDL), Low density lipoprotein (LDL), triglycerides (TG), creatinine, urea and fasting blood glucose using the Cobas Integra Analyzer with reagents for fortress diagnostics (UK).

Effect of AIE on Relative Organ Weights: Major body organs which include liver, heart, kidney, spleen, stomach, testes (male) and uterus (female) were excised washed with buffered normal saline solution, blotted dry with tissue paper, observed macroscopically and weighed to obtain the Absolute Organ Weight (AOW). The Relative Organ Weight of each organ was calculated using the formula:

$$Relative\ Organ\ \ Weight = \frac{Absolute\ Organ\ Weight}{Body\ Weight\ at\ sacrifice}\ X\ 100\%$$

Statistical Analysis: Experimental results were expressed as mean ± SEM. Differences in mean were assessed using one-way ANOVA followed by the Neuman-Keuls multiple comparison test at significance level of $p<0.05$. All data was evaluated using the GraphPad Prism 6 for Windows.

RESULTS:

Acute toxicity of AIE: In the acute toxicity study, no adverse effects were observed at all doses of 100 mg/kg, 1000 mg/kg, 2500 mg/kg and 5000 mg/kg b.wt. There were no signs of paw licking,

stretching, change in eye colour and nature of stool. No mortality was also observed up to 5000 mg/kg of AIE. Therefore, the LD_{50} is estimated at $LD_{50} \geq$ 5000 mg/kg in mice.

Subchronic Toxicity of AIE: In subchronic toxicity, diarrhoeal symptoms were observed in female rats administered doses of 250 mg/kg and 500 mg/kg on Day 4 which diminished by Day 8.

Effect of Treatment on Percentage Change in Body Weight of Animals: From **Table 1**, significant increase in weight was observed at all doses in male rats with 100 mg showing the highest significance, followed by 250 mg then 500 mg. In female rats a significant decrease was observed at 500 mg by the end of the treatment period, whereas no significant changes were observed at 100 mg and 250 mg.

Effect of Treatment on Relative Organ Weight: From **Table 2** no significant changes were observed in all organs except for a significant increase in the weight of the uterus at 100 mg ($p<0.05$) and 250 mg ($p < 0.01$).

TABLE 1: EFFECT OF AIE ON THE PERCENTAGE CHANGE IN BODY WEIGHTSOF ANIMALS

Male	Normal	100 mg	250 mg	500 mg
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	7.55 ± 2.17	21.07 ± 2.07*	14.25 ± 3.04	13.78 ± 3.23
8	13.08 ± 0.96	34.10 ± 3.14****	23.31 ± 3.18	22.23 ± 3.31
12	15.83±3.10	36.95 ± 3.32****	27.34 ± 3.96	24.66 ± 2.90
16	16.32 ± 4.46	52.04 ± 4.34****	38.11 ± 3.98****	33.98 ± 3.70***
20	22.71 ± 4.38	53.83 ± 3.22****	40.05 ± 3.80***	38.45 ± 2.58**
24	24.99 ± 3.13	43.95 ± 2.93***	30.97 ± 3.80	29.56 ± 2.44
28	26.66 ± 2.82	57.49 ± 3.89****	40.99 ± 4.58**	41.74 ± 2.69**
Female	**Normal**	**100 mg**	**250 mg**	**500 mg**
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	14.28 ± 1.58	14.88 ± 2.93	11.60 ± 0.94	8.48 ± 1.06
8	18.68 ± 1.91	17.70 ± 2.90	13.51 ± 1.73	9.83 ± 2.59
12	18.93 ± 1.65	16.65 ± 1.95	15.38 ± 1.86	10.51 ± 3.69
16	16.06 ± 1.48	13.66 ± 2.65	14.20 ± 1.60	9.14 ± 2.85
20	30.32 ± 1.61	26.39 ± 3.74	20.11 ± 3.22	15.55 ± 4.64**
24	28.50 ± 2.00	24.45 ± 4.99	19.42 ± 4.37	13.72 ± 4.01**
28	36.01 ± 1.20	31.41 ± 5.43	25.81 ± 5.28	20.37 ± 3.78**

Mean ± SEM; Statistical significance; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

TABLE 2: EFFECT OF AIE ON THE RELATIVE ORGAN WEIGHT (ROW) OF ANIMALS

Male	Normal	100 mg	250 mg	500 mg
Liver	2.69 ± 0.03	2.62 ± 0.14	2.44 ± 0.04	2.56 ± 0.15
Lungs	0.66 ± 0.05	0.64 ± 0.07	0.77 ± 0.07	0.79 ± 0.07
Stomach	0.59 ± 0.01	0.68 ± 0.02	0.63 ± 0.04	0.70 ± 0.03
Kidneys	0.62 ± 0.04	0.71 ± 0.01	0.71 ± 0.03	0.69 ± 0.03
Testes	1.22 ± 0.08	1.25 ± 0.13	1.18 ± 0.08	1.31 ± 0.09
Heart	0.33 ± 0.01	0.35 ± 0.01	0.39 ± 0.01	0.34 ± 0.01
Spleen	0.22 ± 0.014	0.25 ± 0.02	0.26 ± 0.02	0.25 ± 0.02
Female	**Normal**	**100 mg**	**250 mg**	**500 mg**
Liver	2.48 ± 0.06	2.45 ± 0.04	2.52 ± 0.06	2.61 ± 0.09
Lung	0.92 ± 0.12	0.83 ± 0.17	0.83 ± 0.09	0.66 ± 0.04
Stomach	0.71 ± 0.04	0.65 ± 0.04	0.68 ± 0.04	0.75 ± 0.05
Kidneys	0.65 ± 0.03	0.66 ± 0.02	0.64 ± 0.05	0.74 ± 0.03
Uterus	0.30 ± 0.03	0.44 ± 0.07*	0.46 ± 0.04**	0.32 ± 0.04^{+}
Heart	0.36 ± 0.02	0.37 ± 0.01	0.38 ± 0.01	0.41 ± 0.01
Spleen	0.30 ± 0.04	0.23 ± 0.02	0.27 ± 0.01	0.27 ± 0.02

Mean ± SEM; Statistical significance; *p<0.05, **p<0.01 compared with Normal, +p<0.05 compared with 100 mg group.

Effect of Treatment on Haematological Indices of Animals: There was significant decrease in WBC count at 500mg ($p<0.05$) amongst male rats, whereas amongst female rats significant increase in platelet levels was recorded at 250 mg ($p<0.05$) and 500 mg ($p<0.05$) with significant increase in Platelet Larger cell ratio (P-LCR) at 100 mg ($p<0.05$).

TABLE 3: EFFECT OF AIE ON THE HAEMATOLOGICAL PARAMETERS OF ANIMALS

Male	Normal	100 mg	250 mg	500 mg
WBCx10^3/µL	8.82 ± 1.55	7.50 ± 0.87	9.33 ± 0.96$^+$	6.13 ± 1.14*
RBCx10^6/µL	7.44 ± 0.38	7.16 ± 0.23	7.74 ± 0.20	7.41 ± 0.14
HGB g/dL	12.58 ± 0.25	12.08 ± 0.12	12.58 ± 0.39	12.65 ± 0.21
HCT%	43.58 ± 1.82	41.90 ± 0.52	44.10 ± 1.43	43.40 ± 0.80
MCV fL	58.70 ± 0.83	58.65 ± 1.26	56.93 ±0.42	58.60 ± 0.72
MCH pg	16.98 ± 0.65	16.90 ± 0.47	16.23 ± 0.17	17.08 ± 0.19
MCHC g/dL	28.98 ± 0.78	28.83 ± 0.36	28.53 ± 0.20	29.15 ± 0.09
PLT	794.80 ± 59.19	899.80 ± 50.52	859.80 ± 38.89	825.50 ± 74.01
%LYM	74.95 ± 1.70	74.15 ± 2.82	70.25 ± 2.05	69.6 ± 32.55
%LEU	25.05 ± 1.70	33.28 ± 8.78	29.75 ± 2.05	30.3 ± 82.55
RDW-SD	29.75 ± 0.63	29.43 ± 0.34	29.75 ± 0.56	28.5 ± 80.41
RDW-CV	12.45 ± 0.51	12.18 ± 0.23	12.93 ± 0.43	11.6 ± 50.25
PDW	7.95 ± 0.34	7.70 ± 0.11	8.10 ± 0.11	8.05 ± 0.087
MPV	7.00 ± 0.21	6.88 ± 0.06	6.90 ± 0.00	7.03 ± 0.07
P-LCR	5.73 ± 0.92	4.88 ± 0.26	5.10 ± 0.18	5.68 ± 0.18
PCT	0.55 ± 0.03	0.62 ± 0.04	0.59 ± 0.03	0.58 ± 0.05
Female	**Normal**	**100 mg**	**250 mg**	**500 mg**
WBCx10^3/µL	5.43 ± 0.28	6.30 ±1.05	5.83 ± 0.53	5.18 ± 0.61
RBCx10^6/µL	7.05 ± 0.24	6.96 ± 0.27	7.36 ± 0.24	7.44 ± 0.18
HGB g/dL	12.38 ± 0.27	11.56 ± 0.60	13.23 ± 0.45	13.15 ± 0.25
HCT%	41.85 ± 1.00	40.90 ± 1.80	44.03 ± 1.12$^+$	44.05 ± 1.31$^+$
MCV fL	59.45 ± 0.96	58.78 ± 0.50	59.85 ± 0.58	59.20 ± 0.43
MCH pg	17.60 ± 0.44	16.60 ± 0.42	18.00 ± 0.19	17.70 ± 0.17
MCHC g/dL	29.55 ± 0.27	28.28 ± 0.77	30.03 ± 0.46	29.88 ± 0.33
PLT	827.00 ± 48.78	818.30 ± 52.59	1152.00 ± 77.68*	1113.00 ± 99.36*
%LYM	79.98 ± 5.82	81.60 ± 6.04	76.95 ± 5.89	74.25 ± 1.11
%NEU	20.03 ± 5.82	21.48 ± 5.04	23.05 ± 5.89	25.75 ± 1.11
RDW-SD	28.48 ± 0.24	28.63 ± 0.57	28.15 ± 0.25	27.78 ± 0.13
RDW-CV	11.23 ± 0.35	11.58 ± 0.54	10.95 ± 0.06	10.78 ± 0.19
PDW	7.95 ± 0.09	8.33 ± 0.08	7.93 ± 0.11	7.43 ± 0.21
MPV	6.95 ± 0.03	7.08 ± 0.07	6.83 ± 0.13	6.58 ± 0.11
P-LCR	5.88 ± 0.32	6.63 ± 0.57*	4.95 ± 0.47$^+$	4.50 ± 0.61$^+$
PCT	0.58 ± 0.03	0.58 ± 0.04	0.79 ± 0.04	0.73 ± 0.06

Mean ± SEM; Statistical significance; *p<0.05 compared with Normal, +p<0.05 compared with 100 mg group.

TABLE 4: EFFECT OF AIE ON THE BIOCHEMICAL PARAMETERS OF ANIMALS

Male	Normal	100 mg	250 mg	500 mg
Creat µmol/L	44.98 ± 1.40	38.23 ± 5.93	42.03 ± 4.71	33.70 ± 4.24
Urea mmol/L	9.14 ± 0.29	5.41 ± 0.79	5.28 ± 0.40	5.79 ± 0.31
ALT U/L	48.40 ± 6.46	33.33 ± 2.21	31.93 ± 1.28	35.68 ± 4.13
AST U/L	127.33 ± 2.18	92.05 ± 8.00***	118.55 ± 6.84	125.40 ± 6.43
TBil mmol/L	17.98 ± 2.10	21.60 ± 0.98	37.30 ± 0.95*	35.88 ± 3.59*
TChol	1.25 ± 0.05	1.20 ± 0.09	1.30 ± 0.09	1.32 ± 0.06
TTrigs	0.69 ± 0.12	0.58 ± 0.04	0.72 ± 0.09	0.46 ± 0.04
HDL	0.27 ± 0.02	0.35 ± 0.05	0.21 ± 0.03	0.23 ± 0.02
LDL	0.67 ± 0.02	0.58 ± 0.12	0.77 ± 0.07	0.88 ± 0.04$^+$
Glucose	3.34 ± 0.26	4.09 ± 0.64	4.31 ± 0.12	4.92 ± 0.26*
Male	**Normal**	**100 mg**	**250 mg**	**500 mg**
Creat µmol/L	50.28 ± 2.38	40.13 ± 8.13	37.45 ± 9.39	60.25 ± 3.60
Urea mmol/L	7.92 ± 0.55	5.94 ± 0.71	7.35 ± 0.41	7.38 ± 0.82
ALT U/L	42.43 ± 1.89	33.65 ± 3.76$^+$	31.95 ± 2.88	14.08 ± 1.65*
AST U/L	143.08 ±3.69	127.43 ± 2.14	104.48 ± 5.86**	99.901 ± 2.34***
TBil mmol/L	12.50 ± 0.74	13.05 ± 1.10	11.68 ± 1.28	8.38 ± 0.89*
TChol	1.35 ± 0.12	1.38 ± 0.14	1.20 ± 0.03	1.30 ± 0.09
TTrigs	0.95 ± 0.09	0.87 ± 0.18	0.93 ± 0.14	0.91 ± 0.23
HDL	0.22 ± 0.03	0.26 ± 0.04	0.22 ± 0.04	0.27 ± 0.07
LDL	0.70 ± 0.12	0.72 ± 0.14	0.56 ± 0.08	0.61 ± 0.09
Glucose	3.65 ± 0.13	3.91 ± 0.21	4.10 ± 0.24	3.53 ± 0.07

Mean ± SEM; Statistical significance; *p<0.05, **p<0.01, ***p<0.001 compared with Normal.

Effect of treatment on Biochemical Parameters of animals: From **Table 4**, significant decrease in hepatic biomarkers (AST and ALT) were recorded at 250 mg and 500 mg/kg groups respectively amongst female rats, whereas significant decrease was recorded in AST levels at only 100mg in male rats. Total bilirubin was also significantly decreased at 500 mg in female rats whilst levels significantly increased at 250 mg and 500 mg in male rats. Notwithstanding, significant increase in blood glucose level at 500 mg amongst male rats was observed.

DISCUSSION: Medicinal plants have been duly accepted over the years for their exceeding amounts of therapeutic benefits. Although useful, some might be lethal when misused. As a matter of fact, it is highly expedient that toxicity studies be carried out to ensure the safety of consumers.

In the acute toxicity study, no adverse or toxic effects were observed and LD_{50} was estimated at $LD_{50} \geq 5000$ mg/kg indicating the extract is safe at the acute level. In the subchronic toxicity study, diarrhoeal symptoms observed on the fourth day amongst female rats at 250 mg and 500 mg could be because of side effects of the extract administered. The symptoms, which however, diminished by the eight day could be attributed to the active role played by zinc as discovered in a heavy metal analysis (unpublished data). In a meta-analyses by Salvatore *et al.*,[16] and Hoque and Binder [17], zinc supplementation was confirmed to lessen the duration and severity of acute and chronic diarrhoea and reduce the risk of a recurrent episode for the next 2-3 months. These symptoms being present in only female rats could be because they are more sensitive to acute toxic effects of chemicals than male rats [18].

At termination, a significant increase in body weight was recorded at all doses for male rats with 100 mg recording the highest increase in weights. This suggests AIE had no adverse effects on the diet of male animals, which showed normal appetite for food, hence weight gain. Conversely, there were no significant changes in weight amongst female rats at 100 mg and 250 mg, which also indicates no adverse effect on diet. However significant decrease at 500 mg, as compared to the normal, could be attributed to some physiological process, for instance with the diarrhoea observed, which could alter the appetite, diet and ability to retain the nutritional benefits, leading to poor nutrition and reduced weight.

In the macroscopic examination of organs, no colour change or serrations was observed with respect to the normal. There were also no significant changes in weight observed in all organs except for a significant increase in uterus at 100 mg and 250 mg. Organ hypertrophy is usually an indication of major toxicity of a chemical substance as well as accumulation of a chemical substance in the target organ [19, 20]. It is also a sensitive indicator of chemical changes to the organs [21]. Enlarged uterus is also usually associated with hormonal imbalance, oestrogen dominance, fibroids and adenomyosis[22]. Therefore results obtained indicates possible toxicity to the uterus and suggests further reproductive and teratogenic studies.

Several different prescribed drugs, including antibiotics and chemotherapy, are known to decrease WBC production or destroy WBC [23]. In this study, decreased WBC count at 500 mg could indicate the extract, when overdosed could have a negative effect on WBC production and break down. Significant increase in platelet levels observed at 250 mg and 500 mg could be because of the presence of zinc, which is known to enhance platelet activation. This could however lead to abnormal blood clotting in the cardiovascular system, which could block the flow of blood to vital organs, depriving them of oxygen and nutrients.

Elevated levels of hepatic biomarkers ALT and AST are usually indicators of a damaged liver whereas low levels usually have no clinical significance and are associated with a healthy liver [24]. Therefore in this study, decreased levels of these markers as shown in **Table 4** indicates the extract has no significant effect on the liver. Also, low bilirubin levels in female rats at 500 mg indicates a healthy liver performing its normal function of conjugation and elimination of bilirubin. However, the significant increase in bilirubin levels at 250 mg and 500 mg in male rats could be related to normal physiological activities such as haemolysis**.**

In the renal function tests, no significant changes were observed in both urea and creatinine levels at

all doses, but for a significant decrease in creatinine level at 500 mg in male rats. High clearance of creatinine and urea from the blood is an indication a proper functioning kidney. Therefore, suggest AIE has no adverse effect on the kidney and even possesses some nephroprotective activity. In lipid profile, there were no significant changes in LDL, HDL total cholesterol and triglycerides at all doses in both male and female rats. These are usually markers of cardiovascular diseases. Hence result indicates the extract, AIE poses no risk of causing or aggravating cardiovascular diseases.

High blood glucose level as observed at 500 mg in male rats is associated with hormone action. Whilst insulin, facilitates the uptake of glucose from food ingested into the body cells for energy production, glucagon breaks down glycogen stored in the liver and muscles, releasing glucose to provide energy when levels from food is low or not available [25]. Result obtained in this study could be due to some imbalance in hormonal action at the threshold dose of 500 mg in these non-diabetic male rats.

CONCLUSION: *Acalypha inferno* had an $LD_{50} \geq$ 5000 mg/kg indicating its safety at the acute level. It also possesses some hepato and nephroprotective properties although it could induce some hyper-glycemic activity at high doses and also induce some negative effect on uterine activity. In general, findings show *Acalypha inferno* has no prospective adverse or toxicological effect and suggests it would be safe with controlled use.

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