

ORAL ACUTE TOXICITY STUDIES OF THE AQUEOUS FRUIT EXTRACT OF *ANNONA MURICATA* IN ALBINO RATS

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Abstract: The acute toxicity of aqueous fruit extract of *Annona muricata* was evaluated in Swiss albino rats. The rats were randomly distributed into four groups of three animals each for the first phase. The groups were respectively administered orally aqueous fruit extract of *Annona muricata* at 0, 10, 100, and 1000 mg/kg body weight in a single dose. In the second phase of the experiment, the albino rats were divided into four groups of 3 animals each and were orally administered single doses of the extract at 0, 1600, 2900, and 5000mg/kg body weight and monitored frequently for 24 hours and 14 days. No death was recorded in each group. After the oral intubation, no mortality was observed in the albino rats. All the animals showed a positive gain in body weight throughout the study. The oral LD₅₀ of aqueous fruit extract of *Annona muricata* was estimated to be equal to or below 5000mg/kg body weight, and the plant extract was concluded to be safe and non-toxic when taken through the oral route. The LD₅₀ value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicologic classification of chemicals.

Keywords: Oral Acute Toxicity, *Annona muricata*, Aqueous Fruit Extract, Albino Rats.

1. INTRODUCTION

Acute toxicity refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours.

Acute toxicity tests assess a chemical's capacity to produce acute toxicity in a test organism after a single dosage, particularly when the material is administered in high concentrations and short amounts of time. The indicators of harmful behavior can appear right away or later [21]. These tests offer a screening technique for toxicity assessment, especially for recently discovered, unidentified compounds. The measurable measure of an agent's potential to cause harm is the LD₅₀ (lethal dose 50%), or the dose that will kill 50% of test subjects. These tests can also be used to determine other important parameters, such as the therapeutic dose and therapeutic index of the test compounds. The data gathered from acute toxicity studies serves as the foundation for categorization and hazard evaluation of novel chemical compounds, thereby furnishing the information required for warning labels of chemical substances that may be hazardous. [7]. Comparing the potencies of medications in clinical and experimental contexts is another use for these data. [27].

1.2 OVERVIEW OF *ANNONA MURICATA* (SOUSOP)

With over 2300 species and 130 genera, the Annonaceae family most likely originated in North, Central, and South America [18]. Five *Annona* species—*Annona muricata*, *Annona reticulata*, *Annona cherimola*, *Annona squamosa*, and *Annona macrophyllata*—were found to have anti-diabetic qualities in a review of nine *Annona* species [5]. There are several names for *A. muricata* L., including guanabana, paw-paw, graviola, sirsak, and soursop. The name soursop refers to the sour and sweet flavors of the fruit [18].

Many studies reported the therapeutic effects of *A. muricata*, such as anti-tumor, anti-helminth, anti-fungal, anti-bacterial, hypotensive, anti-viral, and anti-inflammatory effects [20]. Various parts of *A. muricata*, such as leaves and bark, have been used for medicinal purposes. Over 200 chemical compounds have been discovered and extracted, including phenolics, acetogenins, and alkaloids [6]. Due to its medicinal and pharmacological effects, this plant is considered a potential alternative treatment for diabetes mellitus (DM), hypertension, cancer, and bacterial infections [24]. Furthermore, in comparison to currently available commercialized treatments, it is inexpensive, easily accessible, and environmentally friendly, making it an excellent package to take into consideration for future possible medications [19].



Figure 1. *Annona muricata* (A) Habit; (B) Fruit; (C) Rind; (D) Pulp; (E) Seed (F) Leaves; (G) Bark flowers; (H) Root [6].

Toxicity testing is paramount in the screening of newly developed drugs before it can be used on humans. Toxicity testing is the determination of potential hazards a test substance may likely produce and the characterization of its action; most of the toxicity testing is carried out on experimental animals [8]. The advantages of using animal models in toxicity testing are enormous. The benefits encompass the potential for a distinct genetic makeup, the ability to be exposed under controlled conditions for a predetermined amount of time, and the opportunity to thoroughly inspect every tissue after necropsy [8]. Chemicals sold in commerce can be classified and labeled according to their hazards using the information that has been gathered [8]. A test substance's potential hazardous consequences must be described in addition to its safety, as this is the fundamental purpose of toxicity testing. Following the thalidomide tragedy in the early 1960s, when thousands of infants were born with serious birth deformities worldwide, toxicity testing received a lot of attention [2].

Following this incident, many nations have decided to screen for toxicity and teratogenicity in both sexes to stop such catastrophes from happening in the future. Toxicity testing employed a wide range of tests in different species of animals with long-term administration of the drug, regular monitoring of physiological, biochemical abnormalities, and detailed post-mortem examination at the end of the trial to detect gross or histological abnormalities [14]. Doses above the therapeutic range are used in toxicity testing to ascertain the toxic signs of action of the drug [2]. This work seeks to highlight the importance of toxicity testing in the development of therapeutic agents. A drug's toxic effects might range from minimal to so severe as to hinder the compound's continued growth. Clinical analysis, autophic analysis, hemological and hemochemical analysis, histopathological analysis, statistical presentation, and data interpretation all support every toxicity study.

2. MATERIALS AND METHODS

2.1 FRUIT COLLECTION PROCESS AND IDENTIFICATION

Fresh fruits of *Annona muricata* were bought from Ezio-bodo Elu, Owerri, Imo state, Nigeria, in February 2024, and identified by a taxonomist, Mr Iwueze Francis of the Department of Forestry and Wildlife Technology, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri, FUTO. The plant was prepared and kept in the University herbarium with voucher number FUTO/FWT/ERB/2022/71.

2.2 FRUIT MATERIAL AND EXTRACT PREPARATION

The fruits of *Annona muricata* were cut open, and the pulp was separated from its rind and seeds. The pulp of the fruit was air-dried under shade, inside the laboratory, for four weeks, and ground into powdered form [13]. This powdered sample was stored in airtight polythene bags to protect the sample from sunlight. The aqueous extract of the fruit powder was prepared by quantitative extraction, i.e., 50 g of this powder was soaked in 600 ml of distilled water in a 1000 ml conical flask sealed tightly with cotton wool and foil for 72 hours, and thereafter filtered with Whatman no 1 filter paper. The decoction liquid obtained was concentrated in a water bath at 450 °C to produce a semi-solid residue. The extract was stored in the refrigerator at 40°C until it was used for evaluation [22].

2.3 EXPERIMENTAL ANIMALS

The female albino rats used for the study were purchased from the Department of Biochemistry, Abia State University, Uturu. They were kept in standard cages in the animal house of the Department of Biochemistry, Federal University of Technology, Owerri, Imo state. The animals were about 8-9 weeks old and weighed between 40-110g initially. They were allowed to acclimatize for two weeks before the commencement of the study. They were fed growers' pellet feeds with tap water ad libitum. The animals were starved for some hours before the commencement of the experiment [10].

2.4 ACUTE TOXICITY EVALUATION/DETERMINATION OF MEDIAN LETHAL DOSE

The acute toxicity of aqueous fruit extract of *Annona muricata* was conducted following Lorke's method [15]. One route of administration was used for this study: oral intubation. The method involved a two-phased administration. For the first phase, 12 rats were randomly distributed into four groups of three animals each. The groups were administered orally, single doses of aqueous fruit extract of *Annona muricata* at 10, 100, and 1000 mg/kg body weight; the fourth group, which was the control, was administered normal saline only. The animals were observed closely for the first hour and then every 30 minutes for the first 24 hours for the onset of any immediate signs of toxicity and daily during the 14-day observation period to record any delayed acute effects. During this period, their weights were recorded daily, and clinical signs of toxicity such as loss of appetite, immobility, convulsion, etc., were observed, and no mortality was recorded [9].

In the second phase of the experiment of oral administration, 12 rats were divided into four groups of 3 animals each and were administered single doses of the extract at 1600, 2900, and 5000 mg/kg body weight. The fourth group, which was the control, received normal saline only. The second phase doses were chosen based on the results of the first phase treatment. The animals were observed as frequently as previously described in the first phase, and no mortality was recorded. The animals were weighed daily and monitored for two weeks for clinical signs of acute toxicity such as writhing, gasping, palpitation, increased or decreased respiration rate. The result of the first and second phases of the experiment was used to calculate the LD₅₀ of the fruit extract. The LD₅₀ was calculated according to the method outlined by Lorke [15] as: $\sqrt{\text{Geometric mean of the maximal dose without mortality} \times \text{Geometric mean of the minimal dose with mortality}} = \text{Xmg/kg}$ [9].

2.5 STATISTICAL ANALYSIS

All data generated during the course of the research were expressed as mean \pm standard deviation (SD) and analyzed statistically by analysis of variance (ANOVA). Means were compared using the LCD post hoc test, and differences between treatment and control groups were significant at $p \leq 0.05$

3. RESULTS

3.1 RESULTS OF QUANTITATIVE EXTRACTION

$$\% \text{ Fruit yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry fruit}} \times \frac{100}{1}$$

$$\% \text{ Fruit yield} = \frac{38.24}{50} \times \frac{100}{1}$$

$$= 76.48\%$$

The percentage yield of the extract was calculated to be 76.48%.

For the animals administered the extract via the oral route

The results of the oral LD₅₀ determination of aqueous fruit extract of *Annona muricata* are shown in Table 1 below. No mortality was observed in either the control or treated groups during both phases of treatment. No clinical signs of toxicity were noted in any of the animal groups during phase one. In phase two, animals treated with 2900 mg/kg and 5000 mg/kg body weight of the plant extract exhibited signs of irritability and weakness one hour after administration. However, these animals recovered within two hours. The absence of mortality during the study suggests that the LD₅₀ is greater than 5000 mg/kg body weight.

Table 1: LD₅₀ Determination of Aqueous Fruit Extract of *Annona muricata*

Experiment	Dose(mg/kg)	Number of mortalities
Phase 1	Control	0/3
	10	0/3
	100	0/3
	1000	0/3
Phase 2	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3

The LD₅₀ of the aqueous fruit extract of *A. muricata* was calculated to be ≤ 5000 mg/kg.

Phase 1

From Table 2 below, it was shown that the animals gained weight after 14 days. There was 15, 22, 25, and 44% weight gain in the animals administered 10, 100, 1000, and 0mg of the extract. The control group had more weight gain which was 41% weight gain, followed by the group administered 1000mg of the fruit extract which had 25% weight gain, followed by the group administered 100mg of the fruit extract which had 22% weight gain, and lastly the group administered 10mg of the fruit extract which had 15% weight gain.

Table 2: Percentage weight gain of animals in phase 1

Dose (mg/kg)	Average body weight (g) on day 1 (I)	Average body weight (g) on day 14 (F)	X= (F-I)/I	% Weight Gain (%)
10	72.76	83.65	0.150	15
100	94.13	114.44	0.216	22
1000	110.05	137.62	0.250	25
Control	49.56	69.91	0.410	41

The results of the 14 days weight measurement of the animals during the phase 1 is presented in Figure 4.1. The change in body weight of the animals during the phase 1 is plotted in Figure 1 below. Significant difference ($p < 0.05$) in body weight was noted in every group. The body weight of the rats given 100 and 1000mg/kg of the extract showed a notable difference throughout the 14 days compared with the control animals. Every group displayed an upward trend in body weight and a favorable shift during the first seven days of the test, then a decrease from day seven to day fourteen for the rats given 10mg/kg of extract (Figure 4.1).

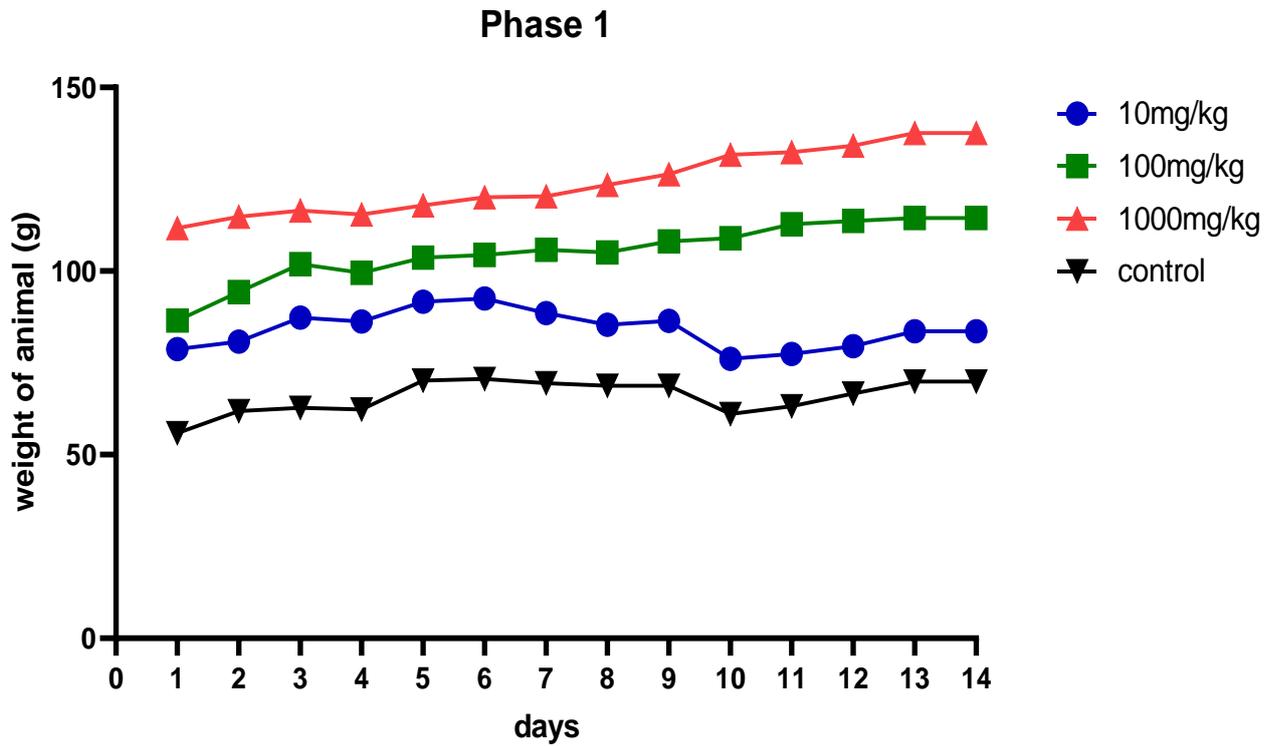


Figure 3.1. Change in body weights (g) of animals in phase one administered orally with 0, 10, 100, 1000 mg/kg body weight of aqueous fruit extract of *Annona muricata*

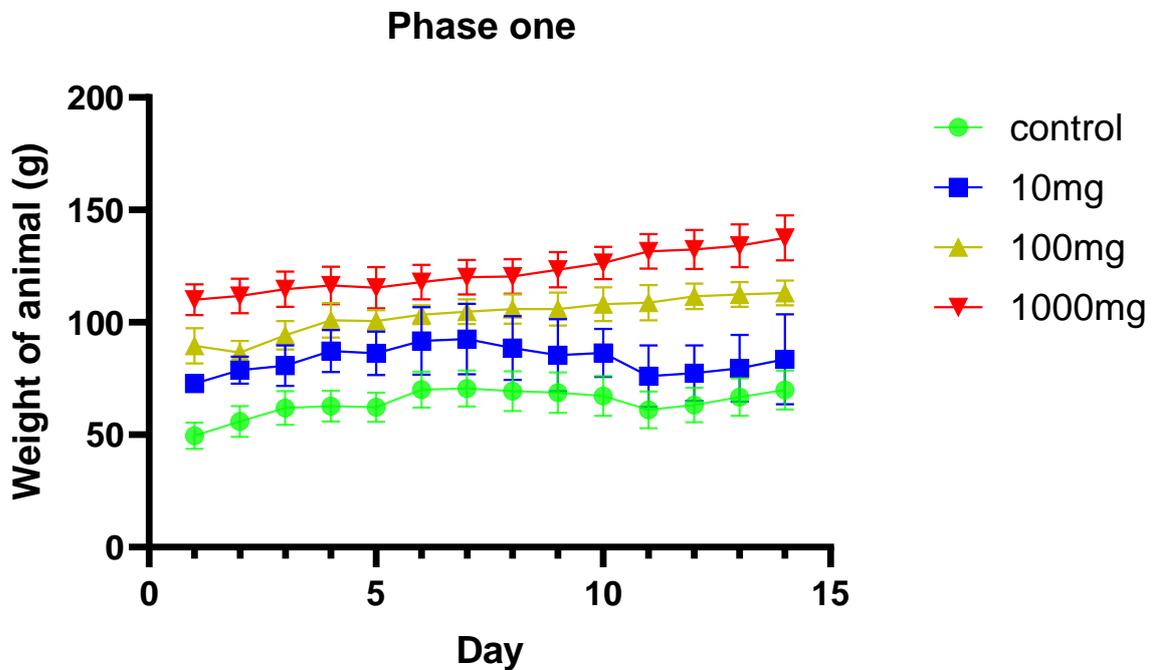


Figure 3.2. Change in body weights (g) of animals in phase one administered orally with 0, 10, 100, 1000 mg/kg body weight of aqueous fruit extract of *Annona muricata* showing the standard error bars

Phase 2

From Table 3 below, it was shown that the animals gained weight after 14 days. There was 36, 47, 8 and 45% weight gain in the animals administered 1600, 2900, 5000 and 0mg of the extract. The group administered 2900mg of the fruit extract had more weight gain which was 47% weight gain, followed by the control group which had 45% weight gain, followed by the group administered 1600mg of the fruit extract which had 36% weight gain, and lastly the group administered 5000mg of the fruit extract which had 8% weight gain.

Table 3: percentage weight gain of animals in phase 2

Dose (mg/kg)	Average body weight (g) in day 1 (I)	Average body weight (g) in day 14 (F)	X= (F-I)/I	%Weight Gain (%)
1600	111.93	152.58	0.363	36
2900	74.73	110.18	0.474	47
5000	136.04	147.23	0.082	8
Control	86.44	125.27	0.447	45

The animal's body weight distribution during phase 2 is shown in Figure 4.2 below. There was a decrease in the body weight of rats given 1600 and 5000mg/kg of the extract after injection, after which it began to display an upward trend in body weight. When comparing the body weight of the treated groups to the control animals from day 6 to day 14, there is a significant difference ($p < 0.05$). significant variation was seen from day 12 to day 14 between the animals in the control group, those administered 1600mg/kg body weight, and those administered 2900 mg/kg body weight. Figure 4.2 illustrates how the animals' body weight changed during phase 2. The group given 1600, 2900 and 5000mg/kg body weight of the extract and the control group showed an increase in the animals' body weight over the course of the investigation.

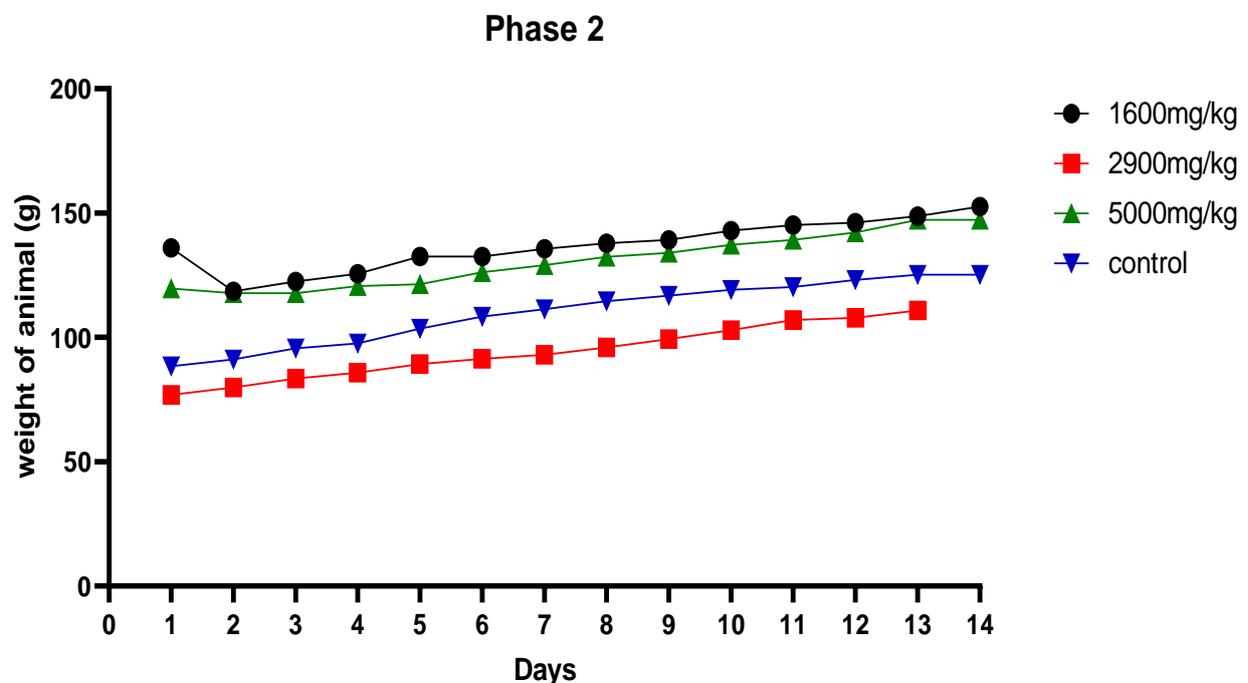


Figure 3.3: Change in body weights (g) of animals in phase two administered orally with 0, 1600, 2900, 5000 mg/kg body weight of aqueous fruit extract of *Annona muricata*.

Phase Two

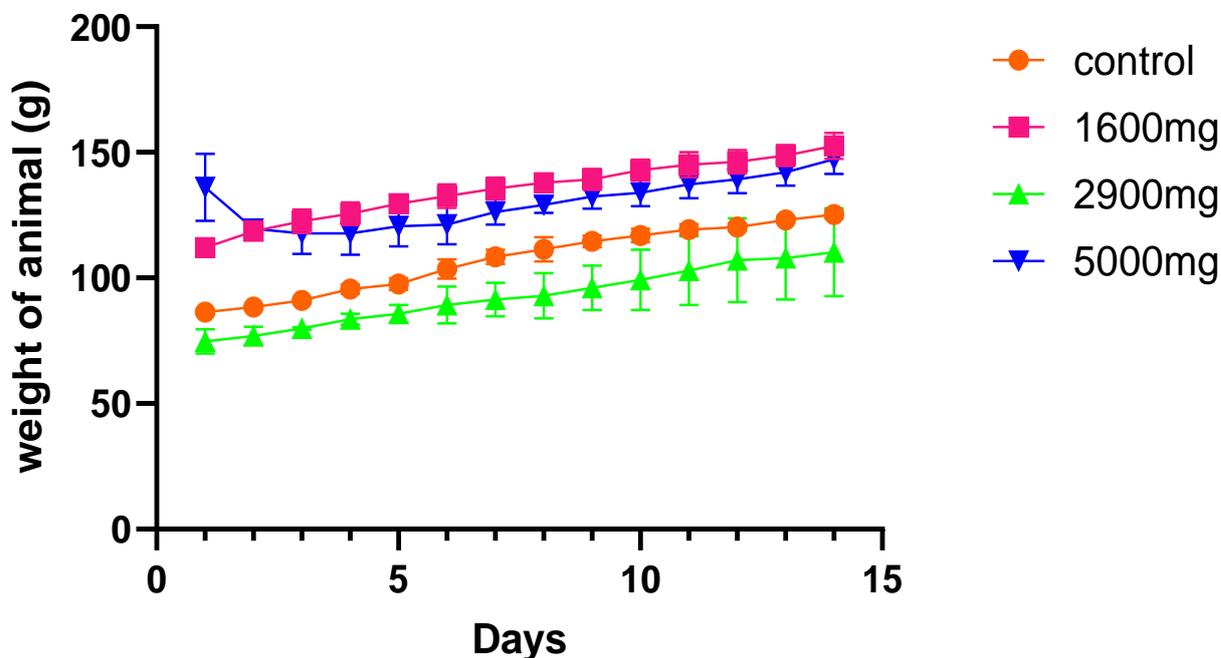


Figure 3.4: Change in body weights (g) of animals in phase two administered orally with 0, 1600, 2900, and 5000 mg/kg body weight of aqueous fruit extract of *Annona muricata*, showing the standard error bars of the data.

4. DISCUSSION

Being poisonous is expressed as toxicity, which is a state of adverse effects caused by interactions between toxicants and cells. The goal of a plant extract's toxicological assessment is to determine its potential side effects, ensuring the safety of consumption. Certain elements have the potential to influence the hazardous nature of an extract from a therapeutic plant. This toxicity may occur during the manufacturing of the extract or may be intrinsic in the vegetable medication. The drug's toxicity is associated with its compound(s) and may or may not be related to the active ingredient. The many useful properties of *A. muricata* are a pointer to its potential as a natural source of drugs, but also make it a subject of potential abuse, especially among the local consumers [17]. Hence, the present study was particularly designed to investigate the acute toxicity of aqueous fruit extract of *A. muricata* by using oral toxicity analysis. A recent study by [16] revealed that tannins present in medicinal plants are potent in increasing body mass. Also, a phytochemical analysis of *A. muricata* has shown the presence of small quantities of tannins, among other components, as one of its active components. This could be responsible for the increased body weight observed in this study [3].

In the oral acute toxicity evaluation, The LD₅₀ of the aqueous extract of *A. muricata* was estimated to be more than 5000 mg/kg. The dose produced no mortality after 24 hours and a 14-day observation period. It also had no adverse effects on the behavioral responses of the tested rats after 14 days of observation. It has been suggested that any substance with an oral LD₅₀ of above 5000 mg/kg should be regarded as safe [11]. It can therefore be inferred that the plant under study is non-toxic and safe. Although the extract can be deduced to be safe, some dose-dependent toxic manifestations were observed in the groups treated with 2900 mg/kg and 5000 mg/kg body weight of the extract following oral administration. This may be due to the effect of one or more of the chemical constituents present in the extract. The non-toxic observation that was made in the current studies following the evaluation of the acute toxicity aligns very well with other studies on the toxicity of *A. muricata*. [1] reported a nontoxic LD₅₀ of aqueous extract of *A. muricata*. The non-toxic effect of the plant could be due to its rich content of nutritional molecules. [6] reported that the whole plant is rich in phytochemical contents. Available evidence has shown that body weight changes are important and sensitive indices of toxic effects [23]. The monitoring of

body weight of the experimental animals is important while studying the toxicity and safety of a natural product, since it hints at the physiological and metabolic status of the animals and gets rid of the researcher from deriving any “false” observations due to nutritional abnormalities of the rats. In this current study, the positive change in body weight shown by all the rats in Tables 2 and 3 was comparable and followed a general trend. The slight elevation in body weight after the 14-day treatment can be attributed to normal growth of the animals over the period [17]. None of the experimental groups suffered loss in weight or gained overweight, which suggested that the plant extracts did not induce significant changes in the appetite and did not exert any deleterious effects on the general health status and metabolic growth of the rats. The animals in the control group showed a negative change in body weight towards the end of the observations. This change could be merely a result of environmental factors, as the control group only received water and food. The pattern of body weight was altered after day 7 during the phase 1 experiment. However, this change was also observed with the control group and is therefore not caused by the administration of aqueous extract. This suggested that the fruit extracts did not induce any deleterious effects on growth and development of the rats. A positive change in body weight of animals has also been reported by [1] after oral administration of non-toxic plant extracts.

The current study demonstrates a high safety profile of the aqueous extract from *A. muricata* fruits, with no observable toxic effects or mortality up to the limit dose of 5000 mg/kg upon acute oral exposure in rats. These findings corroborate earlier reports of the low acute toxicity potential of leaf extracts in rodents [26]. *A. muricata* is widely used in traditional medicine to treat a variety of ailments, such as hypertension, diabetes, and cancer. Research also stated that these plants contain various types of bioactive compounds from certain classes, such as acetogenins, flavonoids, phenols, alkaloids, and megastigmane. In vivo and in vitro research showed that it has the potential to treat various conditions, such as wound healing, ulcer, inflammation, cancer, diabetes, and hypertension, which corroborates with the result in Table 1, having no mortality in all the groups of animals in phases 1 and 2 [9].

Biologically active plant chemicals other than traditional nutrients that have a beneficial effect on human health have been termed phytochemicals. In the preliminary qualitative phytochemical analysis of *A. muricata*, it contained different phytochemicals such as flavonoids, alkaloids, phenols, saponins, tannins, etc. This attests to the potential medicinal values of the plant materials. This corresponds with the work of [6].

Absence of adverse clinical manifestations, unaltered body/organ weights, and normal hematological parameters suggest the extract does not induce systemic toxicity. [25].

The oral LD₅₀ could not be determined due to lack of mortality, signifying this ethnomedicinal fruit extract has a high therapeutic index and wide safety margins for acute consumption, aligning with traditional practices [4]. The results validate its relatively safe applications and encourage further studies to explore pharmacological properties.

Also, Table 2 showed a percentage weight gain of the test animals in phase one as follows: 15, 22, 25, and 44% in the animals administered 10, 100, 1000, and 0mg of the fruit extract. Table 3 also showed a percentage weight gain of the test animals in phase one as follows: 36, 47, 8, and 45% weight gain in the animals administered 1600, 2900, 5000, and 0mg of the extract. This weight gain is prompted by the nutritional and therapeutic values of *A. muricata* fruit; this is in accordance with [12] *Annona muricata* (soursop) is known for its nutritional fruits and has shown therapeutic promise.

5. CONCLUSION

Within the limits of this acute oral study design and parameters evaluated, the results conclusively demonstrate that *A. muricata* aqueous fruit extract does not exhibit toxic effects or potential health risks from single-dose exposure up to 5000 mg/kg. This substantiates the traditional safe consumption and encourages additional studies, including subacute and chronic toxicity evaluations, as well as clinical trials and pharmacological characterization to develop evidence-based therapeutic applications.

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