American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Biochemistry and Molecular Biology

ISSN 2150-4210
DOI: 10.3923/ajbmb.2023.11.19

Research Article

Effect of Ethanol Root Extract of *Physalis angulata* on Kidney Function Biomarkers Following Carbon Tetrachloride (CCl_4) Induced Toxicity in Albino Rats

[1]Solomon Ochepo Adole and [2]J.J. Adanu

[1]University of Nigeria, Nsukka, Enugu, Nigeria
[2]Department of Biological Sciences, Baze University, Abuja, Nigeria

Abstract

Background and Objective: Oxidative stress caused by activities of free radicals has been implicated to play a major role in different disease conditions including organ related diseases such as kidney disease. This study was designed to investigate the effects of *Physalis angulata* on CCl_4 induced oxidative stress. **Materials and Methods:** The ethanol root extract of *Physalis angulata* was administered to thirty made albino rats divided into six caged of five rats each. On the 14th day of the experiment, carbon tetrachloride was used to induce oxidative stress in the animal. Samples were collected and assayed for some kidney function biomarkers. **Results:** The results show that carbon tetrachloride significantly ($p<0.05$) deteriorated the renal functions of rats. This is seen in the high elevation of renal function biomarkers such as uric acid, urea and creatinine in the positive control group. Lipid peroxidation products such as malondialdehyde (MDA) and lipid hydroperoxides (LHP) was also significantly elevated ($p<0.05$) in the positive control when compared to the normal control, standard (silymarin) control and all the test groups. The ethanol root extract of *Physalis angulata* and the standard drug (silymarin) significantly protected the kidney from oxidative stress caused by CCl_4. The plant also showed the capacity to stabilize the levels of vitamin C and E following the CCl_4 attack. **Conclusion:** The administration of *Physalis angulata* root extract showed the potential of quenching or reducing the activities of free radicals thereby reducing oxidative stress.

Key words: *Physalis angulate*, silymarin, carbon tetrachloride, lipid peroxidation, oxidative stress

Citation: Adole, S.O. and J.J. Adanu, 2023. Effect of ethanol root extract of *Physalis angulata* on kidney function biomarkers following carbon tetrachloride (CCl_4) induced toxicity in Albino rats. Am. J. Biochem. Mol. Biol., 13: 11-19.

Corresponding Author: Solomon Ochepo Adole, University of Nigeria, Nsukka, Enugu, Nigeria

Copyright: © 2023 Adole, S.O. and J.J. Adanu. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Various diseases condition such as cancer, organ malfunction, atherosclerosis, malaria, chronic fatigue syndrome and neurodegenerative diseases including cognitive disease, Alzheimer's disease and Parkinson's disease are associated with oxidative stress[1]. Oxidative stress is also associated with premature ageing of cells and can eventually result from autoimmunity, tissue inflammation, damaged cell membranes and ultimately, cell death[2]. Free radicals can interact with molecules in the body and damage various cell components such as DNA, protein and lipids, giving rise to various disease states. When the presence of free radicals causes a change in the redox potential of a cell, the cell's antioxidant system is stimulated and protects the body from the damage caused by free radicals[3]. In more severe cases, however, a cell can become necrotic and die. Exposure to some environmental pollutants and drugs may result in cellular damages. This cellular damage arises from metabolic activation of compounds to a highly reactive oxygen species (ROS) which plays a major role in many health conditions. A lot of persons are environmentally or occupationally exposed to carbon tetrachloride which is a volatile organic compound[4].

Studies have shown that exposure to CCl_4 may increase the generation of free radicals in the kidney and other organs of the body. Different scientific evidence have revealed that exposure to CCl_4 may induce oxidative stress in cells of living organism[4]. In addition to hepatic toxicity, many researchers have shown that CCl_4 may also induce a different forms of disorders in organs such as kidneys, lungs, testes and generate free radicals in the blood cells as well[5]. Other studies also reveal that exposure to this solvent causes acute and chronic renal injuries[6,7], increase lipid peroxidation, reduces renal microsomal NADPH and renal reduced/oxidized glutathione ratio in the kidney cortex as well as renal microsomes and mitochondria[8]. Increased exposure to CCl_4 results in damages to body organs[9]. Despite these reports, the mechanism of carbon tetrachloride (CCl_4) action on the kidney is not completely elucidated.

Plant products are very essential to health care. Nature has provided man with the necessities of life, which includes food, medicine, shelter and raw materials[10]. The majority of the phytochemicals present in plant sources have been reported to have a positive impact on health and disease prevention[11].

Physalis angulata L. belong to the family Solanaceae. It has been reported to contain secondary metabolites such as phytosterols, physalis, flavonols glycoside and angulatin A.[12]. Various parts of the plants are traditionally used as medicine to cure various disorders like asthma, kidney disease, jaundice, gout, inflammations, cancer, digestive problems and diabetes[13]. This study focused on the nephroprotective activity of *Physalis angulata* consumption following induction of oxidative stress using CCl_4.

MATERIALS AND METHODS

Plant material, collection and preparation: The roots of *Physalis angulata* were collected from Yola South Local Government Area of Adamawa State, Nigeria, in August 2016. The taxonomy of the plants was identified and authenticated by a botanist in the department of plant sciences, University of Nigeria, Nsukka.

The fresh root of *P. angulata* was washed and shade dried for 2 weeks. It was then ground into powder using the mechanical grinder and sieved with 1 mm mesh size and stored in an airtight container until ready for use. Dried root powder of *Physalis angulata* (1500 g) was extracted with ethanol using a soxhlet extractor. The soxhlet extraction heats the solvent to boiling temperature (>78°C). The extract was filtered using Whatman no. 1 filter paper and concentrated using a water bath with a constant temperature of 50°C to obtain a solid mass which was refrigerated.

Animals: Male albino rats, with average body weights of 130-190 kg, were obtained from the Animal House of Veterinary Medicine, University of Nigeria, Nsukka. The animals were housed in aluminium cages and placed in a well-ventilated house and exposed to 12 hrs natural light and 12 hrs dark cycle. They were acclimatized for 7 days and given access to grower feed (Vital Feeds Jos, Nigeria) and tap water *ad libitum*.

Experimental design: A total of 30 male albino rats were randomly divided into six groups of five rats each. Group 1 served as normal control, Group 2 (positive control), Group 3 serve as standard control and Groups 4, 5, 6 were the test groups. Groups 1 and 2 were fed a normal diet with normal saline daily. The standard Group 3 was administered a reference drug (Silymarin 100 mg kg^{-1}) daily. The test Groups 4,5,6 received a daily dose of 100, 200 and 500 mg kg^{-1} body weight of ethanol root extract of *Physalis angulata*. Rats in all the groups were induced with 0.5 mL CCl_4 (prepared in the ratio 2:1, olive oil and CCl_4) except for the normal control Group 1. Extract and reference drugs were administered via oral intubation for 14 days. The rats were induced with a single dose intraperitoneal injection of carbon tetrachloride on the 14th day.

Collection of sample from animal: Blood samples were collected from the animals following an overnight fast through ocular puncture. The samples were put in plain (without anticoagulant) specimen bottles. The rats have sacrificed 24 hrs after their last daily doses using chloroform as anaesthesia and the kidney harvested. The kidneys were then excised, perfuse with 10% formal saline and homogenized in phosphate buffer (0.2 M, pH 7.4).

Preparation of kidney homogenate: Kidney tissues were homogenized in 3 mL phosphate buffer and centrifuged at 4000 rpm for 5 min. The supernatant was used for the measurement of vitamin C and E, malondialdehyde and lipid hydroperoxides.

Determination of biochemical parameters: Biochemical parameters assayed include serum creatinine, blood urea nitrogen and uric acid. Other parameters include non enzymic antioxidant such as vitamin C and E. Lipid peroxidized products such as malondialdehyde and lipid hydroperoxide were also assayed for.

Determination of vitamin E: Vitamin E content was estimated by the method of Pearson as described by Nwabueze and Emenonye[14]. Ethanol (1.5 mL) was added to 0.5 mL of diluted sample and mixed. To the mixture was added 1.0 mL of α,α-dipyridyl solution and 1.0 mL of ferric chloride solution and mixed properly. The absorbance was read at 520 nm in the spectrophotometer. The values obtained were read from a standard curve as mg dL^{-1} of serum.

Determination of vitamin C: Vitamin C level was determined by the method of Pearson as described by Nwabueze and Emenonye[14]. To 0.1 mL of the sample was added 0.9 mL of distilled water. Also, 1 mL of 10% TCA was added. Shake vigorously and centrifuge for 10 min. at 3000 rpm. The clear supernatant (1.0 mL) was pipetted into a test tube and 0.4 mL of combined colour reagent was added. The tube was put in a water bath at 56°C for 1 hr and cooled in an ice bath for 5 min. Ice cold H_2SO_4 (2 mL) was slowly added and allowed to stand for 3 min. Absorbance was read at 490 nm.

Determination of lipid peroxidation (malondialdehyde): Malondialdehyde was estimated by the method described by Ogara *et al.*[15]. A volume of sample (0.1 mL) was mixed with 0.9 mL of H_2O in a test tube. Twenty five percent trichloroacetic acid (0.5 mL) and 0.5 mL of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was heated for 40 min in water-bath and then cooled in cold water. After which 0.1 mL of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken with a spectrophotometer at wavelength 532 and 600 nm against a blank.

Determination of urea: Urea concentration was determined using the method of Bartels and Bohmer as stated by Mona *et al.*[16] and described in Randox Kit.

Procedure: Same volume (10 µL) of distilled water which acts as blank, standard calibrator (urea) and sample were added to three separate test tubes. To this test tubes, 100 µL of reagent was added to each and mixed properly. The samples were incubated at 37°C for 10 min.

The absorbance of the sample and standard against the blank was read at 546 nm.

Determination of creatinine: Serum creatinine was determined using the method of Bartels and Bohmer as stated by Mona *et al.*[16]. Reagent (2.0 mL) was added and mixed with 1 mL of blood sample and incubated for 30 min. Reagent (2 mL) was also mixed with 1 mL of standard (creatinine) and incubated for 30 sec. The absorbance of the sample and standard were taken at 492 nm.

Determination of uric acid: This was done using the method of Trinder as mentioned by Ekun *et al.*[17]. In this method, 2 test tubes were set up for sample and standard respectively. A reagent (1 mL) was added into all tubes. Furthermore, 25 µL of sample and standard were added to appropriate tubes, mixed properly and incubated at room temperature for 10 min. Absorbances were taken at 520 nm using spectrophotometer.

Statistical analysis: The data obtained were analyzed using Statistical Product and Service Solution (SPSS) version 22.0 and the results were expressed as Mean±SD. Significant difference were established by One-way Analyses of Variance (ANOVA) using Duncan and LSD multiple comparison statistics and the accepted level of significance was $p<0.05$ for all the result.

RESULTS

Figure 1 show the vitamin C (mg dL^{-1}) level. A dose-related significant increase ($p<0.05$) was observed in the test Group 5 (1.69±0.07) and 6 (1.83±0.08) when compared

to Group 2 (1.48±0.07). No significant difference (p>0.05) was shown between the positives control Group 2 (1.48±0.07) and test Group 4 (1.51±0.15). This suggests that the test Group 4 administered with 100 mg kg^{-1} extract was not effective in maintaining vitamin C activity following CCl_4 induction.

Figure 2 represent vitamin E (mg dL^{-1}) level. No significant difference (p>0.05) in the levels of vitamin E across the text groups (1.28±0.07, 1.28±0.05 and 1.29±0.03) when compared to the positive control Groups 2 (1.21±0.15) and standard control (1.29±0.10). A significant elevation (p<0.05) was observed in the normal control Group 1 (1.34±0.18) when compared to all the test groups.

Figure 3 show the effects of CCl_4 on malondialdehyde (nmol mg^{-1}) following treatment with ethanol root extract of *Physalis angulata*. There was no significant increase (p>0.05) in the level of MDA in the test Groups 4, 5 and 6 (3.89±0.22, 3.81±0.21 and 3.55±0.07) when compared to Group 1 (3.30±0.30). However, a significant increase (p<0.05) was observed in the positive control Group 2 (5.21±0.15) when compared to the standard Group 3 (4.55±0.33) and the test groups. Group 3 (4.55±0.33) also showed a significant increased (p<0.05) in the concentration of malondialdehyde when compared to Group 1 (3.30±0.30) and all the test groups.

Figure 4 indicates an increase (p<0.05) in the value of lipid hydroperoxides (LHP) (nmol mg^{-1}) in Group 2 (0.040±0.00) when compared to Group 1 (0.020±0.00) and all the test groups (0.026±0.00, 0.027±0.00 and 0.026±0.00). No significant difference (p>0.05) was observed in Group 3 (0.024±0.00) when compared to the test groups. However, the test groups were observed to be significantly elevated (P<0.05) when compared to Group 1 (0.011±0.00).

Figure 5 (Uric acid level) (mg dL^{-1}). A significant increase (p<0.05) was observed in the uric acid level of positive control Group 2 (3.88±0.28) when compared to the normal control Group 1 (3.40±0.16), standard three (3.68±0.38) and the test groups (3.35±0.10, 3.46±0.18 and 3.39±0.29). However, no significant difference (p>0.05) was observed between the test groups when compared to Groups 1 and 3.

Figure 6 represents Urea level (mg dL^{-1}). The ethanol root extract of *Physalis angulata* showed a significant decrease (p<0.05) in the urea level of rats in the test groups (32.00±1.82, 31.50±2.08 and 29.25±0.95) when compared to the positive control (37.25±1.89). There was also a significant decrease (p<0.05) across the test groups with Group 6 (29.25±0.95) having the most significant decrease (p<0.05) when compared to Group 2 (37.25±1.89).

Figure 7 show Creatinine level (mg dL^{-1}). There was no significant difference (p>0.05) between the test groups (1.01±0.01, 1.00±0.06 and 1.07±0.02) when compared to Group 1 (1.06±0.03). A significant decrease (p<0.05) was observed in Group 3 (0.94 ± 0.00) when compared to Group 1 (1.06±0.03) and all the test groups. However, a significant increase (p<0.05) was observed in the positive control (1.32±0.01) when compare to the standard (0.94±0.00) and test groups.

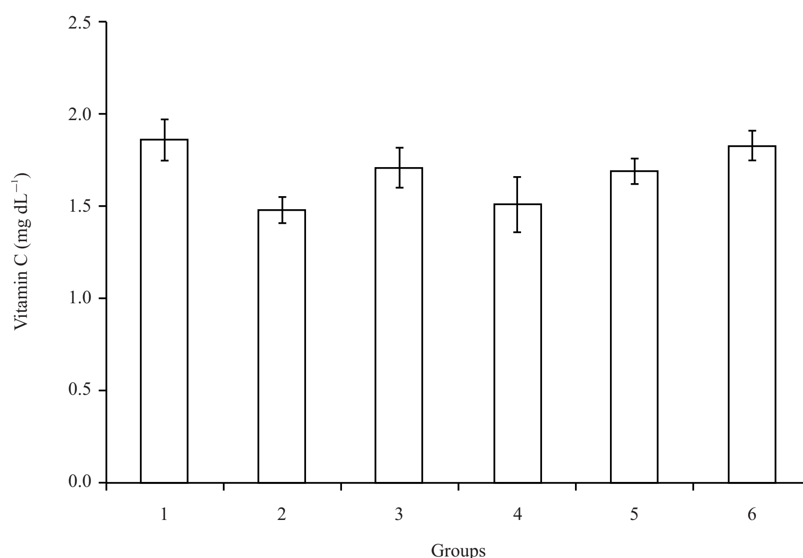

Fig. 1: Effect of ethanol root extract of *Physalis angulata* on vitamin C level in rat

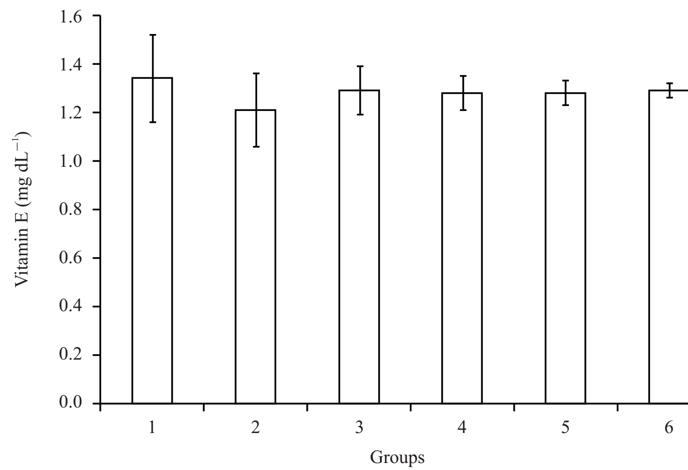

Fig. 2: Effect of ethanol root extract of *Physalis angulata* on vitamin E level of rat

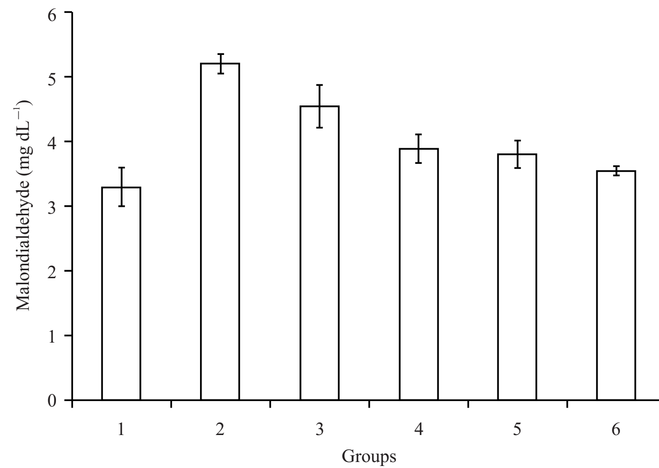

Fig. 3: Effects of ethanol root extract of *Physalis angulata* on malondialdehyde concentration in rat

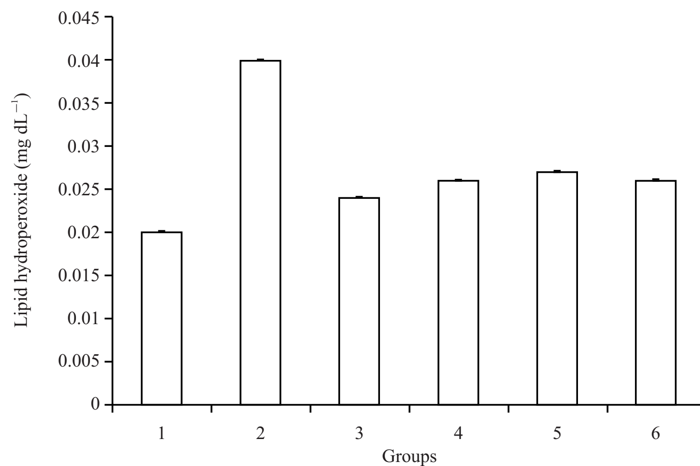

Fig. 4: Effect of ethanol root extract of *Physalis angulata* on lipid hydroperoxides activities of rat

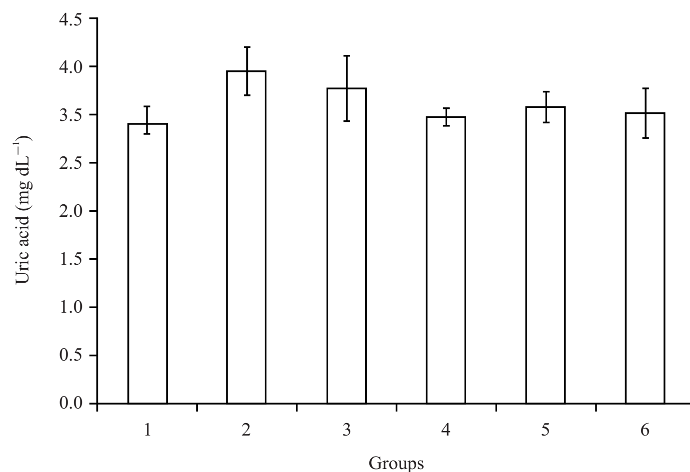

Fig. 5: Effect of ethanol root extract of *Physalis angulata* on uric acid level of rat

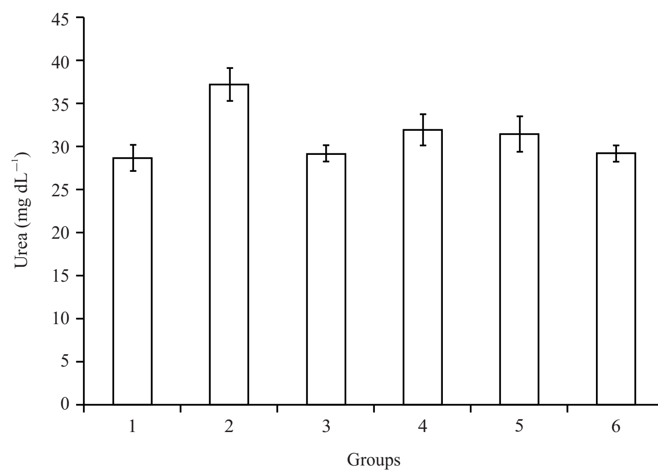

Fig. 6: Effect of ethanol root extract of *Physalis angulata* on urea level of rat

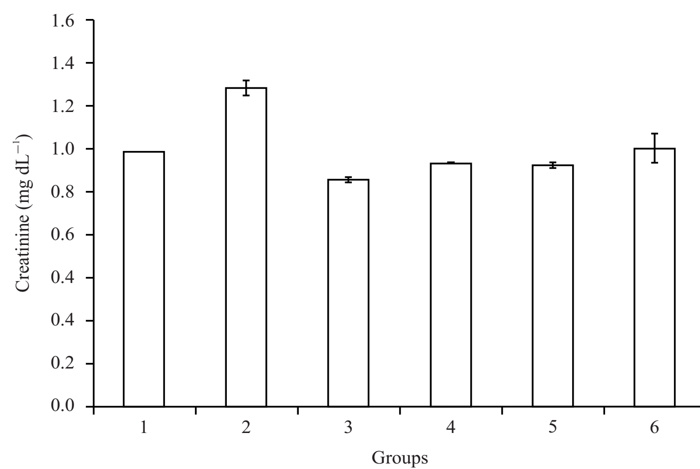

Fig. 7: Effect of ethanol root extract of *Physalis angulata* on creatinine activities of rat

DISCUSSION

Plants are good sources of medicine and today they continue to play several roles in health care[11]. Some of the major problems associated with the use of plants or plant parts as the medicinal alternatives is the inadequate information available on the phytochemistry, pharmacology, antioxidant property and toxicity of some of these plant parts[18].

The non-enzymic antioxidants complement the activity of the enzymic antioxidants in preventing animals from excess oxidative stress by free radicals. Vitamins C and E prevents oxidative stress in cells by acting as free radical scavengers[19]. In this study, a dose-related increase was observed across the test group with the group administered 500 mg kg^{-1} showing the highest significant increase in vitamin C level when compared across the groups. This suggests that high consumption of *P. angulata* root extract either for nutritional or therapeutic purpose can maintain the level vitamin C when the kidney is confronted with oxidative stress. No significant difference ($p>0.05$) was observed in the levels of vitamin E across the groups. However, the level of vitamin E was observed to be higher in the normal control (Group 1) when compared across the groups. Carbon tetrachloride slightly reduced the levels of vitamin E across the group. *Physalis angulata* root extract and the standard drug silymarin as used in this study may not have effectively protected the rat kidney from CCl_4 induced oxidative stress.

Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake and propagation of lipid radicals, the uptake of molecular oxygen and arrangement of double bonds in the unsaturated lipids and eventually their destruction with subsequent production of a variety of breakdown products, including alcohols, ketones, aldehydes and ethers[20]. Lipid peroxidation product accumulation in human tissues is a major cause of tissue and cellular dysfunction that plays a major role in ageing and most age-related and oxidative stress-related diseases[21]. Malondialdehyde (MDA) and lipid hydroperoxides (LHP) are compounds derived from lipid peroxidation and exist in biological matrices both in the free and bound forms. The measurement of the levels of MDA and LHP are widely used to monitor oxidative stress[22]. From the results of the experiment, there was a significant increase in the positive controls of both MDA and LHP when compared with the test groups. This indicates that there was a disruption in plasma membrane of rat kidney. This can result in the loss of the membrane-bound and cytosolic enzymes from the kidney cells to the serum as a consequence of peroxidation of unsaturated fatty acids. However no significant increase in the levels of MDA and LHP was observed across the extract test groups when compare to the normal control. This suggests that the root extract of *P. angulata* may be effective against lipid peroxidation. Previous studies carried out by Reddy *et al.*[23] reveal that *Physalis angulata* root extract was able to normalize the elevated level of MDA in streptozotocin (STZ) induced diabetic rats treated rats.

In the renal function test, the result suggests that the ethanol root extract of *Physalis angulata* is effective in protecting the kidney from oxidative attacks that can result in degeneration of the kidney and increase the uric acid level of the blood. The nitrogen component of urea (blood urea nitrogen-BUN) is the end product of protein. The concentration of blood urea nitrogen is elevated in kidney damage, excessive protein intake and low fluid[24]. It was observed from the result that the extract was able to attenuate the high levels of blood urea caused by the induction of CCl_4 in all the test groups. The test Group 6 which was administered 500 mg kg^{-1} extract showed the highest reduction in urea level when compared across the group. This suggests that a high consumption of *P. angulata* root extract may enhance the ability of the kidney to excrete excess urea. Creatinine is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for high-energy phosphate that can be utilized by tissues for the production of ATP[25]. Creatinine forms spontaneously from p-creatine and its formation occurs at a rate that is relatively constant[26]. Creatinine is a useful tool for normalizing the levels of other molecules found in urine. Elevated levels of creatinine, however, is associated with conditions that result in decreased renal blood flow such as diabetes and cardiovascular disease[27]. The result indicates that the root extract of *Physalis angulata* was able to maintain the normal creatinine concentration at the various dose-treated.

Uric acid, serum urea and creatinine are toxic substances that can cause various types and degrees of harm to the organs of the body including the kidney when present in excess in circulation. Their level is kept in a stable range by the kidneys which help to discharge them when present in excess. In cases of kidney failure, kidney function is affected seriously and kidneys fail to discharge excess uric acid, urea, creatinine and other byproducts of metabolism. This causes an elevated amount in serum. Therefore, serum urea, creatinine, uric acid and other by-product (s) can help to measure kidney function.

CONCLUSION

Traditional medicine has been claimed to be vital in prevention and curing of various diseases in most part of Nigeria, thereby playing an important role in health care

delivery. The study showed that the root of *Physalis angulata* may be used for the management of kidney-related diseases caused by oxidative damage in the kidney. This gives some evidence to the claims that the plant is used in the management of kidney disease. This also indicates that the extract may be effective in enhancing the normal functioning of the kidney. The extract also showed good antioxidant potentials and may be safe for human consumption.

SIGNIFICANCE STATEMENT

Different parts of the plant *Physalis angulata* have been used by many traditional healers for the treatment of different diseases including kidney disease. This study show that the ethanol root extract of *Physalis angulata* can be used in the management of kidney related disease. Therefore, consumption of the plant either for nutritional or therapeutic purpose is recommended for proper kidney function and antioxidant activities.

ACKNOWLEDGMENT

The authors sincerely acknowledge and appreciate everyone who contributed in any way to the success of this research work. Thank you.

REFERENCES

1. Chaitanya, K.V., A.A.K. Pathan, S.S. Mazumdar, G.P. Chakravarthi, N. Parine and V. Bobbarala, 2010. Role of oxidative stress in human health: An overview. J. Pharm. Res., 3: 1330-1333.
2. Klein, J.A. and S.L. Ackerman, 2003. Oxidative stress, cell cycle and neurodegeneration. J. Clin. Invest., 111: 785-793.
3. Pham-Huy, L.A., H. He and C. Pham-Huy, 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 4: 89-96.
4. Adewole, S.O., A.A. Salako, O.W. Doherty and T. Naicker, 2007. Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. Afr. J. Biomed. Res., 10: 153-164.
5. Ozturk, F., M. Ucar, I.C. Ozturk, N. Vardi and K. Batcioglu, 2003. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology, 62: 353-356.
6. Ogeturk, M., I. Kus, N. Colakoglu, I. Zararsiz, N. Ilhan and M. Sarsilmaz, 2005. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J. Ethnopharmacol., 97: 273-280.
7. Manna, P., M. Sinha and P.C. Sil, 2006. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complementry Altern. Med., Vol. 6. 10.1186/1472-6882-6-33.
8. Yilmaz-Ozden, T., A. Can, A. Karatug, Z. Pala-Kara, A. Okyar and S. Bolkent, 2014. Carbon tetrachloride-induced kidney damage and protective effect of *Amaranthus lividus* L. in rats. Toxicol. Ind. Health, 32: 1143-1152.
9. Khan, R.A., M.R. Khan, S. Sahreen and J. Bokhari, 2010. Prevention of CCl_4-induced nephrotoxicity with *Sonchus asper* in rat. Food Chem. Toxicol., 48: 2469-2476.
10. Geidam, M.A. and O.S. Adole, 2014. Effects of the aqueous ethanolic leaves extract of celtis integrifolia on liver function of Wister strain Albino rats. Int. J. Sci. Res. Manage., 2: 713-718.
11. Mukherjee, P.K., N. Maity, N.K. Nema and B.K. Sarkar, 2011. Bioactive compounds from natural resources against skin aging. Phytomedicine, 19: 64-73.
12. Puente, L.A., C.A. Pinto-Munoz, E.S. Castro and M. Cortes, 2011. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. Food Res. Int., 44: 1733-1740.
13. Hseu, Y.C., C.R. Wu, H.W. Chang, K.J.S. Kumar and M.K. Lin *et al*., 2011. Inhibitory effects of *Physalis angulata* on tumor metastasis and angiogenesis. J. Ethnopharmacol., 135: 762-771.
14. Nwabueze, T.U. and A.G. Emenonye, 2016. Processing effect on the physicochemical and volatile fatty acid profile of African breadfruit (*Treculia africana*) seed oil. Food Nutri. Sci., 7: 627-635.
15. Ogara, A.L., P.E. Joshua, K.O. Omeje and I.N.E. Onwurah, 2016. Effects of ingested crude oil contaminated diets on antioxidant enzyme and lipid profile in Wistar Albino rat. J. Appl. Sci. Environ. Manage., 20: 927-932.
16. Mona, E.M.Y., M.A. El-Edel, S.M. Nasr, U.E. Mahrous and M.M. Aboghanima, 2016. Response of Cobb and Sasso broilers to feeding restriction and tryptophan supplementation. Alexand. J. Vet. Sci., 51: 127-134.
17. Ekun, O.A., G.A. Ogunyemi, A. Azenabor and O. Akinloye, 2018. A comparative analysis of glucose oxidase method and three point-of-care measuring devices for glucose determination. Ife J. Sci., 20: 43-49.
18. Okonkwo, E.E., 2012. Traditional healing systems among Nsukka Igbo. J. Tourism Heritage Stud., 1: 69-81.
19. Ajboye, T.O., M.T. Yakubu, A.K. Salau, A.T. Oladiji, M.A. Akanji and J.I. Okogun, 2010. Antioxidant and drug detoxification potential of aqueous extract of *Annona senegalensis* leaves in carbon tetrachloride-induced hepatocellular damage. Pharm. Biol., 48: 1361-1370.
20. Bhattacharjee. S., 2014. Membrane lipid peroxidation and its conflict of interest: The two faces of oxidative stress. Curr. Sci., 107: 1811-1823.

21. Negre-Salvayre, A., N. Auge, V. Ayala, H. Basaga and J. Boada *et al*., 2010. Pathological aspects of lipid peroxidation. Free Radical Res., 44: 1125-1171.
22. Colagar, A.H., M. Pouramir, E.T. Marzony and S.G.A. Jorsaraei, 2009. Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. Braz. Arch. Biol. Technol., 52: 1387-1392.
23. Reddy, P.A., K.R. Vijay, G.V. Reddy, M.K. Reddy and Y.N. Reddy, 2014. Anti-diabetic and hypolipidemic effect of aqueous and methanolic root extracts of *Physalis angulata* in streptozotocin (STZ) induced diabetic rats. Int. J. Pharma. Res. Scholars, 3: 402-409.
24. Abajingin, D.D. and O.E. Ekun, 2019. Haematological and biochemical parameters of rats fed with heavy metaled fish after exposure to a 47mT oscillating magnetic field. Am. J. Med. Sci. Med., 7: 30-35.
25. Wallimann, T., M. Tokarska-Schlattner, D. Neumann, R.M. Epand and R.F. Epand *et al*., 2007. The phosphocreatine circuit: Molecular and cellular physiology of creatine kinases, sensitivity to free radicals and enhancement by creatine supplementation. In: Molecular System Bioenergetics: Energy for Life, Saks, V. (Ed.), Wiley-VCH Verlag GmbH & Co. KGaA, Germany, ISBN-13: 9783527317875, pp: 195-264.
26. Anavekar, N.S., J.J.V. McMurray, E.J. Velazquez, S.D. Solomon and L. Kober *et al*., 2004. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. N. Engl. J. Med., 351: 1285-1295.
27. Gross, J.L., M.J. de Azevedo, S.P. Silveiro, L.H. Canani, M.L. Caramori and T. Zelmanovitz, 2005. Diabetic nephropathy: Diagnosis, prevention and treatment. Diabetes Care, 28: 164-176.