

Evaluation of acute and subacute toxicity of the extract of *Allium cepa* peels in Wistar rats

Modupe Iretila Builders ^{1,*}, Glory Zidyep Michael ¹, Samuel Otokpa Ede ², Simeon Oyepata Joseph ³ and Peter Uduak Ise ¹

¹ Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nasarawa State, Nigeria.

² Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria.

³ Department of Pharmacology and Toxicology, Faculty of Pharmacy, Federal University, Oye-Ekiti, Ekiti State, Nigeria.

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Abstract

Allium cepa is a popular recipe used traditionally to treat diabetes, cardiovascular diseases, cancer, wounds and infections. The acute and sub-acute oral toxicity profile of the aqueous extract of *Allium cepa* peels were evaluated in wistar albino rats in order to determine its safety for use in traditional medicine. The median lethal dose (LD₅₀) body weights and food intake were determined. The acute oral toxicity of the peel extract was carried out in accordance with OECD guideline 423. In addition to this, the haematological parameters and biochemical parameters were also evaluated. The median lethal dose (LD₅₀) of the extract was greater than 2000 mg/kg when given orally in the rats. There were no significant changes in the level of haematological parameters and biochemical parameters. The results from this study indicates that *Allium cepa* peels are relatively safe, hence supporting its use in traditional medicine.

keywords: *Allium cepa* peels; Acute toxicity; Subacute toxicity; Haematological parameters; Biochemical parameters

1. Introduction

About 80% of the World's population both in the developing and developed countries used traditional medicines according to a research conducted by [1]. Thus, World Health Organization, in support of these trends in healthcare, World Health Organization (WHO) recommended these natural products, but advised that safety and efficacy should always be evaluated and considered [2].

Plant kingdom has proven an effective source of drugs for the prevention and treatment of various diseases, because medicinal plants are accessible and inexpensive, therefore have fitted the immediate personal need and commonly used in medicine from time immemorial [3], but there is urgent need to communicate potentially harmful toxins present in natural products to consumers who increasingly make the switch to natural based products because they believe them to be safer [4].

Toxicology deals with adverse effects of chemical substances on living organisms, it involves acute toxicity studies which are carried out to determine the short-term adverse effects of a drug when administered in a single dose, or in multiple doses during a period of 24 hrs to 14 days [5]. These studies are also useful for determination of the dose that will produce mortality or serious toxicological effects when given once or administered in multiple doses [6].

* Corresponding author: Modupe Iretila Builders

In addition to this, Sub-acute toxicity studies are carried out to evaluate a new drug's potential adverse effects following a treatment period of 28 days' duration. Sub-acute toxicity studies are carried out as range-finding studies in order to choose dosage levels to be used in subsequent sub-chronic and chronic toxicity studies. These studies may support initial clinical trials where the duration of treatment may be up to 4 weeks. It is of insufficient duration to identify all secondary effects that may arise during long-term clinical use or during chronic toxicity and carcinogenicity testing [5].

Chronic toxicity is referred to as adverse effects that occur after the repeated or continuous administration of a sample drug for a major part of the life span. This is usually considered to be six months in duration in rodents [7].

Allium cepa belongs to Family: Lillaceae (lilies) which is popularly known as onion, a medicinal plant used as a traditional spice and has tremendous health benefits [8]. *Allium cepa* is an aromatic vegetable that is easily digested and used throughout the whole world. It is referred to as common onion, garden onion and white onion. Traditional names given to it in Nigeria is Alubosa (Yoruba), Albasa (Hausa), Yabasi (Ibo). It is cultivated annually and biennially for vegetable use. The plant grows to a height of 15-45 cm (6-18 inch) and it has yellowish-green leaves with flattened, fan shaped swathe. The inflorescence of onion plant is globular umbel type and bear white flowers with parts in sixes [9]

A. cepa is used ethnomedicinally for the treatment of various diseases such as diabetes, cancer, pain, inflammatory diseases, cardiovascular diseases and infections [10]. However, there is limited scientific information on the acute and sub-acute toxicity profile of *Allium cepa* peels. There are also numerous reports on the efficacy of *Allium cepa* peels, with limited reports on its safety. In this present study, the acute and subacute toxicity of *Allium cepa* peels will be evaluated in order to validate its safety .



Accessed from <https://www.helios.co.uk/shop/allium-cepa>

Figure 1 *A. cepa* plant with the onion bulb

2. Materials and method

2.1. Drugs and chemicals

Normal Saline 500 mL (Storge Pharmacy, Nigeria), Distilled water, formaldehyde, diethyl ether

2.1.1. Equipment

Weighing balance, refrigerator, hand gloves, cotton wool, cannulas, beakers, lithium heparin bottles, EDTA bottles, sample bottles, plain bottles, syringe and Needle (1 mL)

2.2. Collection and extraction of the plant material

Allium cepa peels were purchased in May, 2022 from a local market in Gwagwalada, Abuja. The peels were washed and dried at room temperature, after which they were stored at room temperature. The *Allium cepa* peels (450g) was weighed into a clean jar, and extracted by maceration with 1 litre of distilled water for 48 hours. The resultant extract was filtered using Whatman filter paper 125 mm (No 1) and evaporated to dryness on a water bath at 45°C. The percentage yield was calculated and the concentrated extract stored in the refrigerator at -4°C [10].

2.3. Animals

Thirty wistar albino rats obtained from the animal house of Bingham University, Karu were used for this study. They were given a period of acclimatization and given free access to water and food ad libitum in the animal house of department of pharmacology and toxicology, Faculty of Pharmaceutical sciences, Bingham University.

2.4. Ethical approval

Clearance was gotten from the Bingham University Ethics Review Committee, and the experiment followed the Guidelines for laboratory Procedures laid down by the Bingham University Ethics Committee on Research.

2.5. Acute oral toxicity

The acute oral toxicity was carried out in accordance with OECD guideline 423, which requires only three animals [12, 13]. The three test animals were fasted overnight for 12 hours and their weight taken afterwards. The extract was dissolved in distilled water. The doses of aqueous extract of *Allium cepa* peels (AEAC) were determined in relation to the body weight of each animal. The extract (2000 mg/kg) was administered orally to the animals. The animals were monitored and observed individually for behavioral changes and signs of toxicity at intervals for the first 4 hours, and the observation continued for the next 24 hours. The observation continued for the next 7 days [14]. No death occurred within 48 hours hence the procedure was repeated with another set of three animals.

2.6. Sub-acute toxicity study

The rats were divided into four groups of six rats each. The first group of animals, which served as the control group, was administered with 1 ml/kg of normal saline. The second, third and fourth groups of animals which served as the treatment group, were administered with 125, 250 and 500 mg/kg body weight of *Allium cepa* peel extract. All the doses were administered orally on daily basis for 28 days [15]. Food intake, water intake and body weight were closely monitored weekly. The animals were anaesthetized on day 29 in an airtight container saturated with formaldehyde. Blood samples were collected through cardiac puncture using a needle and syringe into ethylenediaminetetraacetic acid (EDTA) bottles for haematological studies on the following parameters (White blood cells (WBC), Red blood cells (RBC), packed cell volume (PCV), haemoglobin (HGB), platelets (PLT), lymphocytes (LYM), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV). Hepatic indices such as (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin. The livers of the animals were collected, rinsed in 0.9% normal saline and it was fixed in 10% formo-saline for histological studies [11].

2.7. Statistical analysis

The data were presented as the mean \pm S.E.M. Results were analyzed statistically using SPSS (statistical package for social sciences). One-way analysis of one-way variance (ANOVA) was also used, after which Dunnett's test for parametric comparisons between the control and the treatment groups was used. The minimum level of significance was set at $P < 0.05$.

3. Results

3.1. Plant extract

The percentage yield of the aqueous extract of *Allium cepa* peels was determined and calculated as 6.8 % w/w

$$\begin{aligned} \text{Percentage yield} &= \frac{\text{weight of dried extract}}{\text{weight of dried plant sample}} \times 100\% \\ &= \frac{46.5\text{g}}{680\text{g}} \times 100\% \\ &= 6.8\% \text{ w/w} \end{aligned}$$

3.2. Acute toxicity

No toxic reactions were observed in the animals neither was death recorded after administering an oral dose of 2000 mg/kg to three test animals in the first and second phase of the acute toxicity study. The median lethal dose (LD₅₀) of

the aqueous extract of *Allium cepa* peels was estimated to be greater than 2000 mg/kg in rats. During the 14 days of observation, all animals were observed to be active and healthy.

3.3. Sub-acute toxicity

3.3.1. *Allium cepa* peels on Body Weight

During the experiment, mortality of the rats was recorded. Consumption of food and water in the control group decreased but remained normal in the treatment group except for group 5 which consumed more food and water. The control group and group administered with 500 mg/kg of the extract exhibited slight decrease in weight of the animals when compared to the other groups. The milligram of extracts given to the experimental group was based on their individual weight and was administered accurately. The effect of *A. cepa* peels on body weight is shown in table 1 below

Table 1 Effect of Aqueous extract of *Allium cepa* peels on body weight of rats

Weight of rats	Control	125 mg/kg	250 mg/kg	500 mg/kg
Day 0(g)	224.57± 7.15	233.07± 10.90	238.12± 7.12	211.55± 10.84
Day 28(g)	198.02± 8.20	229.50± 9.95*	226.38± 7.20*	204.42± 10.744
Weight loss	-26.55	-3.57	-11.74	-7.13
(%)Weight loss	-13.41	-1.60	-5.19	-3.49

n=6 per group, Data are mean ± SEM, statistically significant * $p < 0.05$.

3.3.2. *Allium cepa* peels on haematological parameters in rats

There was no significant difference ($p > 0.05$) in the haematological parameters of the groups administered with (125 and 500 mg/kg) of the extract when compared with the control group. Though there was significant difference ($p < 0.05$) in the level of red blood cells of the group administered with 500 mg/kg when compared to the other treatment groups as indicated in the table 2 below.

Table 2 Effect of Aqueous extract of *Allium cepa* peels on haematological parameters in rats

Parameters	Control	125mg/kg	250mg/kg	500mg/kg
PCV(%)	41.75±1.31	46.00±3.74	42.75±2.63	43.25±3.84
WBC (x10 ⁹ /L)	7.73±1.79	6.00±1.68	5.83±0.83	4.38±0.31**
PLT(x10 ⁹ /L)	224.75±27.43	287.50±82.40	349.50±50.70**	273.50±55.18
Hb(g/dl)	13.48±0.36	14.58±0.46**	13.40±0.61	13.98±1.10
RBC(x10 ¹² /L)	4.48±0.35	5.20±0.27**	5.10±0.24**	4.52±0.23
MCV(FL)	94.25±5.0	89.00±2.70	84.00±3.00**	96.75±4.61
MCH(Pg)	30.53±1.81	28.88±1.27	26.25±1.27**	30.83±1.30
MCHC(g/dl)	32.28±0.24	31.53±0.48**	31.20±0.60**	31.83±0.34
N(%)	27.50±3.71	38.00±3.70**	40.75±6.82	27.00±2.86
L(%)	66.75±3.94	54.50±3.33**	51.00±6.03**	65.00±2.86
M(%)	4.00±0.71	4.25±0.85	5.00±0.91	6.00±0.41**
E(%)	2.75±0.63	2.75±0.75	2.00±0.0**	2.00±0.41

n=6 per group, Data are mean ±SEM, statistically significant* (P<0.05)

3.3.3. *Allium cepa* peels on liver enzymes in wistar rats

Table 3 shows the effect of aqueous extract of *Allium cepa* on liver enzymes in wistar rats. No significant difference ($p > 0.05$) was recorded in the level of GPT, GOT, ALP and Albumin in all the groups administered *Allium cepa* compared to the control group.

Table 3 Effect of aqueous extract of *Allium cepa* peels on liver enzymes in wistar rats

Enzymes	Aqueous extract of <i>Allium cepa</i> peels(mg/kg)			
	Control	125	250	500
GPT	29.15± 16.84	41.13± 9.57	45.23± 9.97	17.48± 2.87
GOT	45.30± 19.24	52.60± 12.98	55.48± 11.67	25.78 ± 3.79
ALP	57.93± 8.73	46.93± 2.55	61.08± 4.15	47.35± 2.0
ALBUMIN	42.68± 3.08	45.60± 1.57	41.55± 3.49	44.80± 1.44

n= 6, Data are mean ± SEM, Statistically significant*($p < 0.05$)

3.4. Morphological observation

At the end of the experiment, no morphological abnormality was observed on any rat both in the control group and experimental groups. Morphological observation shows that there were no morphological changes in the overall character of the rat except for alteration in the general cell Morphology. There was no physical deformity either at the lower or upper extremities.

3.4 Histopathological study

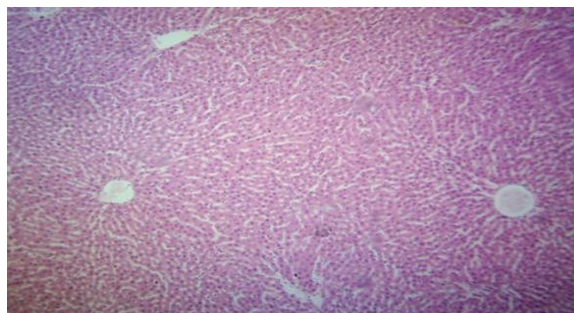


Figure 2 Photomicrograph of Liver section from control group which were administered Normal saline(1ml/kg) with normal histology showing central vein , hepatic cells and sinusoid (H&E x100).

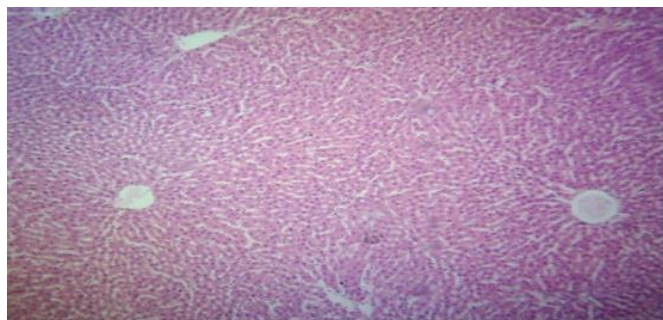


Figure 3 Photomicrograph of Liver section from group treated with aqueous extract of *Allium Cepa* (125mg/kg) with normal histology showing central vein , hepatic cells and sinusoid (H&E x100)

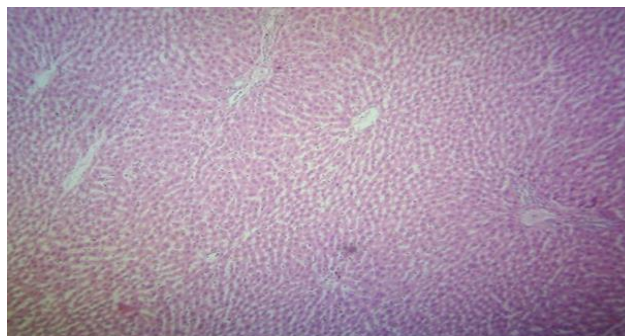


Figure 4 Photomicrograph of Liver section from group treated with aqueous extract of *Allium cepa* (250mg/kg) showing central vein dilatation, hepatic cells and sinusoid (H&E x100)

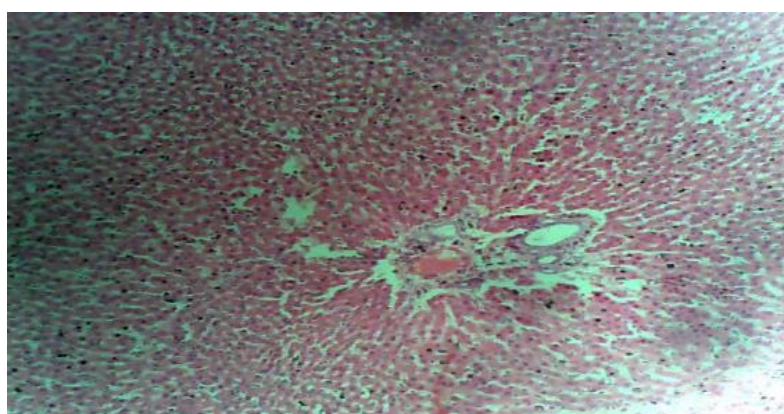


Figure 5 Photomicrograph of liver section from group treated with aqueous extract of *Allium Cepa* (500mg/kg) showing slight degenerative changes in within the cells, central vein dilatation and inflammation.

4. Discussion

No sign of toxicity was observed in the animals employed in the two phases of the acute oral toxicity study. The animals were active and alive during the period of observation. The median lethal dose (LD_{50}) for the aqueous extract of *Allium cepa* was estimated to be greater than 2000 mg/kg in rats which is in accordance with the research carried out [14]. From the observation of the oral acute toxicity studies result, doses of 125, 250, 500 mg/kg were employed for the sub-acute toxicity studies.

Several studies have shown that the major bioactive compounds present in the *A. cepa* peels are flavonoids, these are phenolic compounds which may be attributed to the pharmacological activities including diabetes, cancer, infections, hepatoprotection, cardiovascular diseases anti-inflammatory activities of this plant [15].

Consumption of food and water in the control group and experimented groups remained normal except for the group treated with 500mg/kg (high dose) of aqueous extract of *A. cepa*, which consumed more food and water. On the 28th day, slight decrease in weight of the animal treated with high dose of the aqueous extract was observed when compared to other groups. This shows that the extract may probably has suppressant appetite activity. The study carried out by Mootoosamy *et al* in 2014 indicated that the extract of *A. cepa* exhibited anti-obesity effect in Zucker diabetic fatty rats [16].

In the liver, there was slight decrease in size for the group treated with 500 mg/kg, and this may be indicative that the liver function can be preserved by oral administration of the extract as demonstrated in the sub-acute toxicity study of hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) in mice [17].

Analysis of the haematological parameters showed that the extract was not toxic on the blood forming constituents, because there was no significant difference in comparison to the control group. The slight decrease in the red blood cells

for the group administered with 500 mg/kg may be indicative of normocytic anaemia as demonstrated in the sub-acute toxicity study of *Cassia occidentalis L* [18]. This is a type of anaemia that occurs when the red blood cells are normal in size and hemoglobin content, but they are too few of them. This was confirmed by the components of red blood cell indices (MCV, MCH and MCHC) of the group administered with 500 mg/kg having no significant difference to the control group.

The liver enzymes (ALP and AST) were not significantly affected by the different doses of the extract, in comparison with the control group. The serum transaminases AST and ALT are used in the clinical setting for monitoring the hepatic function. Hepatocellular injuries are depicted by elevations in transaminases that are at least two times normal. If there is elevation in the concentration of ALP, a hepatocellular lesion is still suspected when the elevation in the concentration of ALT is notably higher than the elevation in the concentration of alkaline phosphatase. The lesion is likely to be cholestasis if the magnitude of elevation is nearly equal between ALT and ALP. A liver injury can be termed acute if it lasts less than 3 months; it is chronic after 3 months with consistent symptoms or elevations in enzyme concentration [19]. The reduction in the concentration of ALT in the group administered with 500 mg/kg when compared to the control group is as a result of the constituents in the extract which inhibited the enzyme activity or diminution of important molecules needed for the enzyme activities. The reduced ALT will adversely influence amino acid and carbohydrate metabolism with diminishing effect on ATP generation as shown in the sub-acute toxicity study of *Allium sativum* [20].

In addition to this, insignificant change in the level of albumin shows that the aqueous extract of *A. cepa* peel does not affect the hepatic function of the liver, which confirms the normal histological structure of the treated animals in comparable to the animals that were not treated with the extract.

5. Conclusion

Based on the study, *Allium cepa* was found to be safe up to a dose of 2000 mg/kg during the oral acute toxicity studies. The sub-acute toxicity studies indicated the safety of *Allium cepa* extract, but it is further recommended for chronic toxicity studies which allow for observation of long term effect of this medicinal plant because of the reduced liver size, reduced ALT levels, reduced RBC count.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflicts of interest.

Statement of ethical approval

Ethical approval was obtained from Bingham University Ethics Review Committee.

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