Iranian Journal of Pharmaceutical Sciences
2021: 17 (2): 51-66
www.ijps.ir

Original Article

Acute and Subacute Toxicity of Aqueous Extract of *Allium Cepa* Peels in Wistar Albino Rats

Fatimoh Idowu Ojuade[a*], Rashidat Oluwafunke Ayanniyi[a], Hidayah Ayodeji Olumoh-Abdul[a], Adedolapo Rhoda Ayeni[a], Mumini Wemimo Rasheed[b]

[a]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria, [b]Department of Anatomic Pathology, University of Ilorin Teaching Hospital, Ilorin, Ilorin, Kwara State, Nigeria.

Abstract

Allium cepa (*A. cepa*) is a medicinal plant widely used as spice in food and has been reported to have antiinflammatory, antiulcer, antidiabetic, antihypertensive, anticancer, and antioxidant properties amongst others. The peels from this vegetable also possess antioxidant, antiulcer, antidiabetic, antihypertensive activities to mention few. *A. cepa* peels is used by traditional healers to treat and or manage different ailments but little is known about the safety of *A. cepa* peels. This study evaluated the safety of aqueous extract of *A. cepa* peels (AEACP) in female Wistar albino rats. Oral acute toxicity was evaluated using Organization for Economic Cooperation and Development (OECD) guideline 423, three (3) oral doses of the extract (125, 250, and 500 mg/kg) were used and administered for 28 days for the subacute toxicity study. The effect of AEACP was evaluated on the: body weight, relative organ weight, hematological parameters, hepatic and renal parameters. The effect of AEACP was also evaluated on the histology of the kidney and liver. The median lethal dose (LD50) was estimated to be greater than 2000 mg/kg and administration of AEACP produced no significant ($p<0.05$) differences in body weight, relative kidney weight, creatinine, and uric acid when compared with control group. There were significant ($p<0.05$) reduction in relative liver weight, serum sodium, and serum chloride level in 500 mg/kg group and the percentage reduction in comparison with control was 15.24 ± 1.98, 42.45 ± 2.40, and 9. 65 ± 1.07 respectively. The PLT and ALT values in 125 mg/kg group were significantly ($p<0.05$) lowered by 26.26 ± 2.96 and 39.46 ± 3.04% when compared with control. The WBC, uric acid, Albumin, and D. bilirubin values in 500 mg/kg group were significantly reduced ($p<0.05$) compared with 125 mg/kg group with percentage reductions of 32.10 ± 2.31, 7.79 ± 1.03, 17.89 ± 2.34, and 27.37 ± 2.79 respectively. The urea level in groups treated with 125 and 250 mg/kg of AEACP was significantly lower than the control group and the percentage reduction were found to be 54.17 ± 2.10 and 37.15 ± 1.98 respectively. The histopathological examinations showed no traces of toxicity as the architecture of the liver and kidney were preserved. Acute and subacute use of Allium cepa peels produced no toxicity, its folkloric use is safe and should be encouraged..

Keywords: Antidiabetic, Biochemical, Hematology, Histology, Kidney, Liver, Traditional.

Corresponding Authors: Fatimoh Idowu Ojuade, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria
Tel: (+234) 8075569192
Email: ojuade.fi@unilorin.edu.ng
Cite this article as Ojuade F. I., Ayanniyi R. O., Olumoh-Abdul H. A., Ayeni A. R., Rasheed M. W., Acute and Subacute Toxicity of Aqueous Extract of Allium Cepa Peels in Wistar Albino Rats, 2021, 17 (2): 51-66.

1. Introduction

There is an increasing rate in the acceptance and consumption of traditional medicine (TM) in both developing and developed countries [1]. About 60-80% population of the third world countries are dependent on nature's products to satisfy their daily health care needs [2]. World Health Organization (WHO) has recommended the evaluation of TM to enhance the use of effective ones and discourage the use of toxic ones [3]. Effects of medicinal plants are usually synergistic or additive because they contain several phytochemicals like alkaloids, phenolics, tannins, saponins, and glycosides amongst others [4]. *Allium cepa* (*A. cepa*) belongs to the family called Amaryllidaceae, it's an aromatic vegetable that is easily digested when eaten and it is used throughout the world as a spice [5]. *A. cepa* is referred to as onion, garden onion, and white onion [6]. The plant grows to a height of 15-45 cm (6-18 inches) and has yellowish-green leaves with flattened, fan shaped swathe [5].

Onion peel is the external part of the onion bulb and has several folds, the peels are thin, light weight, strong and often translucent in appearance [7]. It is used traditionally for its medicinal virtues in a plethora of indigenous cultures and the vegetable has been reported to have antioxidant, antibacterial, anti-inflammatory, anti-cholesterol, and anticancer activities [8-10]. The plant is used to prepare ayurvedic formulations used for wound healing and also in treating heart related diseases, diabetes, and cancer of the stomach [11-12]. Onion is prescribed to facilitate bowel movements, it relieves headaches, coughs, snakebite, and improves hair loss. Onion extract is also used for prevention of presternal hypertrophic scar [13-15]. Increase intake of *Allium* vegetables has been studied to reduce risk for hypertension, diabetes, gastric and prostate cancer [16-18].

Onion peels contain a number of constituents like sulphur, qurcertin, protocatechuic acid (PCA), calcium, flavonoids, and phenolics to mention few [19]. The PCA has been reported to possess antioxidant, antibacterial, anticancer, antiulcer, antidiabetic, antiageing, antifibrotic, antiviral, anti-inflammatory, antiatherosclerotic, cardioprotective, hepatoprotective, and nephroprotective activities [14, 19-23,]. There are reports on the efficacy of *A. cepa* peels, with limited reports on its safety. This study evaluated the acute and subacute toxicity of *Allium cepa* peels in wistar albino rats.

2. Materials and Methods

2.1. Experimental Animals

Thirty female Wistar rats (120-130 g) were obtained from the animal house of University

of Ilorin, Ilorin. Rats were allowed to acclimatize for one week and maintained under standard conditions of light and darkness (12/12hrs) with free access to food and water *ad libitium* at Department of Pharmacology and Toxicology, University of Ilorin, Ilorin. The experiment was performed in accordance with the principles of laboratory animal care by National Institute of Health (NIH publication No. 85-23, which was revised in 1985) and the principles laid down by the University of Ilorin Ethics Committee for care and use of laboratory animals. Ethical clearance was obtained from the Ethical Review Committee of the University of Ilorin with an approval no: UERC/ASN/2019/1863.

2.2. Plant Material and Extract Preparation

Allium cepa peels was purchased from a local market (Mandate Market) in Ilorin area of Kwara State in December 2019. It was cleaned and dried at room temperature, two hundred grams (200 g) of *Allium cepa* peels was weighed and soaked in two liters (2 L) of distilled water for 72 hours with intermittent agitation. After 72 hours, the supernatant was decanted, allowed to settled, and filtered with a Whaltman paper (No 1). The filtrate was evaporated to dryness on a water bath at a temperature of 40°C [24]. The concentrated extract was named aqueous extract of *Allium cepa* peels (AEACP) and was stored at 4°C throughout and use for this experiment.

2.3. Oral Acute Toxicity Test

The oral acute toxicity test was carried out in accordance with OECD guideline 423, which requires the use of three (3) female animals in two (2) phases. For the first phase, three (3) female wistar rats were fasted overnight for 12 hours and their weight taken afterwards. The extract was dissolved in normal saline and the animals were orally fed with 2000 mg of the extract per kilogram body weight of the animals (2000 mg/kg). After the administration of extract, the animals were closely monitored individually for behavioral changes and signs of toxicity for 4 hours, and the observation continued for the next 24 hours. In the absence of death, the second phase was repeated with another three (3) set of female animals and same dose of extract. The observation continued for a total of 14 days [25-27].

2.4. Experimental Design for Sub-chronic Study

Twenty four (24) female Wistar rats were randomly divided into four groups of six (6) rats each as below;

Group 1: Normal saline (1 mL/kg),

Group 2: AEACP (125 mg/kg),

Group 3: AEACP (250 mg/kg),

Group 4: AEACP (500 mg/kg). These doses of extract were selected based on the result of oral acute toxicity test. All the doses were administered orally and daily for a period of 28 days and the animals were closely monitored throughout the duration of the experiment [2].

2.5. Blood Collection

Twenty four hours (24 hrs.) after the completion of the experiment, animals were

anaesthetized in an airtight container saturated with diethyl ether (ether). Blood samples were collected through cardiac puncture with the aid of 2 mLs needle and syringe into Ethylenediaminetetraacetic acid (EDTA) bottles and Lithium heparinized bottles.

2.6. Determination of Hematological Parameters

The blood collected inside EDTA bottles were used for the analysis of hematological parameters. The White blood cells (WBC), Red blood cells (RBC), Haemoglobin (HGB), Platelets (PLT), Lymphocytes (LYM), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH), and Mean corpuscular volume (MCV), Hematocrit (HCT), Mean platelet volume (MPV), Platelet distributing width (PDW), and Platelet large cell ratio (P- LCR) were analyzed using Sysmex KX-21N automated hematology analyzer (Sysmex America Inc, USA).

2.7. Determination of Serum Biochemical Parameters

Blood samples collected inside the lithium heparinized bottles were used for serum biochemical parameters. Blood samples were centrifuged 3000 rpm for 15 minutes to separates blood cells from the plasma after which the serum was collected with a Pasteur pipette into clean sample bottles. Concentration of serum electrolytes (Sodium, Chloride and Potassium), liver and kidney enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), Albumin (ALB), direct bilirubin (DB), and Total bilirubin (TB)), Creatinine Urea, and Uric acid were analyzed using diagnostic kits purchased from Ray Biotech, Norcross, GA, USA.

2.8. Determination of Body Weight and Relative Organ Weight

The weight of the animals on the first (day 1) and last day (day 29) of the experiment were taken, the weight of the organs were taken and the changes in body weight and the relative organ weight were calculated using these formula;

$$\%\ \text{weight change} = \frac{\text{weight of animal on day 29} - \text{weight of animal on day 1}}{\text{weight of animal on day 29}} \times 100$$

$$\text{Relative liver or kidney weight} = \frac{\text{weight of organ (g)}}{\text{weight of animal (g)}} \times 100$$

2.9. Organs Preparation and Histology of Tissue

Liver and kidney were removed from the animals, rinsed in 0.9 % normal saline and fixed in 10 % formo-saline for histology. Liver and kidney tissues were processed and embedded in paraffin wax. Organs sections were cut, hematoxylin and eosin staining technique was used and the prepared slides were viewed with a light microscope at a magnification of 40×.

2.10. Statistical Analysis

Data obtained were presented as means ± SEM and analyzed using GraphPad Prism (version 8.03, Graphpad Software Inc.) for window. Statistical analysis was carried out using one-way ANOVA test, this was

followed by Dunnett's Test used for multiple comparisons between groups. Analysis was considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Result of Oral Acute Toxicity Test

No toxic reactions were observed in the animals neither was death recorded after administering an oral dose of 2000 mg/kg in both phases of the oral acute toxicity study. The animals were noted to be active and healthy and the median lethal dose (LD_{50}) of AEACP was estimated to be greater than 2000 mg/kg in rats.

3.1.2. Effect of AEACP on Body Weight, Relative Liver and Kidney Weight

Administration of AEACP produced no significant difference ($p>0.05$) in the weight of the animals when compared to control group. The percentage weight gain in the extract-treated groups were; 28.60 ± 5.12, 26.47 ± 2.34, 31.23 ± 4.78 as compared to 31.36 ± 6.01 observed in control group (Table 1). There was a significant decrease in the relative liver weight for group treated with 500 mg/kg of AEACP when compared with control group, and the percentage decrease was found to be 15.24 ± 1.98. There was no significant difference ($p>0.05$) in the relative kidney weight of the extract groups (6.15 ± 1.35, 6.24 ± 0.64, 6.70 ± 0.42) in comparison with control group (7.01 ± 0.95) as shown in Table 2.

3.1.3. Effect of AEACP on Hematological Parameters

There was a significant reduction ($p<0.05$) in the PLT for group treated with 125 mg/kg of AEACP when compared with control group, the percentage reduction was found to be 26.26 ± 2.96. A significant reduction ($p<0.05$) was observed in WBC of 500 mg/kg group when compare 125 mg/kg group with percentage reduction of 32.10 ± 2.31 (Table 3). Although, there was no significant variation in the WBC of extract treated groups when compared with control group. Administration of different dosses of the extract does not alter other hematological parameters (HGB, HCT, LYM, MCH, MCHC, MCV, MPV, PDW, P-LCR, and RBC) as there was no significant difference ($p<0.05$) when compared with control (Table 3).

3.1.4. Effect of AEACP on Liver Enzymes

Administration of 125 mg/kg of AEACP produced a significant reduction ($p> 0.05$) in the level of ALT (34.87 ± 2.05) when compared with control group (57.60 ± 3.78) and the percentage reduction was found to be 39.46 ± 3.04 (Table 4). A significant reduction in AST level was observed for group treated with 250 mg/kg of the extract when compared with 125 mg/kg and the reduction was 8.97 ± 1.46. There was also significant reduction in Albumin and D. Bilirubin levels for group treated with 500 mg/kg of AEACP when compared with 125 mg/kg group, the percentage reduction were found to be 17.89 ± 2.34, 27.37 ± 2.79 respectively (Table 4).

3.1.5. Effect of AEACP on Serum Electrolyte and Renal Markers

Administration of 500 mg/kg of AEACP produced a significant reduction ($p<0.05$) in serum chloride (9.65 ± 1.07), there was no significant variation in the serum potassium and a significant reduction in the sodium concentration (42.45 ± 2.40) when compared with the control group as seen in Fig.1a, Fig.1.b, and Fig. 1.c, respectively). There was no significant variation in the creatinine level for all the treatment groups (Figure 2a.) but a significant reduction ($p<0.05$) in the level of urea was observed for groups treated with 125 and 250 mg/kg of AEACP when compared with the control group, the percentage reduction were 54.17 ± 2.10 and 37.15 ± 1.98 respectively (Figure 2b.). Group treated with 500 mg/kg of extract also had a significant reduction in uric acid when compared with 125 mg/kg group, the percentage reduction was found to be 7.79 ± 1.03 (Figure 2c).

3.1.6. Effect of AEPN on Histology of Organs

Micrograph section (liver) for control group showed hexagonal plates with central vein containing red blood cells, the plates of hepatocyte appeared normal with no abnormalities. There are no features of mononuclear inflammatory cells or infiltration at the portal triad and hepatic lobules (Figure 3). The liver sections for the groups administered with 125, 250 and 500 mg/kg of AEACP showed hexagonal plates of hepatocyte admixed with central vein and portal triad (B, C, and D), and there were no features of toxicity. Kidney section of the control group showed glomeruli of varying sizes, there was no alteration of glomeruli architecture (Figure 4). The kidney sections of the extract groups showed tubules which are lined by simple cuboidal epithelium. The renal arterioles present are with normal interstitial spaces with no renal tubular necrosis or cellular infiltration within the interstitial spaces for all the treatment groups.

3.2. Discussion

Acute and subacute toxicity testing in experimental animals is relevant to determine the potential toxic effects of any agent in humans [28]. In this study, the extract was found to be safe at a dose of 2000 mg/kg as no signs of behavioral or neurological toxicities were recorded within the period of observation. Xenobiotic with LD_{50} of greater than 2000 mg/kg to less than 5000 mg/kg has been said to have the lowest or no toxicity [29]. Hence, aqueous extract of *A. cepa* peels is safe when used within the limit of its LD_{50} value. Oral acute toxicity testing is not enough to evaluate the toxicity of any agent as the long term effect of such an agent wouldn't be evident with a single dose administration. Therefore, the assessment of subacute oral toxicity is vital as it helps to study the morphological and physiological alterations in organs after repeated low doses of extract or chemicals [30, 31]. Administration of AEACP for 28 days produced no significant changes in the body weight of the animals (Table 1). The extract therefore, has no toxic effects on metabolic processes and hence non-toxic since weight loss is a good marker of toxicity [32].

Liver and kidney are vital organs in the metabolism of drugs, extracts, and or chemicals. Significant alterations in the weight of these important organs are clear signs of toxicity [33]. There was no significant variation in the relative kidney weight for extract-treated groups when compared with control group (Table 2), this was further evident by the histological findings which showed normal architecture. The significant reduction in relative liver weight for group treated with 500 mg/kg of AEACP (Table 2) may be due to differences in the size and/or weight of animals' organs used [34]. This finding is in agreement with the work of Demle *et al*, 2019 where a hydroalcoholic extract of onion peels was found to be hepatoprotective.

The hematopoietic system is a vital target for assessing the toxicity of plant extract in animal model [35]. This system can be damaged by toxicants via indirect pathways as seen in oxidative hemolysis within the circulation and or immunotoxic reactions with the components of blood [36]. The significant reduction in PLT and WBC values observed in some groups treated with extract (Table 3) may not be indicative of toxicity because the values of these parameters fell within the normal range stipulated for Wistar rats [37, 38]. Conditions that could alter these values include but not limited to; anemia, infection, and or immune system dysfunction [35]. Since none of these conditions could be ascertained, the extract can be concluded to be safe on hematological parameters when used within this duration. A similar study had confirmed the antioxidant, hepatoprotective, and neuroprotective effects of PCA which is a constituent of *Allium cepa* peels [22].

The liver is susceptible to the toxic effects of xenobiotics because most chemicals are metabolized by the liver before elimination, most times through the bile [2]. When the liver membrane gets damaged, enzymes located in the cytosol are most times released into the bloodstream and this causes an increase in the values of these enzymes [39, 40]. Serum ALP, ALT, AST, albumin and bilirubin are important markers for assessment of intrahepatic and extrahepatic bile obstruction, liver cells infiltration, acute and chronic hepatocellular injury [41]. The concentrations of liver enzymes assayed in this study varied within and across the groups (Table 4). The concentration of alanine transaminase (ALT) is usually low in the blood, alongside with aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin [40]. The low levels observed from this study was in tandem with the work of Damle *et al*, 2019 and Nutila *et al*, 2003.

Data from this study showed that AEACP has positive chloride and sodium modulatory effect (Figure 1a & 1c). The reduction of chloride and sodium points to its potential to lower salt level in the body which can ameliorate hypertensive conditions. This finding is in agreement with the work of Naseri *et al*, 2008, where a hydroalcoholic extract of *Allium cepa* peels was found to have a vasorelaxant and hypotensive effects. Electrolyte level especially sodium (Na) and Chloride (Cl), Creatinine, Urea, and Uric acid

are parameters that can be used to assess kidney function, elevated level of these parameters are indications for a potential disease condition of the kidney [44]. Increasing the level of urea can occur with an increase in age and an increase in protein content of food or feed.

Low concentration of urea cannot be used alone to diagnose liver or kidney problem as low level of this parameter can be observed in normal individuals [45, 46]. Since other parameters aside from the urea level are normal, the extract can be regarded to be safe on kidney. This was further supported by the histopathological finding which showed normal architecture of the kidney for the treatment groups (Figure 4). The extract-treated groups showed tubules that are lined with simple cuboidal epithelium cells but this is not of toxicological importance as the cuboidal epithelium aid absorptive and secretory functions [47].

4. Conclusion

Data from this study showed that *Allium cepa peels* is non-toxic and it is safe for short term use in traditional medicine for management of various ailments. Subchronic and chronic toxicity studies need to be conducted on *Allium cepa* peels to evaluate its long term safety.

Acknowledgments

The authors acknowledge the technical staff at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, for their support during the course of this work.

References

[1] Ayanniyi RO, Ojuade FI, Olumoh-Abdul H, Salawu KM, Olufadi-Ahmed H, Adebisi-Jose GO. Evaluation of anti-nociceptive and anti-inflammatory activities of leaf extract of Turraea vogelli Hook. f. ex. Benth. *Pak. J. Pharm. Sci.* (2019) 32: 241-245.

[2] Ayanniyi RO, Abdul-Olumoh HA, Ojuade FI, Adebisi-jose GO, Rasheed MW. Acute and subacute toxicity studies on methanol leaf extract of Turraea vogelli Hook. f. ex. Benth. *Niger. J. Pharm. Sci.* (2018) 17: 26-32.

[3] World Health Organization. The WHO strategy for traditional medicine: Review of the global situation and strategy implementation in the Eastern Mediterranean Region, (2002).

[4] Haque N, Salma U, Nurunnabi TR, Uddin MJ, Jahangir MF, Islam SM, Kamruzzaman M. Management of type 2 diabetes mellitus by lifestyle, diet and medicinal plants. *Pak. J. Biol. Sci.* (2011) 14: 13-26.

[5] Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK, Fawzi MM. Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review. *Crit. Rev. Food Sci. Nutri.* (2019) 59: 39-70.

[6] Upadhyay RK. Nutritional and therapeutic potential of Allium vegetables. *J. Nutr. Ther*. (2017) 6: 18-37.

[7] Kakkar S and Bais S. A review on protocatechuic acid and its pharmacological potential. *ISRN Pharmacol*. (2014) 26: 6-9.

[8] Upadhyay RK. Nutraceutical uses of Allium cepa: A review. *Int. J. Green Pharm.* (2016) 10: 60-84.

[9] Krishnaswamy K. Traditional Indian spices and their health significance. *Asia Pac. J. Clin. Nutr.* (2008) 17: 265-68.

[10] Gangopadhyay KS, Khan M, Pandit S, Chakrabarti S, Mondal TK, Biswas TK. Pharmacological evaluation and chemical

standardization of an ayurvedic formulation for wound healing activity. *Int. J. Low. Extrem. Wounds* (2014) 13: 41-9.

[11] Jenwitheesuk K, Surakunprapha P, Jenwitheesuk K, Kuptarnond C, Prathanee S, Intanoo W. Role of silicone derivative plus onion extract gel in presternal hypertrophic scar protection: A prospective randomized, double blinded, controlled trial. *Int. Wound J*. (2012) 9: 397-402.

[12] Park J, Kim J, Kim MK. Onion flesh and onion peel enhance antioxidant status in aged rats. *J. Nutr. Sci. Vitaminol.* (2007) 53: 21-9.

[13] Abdel-Maksouda G, El-Aminb AR. A review on the materials used during the mummification process in ancient Egypt. *Med. Archaeol. Archaeometr*. (2011) 11: 129-50.

[14] Zhou Y, Zhuang W, Hu W, Liu GJ, Wu TX, Wu XT. Consumption of large amounts of *Allium* vegetables reduces risk for gastric cancer in a meta-analysis. *Gastroenterol.* (2011) 141: 80-89.

[15] Khadzhiĭski O, Diakov R, Petrova M. Contractubex used in the treatment of post burn scars and keloids. *Khirurgiia* (2001) 57: 44.

[16] Wang Y, Tian WX, Ma XF. Inhibitory effects of onion (*Allium cepa* L.) extract on proliferation of cancer cells and adipocytes via inhibiting fatty acid synthase. *Asian Pac. J. Cancer Prev.* (2012) 13: 5573-79.

[17] Otoom SA, Al-Safi SA, Kerem ZK, Alkofahi A. The use of medicinal herbs by diabetic Jordanian patients. *J. Herb. Pharmacother.* (2006) 6: 31-41.

[18] Zurada JM, Kriegel D, Davis IC. Topical treatments for hypertrophic scars. *J. Am. Acad. Dermatol.* (2006) 55: 1024-31.

[19] Shivanand P, Mahalaxmi R. Identification and determination of protocatechuic acid present in greater cardamom fruit extracts by HPTLC technique. *Inter. J. Pharm. Sci. Rev. Res*. (2010) 1: 27-32.

[20] Arslan R, Aydin S, Samur DN, Bektas N. The possible mechanisms of protocatechuic acid-induced central analgesia. *Saudi Pharm. J.* (2018) 26: 541-5.

[21] Singh BN, Singh BR, Singh RL, Prakash D, Singh DP, Sarma BK, Upadhyay G, Singh HB. Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food Chem. Toxicol.* (2009) 47: 1161-7.

[22] Damle MC, Tembhurne SV, Todkar SR. Evaluation of hepatoprotective activity of hydroalcoholic extract of onion peels containing protocatechuic acid. *J. Drug Deliv. Therap.* (2019) 9: 82-89.

[23] Park J, Kim J, Kim MK. Onion flesh and onion peel enhance antioxidant status in aged rats. *J. Nutri. Sci. Vitaminol.* (2007) 53: 21-9.

[24] Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Nigeria, (1993) 150-156.

[25] Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, Karhunen P, Peltonen K. Application of OECD Guideline 423 in assessing the acute oral toxicity of Moniliformin. *Food Chem. Toxicol.* (2013) 53: 27-32.

[26] Sankhari JM, Jadeja RN, Thounaojam MC, Devkar RV, Ramachandran AV. Safety evaluation of Eugenia jambolana seed extract. *Asian Pac. J. Trop. Med.* (2010) 3: 982-987.

[27] Nana HM, Ngane RN, Kuiate JR, Mogtomo LK, Tamokou JD, Ndifor F, Mouokeu RS, Etame RE, Biyiti L, Zollo PA. Acute and sub-acute toxicity of the methanolic extract of Pteleopsis hylodendron stem bark. *J. Ethnopharmacol.* (2011) 137: 70-76.

[28] Bello I, Bakkouri AS, Tabana YM, Al-Hindi B, Al-Mansoub MA, Mahmud R, Asmawi M. Acute and sub-acute toxicity evaluation of the methanolic extract of Alstonia scholaris stem bark. *Med. Sci.* (2016) 4: 4-12.

[29] Mekonnen A, Tesfaye S, Christos SG, Dires K, Zenebe T, Zegeye N, Shiferaw Y, Lulekal E. Evaluation of skin irritation and acute and subacute oral toxicity of lavandula angustifolia essential oils in rabbit and mice. *J. Toxicol.* (2019).

[30] Abdulrahman FI, Onyeyili PA, Sanni S, Ogugbuaja VO. Toxic effect of aqueous root bark extract of Vitex doniana on liver and kidney functions. *Int. J. Biol. Chem.* (2007) 1: 184-95.

[31] Prasanth KM, Suba V, Ramireddy B, Srinivasa BP. Acute and subchronic oral toxicity assessment of the ethanolic extract of the root of Oncoba spinosa (Flacourtiaceae) in rodents. *Trop. J. Pharm. Res.* (2015) 14: 1849-55.

[32] Aiyalu R, Ramasamy A. Acute and sub-acute Toxicity study of Aqueous extracts of Canscora heteroclita (L) Gilg in Rodents. *Pharmacogn. J.* (2016) 8: 23-28.

[33] Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz WO, Muzzi RM, Oesterreich SA, Arena AC. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from Acrocomia aculeata pulp in rats. *Food Chem. Toxicol.* (2014) 74: 320-5.

[34] Auletta CS, RAC M. Acute, subchronic, and chronic toxicology. *Handbook of Toxicology.* (1995) 51-162.

[35] Ashafa AO, Yakubu MT, Grierson DS, Afolayan AJ. Toxicological evaluation of the aqueous extract of Felicia muricata Thunb. leaves in Wistar rats. *Afri. J. Biotechnol.* (2009) 8: 42-48

[36] Irons RD. Quinones as toxic metabolites of benzene. *J. Toxicol. Environ. Health Sci.* (1985) 16: 673-678.

[37] Levine BS. Animal Clinical Pathology. *CRC Handbook of Toxicology*, (1995) 517-37.

[38] Jones ML, Allison RW. Evaluation of the ruminant complete blood cell count. *Vet. Clin. North Am.:* Food Animal Practice (2007) 23: 377-402.

[39] Owu DU, Osim EE, Ebong PE. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta Trop.* (1998) 69: 65-73.

[40] Fischbach FT, Dunning MB. A manual of laboratory and diagnostic tests. Lippincott Williams & Wilkins (2009).

[41] Ezeji EU, Chukwudi P. Effects of Mixtures of Lambda-Cyhalothrin and Dimethoate on Liver and Antioxidant Enzyme Systems of Adult Male Albino Rats. *Fut. J. Ser.* (2019) 5: 228-235.

[42] Nuutila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey KM. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* (2003) 81: 485-493.

[43] Naseri MK, Arabian M, Badavi M, Ahangarpour A. Vasorelaxant and Hypotensive Effects of Allium cepa peel hydroalcoholic extract in rat. *Pak. J. Biol. Sci.* (2008) 11: 1569-1575.

[44] Bang M, Kim HA, Cho YJ. Alterations in the blood glucose, serum lipids and renal oxidative stress in diabetic rats by supplementation of onion (Allium cepa. Linn). *Nutr. Res. Pract.* (2009) 3: 242-246.

[45] Mishra S, Pani SR, Sahoo S. Anti-nephrotoxic activity of some medicinal plants from tribal rich pockets of Odisha. *Pharmacogn. Res.* (2014) 6: 210-217.

[46] Okokon JE, Nwafor PA, Noah KJ. Nephroprotective effect of Croton zambesicus root extract against gentamycin-induced kidney injury. *Asian Pac. J. Trop. Med.* (2011) 4: 969-972.

[47] Adams M, Smith UM, Logan CV, Johnson CA. Recent advances in the molecular pathology, cell biology and genetics of ciliopathies. *J. Med. Genet.* (2008) 45: 257-267.

Tables:

Table 1. Effect of AEACP on body weight.

Days	**Treatment groups**			
	Control	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**
Day 1 (g)	116.0 ± 4.01	117.10 ± 2.40	133.9 ± 6.50	120.70 ± 5.3
Day 28 (g)	169.0 ± 13.5	164.0 ± 9.96	182.10 ± 11.29	175.50 ± 13.9
Weight gain (g)	53.0 ± 4.32	46.90 ± 3.69	48.20 ± 1.89	54.80 ± 7.67
Weight gain (%)	31.36 ± 6.01	28.60 ± 5.12	26.47 ± 2.34	31.23 ± 4.78

Data are expressed as mean ± SEM, n=6, $^{**}p < 0.05$ is significant difference in comparison with control, $^{\#\#}p < 0.05$ is significant difference between the extract groups.

Table 2. Effect of AEACP on relative liver and kidney weight.

Relative organ weight	**Treatment groups**			
	Control	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**
Liver ($\times 10^{-3}$ g)	46.84 ± 1.60	43.83 ± 1.80	41.03 ± 1.80	$39.7 \pm 2.92^{**}$
Kidney ($\times 10^{-3}$ g)	7.01 ± 0.95	6.15 ± 1.35	6.24 ± 0.64	6.70 ± 0.42

Data are expressed as mean ± SEM, n=6, $^{**}p < 0.05$ is significant difference in comparison with control, $^{\#\#}p < 0.05$ is significant difference between the extract groups.

Table 3. Effect of AEACP on hematological parameters.

Parameters	**Treatment groups**			
	Control	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**
HGB (g/dl)	11.87 ± 0.13	11.60 ± 0.26	10.93 ± 0.33	10.80 ± 0.10
HCT (%)	44.43 ± 0.90	44.30 ± 1.05	41.87 ± 0.84	43.00 ± 0.60
LYM %	91.40 ± 0.72	90.93 ± 0.23	87.97 ± 3.77	89.60 ± 0.40
MCH (pg)	13.67 ± 0.32	14.47 ± 0.33	14.47 ± 0.34	13.55 ± 0.35
MCHC (g/dL)	26.70 ± 0.35	26.20 ± 0.21	26.13 ± 1.07	25.10 ± 0.60
MCV (fL)	51.23 ± 0.59	55.27 ± 1.13	55.40 ± 1.36	53.90 ± 0.30
MPV (fL)	7.03 ± 0.033	7.13 ± 0.09	7.07 ± 0.15	6.85 ± 0.05
PDW (fL)	9.77 ± 0.22	9.97 ± 0.22	9.30 ± 0.36	9.15 ± 0.25
P-LCR (%)	8.70 ± 0.44	9.20 ± 0.74	8.63 ± 0.69	7.70 ± 0.42
PLT ($\times 10^3/\mu L$)	894.70 ± 24.66	659.70 ± 56.69**	841.0 ± 31.39	862.0 ± 74.00
RBC ($\times 10^6/\mu L$)	8.68 ± 0.23	8.02 ± 0.13	7.56 ± 0.05	7.98 ± 0.15
WBC ($\times 10^3/\mu L$)	14.57 ± 0.84	16.73 ± 1.74	13.23 ± 0.50	11.35 ± 0.45$^{\#\#}$

Data are expressed as mean ± SEM, n=6, $^{**}p < 0.05$ is significant difference in comparison with control, $^{\#\#}p < 0.05$ is significant difference between the extract groups.

Table 4. Effect of AEACP on liver enzymes.

Liver enzymes (U/L)	**Treatment groups**			
	Control	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**
ALP	97.67 ± 13.72	95.00 ± 6.11	98.70 ± 2.03	94.50 ± 2.50
ALT	57.60 ± 3.78	34.87 ± 2.05**	45.93 ± 4.10	55.30 ± 2.40
AST	138.30 ± 36.34	142.7 ± 36.07	129.90 ± 16.93$^{\#\#}$	139.90 ± 10.03
Albumin	30.41 ± 1.84	32.03 ± 2.16	26.91 ± 0.42	26.30 ± 4.41$^{\#\#}$
D. bilirubin	4.42 ± 2.06	4.64 ± 0.61	3.48 ± 1.60	3.37 ± 1.85$^{\#\#}$
T. bilirubin	36.45 ± 0.86	36.21 ± 0.39	36.88 ± 0.26	36.15 ± 0.08

Data are expressed as mean ± SEM, n=6, $^{**}p < 0.05$ is significant difference in comparison with control, $^{\#\#}p < 0.05$ is significant difference between the extract groups.

Figures:

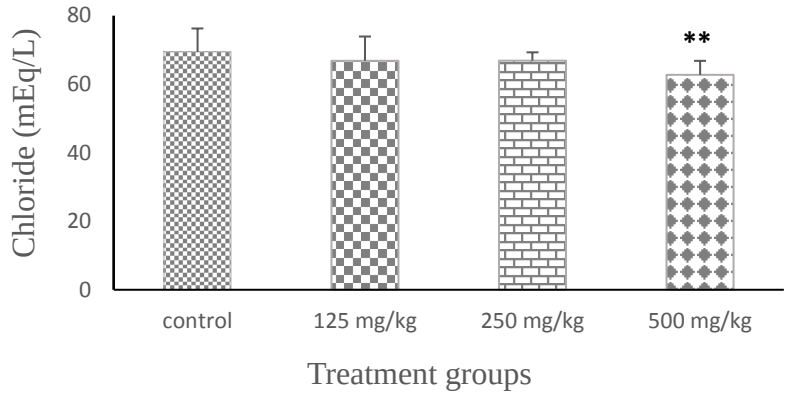

Figure 1a. Effect of AEACP on serum chloride. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between the extract groups.

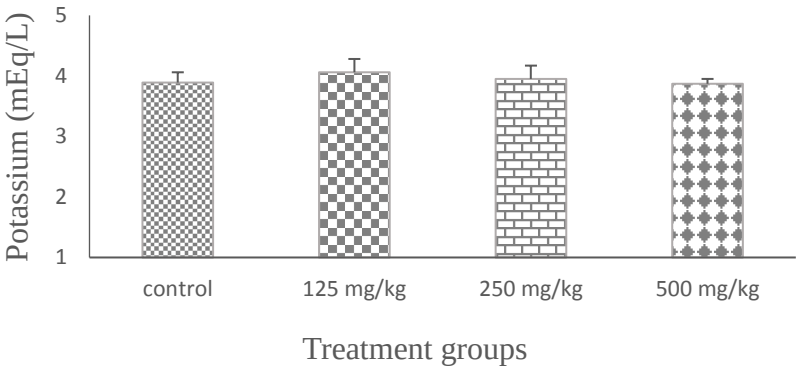

Figure 1b. Effect of AEACP on serum potassium. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between the extract groups.

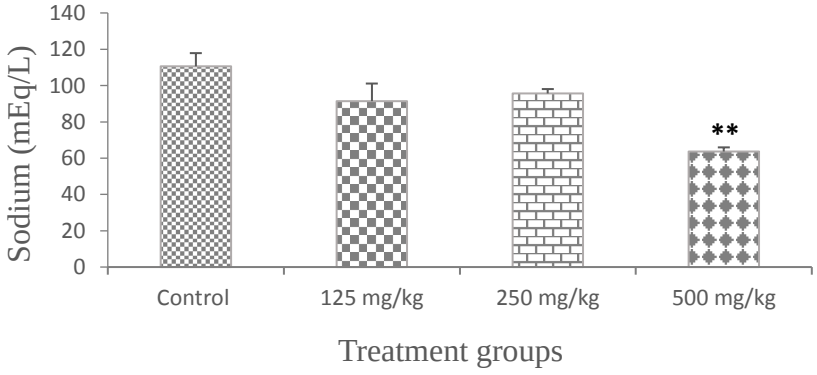

Figure 1c. Effect of AEACP on serum sodium. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between the extract groups.

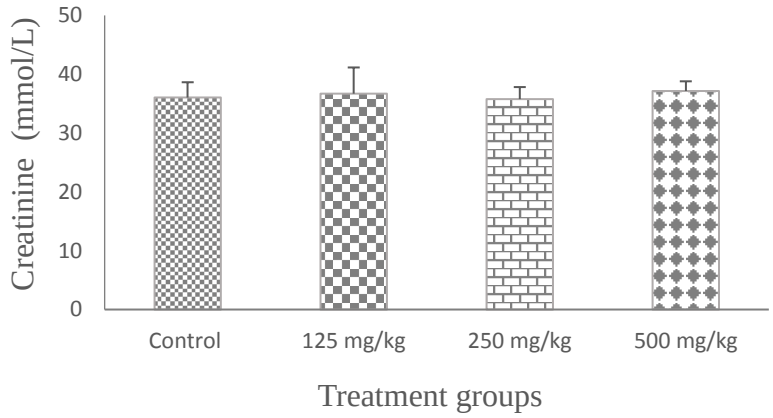

Figure 2a. Effect of AEACP on creatinine. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between extract groups.

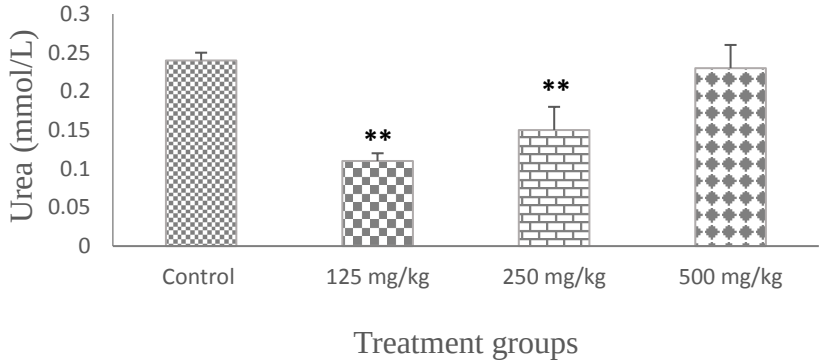

Figure 2b. Effect of AEACP on urea. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between the extract groups.

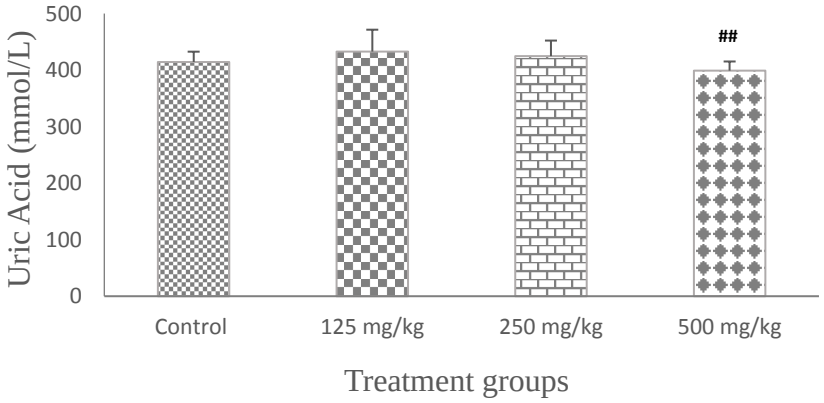

Figure 2c. Effect of AEACP on uric acid. Data are expressed as mean ± SEM, n=6, ***p* < 0.05 is a significant difference in comparison with control, ##*p* < 0.05 is significant difference between the extract groups.

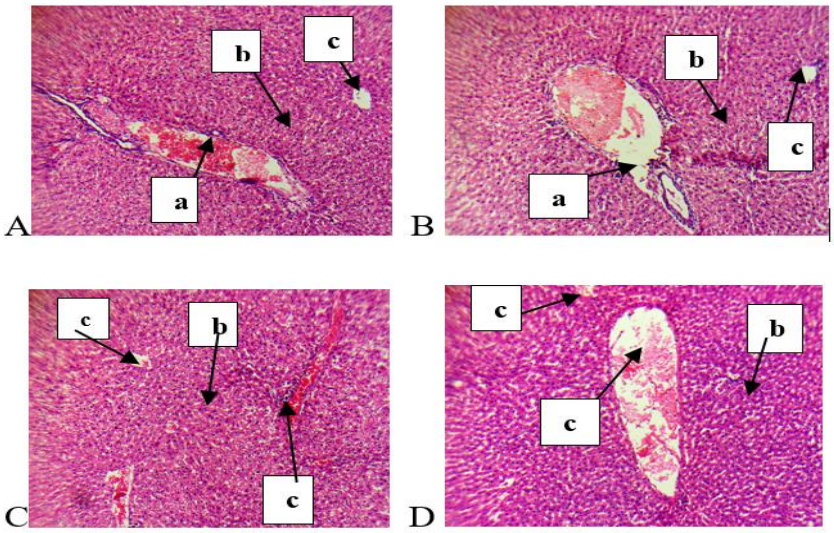

Figure 3. Micrograph sections of liver (40 X, H & E).A: liver section shows tissue with preserved architecture; B: liver section shows hexagonal plates of hepatocyte, there were no traces of cellular infiltration or necrosis; C: same as A; D: same as B. a = portal area, b = hepatocytes, c = central vein. A-D are normal saline (1 ml/kg), 125 mg/kg of AEACP, 250 mg/kg of AEACP, and 250 mg/kg of AEACP respectively.

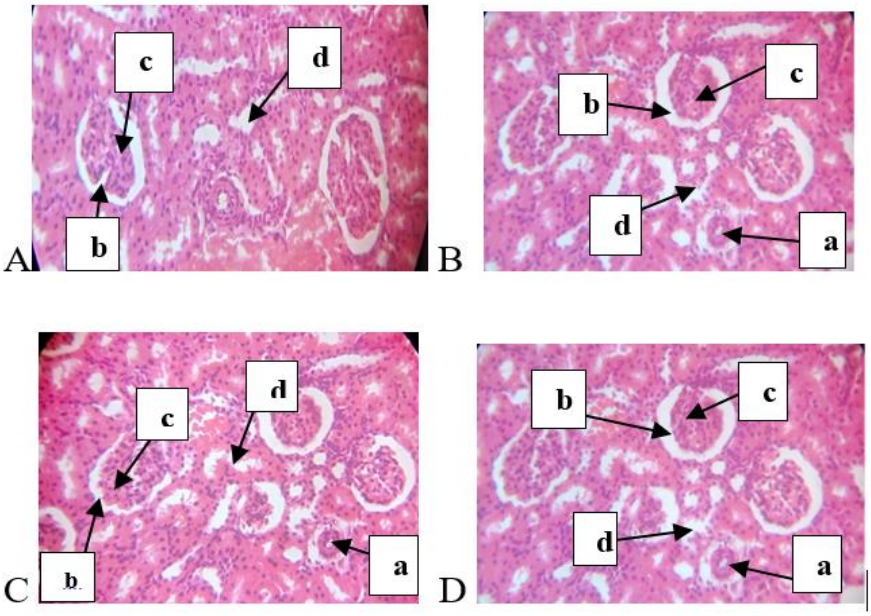

Figure 4. Micrograph sections of kidney (40 X, H & E). A: kidney section showed tissue with preserved architecture, there was no necrosis and or cellular infiltration; B: section showed tubules which are lined by simple cuboidal epithelium; C: same as A; D: same as B. a = cuboidal epithelium cell, b = bowman capsule, c = glomeruli, d = distal convoluted tubules.

ONLINE SUBMISSION

www.ijps.ir