Amelioration potentials of *Vernonia calvaona* ethanol leaf extract in paracetamol-treated rats

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Paracetamol toxicity due to overdose amongst people living in the developing countries has been on the increase. This study investigated the amelioration potentials of *Vernonia calvaona* ethanol leaf extract in paracetamol-treated rats. Thirty five Wistar rats were divided into five groups of seven rats each. Hepatic damage was induced by administering 2 g/kg b.wt of paracetamol p.o for four day to all the groups except group 1 (normal control). This was followed by 21 days treatment with crude ethanol extract of *V. calvaona* (VC) leaf as well as vitamin E. Group 2 received 2 g/kg b.wt of paracetamol while groups 3 and 4 received 200 and 400 mg VC extracts/kg b.wt, respectively. Group 5 received vitamin E 100 mg/kg b.wt. and histology of the liver, respectively. The result showed a significant (p<0.05) increase in serum alanine transferase (ALT), aspartate transferase (AST) and ALP during the pre-treatment phase while the treatment phase indicated a significant (p<0.05) reduction in the serum ALT, AST and alkaline phosphatase (ALP) levels in a dose dependent pattern relative to the controls. The 400 mg VC extract/kg b.wt and the vitamin E 100 mg/kg b.wt reversed the levels of these enzymes to a non-significant (p>0.05) difference relative to the controls. The histology of the liver in the VC extract treated groups indicated regeneration of the hepatocytes when compared to the control. It is therefore suggested that crude extract of *V. calvaona* leaf possesses protective effect against paracetamol induced hepatic damage in rats.

**Key words:** Amelioration potentials, liver function biomarkers, *Vernonia calvoana*.

INTRODUCTION

Medicinal plants have often played very significant roles in African societies and the world over. The rich biodiversity of the tropics makes it a potential source of medicinal phytochemicals. There are nearly about 12,000 secondary compounds identified in science as reported by Obeten et al. (2017). Therefore necessitating, the current research interests in ethno-medicine, ethno-botany, and ethno-pharmacology (Olowokudejo et al., 2008). About 250,000 species of flowering plants have been reported to occur globally, and approximately half (125,000) of these species are found in the tropical forests. Yet, only about one percent of these tropical species have been investigated for their chemical and therapeutic potentials (Jachak and Saklani, 2008). The
tropical rain forest, of which Nigeria is rich as a reservoir of phytomedicines with most of these plants containing substances that could be exploited for therapeutic purposes by man (Sofowora, 1982; Iwu, 1993).

The prevalence of drug abuse and self-medication especially for over the counter analgesic drugs has gone on unabated for years. Alcohol consumption also is considered as one of the major causes of liver damage (Dominic et al., 2011), however, intake of other substance of abuse has been on the increase especially paracetamol. This practice may predispose consumers of such drugs to liver damage.

One of such analgesics that are often abused due to over dose is acetaminophen (N-Acetyl-p-aminophenol) with the trade name paracetamol. It possesses both analgesic and antipyretic properties.

The administration of paracetamol is safe when therapeutic dose is not exceeded, however, it can cause hepatic necrosis, nephrotoxicity, extra-hepatic lesions and even death in humans and experimental animals when taken in overdose (Ray et al., 1999; Webster et al., 1996; Mohanraj et al., 2013). Acetaminophen –induced renotoxicity at 750 mg/kg body weight had been established by Sathishkumar and Baskar (2014). Liver damage induced with acetaminophen at 100 mg/100 g in Wistar rats has been reported by Yakubu et al. (2013). Paracetamol hepato-toxicity is mediated by an initial metabolic oxidation, covalent binding, and subsequent activation of macrophages to form reactive oxygen and nitrogen species in which the central portal for handling its metabolism is the liver.

Medicinal plants are considered to be safe with minimal side effects and due to its low-cost, most persons living in developing countries tend to use them as alternatives to synthethic drugs in managing most diseases. The therapeutic effects of several medicinal plants and vegetables used commonly in folk medicine against many diseases are attributed to the presence of phenolic content which is responsible for their antioxidant activity (Okwu, 2004; Sofowora, 1993; Iwu, 1993). One of such medicinal plants is *Vernonia calvaona Hook (Asteraceae)*, it is a small tropical plant with height of less than 1 m and has been indicated in the management of diabetes, heart disease, malaria and stomach ache (Igile et al., 2013).

The hypolipidemic and antioxidant potentials of *V. calvoana* leaf extracts in diabetic rats has been reported by Iwara et al. (2015) while the *in vitro* antioxidant properties of the inflorescent of *V. calvoana* indicated increased concentration of flavonoids (Egbung et al., 2016) thereby supporting its anti-oxidation role as previously reported. Despite the wide application of *Vernonia* genus in traditional medicine, there is still paucity of information on *V. calvoana*. This work was designed therefore to evaluate the effect of crude extract of *V. calvoana* leaves on some serum enzyme activity in paracetamol treated Wistar rats.

### MATERIALS AND METHODS

#### Equipments

The equipment used in this study include: rotary evaporator, animal cages, precision pipette (Syntron Bioresearch Inc. USA), spectrophotometer (SAEC, Italy), automated chemical analyzer (BC2600 KX-21N) and centrifuge (Everest, China).

#### Chemicals and reagents

All the chemicals used in this study were of analytical grade. Reagent kits used for all the biochemical analysis were purchased from Agappe diagnostics, Switzerland. Ethanol and chloroform were purchased from Globus Chemicals, Mayne Avenue, Calabar, Cross River State, Nigeria. Normal saline was purchased from Bez pharmacy, Etta-agbor, Calabar. M&B paracetamol (500 mg) and evitrol vitamin E capsules (100 mg) were obtained from Anijah pharmacy, Etta Agbor road, Calabar, Cross River State, Nigeria. Formalin and paraffin wax were obtained from Aldrich Chemical Co. Ltd. England, while Haematoxylin and Eosin staining dyes were obtained from Sigma, USA. Agape diagnostic kits were employed in the determination of biochemical parameters including AST, ALT, and ALP, total protein, total bilirubin and albumin.

#### Collection of plant materials and extract preparation

Fresh leaves of *V. calvoana* were obtained from the farm in Ugep, Yakurr local government area of Cross River state. A sample of the plant was taken to the herbarium in the Department of Botany, University of Calabar for identification. Authentication was done by Mr Frank Apejoye and a voucher specimen deposited and assigned herbarium number 126. The leaves were washed with clean water to remove debris and dust particles, thereafter shade dried. The dried leaves were then homogenized to fine powder using an electric blender. The powdered sample was soaked in ethanol for 48 h. This was then filtered using a cheese cloth and filter paper (Whatman No.1) and the ethanol evaporated using a rotary evaporator. The pasty concentrate was then collected into clean sample bottles using a spatula and stored in a refrigerator until required for use.

#### Experimental animals

Thirty five albino Wistar rats weighing between 150 to 220 g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar and used for the experimental investigation. The animals were housed in standard plastic cages in the animal house of the Department of Biochemistry. They were allowed free access to normal rat chow and tap water *ad libitum* throughout the experimental period. The animals were kept at room temperature with 12 h light /dark cycle. They were allowed one week acclimatization before commencement of experimental treatment.

#### Acute toxicity test (LD₅₀)

Oral acute toxicity of crude extract of *V. calvoana* was determined in mice as described by Lorke (1983). This was done in two phases; in the first phase of the test, nine animals were divided into three groups of three animals each. Each group of animals was given different doses of the extract. The animals were then observed for a period of 24 h for any sign of toxicity as well as mortality. The second phase requires three animals divided into
Table 1. Result of acute toxicity evaluation (LD50) of Vernonia calvoana ethanol extracts.

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of mice</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg V. calvaona extract/kg b.wt</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>800 mg V. calvaona extract/kg b.wt</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2400 mg V. calvaona extract/kg b.wt</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3000 mg V. calvaona extract/kg b.wt</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4500 mg V. calvaona extract/kg b.wt</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6000 mg V. calvaona extract/kg b.wt</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

No death was recorded in both phases. V. calvaona crude extract showed no sign of toxicity even at a dose of 6000 mg/kg b.wt of animal via oral administration in mice. Hence doses of 200 mg/kg b.wt and 400 mg/kg b.wt of the extract were chosen for further experiment.

three groups of one animal each. The animals were given higher doses of the extract and observed for 24 h for signs of toxicity and mortality. The LD50 is calculated using the formula:

\[
LD_{50} = \sqrt{D_0 \times D_{100}}
\]

Where \( D_0 \) = highest dose that gave no mortality
\( D_{100} \) = lowest dose that produced mortality

Experimental design

Thirty five albino Wistar rats were divided into five groups of seven animals each. Group one served as control and received 1 ml each of normal saline. Group two (toxic group) received paracetamol (2 g/kg b.wt) alone. Group 3 (treatment group) received paracetamol (2 g/kg b.wt) plus (200 mg VC extract/kg b.wt). Group 4 (treatment group) received paracetamol (2 g/kg b.wt) plus (400 mg VC extract/kg b.wt). While group 5 (positive control) received paracetamol (2 g/kg b.wt) plus vitamin E (100 mg/kg b.wt). The treatment lasted for 21 days.

Induction of hepatotoxicity

Hepatotoxicity was induced with paracetamol (2 g/kg b.wt) prepared in normal saline and administered to the animals in all the groups except the control group, once a day for four days. Three days after paracetamol administration, one animal was selected at random from all the groups anaesthetized under chloroform vapour and dissected. Whole blood was collected and used for the estimations of serum enzyme levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase.

Collection of blood and tissue samples for analyses

At the end of the 21 days treatment period, the animals were fasted for 12 h but allowed free access to water, anaesthetized under chloroform vapour and dissected. Whole blood was collected from the heart by cardiac puncture into sterile plain tubes using sterile syringes and needles, serum was obtained using the centrifuge and stored at 4°C in a refrigerator for subsequent biochemical analyses. The organ (liver) was surgically removed, suspended in 10 percent buffered formalin (formal saline) and used for histopathological study.

Biochemical analyses

Biochemical assay estimations of serum AST, ALT, and ALP were done using Agape diagnostic kits, Switzerland, according to manufacturer’s protocol.

Histology of the liver

The fixed liver sections were processed for histological examination using haematoxylin and eosin (H and E) basic histological procedures (Parry, 2012).

Statistical analysis

Data was expressed as means ± standard error of mean (SEM). The statistical tools used for the analysis was one way analysis of variance (ANOVA) and the post hoc Newmann Keul’s multiple comparisons test. The computer software utilized were Microsoft excel 2013 edition and SPSS 16.0 for windows. Differences between means were considered significant at \( p<0.05 \).

RESULTS

The results of this study on the amelioration of paracetamol induced hepatotoxicity in albino Wistar rats using crude extract of V. calvoana leaves are presented in Table 1 (acute toxicity test), Figures 1 to 3 (effect of treatment on serum enzymes) and Plates 1 to 5 (effect of treatment on the integrity of the liver). The effect of treatment on serum AST levels of paracetamol treated rats showed that the AST levels of the paracetamol (2 g/kg b.wt) treated group and 200 mg V. calvoana extract/kg b.wt treated group were significantly (\( p<0.05 \)) high compared to the control group. However there was no significant (\( p>0.05 \)) difference between the AST levels of the 400 mg V. calvoana extract/kg b.wt treated group and Vitamin E (100 mg/kg b.wt) treated groups compared to the control group.

Effect of V. calvoana extracts on serum ALT levels of paracetamol treated rats showed that the ALT levels of
Figure 1. AST levels in rats treated with PCM (2 g/kg b.w), PCM 2 g/kg + 200 mg V. calvaona extract/kg b.wt, PCM 2 g/kg + 400 mg V. calvaona extract/kg b.wt, PCM 2 g/kg + Vitamin E (100 mg/kg b.wt) and control. * = p<0.05 vs. Control; a = p<0.05 vs. PCM 2 g/kg b.w; b = p<0.05 vs. PCM 2 g/kg + 200 mg VC extract/kg b.w.

Photomicrographs showing sections of the liver in the control group and the treated groups

The liver section of the control group shows a normal liver architecture as indicated by the presence of prominent congested central vein with hepatocytes radiating outward. The hepatocytes have regular cytoplasmic and nuclei outline with prominent nucleoli. The separating sinusoidal spaces are dilated and congested. The portal area contains mild mononuclear inflammatory cells with intact limiting plates.

Section of the liver shows damage to the liver tissue as evidenced by the presence of swollen hepatocytes with clear vacuolation and indistinct cytoplasmic outline. The separating sinusoidal spaces are dilated. The portal area is expanded and contains severe mononuclear cellular
Figure 2. Alanine aminotransferase levels in rats treated with PCM (2 g/kg b.wt), PCM 2 g/kg + 200 mg V. calvaona extract/kg b.wt, PCM 2 g/kg + 400 mg V. calvaona extract/kg b.wt, PCM + Vitamin E (100 mg/kg b.wt) and control. * = p<0.05 vs. Control; a = p<0.05 vs. PCM 2 g/kg b.wt; b = p<0.05 vs. PCM 2 g/kg + 200 mg VC extract/kg b.w.

DISCUSSION

The liver is critical in the regulation, synthesis, storage, secretion and excretion of biomolecules in the body as well as in the detoxification of xenobiotics and drugs in the biological system. Oxidative stress due to paracetamol intoxication involves membrane perturbation via NAPQI (N-acetyl-p-benzoquinone imine) binding to parenchyma cell proteins (forming protein adducts which are responsible for the dysfunction and death of hepatocytes, leading to liver necrosis) and is mediated by lipid modification, inhibition of transcription, translation and fragmentation of DNA (Vermeulen et al., 1992; Ray et al., 1999). The acute toxicity evaluation showed neither toxicity nor mortality even at a dose of 6000 mg V. calvaona extract/kg b.wt of animal. The crude extract of inflammatory cells with intact limiting plates and patchy areas of necrosis.

Section of the liver shows a prominent central vein with plates of hepatocytes radiating outward. The hepatocytes are swollen with clear vacoulation and the cytoplasmic and nuclei outline prominent. The separating sinusoidal spaces are dilated. The portal area contains moderate mononuclear cellular inflammatory cells with intact limiting plates.

Section of the liver shows a prominent central vein with plates of hepatocytes radiating outward. The hepatocytes have regular cytoplasmic and nuclei outline with prominent nucleoli. The separating sinusoidal spaces are dilated. The portal areas are moderately expanded and contain mononuclear cellular inflammatory cells with intact limiting plates. Section of the liver shows a prominent central vein with plates of hepatocytes radiating outward. The hepatocytes have regular cytoplasmic and nuclei outline with prominent nucleoli. The separating sinusoidal spaces are dilated. The portal area contains moderate mononuclear cellular inflammatory cells with intact limiting plates.
Figure 3. Alkaline Phosphatase (ALP) levels in rats treated with paracetamol (PCM) (2 g/kg b.wt), PCM 2 g/kg + 200 mg V. calvaona extract/kg b.wt, PCM 2 g/kg + 400 mg V. calvaona extract/kg b.wt, PCM 2 g/kg + Vitamin E (100 mg/kg b.wt) and control. * = p<0.05 vs. Control; a = p<0.05 vs. PCM 2 g/kg b.wt.

V. calvaona was found to be safe and could be used for medicinal purposes even at a dose level of 6000 mg/kg b.wt of animal. Increase concentrations of serum AST, ALT and ALP were seen on paracetamol induced liver injury. Excessive levels of serum enzymes in the blood have been attributed to hepatic damage as well as organ turnover (Pendota et al., 2010). Therefore the increased levels of these bio-markers in the blood of paracetamol intoxicated animals imply that the plasma membrane of the hepatic cells were damaged, resulting in their leakage into extra-cellular fluid. The administration of paracetamol (2 g/kg b.wt of animal) caused a marked significant increase in the blood levels of AST, ALT, and ALP when compared to normal control.

However, the treatment of animals with crude extract of V. calvaona leaves after paracetamol intoxication caused a significant reduction in the levels of these enzymes in a dose dependent manner. The administration of 200 mg V. calvaona extract/kg b.wt of animal caused a significant (p<0.05) reduction in the serum AST, ALT and ALP levels compared to the paracetamol intoxicated group, while administration of the extract (400 mg/kg b.wt of animal) and the standard drug (Vitamin E) at 100 mg/kg b.wt of animal) caused a more significant reduction in AST, ALT and ALP which showed no significant (p>0.05) difference when compared to the control group. Our findings correlates with the report of Yakubu et al. (2013) where serum levels of AST, ALT, ALT and LDH were modulated by soymilk in acetaminophen treated rats.

The extent of damage to the liver by paracetamol was further supported by morphological changes in the liver of the experimental animals. The photo micrographs of
normal control showed normal cellular architecture (plate 1) with normal structure of the hepatocytes. Histological examination of the liver sections of the treated groups showed that the normal liver architecture was however disturbed by paracetamol intoxication. There were signs of necrosis and derangement of hepatic cells, cellular infiltration and inflammation in the liver section of animals treated with paracetamol alone.

However, in the liver section of animals treated with 200 mg V. calvaona extract/kg b.wt, the micrograph showed recovery of the damaged hepatocytes, with reduction in the quantity of necrotic cells and minimal inflammation. The 400 mg/kg b.wt treated group showed more remarkable recovery of hepatocytes while the
vitamin E (100 mg/kg b.wt of animal) treated group indicated almost complete reversal of liver architecture to normal. The histological results showed also that the crude extract of *V. calvaona* leaves ameliorated the

**Plate 3.** Histological section of the liver in group 3 animals treated with 200 mg *V. calvaona* extract/kg b.wt of animal (×400). CV: Central vein; PT: Portal area; HP: Hepatocytes.

**Plate 4.** Histological section of the liver in group 4 animals treated with 400 mg *V. calvaona* extract/kg b.wt of animal (×400). SS: Sinusoidal space; HP: Hepatocytes; CV: Central vein.
paracetamol induced liver damage by restoring the normal architecture of the liver in a dose dependent manner.

The therapeutic activity of medicinal plants usually depends on their bio-active principles that are predominantly alkaloids and phenolics especially in exerting antioxidant activity (Hayase and Kato, 1984; Sofawara, 1993; Yen and Duh, 1993). Dominic et al. (2011) reported the protection of liver damage in alcohol treated rats using extracts of Emilia sonchifolia, a Lamiaceae like V. calvoana. Phenolic compounds, flavonoids, saponins and glycosides have been identified in significant amount as part of the phytochemical composition of V. calvoana leaves (Igile et al., 2013). The presence of these phytochemicals in the ethanol leaf extract of V. calvoana could be a potential source of boosting the body’s glutathione stores which is responsible for the amelioration of hepatic damage induced by paracetamol overdose in Wistar rats.

Conclusion

The findings of this research work elucidate the protective potential of crude extract of V. calvoana against paracetamol induced hepatotoxicity. The 400 mg/kg b.wt dose of V. calvoana extract indicated effective hepatoprotection against paracetamol toxicity and compared well with the standard drug (vitamin E). Further investigation should be carried out to isolate and identify the compounds present in the leaves of V. calvoana that is responsible for its hepato-protective property and subsequent in vivo mechanism of action.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


