Original Article

Aqueous Beetroot Juice Extract Improves Renal Function and Some Biochemical Parameters in Carbon Tetrachloride-Induced Toxicity in Sprague Dawley Rats

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Abstract

Background: The ability of plant extracts to improve toxicity in the kidney is gaining attention. This may be due to the untoward effects and high cost of orthodox drugs used for the management of kidney disease, therefore, the use of aqueous beetroot juice as a potential ameliorative agent was evaluated. **Materials and Methods:** Group 1 received 2 ml/kg distilled water (normal control); Group II received 2 ml/kg olive oil; Group III received 2 ml/kg carbon tetrachloride (CCl_4) in olive oil 48 hourly (negative control); Group IV received 2 ml/kg CCl_4 in olive oil and 250 mg/kg of beetroot/day; Group V received 2 ml/kg CCl_4 in olive oil and 500 mg/kg of beetroot/day; and Group VI received 2 ml/kg CCl_4 in olive oil and 100 mg/kg silymarin/day (positive control) for 14 days. Animals were euthanized, and blood was collected for the evaluation of electrolytes, urea, and creatinine, whereas the kidney tissue was assessed for histopathological changes. **Results:** There was a significant ($P < 0.05$) decrease in the level of K + in the group of rats administered 250 mg and 500 mg/kg of aqueous beetroot extract, when compared to negative control. The levels of Na+ were reduced in the treated groups when compared to normal control. There were no significant changes in Cl − levels in all the group of animals. The urea levels decreased in the groups treated with extract or silymarin, and a similar trend of creatinine was observed in the groups administered beetroot extract. However, creatinine concentration was significantly ($P < 0.05$) increased in the group of rats administered only silymarin when compared to other groups. Histopathological evaluation revealed that the tubular necrosis observed in the kidney was ameliorated after beetroot juice administration. **Conclusion:** Beetroot juice showed nephroprotective effects against CCl_4-induced renal toxicity.

Keywords: Beetroot juice, carbon tetrachloride, nephrotoxicity, silymarin

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Introduction

Herbal medicine is the mainstay of about 75%–80% of the world's population, mainly in developing countries, for primary health care.[1] The interest in functional foods, which are nutrient dense or fortified foods promote optimal health and reduce the risk of diseases examples are beans, nuts, oats, cruciferous vegetables, and fermented dairy products have inherent bioactive components.[2] The kidneys maintain constant extracellular environment by the excretion of catabolites such as urea, creatinine and uric acid, and regulation of water and electrolyte to avoid abnormal concentration which has deleterious effects.[3]

Carbon tetrachloride (CCl_4) affects tissues such as liver, kidney, and central nervous system, thereby undergoing peroxidative degeneration generating radicals that bind lipids, proteins, and DNA.[4] Beetroot (*Beta vulgaris*) is a red-colored bulbous root that is consumed by cooking, in salads or as juice.[5] The aim of this study was to evaluate the ameliorative effects of aqueous extract of beetroot on CCl_4-induced nephrotoxicity in the serum and tissue.

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Materials and Methods

Plant material and extraction

Beetroot (*B. vulgaris*) was purchased from the vegetable market on Airport Road, Benin City, Nigeria from a local vendor. The plant identification was by a Taxonomist at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A voucher number UBH_B 374 was obtained and deposited in the Herbarium of the University. The beetroots were washed under running tap water, peeled with a knife and blended, the juice was extracted using a muslin cloth and distilled water. The juice extracted was stored in an air tight container (10 kg of beetroot yielded approximately three liters [3 L] after extraction) and allowed to settle for 20-30 min. The juice obtained was freeze dried (Armfield vacuum freeze dryer Model FT 33, England) and ready for use. The freeze-dried sample is composed of 10% beetroot juice containing 9808.0 mg GAE/100 ml polyphenols and 8334.0 mg QE/100 ml flavonoids.[6] The freeze dried sample was stored at 0°C until ready for use.

Animals

Thirty apparently healthy Sprague-Dawley male rats of average body weight (160.20 ± 2.54 g) were grouped into six groups of five animals each. They were obtained from the animal house in Anatomy Department, University of Benin, Benin City, Nigeria, and housed in spacious plastic cages floored with animal beddings in the animal house of Department of Medical Biochemistry. The cages were cleaned daily. Animals were acclimatized for 2 weeks and maintained under the standard conditions of temperature (23 ± 2°C) and (12 h light/dark cycle). The rats were fed standard pelleted feed (Top Feeds; Premier Feed Mills Co Ltd, Ibadan, Nigeria) for 2 weeks. Ethical clearance was obtained from the Ethical Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria, with reference number CMS/REC/2016/002.

The six groups were composed as follows: Group I: Apparently healthy rats that received 2 ml/kg distilled water (Normal control); Group II: Apparently healthy rats that received 2 ml/kg olive oil; Group III: Apparently healthy rats which received 2 ml/kg CCl_4 in olive oil (negative control); Group IV: Apparently healthy rats that received 2 ml/kg CCl_4 in olive oil and 250 mg/kg body weight of beetroot extract/day; Group V: Apparently healthy rats which received 2 ml/kg CCl_4 in olive oil and 500 mg/kg body weight of beetroot extract/day; and Group VI: Apparently healthy rats that received 100 mg/kg body weight of silymarin/day and 2 ml/kg CCl_4 in olive oil (positive control). Silymarin [Micro Labs Ltd; 92, SIPCOT HOSUR-635 126, INDIA] and CCL_4 (Guangdon Guanghua Chemical, China) were of analytical grade. The animals were fasted for 12 h before the administration of 40% concentration of CCl_4 in olive oil which served as a vehicle (GOYA Extra Virgin Olive oil, Manufactured by Goya En Espania, S. A. U. Sevilla, Spain), at a dose of 2 ml/kg body weight every 48 h (between 8.00 am and 9.00 am) for 14 days using gastric gavage which was modified.[7] Animals were fasted overnight and euthanized by decapitation after the 14th day. Blood was collected for biochemical evaluation, whereas the kidneys were dissected, freed of adherent tissues, and immediately fixed with 10% buffered formalin.

Determination of serum markers in kidney damage

The serum from each group was used to determine the levels of creatinine (creatinine: Randox Creatinine reagent–[JAFFE method] Cat No CR7948. ready for use according to the manufacturer's instructions), urea (Randox Urea [Berthelot] Berthelot method. Cat No UR1068. Liquid ready-to-use reagents according to the manufacturer's instructions) electrolytes K^+, Cl^- and Na^+ (OPTI™ LION Electrolyte analyzer [OPTI medical systems, Inc. Roswell, Georgia, USA]).

Histopathological examination

The kidney harvested was trimmed of any adherent tissue and placed in sterile containers, containing 10% buffered formalin. They were cut so that they can enter the cassettes, and the morphological areas of suspicion were taken and included in the section (the central region of the renal tissue). It was processed by removing the tissue from buffered formalin, and dehydrated through graded concentration of alcohol starting from the lowest (70%, 90%, 95%, and absolute). The alcohol in the tissues was cleared with xylene which was replaced with paraffin wax by impregnation. Staining was done with hematoxylin and eosin. A microtome was used to section at 10 μ. The processed tissues on the slides were analyzed microscopically.

Statistical analysis

Biochemical data were expressed as mean ± standard error of the mean. The difference between the groups was tested using the ANOVA. Duncan's multiple range test was used to determine the significant difference among the means ($P < 0.05$).

Results

Biochemical assessment

The LD_{50} of aqueous extract of beetroot was >5000 mg/kg body weight. Nevertheless, the 250 mg and 500 mg/kg body weight of the extract doses administered to the animals daily were 5% and 10%, respectively, of the LD_{50}.[8] The graded administration of the aqueous extract of beetroot did not show any significant changes ($P > 0.05$) in the concentration of serum potassium in the rats during the administration of CCl_4. There was, however, a significant reduction ($P < 0.05$) in the concentration of potassium ions in the groups that were administered 250 mg and 500 mg of extract, respectively, when compared to the negative control (Group III, that received only CCl_4), and the positive control [Group VI, that received only 100 mg of silymarin, Figure 1]. There were no significant ($P > 0.05$) changes in the concentration of chloride ions in all the groups of animals exposed to olive oil and CCl_4 extract and silymarin [Figure 2]. There were

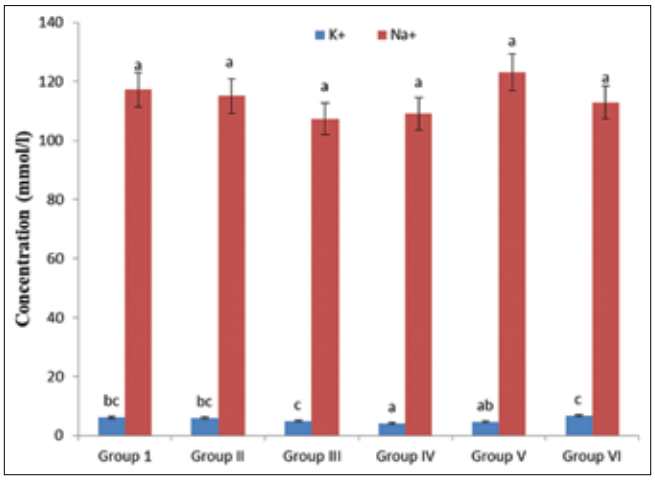

Figure 1: Effect of extract on serum electrolytes in rats. Serum concentrations of potassium and sodium ions were expressed as mean ± standard error of the mean ($n = 5$). Values with different superscripts are significantly different when $P < 0.05$ Na^+ = a; no significant changes ($P > 0.05$) in all the groups. K^+ = a; significantly decreased ($P < 0.05$) in Group IV when compared to Groups I, II, and III c; significantly increased ($P < 0.05$) in Group VI when compared to groups IV and V

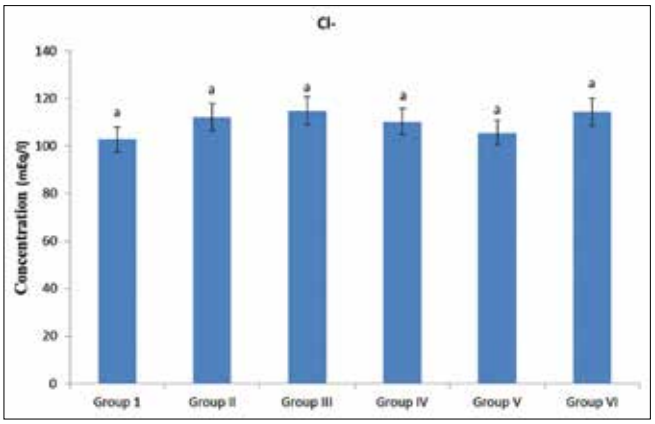

Figure 2: Effect of oral administration of extract on serum chloride ions in normal rats. Serum concentration of chloride ions were expressed as mean ± standard error of the mean ($n = 5$). Values with different superscripts are significantly different when $P < 0.05$ a = No significant changes ($P > 0.05$) in all the groups

no significant ($P > 0.05$) changes in urea concentration in all the groups of animals; however, the creatinine concentration was higher in the negative control than the groups of animals administered 250 and 500 mg/kg body weight of beetroot extract. Nevertheless, the creatinine concentration was significantly ($P < 0.05$) increased in the group of animals that received silymarin (Group VI) when compared to the other groups [Figure 3].

Histopathological analysis

Administration of rats with CCl_4 only (negative control; plate 3) showed focal tubular necrosis, while the treatment of CCl_4-induced nephrotoxicity rats with aqueous extract of beetroot maintained normal tubules and glomeruli.

Discussion

Kidneys are very useful in the metabolism and excretion of chemicals and drugs and have xenobiotic detoxifying enzymes.[9] Xenobiotic-induced renal injury is dependent on the dose, nature and duration of exposure.[10]

Kidneys are majorly responsible for maintaining fluid and electrolyte balance in serum. The derangements of electrolyte and acid-base could emerge with progressive loss of kidney function.[11] Hyperkalemia which is excess of potassium in blood occurs in cases of renal failure because the kidneys loses its ability to excrete the mineral. In situations of severe dehydration, hyperkalemia can also occur.[12] Similarly, abnormal concentration of sodium and/or potassium in serum can affect the osmotic pressure of body fluid which is associated with blood pressure.[13,14] On oral administration of the extract daily for 14 days, we observed a significant ($P < 0.05$) reduction of potassium ion in Groups IV and V [Figure 1] that were treated with 250 mg and 500 mg/kg of aqueous beetroot extract, respectively, when compared to the positive control (Group VI that received silymarin). We proposed that the reduction of potassium ions in the groups treated with the extract may be due to the phytoconstituents and other active compounds which acted on the renal tubules [Figure 1]. While, the increased potassium ions in the group that was administered silymarin (Group VI) may be due to the absence of some active ingredients that are present in the extract but not available in the drug, or it may be due to some physiological changes in the animals. Beetroot extract could mitigate this change by maintaining the electrolyte balance. Chesbrough[15] has showed that hypernatremia is rare but can occur when the body loses fluids that contain less sodium than plasma along with water intake restriction or if there is excessive sodium intake with limited liquid intake. It has been observed that hypernatremia almost always indicate water depletion.[16,17] Some workers,[18] had reported that ethanolic extract of *Lagenaria breviflora* fruit was administered to rats which resulted in the elevation of sodium ion in the test groups without significant ($P > 0.05$) changes, which is similar to this study [Figure 1]. We are proposing that the slight increase in sodium ions in the groups of rats administered 250 mg or 500 mg of extract compared to negative control may be due to its content of sodium like compounds, which we reported in our evaluation of minerals composition in beetroot juice. The minerals we noted in the study may have been responsible for the osmotic pressure and acid-base balance.[19]

Chloride is the most abundant anion in the extracellular fluid. Serum chloride ions can be used to assess renal functions.[20] There were no significant ($P > 0.05$) changes in chloride ions among the groups of animals; however, we noted a decline in the groups that received 250 mg and 500 mg/kg of extract when compared to the negative and positive controls [Figure 2]. This effect may be due to the active compounds which probably had interactions with the tubular cells thereby maintaining the acid–base balance.

Creatinine and urea are the metabolic waste products that are excreted from the body in urine and are markers of kidney function in participants suffering from renal assault. Increase in these markers in serum is an indication of renal dysfunction; it also reflects decreased glomerular filtration rate, while elevated urea indicates dysfunctional reabsorption.[21] The elevated levels of serum creatinine and urea in the negative control (the group of rats administered only CCl_4) when compared to normal control [Figure 3] signifie renal alteration. Increase in serum creatinine level in the present study may indicate abnormality in the glomerular filtration rate, while the increased urea level may imply that there is reduced reabsorption at the renal epithelium of CCl_4 treated rats [negative control, Figure 3]. In this study, we found that there was a reduction in the serum concentration of creatinine and urea in the groups treated with aqueous extract of beetroot, when compared to the negative control. Interestingly, it may be due to the active components and antioxidant properties in the extract. The results from our study are similar to the work of Owumi and Dim, 2019[22] where they reported that there was obvious reduction in the serum urea and creatinine levels in rats co-administered Mn and Chlorpyrifos and organophosphorus pesticide.

Moreover, in the group of animals administered silymarin, there was a reduction in the concentration of urea and a concomitant significant ($P < 0.05$) elevation in serum creatinine when compared to the negative control [Figure 3]. These observations may be as result of the presence of polyphenols, flavonoids, and antioxidant potentials present in the extract. These were able to mitigate the deteriorative effects of CCl_4 by preventing protein denaturation, lipid peroxidation, and oxidative damage which are the features of cell death in renal disease.

Some herbal and natural products have been demonstrated to possess antioxidant properties that can reduce the generation of free radicals and protect the kidney from damage.[23] Beetroot has been shown to possess significant antioxidant and radical-scavenging abilities which correlated positively with natural antioxidants.[24] In our previous studies,[6,25] we demonstrated the overwhelming significant ($P < 0.05$) reduction in serum malondialdehyde on administering the extract of beetroot to rats and the modulation of antioxidant enzymes in the injured rat liver. Histopathologically, normal glomerulus and tubules were noticed in the animals treated with beetroot extract, and there was tubular necrosis in the animals that were exposed to only CCl_4 [Figure 4: plates 4, 5, and 3, respectively]. This observed beneficial role may be due to the protective potentials of the active ingredients such as flavonoids and polyphenols as well as the abundant antioxidants in the aqueous extract of beetroot.[6]

Histopathological evaluation in this study revealed that aqueous extract of beetroot was able to regenerate tubular necrosed cells in the treatment groups (plates 4, 5, and 6 when compared to negative control, plate 3). The regenerated cells are similar to those in the positive control (plate 6). The observed regeneration of tissues may be due to the phytoconstituents of beetroot which can reduce lipid peroxidation, thereby stabilizing the lipid membrane of the kidney and ultimately prevent necrosis.[6] The histopathological findings in this study are similar to what was reported on ethanolic beetroot extract ameliorating gentamicin-induced nephrotoxicity in rats.[26]

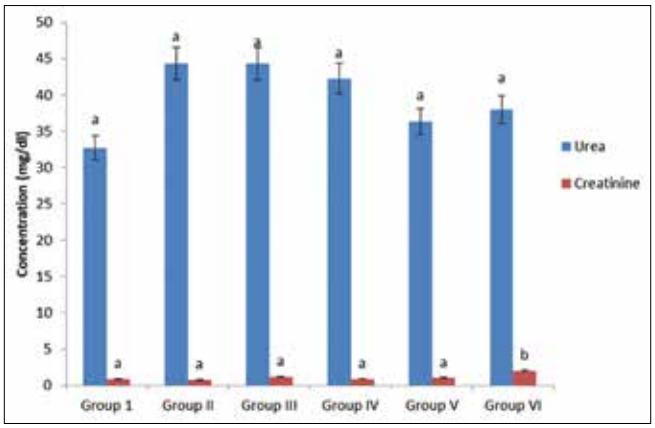

Figure 3: Effect of oral administration of extract on serum urea and creatinine in normal rats. Serum concentrations of urea and creatinine were expressed as mean ± standard error of the mean ($n = 5$). Values with different superscripts are significantly different when $P < 0.05$ urea = a; no significant changes ($P > 0.05$) in all the groups Creatinine = b, significantly increased ($P < 0.05$) in Group VI compared to other groups

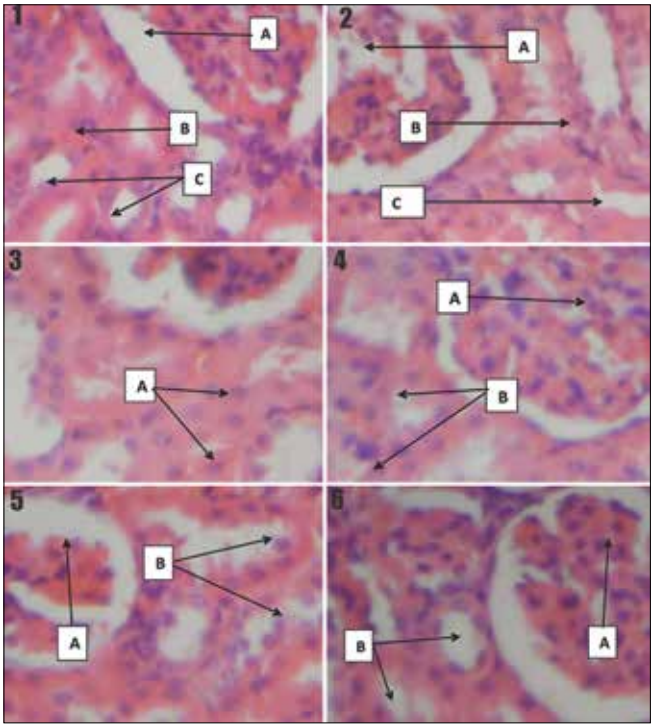

Figure 4: Representative photomicrographs of kidney sections treated with beetroot and CCl4 (H and E staining ×100). Plate 1 (normal rats; normal glomerulus [A], Interstitial space [B], tubules [C]). Plate 2 (normal rats and olive oil; normal glomerulus [A], Interstitial space [B], tubules [C]). Plate 3 (effect of CCl4 [negative control]; Focal tubular necrosis [A]). Plate 4 (250 mg/kg extract; Normal glomerulus [A] and tubules [B]). Plate 5 (500 mg/kg extract; Normal glomerulus [A] and tubules [B]). Plate 6 (100 mg/kg silymarin [positive control]; Normal glomerulus [A] and tubules [B])

Conclusion

The results obtained suggested that nephrotoxicity due to CCl_4 effects were ameliorated by the administration of aqueous beetroot extract, which may be due to the interruption of pathways that can lead to cell death. This study was in rats and could be used to infer what may happen in humans on administering aqueous extract of beetroot juice. There is need for further studies on the active ingredients in the extract.

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Conflicts of interest

There are no conflicts of interest.

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