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European Journal of Experimental Biology, 2011, 1 (4):115-124

ISSN: 2248 –9215

Evaluation of acute and subchronic toxicity of *Annona Muricata* (Linn.) aqueous extract in animals

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ABSTRACT

Annona muricata is an important underutilized plant with established hepatoprotective effect against carbon tetrachloride and acetaminophen and can treat hepatic jaundice. The present study was carried out to evaluate acute and subchronic toxicity in animals and also to evaluate the phytochemical profile of aqueous extract of A. muricata leaves using standard procedures. The aqueous extract contained saponins, general glycosides and flavonoides. The median acute toxicity value (LD_{50}) of the extract of A. muricata was determined to be < 5g/kg body weight. The extract lowered blood plasma glucose and low density lipoprotein (LDL-cholesterol) levels but raised high density lipoprotein (HDL-cholesterol) levels in both male and female rats. Treatment had no effect on liver, kidney, heart and stomach weight while uterus weight were increased in 1000 mg/kg and beyond. Haematological parameters, ALT, AST, ALP, urea and albumin were unaffected while creatinine levels were increased at 2500 mg. The LD_{50} value indicated the drug as being safe. The extract did not produce any toxic effect in the animals' tissues at low and moderate doses but could cause kidney damage in higher doses. Lowering of plasma glucose level and the positive effects of the extract on the cardiovascular risk factors were an indicator that the extract could have some good antidiabetic activity.

Keyword: *Annona muricata*, acute toxicity, subchronic toxicity, phytochemical.

INTRODUCTION

The use of complementary traditional medicine which include herbal medicines in the treatment of various diseases has expanded rapidly in both developed and developing countries, attributable to affordability, accessibility and efficacy [1]. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Some medicinal plants such as opium poppy have been long recognized and widely used, while

others, such as Pacific yew, the original source of the cancer drug, taxol, are relatively new arrivals to the mainstream medicine [2]. Documented and undocumented adverse drug reactions associated with herbal medicines makes it pertinent that pre-clinical toxicological studies be carried out on these natural products.

Annona muricata (Linn.) commonly called soursop or "Apre" in the local Ghanaian Twi language, is a small erect evergreen tropical fruit tree plant belonging to the family Annonaceae, growing 5 to 6 metres in height. It is underutilized [3] and is grown in Ghana mainly for ornamental purposes and for its fruits. The leaves of *A. muricata* have been reported to contain several groups of substances collectively called annonaceous acetogenins including murihexocin and annocuricin [4], annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexocin A and B, (2,4-trans)-annomuricin-D-one, 4-acetyl gigantetrocin and cis-gigantrionin [5], muricatocin A, B and C [6], and annohexocin [5]. The high potency, selectivity, wide chemical and biological diversity, and effectiveness of these compounds against microbial resistance could well make them the next class of useful natural antitumor and pesticidal agents [7] and other pharmacological effects.

The leaves of *A. muricata* have essential oils with parasiticidal, anti-diarrhoeal, rheumatological and anti-neuralgic properties [8]. The boiled water infusion of the leaves has anti-plasmodic, astringent, and gastric properties [9], help treat diabetes and gastric upset [10], jaundice [11] and used in treating kidney aliments [12]. The leaves are also hepatoprotective against carbon tetrachloride and acetaminophen induced liver damage [13] and in streptozotocin-treated diabetic rats [14]. Methanol extract of *A. muricata* exhibited antibacterial activity against some strains of *E. coli* [15].

Subchronic toxicity evaluation is required to establish potential adverse effects of this valuable underutilized fruit plant [16]. The aim of the study was to evaluate the acute and subchronic safety of *A. muricata* in animals and also to carry out the preliminary phytochemical screening.

MATERIALS AND METHODS

Plant preparations and extraction

Leaves of *Annona muricata* were collected in the month of April 2010, from the surrounding fields of Department of Biochemistry and Biotechnology Annex offices and was authenticated at the Department of Herbal Medicine, KNUST and voucher specimen (KNUST/HM1/2011/L057) deposited at the faculty herbarium. The leaves were washed, shade-dried, milled and decocted (1.41 kg with 10 L water). The aqueous extract was freeze-dried to obtain the *A. muricata* aqueous extract (AMAE) weighing 211 g (14.96% w/w yield) which as used in the study. Qualitative phytochemical screening of AMAE for secondary metabolites were carried out using standard methods [17 – 19].

Acute oral toxicity study

The toxicity study was carried out using 25 Swiss albino mice (20 – 25 g) of either sex obtained from the animal facility of the Department of Biochemistry and Biotechnology, KNUST, Kumasi-Ghana. The animals were divided into five groups of five animals per group; control group and 4 treated groups. They were maintained on standard feed (GAFCO, Tema, Ghana) and water and allowed to acclimatise for seven days to the laboratory environment before the experiment. After an overnight fast, the control group received 0.3 ml sterile distilled water while each treated group received 100, 1000, 2500 and 5000 mg/kg b.wt. administered orally

with the aid of a feeding needle connected to syringe at stated doses in appropriate volume of sterile distilled water. Doses were selected based on the fixed dose method [20]. The animals were observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days. Signs of toxicity included paw-licking, stretching, respiratory distress, diarrhoea and death were observed. The oral median lethal dose (LD_{50}) was calculated as the geometric mean of dose that caused 0 % and 100 % mortality respectively. Three dose (100, 1000 and 2500 mg/kg b.wt.) were selected for the subchronic toxicity studies [21].

Sub-acute toxicity studies of AMAE

Twenty males (210 – 260 g) and 20 females (190 – 220 g), were separately divided into four groups of 5 animals. For each sex, group I served as the vehicle control and received 1 ml/100 g b.wt. sterile distilled water daily while groups II, III and IV were administered 100, 1000, and 2500 mg extract/kg b.wt. daily in appropriate volume of distilled water for 14 days. All animals were fasted 12 hours prior to first oral drug administration and had free access to food and water throughout the duration of the experiment. They were observed daily for general signs of toxicity and mortality [1]. Rats in all groups were weighed on the first day (D0) and at the end of day 2 (D2), D4, D6, D8, D10, D12 and D14. The percent change in body weight was calculated using the formula;

$$\text{Percentage change in body weight} = 100 \times \frac{Weight_n - Weight_{initial}}{Weight_{initial}}$$

[$Weight_{initial}$: measurement on the first day (D0); $Weight_n$: measurements at end of D2, D4, …, D14]

At the end of the experiment, animals were fasted overnight and sacrificed by cervical dislocation. Incisions were quickly made in the sacrificed animal's cervical region with the aid of a sterile blade and blood samples collected from the heart were dispensed into EDTA bottles for haematological analysis using Sysmex Haematology System (USA). Determinations included packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, platelets (Plat) count, white blood cell (WBC) count and differentials, mean capsular volume (MCV), mean capsular haemoglobin (MCH), and mean capsular haemoglobin concentration (MCHC).

Portions of the blood were dispensed into plain tubes, allowed to clot and centrifuged at 3500g for 10 minutes. The sera were separated and used for the evaluation of biochemical parameters using the Cobas Integra 400 Clinical Chemistry Analyzer (Roche, USA). Determinations included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin concentrations, total cholesterol, high density lipoproteins (HDL), total triglycerides, glucose, creatinine and urea using standard kits. Low density lipoprotein (LDL) concentration was calculated using the Friedewald's equation [22].

Organs of sacrificed animals, namely liver, heart, spleen, stomach, kidneys, testes or uterus were excised, washed with normal buffered saline, weighed to obtain absolute organ weight (AOW) and observed macroscopically. The relative organ weights (ROW) were calculated for each rat using the formula;

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

Statistics

Data were analysed using GraphPad Prism 5 for Windows. The experimental results were expressed as the Mean ± standard error mean (SEM). Data were assessed by one-way ANOVA followed by Newman-Keuls multiple comparison test. Values for which $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the plant extract (Table 1) revealed the presence of saponins, condensed tannins, glycosides and flavonoids. Alkaloids and sterols were conspicuously absent.

Table 1: Phytochemical screening of the aqueous extract of *A. muricata*

Phytochemical	Presence
Saponins	+++
Condensed Tannins	+++
Flavonoids	+
Alkaloids	-
Glycosides	+++
Sterols	-

(-) Absent (+) Slightly present (+++) Abundantly present

In oral acute toxicity studies, no untoward clinical signs were observed in the rats at all the doses studied (100, 1000, 2500 and 5000 mg). There were no changes in the nature of stool, urine and eye colour. No mortality was observed at all dose levels from the critical 24 hours post administration to the end of the seventh day. Orally, 5000 mg/kg of AMAE was well tolerated in mice even after 7 days. Hence the LD_{50} was estimated to be <5000 mg/kg (orally).

Table 2: The effect of AMAE on body weight changes in the control and treat rats in subchronic toxicity studies

Dose	Vehicle	100 mg/kg	1000 mg/kg	2500 mg/kg
Males				
Day 0	216.0 ± 4.59	235.6 ± 2.29	244.0 ± 1.10	257.4 ± 1.83
Day 2	217.8 ± 4.50	238.8 ± 2.20	249.4 ± 1.94	258.6 ± 4.08
Day 4	220.2 ± 4.44	241.4 ± 2.32	252.2 ± 2.33	263.4 ± 5.53
Day 6	225.6 ± 4.83	245.8 ± 2.67	256.2 ± 1.80	260.2 ± 3.96
Day 8	226.8 ± 4.09	245.6 ± 2.79	253.8 ± 2.65	259.8 ± 4.39
Day 10	228.8 ± 3.93	250.4 ± 2.73	248.2 ± 2.31	260.2 ± 2.54
Day 12	229.6 ± 4.48	251.0 ± 2.17	248.4 ± 1.86	257.6 ± 2.54
Day 14	231.2 ± 4.64	255.6 ± 1.78	252.0 ± 2.80	256.4 ± 3.67
Female				
Day 0	192.4 ± 0.93	198.2 ± 1.50	203.2 ± 1.39	211.2 ± .177
Day 2	191.2 ± 1.20	197.6 ± 3.76	202.8 ± 1.99	212.4 ± 1.47
Day 4	192.8 ± 1.39	200.0 ± 4.10	200.4 ± 1.91	211.0 ± 1.23
Day 6	194.8 ± 1.50	202.8 ± 4.62	202.0 ± 1.38	209.0 ± 3.77
Day 8	195.6 ± 3.27	205.0 ± 4.09	200.2 ± 1.91	208.4 ± 2.64
Day 10	196.6 ± 2.44	206.4 ± 2.11	199.8 ± 0.49	208.6 ± 1.99
Day 12	196.8 ± 3.11	204.6 ± 1.60	196.6 ± 0.93	204.6 ± 2.66
Day 14	200.2 ± 2.40	205.4 ± 1.25	200.6 ± 1.33	208.4 ± 3.46

Mean ± SEM, (n = 5)

In subchronic studies, all rats used for the study appeared normal before, during and post-treatment. Mortality was not recorded at all dose levels used for the study; 100, 1000 and 2500

mg/kg b.wt. The results of the effect of the extract on the body weight of the animals compared with vehicle is as shown in Table 2 and Fig. 1. There were no significant increases in the weight of animals treated with 100 mg AMAE. However, there were significant decreases in percent body weight changes in male rats treated with 1000 mg AMAE on day 12 and 14 ($p<0.05$) and at 2500 mg on day 10 ($p<0.01$) and 12 and 14 ($p<0.001$). For female rats, significant decreases were observed at 1000 mg on day 12 ($p<0.01$) and 14 ($p<0.05$); and at 2500 mg on day 12 and 14 ($p<0.05$).

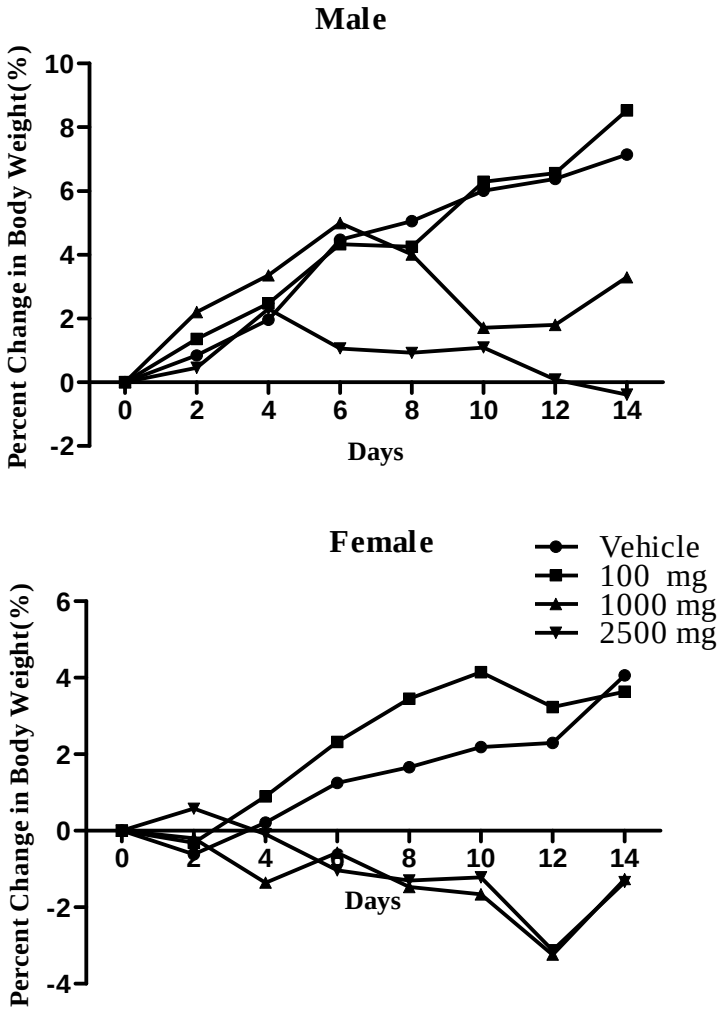

Fig. 1: Mean percentage change in body weight of control and treated animals in subchronic toxicity study. Each point represent a mean of 5 animals

The results of the effect of AMAE on absolute organ weights of male and female rats are as shown in Table 3. Macroscopic examination did not show any changes in the colour of organs of the treated animals compared with vehicle. There were no significant changes in the relative weights of the liver, kidney and heart in both males and females. Treatment had no effect on spleen, stomach and testes of male rats. For females, significant increases were observed in the relative weight of stomach ($p<0.05$) and uterus ($p<0.01$) of 1000 mg treated group compared with vehicle group.

Table 3: The effect of the extract on relative organ weight (ROW) of control and treated animals in the subchronic toxicity study

ROW (%)	Vehicle	100 mg/kg	1000 mg/kg	2500 mg/kg
Male				
Liver	2.71 ± 0.065	2.70 ± 0.072	2.79 ± 0.088	2.74 ± 0.088
Kidney	0.73 ± 0.052	0.69 ± 0.015	0.68 ± 0.019	0.66 ± 0.010
Heart	0.36 ± 0.013	0.37 ± 0.008	0.34 ± 0.004	0.38 ± 0.011
Spleen	0.23 ± 0.007	0.22 ± 0.009	0.24 ± 0.012	0.24 ± 0.007
Stomach	0.70 ± 0.023	0.60 ± 0.059	0.58 ± 0.019	0.72 ± 0.029
Testes	1.35 ± 0.054	1.24 ± 0.044	1.33 ± 0.036	1.24 ± 0.020
Female				
Liver	2.87 ± 0.059	2.65 ± 0.037	2.82 ± 0.057	2.81 ± 0.068
Kidney	0.77 ± 0.050	0.62 ± 0.007	0.64 ± 0.017	0.65 ± 0.016
Heart	0.40 ± 0.010	0.37 ± 0.004	0.40 ± 0.006	0.42 ± 0.012
Spleen	0.30 ± 0.014	0.31 ± 0.016	0.35 ± 0.005	0.31 ± 0.003
Stomach	0.75 ± 0.028	0.70 ± 0.006	0.86 ± 0.017*	0.72 ± 0.054
Uterus	0.31 ± 0.006	0.31 ± 0.023	0.42 ± 0.037**	0.38 ± 0.036

*Mean ± SEM, (n = 5), *p<0.05; **p<0.01 vs. Vehicle*

The results of the effect of AMAE on haematological indices of male and female rats is shown in Table 4. Significant increases (*p<0.01 – p<0.001*) were observed in lymphocyte levels in the animals at high doses. All other determinations showed no significant difference from vehicle group.

Table 5 summarises the effect of extract on biochemical parameters of treated animals. There were significant decreases (*p<0.01 – p<0.001*) in the serum glucose levels of male and female animals especially in rats treated with 1000 mg and beyond compared with vehicle group. There were non-significant decreases in the albumin levels coupled with significant increases (*p<0.001*) in serum creatinine levels only in animals treated with highest dose of extract (2500 mg/kg). There were significant decreases (*p<0.01*) in serum cholesterol and LDL levels in female rats treated with 100 mg/kg extract.

Table 4: Haematological profile on male and female rats administered AMAE

Male				
	Vehicle	**100 mg**	**1000 mg**	**2500 mg**
WBC x 10^3/µL	4.64 ± 0.43	5.84 ± 0.56	6.66 ± 1.33	7.26 ± 0.94
RBC x 10^6/ µL	6.45 ± 0.14	6.14 ± 0.39	6.44 ± 0.10	6.04 ± 0.20
HGB g/dL	12.46 ± 0.12	11.40 ± 0.56	12.38 ± 0.26	11.74 ± 0.72
HCT %	38.90 ± 1.12	35.02 ± 2.00	37.00 ± 0.75	35.04 ± 1.06
MCV fL	60.28 ± 0.83	57.16 ± 0.41	7.42 ± 0.63	58.10 ± 0.89
MCH pg	19.34 ± 0.55	18.64 ± 0.29	19.24 ± 0.34	19.34 ± 0.70
MCHCg/dL	32.14 ± 1.11	32.60 ± 0.31	33.48 ± 0.69	33.44 ± 1.52
%LYM	79.78 ± 2.63	83.30 ± 3.16	79.56 ± 5.62	87.64 ± 3.59**
Female				
WBC x 10^3/µL	5.86 ± 0.49	5.32 ± 0.51	5.70 ± 0.76	6.26 ± 0.53
RBC x 10^6/ µL	5.76 ± 0.17	5.16 ± 0.45	5.54 ± 0.28	5.46 ± 0.20
HGB g/dL	11.74 ± 0.31	10.10 ± 1.34	10.90 ± 0.28	11.08 ± 0.36
HCT %	34.28 ± 1.25	29.46 ± 2.53*	30.86 ± 0.92	31.34 ± 0.89
MCV fL	59.68 ± 0.66	57.12 ± 0.53	57.20 ± 0.60	57.88 ± 0.68
MCH pg	20.40 ± 0.15	19.26 ± 1.40	20.36 ± 0.19	20.66 ± 0.14
MCHC g/dL	34.20 ± 0.43	33.68 ± 2.48	35.36 ± 0.23	35.32 ± 0.30
%LYM	80.38 ± 2.22	78.74 ± 3.16	87.46 ± 2.11***	83.74 ± 2.53

*Mean ± SEM, (n = 5), *p<0.05; **p<0.01; ***p<0.001 vs. Vehicle*

Table 5: Effect of AMAE on biochemical profiles of the control and treated animals in the subchronic toxicity study

Parameter	Vehicle	100 mg/kg	1000 mg/kg	2500 mg/kg
Male				
ALT (IU/L)	55.4 ± 3.64	53.8 ± 5.06	51.4 ± 3.64	62.0 ± 5.50
AST (IU/L)	16.0 ± 0.63	18.8 ± 1.20	20.0 ± 0.63	18.8 ± 0.20
ALP (IU/L)	92.2 ± 3.26	82.4 ± 6.97	74.4 ± 5.05**	85.2 ± 6.45
Albumin (mg/L)	36.6 ± 0.78	36.0 ± 0.78	25.8 ± 1.93	24.8 ± 2.04
Urea (µmol/L)	11.5 ± 1.16	9.8 ± 0.42	8.7 ± 0.67	8.7 ± 0.43
Creatinine (µmol/L)	77.3 ± 8.66	92.1 ± 2.34	91.2 ± 8.51	108.8 ± 7.95***
Cholesterol (µmol/L)	2.08 ± 0.23	2.10 ± 0.12	2.16 ± 0.13	2.14 ± 0.14
HDL (µmol/L)	0.80 ± 0.04	0.82 ± 0.03	0.83 ± 0.02	0.81 ± 0.01
LDL (µmol/L)	1.16 ± 0.19	1.18 ± 0.14	1.24 ± 0.12	1.26 ± 0.13
Triglycerides (µmol/L)	0.26 ± 0.02	0.24 ± 0.04	0.20 ± 0.03	0.16 ± 0.02
Glucose (mg/L)	1.64 ± 0.20	1.20 ± 0.20	0.58 ± 0.09**	0.60 ± 0.12**
Female				
ALT (IU/L)	54.6 ± 6.15	50.0 ± 5.04	59.8 ± 3.23	50.2 ± 6.55
AST (IU/L)	16.0 ± 0.55	18.4 ± 0.98	19.2 ± 0.58	18.8 ± 0.97
ALP (IU/L)	73.6 ± 4.43	75.8 ± 6.45	75.8 ± 4.16	65.6 ± 1.17
Albumin (mg/L)	39.4 ± 0.60	36.4 ± 0.75	25.0 ± 2.19*	29.2 ± 2.35
Urea (µmol/L)	11.9 ± 0.97	9.5 ± 0.60	10.3 ± 0.73	9.3 ± 0.40
Creatinine (µmol/L)	68.3 ± 5.28	58.8 ± 9.83	70.3 ± 3.35	106.5 ± 2.81***
Cholesterol (µmol/L)	2.02 ± 0.21	1.50 ± 0.18**	1.66 ± 0.05	1.68 ± 0.11
HDL (µmol/L)	0.72 ± 0.04	0.81 ± 0.02	0.67 ± 0.02	0.75 ± 0.07
LDL (µmol/L)	1.09 ± 0.14	0.55 ± 0.16**	0.74 ± 0.04	0.78 ± 0.05
Triglycerides (µmol/L)	0.46 ± 0.09	0.30 ± 0.00	0.54 ± 0.05	0.32 ± 0.06
Glucose (mg/L)	3.84 ± 0.28	2.08 ± 0.23***	1.50 ± 0.12***	1.76 ± 0.36***

*Mean ±SEM, (n = 5), **p<0.01; ***p<0.001 vs. Vehicle*

The need to evaluate the toxicity profile of *Annona muricata* leaf aqueous extract (AMAE) was prompted by the increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicine. Consequently, herbal medicines have received greater attention as alternatives to orthodox therapy, leading to their increase in demand [22]. Aqueous extract, as directed by ethnomedicinal use in Ghana, contained polar extracts which are responsible for therapeutic effect. Phytochemical analysis helps detect the chemical constituents of plants extract in search of bioactive agents as basis for drug synthesis [16]. The presence of saponins, condensed tannins and glycosides as the major constituents and trace amounts of flavonoids contribute immensely to the bioactivity of *A. muricata* and also to its usage in treating various diseases. These have included antioxidant activity [14], hepatoprotective effect [13], and antibacterial agent [15].

In the acute oral toxicity study of the extract, no changes in the behaviour of rats were observed. Also no adverse gastrointestinal effects were observed in male and female animals administered with up to 5 g/kg b.wt. The median acute toxicity value (LD_{50}) was estimated to be $\leq$ 5 g/kg b.wt., indicating safety [24]. The gram equivalence of LD_{50} in an average adult man (approx. body weight 60 kg) would translate to 300 g dose of the extract. Further, the extract is safe by oral route in relation to its folkloric therapeutic dose (1 tea cup three times a day approximately, 211 mg/kg/day). Thus its high oral therapeutic index (≈1421) might be used as a rough indication of a wide margin between the effective and toxic dose. Earlier reports have shown that if the median lethal dose of a test substance is three times more than the minimum effective dose, the substance is considered a good candidate for further studies [1]. At present the following chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values are recommended by the Organisation of Economic Co-operation and Development (OECD, Paris,

France): very toxic, ≤ 5 mg/kg; toxic, $> 5 \leq 50$ mg/kg; harmful, $> 50 \leq 500$ mg/kg; and no label, $> 500 \leq 2000$ mg/kg [25]. The leaf extract is therefore safe for oral use for the management of several diseases.

The body weight changes serve as a sensitive indication of the general health status of animals. A varied response of male and female rats to AMAE at high dose levels were observed. 1000 and 2500 mg/kg/day resulted in decline in body weight of female rats throughout the duration of experiment whereas at the same dose, increases were observed in male rats with a decline from day 6 (Table 2 and Fig. 1). However weight gains were observed in all animals administered 100 mg/kg/day. It can be stated that at 100 mg, AMAE did not interfere with the normal metabolism of animals as corroborated with non-significant difference from animals in vehicle control group. However at higher dose (1000 and 2500 mg/kg/day), the crude extract may have been metabolised to a toxic end product which could interfere with gastric function and decreased food conversion efficiency [26]. In addition, the diets were well-accepted by animals treated with 100 mg suggesting AMAE did not possibly cause any alterations in carbohydrate, protein or fat metabolism in these experimental animals. It also shows that AMAE at 100 mg did not adversely interfere with the nutritional benefits such as weight gain and stability of appetite expected of animals that are continually supplied with food and water *ad libitum*. However, the same could not be said for animals administered 1000 and 2500 mg AMAE. Thus overdose of AMAE could result in loss of appetite and decrease in body weight.

The macroscopic examinations of the organs of animals treated with various doses of AMAE did not show any changes in colour compared with vehicle control group. Hypertrophy of organs are first hand indication of toxicity of chemical or biological substance. The significant increase in the relative weights of stomach ($p<0.05$) and uterus ($p<0.01$) in female rats administered 1000 mg is a sign of possible toxic effect of AMAE to these organs (Table 3). The absence of organ toxicity in males at all doses gives an indication of the susceptibility of the female system to toxicity of substance that may not affect males. Thus an overdose of AMAE in females should be avoided due to the observed effect on the uterus. This is in agreement with earlier reports which demonstrated a uterine stimulant activity in rats and advised against the use of *A. muricata* during pregnancy [27].

The effect of AMAE on some haematologic parameters of male and female rats is as shown in Table 4. The general lack of significant changes in blood indices is an indication of safety AMAE. The observed non-significant increase in WBC count and lymphocytes could emphasize the beneficial effect of AMAE in improving the immunity and general well-being of the animals [16]. Also, the observed non-significant difference in haemoglobin concentration in male and female rats could be used to justify the fact that AMAE at all doses does not induce anaemia, making it safe. Also contrary to documented use of *A. muricata* in floristic studies in Ghana as a tonic [11], such was not observed in the current study. AMAE did not confer significant increase in the production of red blood cell.

The liver releases alanine aminotransferase (ALT) and an elevation in plasma concentration is an indicator of liver damage. The liver and heart release AST and ALT, and an elevation in the levels of these enzymes are indicators of liver and heart damage [22]. The non-significant changes in ALT and AST in both male and female rats at all doses (Table 5) indicates that the extract had no deleterious effect on liver function. ALP which has both hepatic and bone sources, showed a significant decrease ($p<0.01$) in male rats administered 1000 mg/kg AMAE. The

general lack of significant changes in the aminotransferases and ALP together with normal liver weight is an indication that AMAE is safe and offers no deleterious effect on the liver.

The decrease in the plasma levels of albumin in male and female rats administered 1000 and 2500 mg/kg AMAE may be a sign of impaired renal function [28]. This effect was more pronounced in female rats, as higher doses showed significant decreases ($p<0.05$) (Table 5). Further biochemical changes were observed in creatinine concentration at higher doses which suggest a possible kidney damage, especially by renal infiltration mechanism [29]. However, the combined decrease in albumin and increase creatinine levels occurred in animals treated with higher doses. Thus the extract at 1000 mg and beyond may likely cause kidney damage. Effect of the extract on biochemical parameters showed a remarkable decrease in plasma glucose level especially at 1000 mg in both male and female compared with vehicle group (Table 5). This indicates the presence of hypoglycaemic components in the extract and gives credence to the use of *A. muricata* as hypoglycaemic agent [10].

The decrease in the plasma total cholesterol and triglycerides levels may be attributed to the presence of hypolipidaemic agents in the extract. The general lack of significant changes in HDL and LDL levels indicate that the extract had no effect on lipid metabolism of animals. However, the non-significant increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in female rats administered 100 mg/kg AMAE is an indication that low dose of the extract can reduce the cardiovascular risk factors which contribute to death of diabetic subjects [30]. The reduction of the cardiovascular risk factors can further give support to the traditional use of the herbal formulation of *A. muricata* as a hypoglycaemic agent. Also the general lack of significant difference in lipid profile in male rats indicates that the extract at all doses had no effect on lipid metabolism of animals.

CONCLUSION

The high LD_{50} obtained was a clear indication of safety of *A. muricata* for internal and external use. The study showed that *A. muricata* is hypoglycaemic and hypolipidaemic at lower doses. However, higher doses could cause kidney damage leading to renal failure. High doses could also induce negative effect on uterine function. Thus for long term usage, kidney function should be monitored while avoiding usage during pregnancy.

Acknowledgements
The authors acknowledge the technical assistance of Mr. Thomas Ansah (Department of Pharmcology, KNUST), Mr. Kwadwo Kakraba (Department of Pharmacognosy, KNUST), Mr. Frank Bonsu (Clinical Analysis Laboratory, KNUST) and Mr. Osei Assibey (Department of Biochemistry, KATH).

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