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Research Article
Effect of *Vernonia calvoana* Extract on Selected Serum Kidney Function Biomarkers of Acetaminophen Treated Wistar Rats

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Abstract

Objective: To investigate the renoprotective potentials of *Vernonia calvoana* extract (VCE) in acetaminophen treated Wistar rats. **Materials and Methods:** Thirty-five male Wistar rats (100-150 g) were randomly assigned to 5 groups of 7 rats each. Group 1 (normal control) received normal saline, group 2 received normal saline after oral administration of 2 g kg^{-1} b.wt. of acetaminophen, groups 3 and 4 were administered 200 and 400 mg kg^{-1} b.wt. of VCE, respectively after pre-treatment with acetaminophen. Group 5 was administered 100 mg kg^{-1} b.wt., of Vitamin E. All treatments were given orally and study lasted for 21 days thereafter blood was collected for measurement of kidney function biomarkers. One-way analysis of variance was used for analysis followed by *post hoc* multiple comparisons and SPSS. **Results:** The results showed increased serum sodium concentration in group 3 and 4 administered VCE when compared with the acetaminophen treated control, unlike the Vit E supplemented group which showed a decrease ($p<0.001$). Conversely, serum potassium and urea concentrations were decreased in the VCE treated groups compared to the acetaminophen treated control ($p<0.001$). Further, serum creatinine, a notable marker of kidney function was decreased in VCE treated groups in tandem Vit. E supplemented group when compared to acetaminophen-treated control. **Conclusion:** It was concluded that extracts of *Vernonia calvoana* leaves potently ameliorated imbalance in kidney function biomarkers induced by acetaminophen, hence may possesses renoprotective potentials.

Key words: Acetaminophen, nephro-protective potentials, serum electrolyte balance, vernonia calvoana, vitamin E interaction

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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

Acetaminophen or paracetamol, chemically known as N-acetyl-p-aminophenol (APAP) is a mild, over–the- counter analgesic drug. It is used for the relief of pains such as headaches and in the treatment of flu and common cold. The drug is generally safe when taken in recommended doses, although there are indications than even very small overdoses could be deleterious. Overdose of acetaminophen could produce both renal and liver damage[1]. The drug was shown to induce nephrotoxicity at 750 mg kg^{-1} b.wt., by Sathishkumar co-workers[2].

Acetaminophen breakdown in the liver could result in the formation of a highly toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) by the cytochrome P_{450} enzyme system[3]. Further detoxification to eliminate the metabolite is accomplished by its conjugation with glutathione, such that in overdose conditions acetaminophen depletes glutathione stores and form NAPQI based reactive intermediates that covalently bind many cellular proteins (ref). This leads to apoptosis through caspases and other lysosomal enzyme-mediated mechanisms that finally results in renal tissue damage with severe organ dysfunction[2]. Most ethnobotanicals have been reported to exert renoprotective effects with minimal side effects[4].

Vernonia calvoana is an *Asteraceae* found in the tropics especially South Western Cameroon and South Eastern Nigeria[5]. It is commonly called "Ekeke leaf" and "Uchu nyin" by the indigenes of the central and Northern senatorial districts of Cross River State of Nigeria, respectively[6,7], The hypolipidemic and antioxidant potentials of the plant in diabetic rats models have been reported by Iwara *et al.*[8]. Its use in the treatment of diabetes, fever and obesity has been reported (ref), but with little or no information on the renoprotective potentials of this plant. Consequently, the present study was designed to investigate the renoprotective activity of the extract of this plant, *Vernonia calvoana* in acetaminophen treated Wistar rats.

MATERIALS AND METHODS

The experiment was carried out in November, 2015.

Plant material: Fresh leaves of *Vernonia calvoana* were purchased from a local market in Ugep town, Yakurr Local Government Area of Cross River State, Nigeria. The leaves were authenticated by Mr Frank Apojeye of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria and voucher specimen deposited in the herbarium of the same department (BOT/VC/2/2015).

Chemicals: All chemicals used in this study were of analytical grade. Acetaminophen manufactured by Emzor pharmaceuticals was purchased from Anijah Pharmacy, Etta Agbor Road, Calabar, Cross River State, Nigeria. Diagnostic kits for the estimation of serum urea, creatinine, chloride, sodium and potassium were obtained Randox Laboratories Ltd. (Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom).

Preparation of plant extract: The leaves were washed to remove dust and other forms of dirt and afterwards air-dried at room temperature (27±2°C) for 7 days. The dried leaves were blended to a fine powder using a dry Moulinex super blender and stored in air-tight containers. The coarse power of the leaves (1.5 kg) was extracted using ethanol as solvents. The mixture was filtered first, with a cheese cloth, thereafter by Whatman No. 1 filter paper (24 cm). The filtrate was concentrated using a rotary evaporator (model RE52A, China) to 10% of its original volume within a temperature range of 37-40°C. The filtrate was then concentrated to dryness in a water bath and stored frozen (-4°C) until required for animal treatment.

Experimental animals: Thirty-five male Wistar rats (100-150 g), were obtained from a disease-free stock of the animal house, Department of Zoology, University of Calabar, Calabar. The animals were acclimatized in the Department of Biochemistry animal house for 2 weeks on pelletized rat chow and water provided *ad libitum*. The experiment was conducted in accordance with the internationally accepted principles for laboratory animal use and care[9]. Approval for the use of the animals to carry out the study was obtained from the Faculty of Basic Medical Sciences Animal Ethics Committee, University of Calabar, Calabar. The animals were randomly assigned to five groups of seven rats each and treated as shown in Table 1.

Acetaminophen induced nephrotoxicity: Acetaminophen induced nephrotoxicity was done by oral administeration 2 g kg^{-1} b.wt., of the drug daily for 4 days but equivalent volume of distilled water was given to the control group. At the end of 3 days treatment, an animal from each of the study groups sacrificed and toxicity parameters assayed.

Table 1: Experimental treatment groups

Groups	Number of animals	Treatments
Group 1	7	Normal saline
Group 2	7	2 g kg^{-1} acetaminophen only
Group 3	7	2 g kg^{-1} acetaminophen+200 mg kg^{-1} b.wt., VCE
Group 4	7	2 g kg^{-1} acetaminophen+400 mg kg^{-1} b.wt., VCE
Group 5	7	2 g kg^{-1} acetaminophen+100 mg kg^{-1} b.wt., Vit. E

VC: Extract of *Vernonia calvoana*, Vit. E: Vitamin E and b.wt.: Body weight

Extract administration: The plant extract doses (200 and 400 mg kg^{-1}) used were based on the predetermined LD_{50} values obtained using Lorke's method[10]. The extract was reconstituted in normal saline and administered orally via gastric intubation. The control animals received 0.2 mL of normal saline. Administration of the *Vernonia calvoana* leaf extract carried out consecutively for 21 days. The animals were fasted 12 h overnight prior to the time of sacrifice, anaesthesized and blood collected via cardiac puncture.

Serum kidney parameters: The blood was collected into non-heparinized samples tubes and allowed to clot for 2-3 h after which it was centrifuged at 3000 rpm for 15 min using the Surgifield Centrifuge, Model SM80-2, England, United Kingdom. The obtained serum was stored at 4°C for the estimation of serum urea, creatinine, chloride, sodium and potassium. These estimations were carried out according to the manufacturer's protocol contained in the kits' inserts.

Statistical analysis: Data obtained were expressed as mean±standard error of mean (SEM). One-way analysis of variance was used to determine the differences between means, followed by *post hoc* multiple comparisons. Data were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$. Computer software SPSS (version 17.0 SPSS Inc., Chicago, IL) and Microsoft excel (2007 version) analyser were used for analysis.

RESULTS

Results of the effects of *Vernonia calvoana* leaf extract on some selected kidney function parameters in acetaminophen treated rats are presented in Fig. 1-5.

Figure 1 shows that group 3 has a significantly increased Na^+ concentration at $p<0.001$ compared to the normal control (group 1). Group 3 also shows a significantly higher Na^+ concentration at $p<0.01$ compared to group 2 (nephrotoxic untreated group). The group treated with 400 mg kg^{-1} b.wt. (group 4) of the extract shows a significantly higher value compared to the normal control (group 1) and group 2 at $p<0.001$, respectively. Vitamin E treated group that is group 5, indicated a significant low Na^+ concentration value at $p<0.05$ compared to group 2 and a significantly lower value than group 3 at $p<0.01$, as well as a significantly lower value compared to group 4 at $p<0.001$.

Potassium ion (K^+) concentration of group 2 was significantly lower than the normal control at $p<0.05$, while group 3 has a significantly lower value compared to the normal control at $p<0.001$ and a significantly lower value as well compared to group 2 at $p<0.001$ as shown in Fig. 2. Group 4 also has a significantly lower K^+ concentration, compared to the normal control at $p<0.001$ and a significantly lower value compared to group 2 at $p<0.001$. However, group 5 has a significant value compared to the normal control at $p<0.001$ and significantly lower than group 2 at $p<0.05$. The concentration potassium in group 5 shows a significant increase compared to group 3 at $p<0.05$ and a significant increase compared to group 4 at $p<0.05$.

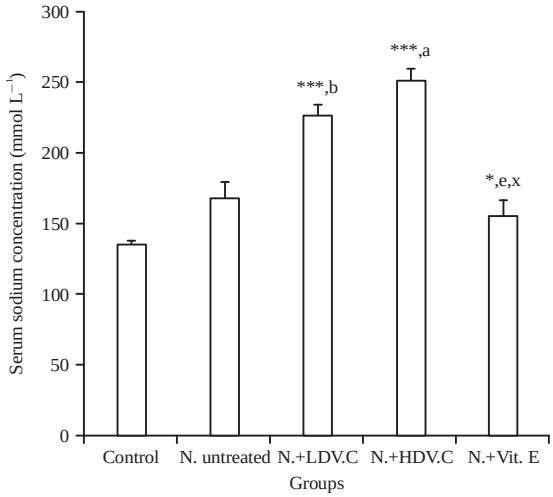

Fig. 1: Effect of *V. calvoana* extract on serum sodium level in acetaminophen treated rats

Values are Mean±SEM, n = 5. *p<0.05, ***p<0.001 vs control, a = p<0.001, b = p<0.01 vs N. untreated, e = p<0.01 vs N+LD V.C, x = p<0.01 vs N+HD V.C

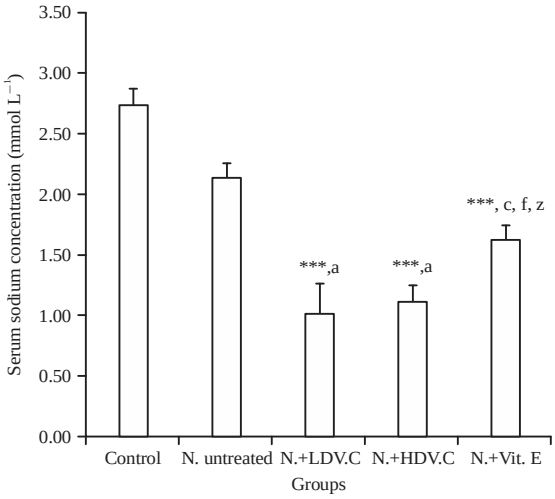

Fig. 2: Effect of *V. calvoana* extract on serum potassium level in acetaminophen treated rats

Values are Mean±SEM, n = 5. *p<0.05, ***p<0.001 vs control, a = p<0.001, c = p<0.01 vs N. untreated, f = p<0.05 vs N+LD V.C, z = p<0.05 vs N+HD V.C

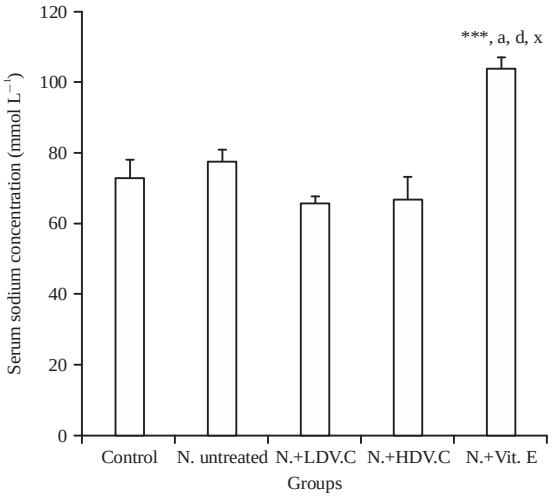

Fig. 3: Effect of *V. calvoana* extract on serum chloride level in acetaminophen treated rats

Values are Mean±SEM, n = 5. ***p<0.001 vs control, a = p<0.001 vs N. untreated, d = p<0.001 vs N+LD V.C, x = p<0.001 vs N+HD V

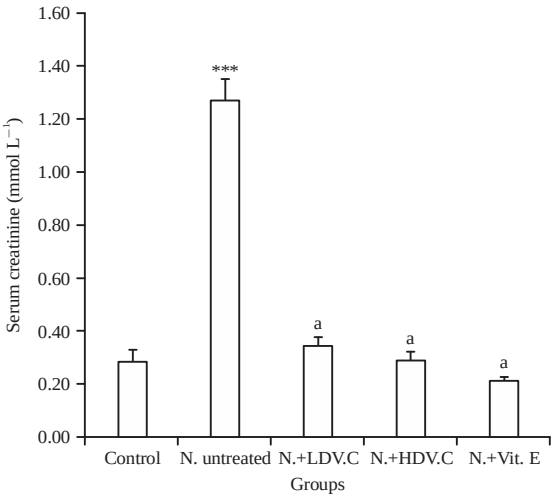

Fig. 4: Effect of *V. calvoana* extract on serum creatinine level in acetaminophen-treated rats

Values are Mean±SEM, n = 5. ***p<0.001 vs control, a = p<0.001 vs N. untreated

Chloride concentration across the experimental groups as shown in Fig. 3. Group 5 shows a significantly higher value compared to the normal control at p<0.001. Compared to group 2. The chloride concentration in group 5 was also significantly higher at p<0.001. Group 5 shows a significantly higher value at p<0.001 compared to group 3 and a significantly higher value as well, compared to group 5 at p<0.001.

There was a significant increase of serum creatinine concentration in group 2 compared to the normal control at

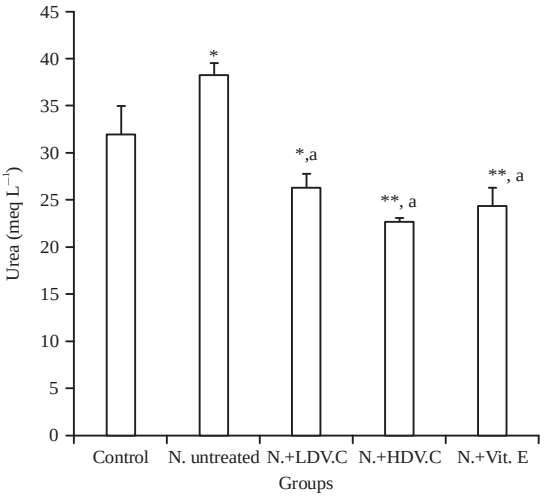

Fig. 5: Effect of *V. calvoana* extract on serum urea level in acetaminophen-treated rats

Values are Mean±SEM, n = 5. *p<0.05, **p<0.01 vs control, a = p<0.001 vs N. untreated

p<0.001, while groups 3, 4 and 5 show a significantly lower values respectively, compared to group 2 at p<0.001 as shown in Fig. 4.

The result in Fig. 5 showed increased blood urea nitrogen concentration in the nephrotoxic untreated group (group 2) at p<0.05 relative to the control group (group 1). Group 2 has a significantly lower blood urea nitrogen value compared to group 1 at p<0.05 and group 2 at p<0.001 respectively. About 200 mg kg^{-1} b.wt., extract treated group (group 3) also has a significantly lower value compared to group 1 at p<0.01 and group 2 at p<0.001, respectively. Vitamin E treated group (group 5) also shows a significant decrease in blood urea nitrogen concentration at p<0.01 compared to the normal control and at p<0.001 compared to group 2.

DISCUSSION

The study investigated the effect of graded doses of extracts of *Vernonia calvoana* on some selected kidney function parameters in acetaminophen treated Wistar rats and it was found that the untreated nephrotoxic group (group 2) showed a significantly lower serum sodium concentration compared to the extract treated groups at 200 and 400 mg kg^{-1} b.wt., respectively. This could indicate a possible electrolyte imbalance, which is a characteristic of acute or chronic renal failure over time[11-12]. The increase in the sodium concentration of the treated groups depicts a possible restorative effect of the plant, according to the report of Palani *et al.*[13] where *Plectranthus amboinicus* plant extract

was administered to acetaminophen induced nephrotoxic rats. Group 2 showed a significantly elevated serum potassium ion level compared to the extract treated groups, which on the other hand showed lower levels of the cation. This could be as a result of decreased filtration of the cation by the kidneys, thus resulting in elevated levels in the blood, which is indicative of possible renal dysfunction[14]. Hyperkalaemia is the most outstanding and life threatening condition and complication of renal failure[12]. Therefore, this implies that the plant may have had a possible rejuvenating effect on the kidneys. No significant change in serum chloride ion concentration was observed in the extract treated groups compared to the nephrotoxic untreated group. However, treatment with vitamin E significantly increased serum chloride concentration.

The serum creatinine concentration of the untreated nephrotoxic group was significantly higher than the extract treated groups and the vitamin E treated group. Atangwho *et al.*[15] reported elevated serum creatinine level as an indicator of possible kidney dysfunction. Gross *et al.*[14] in a study indicated that a rise in serum creatinine level could suggest a possible damage to the functioning nephrons of the kidney. The measurement of creatinine concentration in serum was a useful index for the diagnosis of chronic kidney disease and when serum creatinine level was higher than the normal value, renal failure was most likely a possible outcome[16]. Increased serum creatinine concentration has been considered a marker of assessing nephrotoxicity as reported by Anwar *et al.*[17] and Ali *et al.*[18].

Therefore, the elevated serum creatinine level in the nephrotoxic untreated group could be indicative of the extent of kidney damage caused by the acetaminophen overdose. However, the extract treated groups showed a decrease in serum creatinine level compared to the untreated group (group 2). The serum creatinine values of group 3 and 4 were statistically comparable to the normal control group, thus showing a possible ameliorative effect of the plant. Palani *et al.*[13], Gulnaz *et al.*[19] and Pathan *et al.*[20] also reported a decrease in serum creatinine level, after treatment with various medicinal plant extracts, which was elevated in acetaminophen induced nephrotoxic rats, thus supporting the findings of this study. Reduced serum concentration of creatinine of the extract treated groups indicates a possible kidney risk reversal, which may be accrued to the plant extract, interfering with the metabolism of creatinine and its eventual elimination from blood[21].

Blood urea nitrogen concentration of group 2 increased significantly compared to the extract treated and vitamin E treated groups. Elevated blood urea nitrogen level was indicative of possible renal impairment[22]. The determination of urea concentration of serum was useful in the diagnosis of acute renal failure while elevation of blood urea level is linked to kidney disease/congenital heart failure and indicates the possibility of severe kidney damage[23-25]. Serum urea concentration reduced significantly in both 200 and 400 mg kg^{-1} b.wt., extract of *V. calvoana* treated groups as well as the vitamin E treated groups compared to the nephrotoxic untreated group, that is group 2. The mechanism of urea reduction is unclear, there may have been a probable restoration of glomerular and tubular functions as a result of the extract administered[21]. These findings also agree with the study carried out by Pathan *et al.*[20] which showed a decrease in blood urea nitrogen level of the acetaminophen induced nephrotoxic rats treated with an extract of *Maytenus emarginata*.

CONCLUSION AND FUTURE RECOMMENDATION

The ethanolic leaf extract of *Vernonia calvoana* possesses the potential to prevent kidney dysfunction in acetaminophen induced toxicity, thus emphasising possible renoprotective effect.

Further studies on the isolation of the active principle responsible for the renoprotective effect and mechanism of action is under investigation in our laboratory at University of Calabar.

SIGNIFICANCE STATEMENTS

This present study showed the possible renoprotective effect of *Vernonia calvoana* extracts with Vitamin E supplementation in acetaminophen induced toxicity in Wistar rats. This finding presents novel information as per the use of *Vernonia calvoana* to improve kidney function after exposure to acetaminophen, a typical nephrotoxic agent.

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