



Received on 25 August, 2017; received in revised form, 24 December, 2017; accepted, 30 December, 2017; published 01 June, 2018

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

Christopher Larbie^{*}, H. N. Nyarko, J. J. Tofah and D. Torkornoo

Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

Keywords:

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

Correspondence to Author:

Dr. Christopher Larbie

Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

E-mail: clarbie.cos@knu.edu.gh

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 oils¹. 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, agrochemical residues, phytotoxins, fungal toxins and heavy metals present, pose several health threats when consumed². 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 Island³.

<p style="text-align: center; font-weight: bold; font-size: small;">QUICK RESPONSE CODE</p>	<p style="text-align: center; font-weight: bold; font-size: small;">DOI:</p> <p style="text-align: center; font-size: small;">10.13040/IJPSR.0975-8232.9(6).2273-80</p> <hr style="border: 0; border-top: 1px solid black; margin: 5px 0;"/> <p style="text-align: center; font-size: small;">Article can be accessed online on: www.ijpsr.com</p>
<p style="font-size: x-small;">DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(6).2273-80</p>	

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 soils⁴. 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 infections⁵. In southern Nigeria, the leaves of *Acalypha godseffiana*, known to be rich in saponins, flavonoids, carotenoids and Vitamin C, are eaten as vegetables to manage hypertension and diabetes^{6,7}.



FIG. 1: ACALYPHA INFERNO PLANT¹²

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/kg⁹.

Nonetheless, a study by Inianghe *et al.*,¹⁰ revealed a decline in the Mean Corpuscular Volume (MCV) in wistar 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 respectively¹¹.

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 and 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 °C - 26 °C 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 Animals¹³. 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 method¹⁴. 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 (LD₅₀) 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 earlier¹⁵ 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 overnight 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:

$$\text{Percent Change in Body Weight} = \frac{\text{Weight}_n - \text{Weight}_0}{\text{Weight}_0} \times 100\%$$

Where

Weight_n is the weight on Day 4, D8 ... D28 and Weight₀ 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:

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

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 LD₅₀ is estimated at LD₅₀ ≥ 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 WEIGHTS OF 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 LD₅₀ was estimated at LD₅₀≥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 eighth day could be attributed to the active role played by zinc as discovered in a heavy metal analysis (unpublished data). In a meta-analysis by Salvatore *et al.*,¹⁶ and Hoque and Binder¹⁷, 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 rats¹⁸.

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 organs²¹. Enlarged uterus is also usually associated with hormonal imbalance, oestrogen dominance, fibroids and adenomyosis²². 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 WBC²³. 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 liver²⁴. 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 available²⁵. 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 LD₅₀ ≥ 5000 mg/kg indicating its safety at the acute level. It also possesses some hepato and nephroprotective properties although it could induce some hyperglycemic 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.

ACKNOWLEDGEMENT: The technical assistance of Mr. Frank Bonsu at the Clinical Analyses Laboratory (CANLab) of the Department of Biochemistry and Biotechnology, KNUST is greatly appreciated.

REFERENCES:

- Motaleb MA: Selected Medicinal Plants of Chittagong Hill Tracts. International Union for Conservation of Nature (IUCN), Bangladesh, Edition 1st, 2011.
- Efferth T and Kaina B: Toxicities by herbal medicines with emphasis to traditional Chinese medicine. *Current Drug Metabolism*, 2011; 12(10): 989-996.
- Magnetic Island Plant: *Acalypha inferno*. 2016. <http://www.somemagneticislandplants.com.au/index.php/plants/423-acalypha-inferno> on 16th October, 2016 at 07:45pm.
- Abioye OP, Ekundayo OP and Aransiola SA: Bioremoval of zinc in polluted soil using *Acalypha Inferno*. *Research Journal of Environmental Sciences*, 2015; 9: 249-255.
- Gopalakrishnan S, Saroja K and Elizabeth JD: Chemical investigation of aerial part of *Acalypha fruticosa* Forssk. *Der Pharma Chemica*, 2012; 2(5): 383-389.
- Ikewuchi JC, Anyadiegwu A, Ugono EY and Okungbowa SO: Effect of *Acalypha wilkesiana* Muell. Arg. on plasma sodium and potassium concentration of normal rabbits. *Pakistan Journal of Nutrition*, 2008; 7(1): 130-132.
- Ikewuchi CC and Ikewuchi JC: Comparative study on the Vitamin composition of some common Nigerian medicinal plants. *Pac J Sci Technol.*, 2009; 10: 367-71.
- Onocha PA, Oloyede GK and Afolabi QO: Phytochemical investigation, cytotoxicity and free radical scavenging activities of non-polar fractions of *Acalypha hispida* (leaves and twigs). *Excli Journal*, 2011; 10: 1-8.
- El-Shaibany A, AL-Habori M, Al-Massarani S, Al-Adhl A and Michalsen A: Hypoglycaemic activity of *Acalypha fruticosa* Forssk extracts in normal rabbits. *Tropical Journal of Pharmaceutical Research*, 2015; 14(8): 1445-1450.
- Iniaghe OM, Egharevba O and Oyewo EB: Effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male wistar albino rats. *British Journal of Pharmaceutical Research*, 2013; 3(3): 465-471.
- UCANR: Toxic Plants (by scientific name). 2016. http://ucanr.edu/sites/poisonous_safe_plants/Toxic_Plants_by_Scientific_Name_685/ on 25th October, 2016 at 05:15pm.
- Top Tropicals: Tropical plant catalogue: *Acalypha inferno*. 2016. http://toptropicals.com/catalog/uid/acalypha_inferno.htm on 16th October, 2016 at 06:50pm.
- National Research Council: Guide for care and use of laboratory animal, National Academic Press, Washington, Edition 8th, 2011; 43-45.
- OECD: OECD Guideline for Testing of Chemicals, Acute Oral Toxicity – Fixed Dose. Procedure. 2001 https://ntp.niehs.nih.gov/iccvam/suppdocs/fedddocs/oced/oced_gl420.pdf
- Larbie C, Owusu KP, Torkornoo D and Asibey O: Acute and sub-chronic toxicity of aqueous ethanolic extract of *Ficus pumila* leaves in rats. *European Journal of Biomedical and Pharmaceutical Sciences*, 2016; 3(8): 22-27.
- Salvatore S, Hauser B and Devreker T: Probiotics and zinc in acute infectious gastroenteritis in children: are they effective? *Nutrition*, 2007; 23: 498-506.
- Hoque KM and Binder HJ: Zinc in the treatment of acute diarrhoea: current status and assessment. *Gastroenterology*, 2006; 130(7): 2201-2205.
- Gad SC and Chengelis CP: Acute toxicity testing perspectives and horizons. The Telford Press, Caldwell, 1988; 156.
- OECD: OECD guidelines for testing of chemicals: Repeated Dose 90-day oral toxicity study in rodents. Environmental Health and Safety Monograph Series on Testing and Assessment, No. 408, OECD Publishing, Paris, 1998; 1.
- OECD: OECD guideline for testing of chemicals: Repeated Dose 28-Day Oral Toxicity Study in Rodents. Environmental Health and Safety Monograph Series on Testing and Assessment, No. 407, 2008; 1-2.
- Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK and Schafer K: Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. *Toxicologic Pathology*, 2007; 35(5): 742-750.

22. Lee JR, Hanley J and Hopkins V: Enlarged uterus: fibroids, hormone imbalance, estrogen dominance, 2017. <http://www.johnleemd.com/enlarged-uterus-fibroids-hormone-imbalance-estrogen-dominance.html> on 4th April, 2017 at 05:43.
23. Healthgrades: Low white blood cell count, 2016. <https://www.healthgrades.com/symptoms/low-white-blood-cell-count> on 22nd May, 2017 at 8:16pm.
24. Giannini EG, Testa R and Savarino V: Liver enzyme alteration: a guide for clinicians. CMAJ, 2005; 172(3): 367-379.
25. Warshaw HS: 2017. <http://www.diabeticlivingonline.com/monitoring/blood-sugar/why-are-fasting-blood-glucose-numbers-highon> March 13, 2017 at 10:56am.

How to cite this article:

Larbie C, Nyarko HN, Tofah JJ and Torkornoo D: Aqueous ethanolic extract of *Acalypha inferno* leaves is safe in animals. Int J Pharm Sci Res 2018; 9(6): 2273-80. doi: 10.13040/IJPSR.0975-8232.9(6).2273-80.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)