

TOTAL PROTEIN, ALBUMIN AND GLOBULIN LEVELS FOLLOWING THE ADMINISTRATION OF ACTIVITY DIRECTED FRACTIONS OF *VERNONIA AMYGDALINA* DURING ACETAMINOPHEN INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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(Received 24 February 2011; Revision Accepted 3 February 2012)

ABSTRACT

The effect of treatment with activity directed fractions of *Vernonia amygdalina* during acetaminophen - induced hepatotoxicity in wistar albino rats for 14 days was investigated. The 48 wistar albino rats used were divided into 8 groups of 6 rats each. Group 1 served as the normal control group and received only distilled water. Group 2 served as paracetamol control group and received only paracetamol. Groups 3-8 were treated with acetaminophen and activity directed fractions of *Vernonia amygdalina*. The extracts were obtained by fractionation of the crude ethanolic extract using organic solvents of increasing polarities. Paracetamol was administered at a dose of 171.41mg/kg and the fractions of *vernonia amygdalina* at 200mg/kg. At the end of the treatment with fractions of benzene, chloroform, ethyl acetate, butanol, methanol and residue E produced varying results in the level of total protein, albumin and globulin. Results obtained shows a significant decrease ($P<0.05$) in total protein level (g/dl) in the paracetamol group (23.66 ± 0.59) compared to the normal control group (45.00 ± 1.73). The result also shows a significant increase ($P<0.05$) in total protein (g/dl) in all group treated with the various fractions of *vernonia amygdalina* compared to the paracetamol group, with the group treated with residue E fraction having the highest protein level (g/dl) among all treatment groups (36.50 ± 2.21). The present *in vivo* study has further demonstrated the hepatoprotective potential of this plant. In this study hepatocellular damage induced by acetaminophen intoxication in rats was established based on significant decrease ($P<0.05$) in total protein, globulin activities, and no significant decrease ($P<0.05$) in albumin levels (g/dl) in paracetamol group (25.00 ± 3.36) compared to the normal group (25.33 ± 1.51) as found by previous workers. The albumin and globulin levels (g/dl) show insignificant decrease ($P>0.05$) in groups treated with fractions of *vernonia amygdalina* due to liver dysfunction. From the result significantly increased globulin levels (g/dl) in groups treated with residue E, methanol, and chloroform as well as increased total protein levels in the residue E, methanol, butanol, ethyl acetate, chloroform and butanol groups compared to the paracetamol group is indicative of the fact that the hepatoprotective principles of *vernonia amygdalina* may reside in these fractions.

KEYWORDS: Acetaminophen, *vernonia amygdalina*, hepatotoxicity, total protein, albumin, globulin

INTRODUCTION

Paracetamol or acetaminophen is one of the most commonly used non-narcotic analgesic antipyretic agents. It has only weak anti-inflammatory activity. It is the active metabolite of phenacetin and is derived from coal tar. (Daly *et al*, 2008). Acetaminophen is metabolized in the liver where its major metabolites include inactive sulfate and glucuronide conjugates, which are excreted by the kidneys. Only a small, yet significant amount is metabolized via the hepatic cytochrome P₄₅₀ enzyme system (its CYP2E1 and CYP1A2 isoenzymes), which is responsible for the toxic effects of acetaminophen due to a minor alkylating metabolite, N-acetyl-p-benzo-quinoneimine (Chandrase *et al*, 2002).

Excessive use of acetaminophen damages multiple organs, especially the liver and kidney. In both organs, toxicity from acetaminophen is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine. Acetaminophen overdose leads to the accumulation of N-acetyl-p-benzoquinoneimine, which undergoes conjugation with glutathione.

Conjugation depletes glutathione, a natural antioxidant. This, in combination with direct cellular injury by N-acetyl-p-benzoquinoneimine, leads to cell damage and death. These injuries are known as acetaminophen hepatotoxicity and analgesic nephropathy in the liver and kidney (Lee, 2004).

Vernonia amygdalina, a member of the asteraceae family, is a shrub grown in tropical Africa. The leaves are green with a characteristic odor and a bitter taste which may be consumed either as vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. In the wild, chimpanzees have been observed to instinctively ingest the leaves when suffering from parasitic infections (Izerbigie *et al*, 2004).

Many herbalists and naturopathic doctors recommend aqueous extracts for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced ambrosia, dysentery and other gastrointestinal tract problems. Until the least or so, there were only anecdotal reports and claims to support the health benefits (Ohigashi *et al*, 1991; Jisaka *et al*, 1992). *Vernonia amygdalina* regimen or consumption as dietary

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supplements provides multiple health benefits. (Izerbigie *et al*, 2004). However, saponins present in the leaves may create health hazards such as reduction in red blood cells, white blood cell count and pack cell volume (Igile and Oleszek, 1995).

The pharmaceutical imbalance in remedies that could protect the liver and have antioxidant properties and drugs that induces hepatotoxicity has prompted the research into plants used in folk medicine to treat liver diseases and boost liver functions, and *vernonia amygdalina* is one of such plant that has scientifically been proven to contain phytochemical principles from which its antitumor and antimicrobial properties are derived. However, there have not been adequate scientific data to support the hepatoprotective potentials of *vernonia amygdalina* and provide information on its mechanism of action. Therefore, in this study, the ability of activity directed extracts of *vernonia amygdalina* leaf to protect the liver against acetaminophen-induced hepatocellular damage in rats *in vivo* is investigated.

Materials and methods

Collection and treatment of plant samples

Fresh leaves of *Vernonia amygdalina* were harvested from the endocrine research farm in the University of Calabar. It was identified in the Department of Botany, University of Calabar. They were dried under shade, crushed and soaked in 98% ethanol for 72 hours, then filtered and allowed to evaporate at room temperature to obtain the crude extract of *Vernonia amygdalina*.

The whole extract was subjected to fractionation using organic solvents of varying polarities. It was first soaked in benzene in a separating funnel, shaken and allowed to separate into two fractions. The benzene soluble fraction was obtained and allowed to dry at room temperature to obtain benzene extract, and then the residue was dried and fractionated using chloroform. This procedure was repeated with ethyl acetate, butanol and methanol. And the final residue was labeled Residue E.

Forty-eight Wistar albino rats weighing between 80 to 120g were obtained from the animal house of the Department of Biochemistry, University of Calabar. They were housed in plastic cages in the animal house and fed rat pellets twice a day. The animals were acclimatized for ten days and the average weight of each group was noted before commencement of administration.

The forty-eight wistar albino rats were divided into eight groups of six rats each; group one served as the normal control group, group two served as the paracetamol group, group three to eight served as the treatment groups. The animals in group one which served as the control group received distilled water throughout the treatment period, group two animals were administered with paracetamol and group three to eight were administered with paracetamol and treated with the various fractions of *vernonia amygdalina*.

The Wistar albino rats were given a dose of extract and paracetamol according to their body weight, 250mg/kg and 171.41mg/kg respectively. These were administered twice a day (12 hours part) for a period of 14 days.

After 14 days of administration, the rats were fasted over-night. The following day, they were

anaesthetized in a chloroform fume chamber, and then dissected. Blood was collected through cardiac puncture using sterile needle. The blood was collected into properly labeled tubes. It was centrifuged at 700rpm for 15minutes in an MSE table top centrifuge. A sterile pasture pipette was used to transfer serum from the clotted blood into plane tubes and stored at 4°C till the next day for analysis.

Estimation of serum total protein

The most widely used method for measuring serum protein is the biuret reaction (George, 2009). The principle of this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex. The intensity of the violet color was measured using a DRE 3000 HACH spectrophotometer and is proportional to the concentration of protein (Bjorsten *et al*, 2007).

Estimation of serum albumin

Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye (George, 2009).

The absorbance of the samples and of the standard was measured against reagent blank at 546 nm, and temperature of 37°C. These tubes and their contents were mixed and incubated for 90 minutes at 37°C. Estimation of albumin level (g/dl) was obtained using a DRE 3000 HACH spectrophotometer.

Estimation of serum globulin

Since, bromocresol green (BCG) - albumin complex absorbs light at a different wavelength from the unbound dye, the method may overestimate albumin by binding to other proteins (George, 2009). Hence, the total globulin fraction is generally determined by subtracting the albumin fraction from the total protein fraction.

The data obtained were analyzed statistically using analysis of variance (ANOVA) and students T-Test.

Result

The effects of treatment with activity directed extracts of *vernonia amygdalina* during acetaminophen – induced hepatotoxicity for 14 days on the serum total protein, albumin and globulin levels (g/dl) was investigated. The result shows a significant decrease ($P<0.05$) in total protein level (g/dl) in the paracetamol group (23.66 ± 0.59) compared to the normal control group (45.00 ± 1.73). Also there was a significant increase ($P<0.05$) in total protein in all groups treated with the various fractions of *vernonia amygdalina* compared to the paracetamol group; with the group treated with the residue E fraction having the highest protein level among all treatment groups (36.50 ± 2.12).

While there was a non - significant decrease ($P>0.05$) in albumin level in the paracetamol group (25.00 ± 3.36) compared to the normal control group (25.33 ± 1.51), albumin levels (g/dl) in all groups treated with various fractions of *vernonia amygdalina* were insignificantly decreased ($P>0.05$).

There was a significant decrease ($P<0.05$) in globulin level (g/dl) in the paracetamol group (13.75 ± 4.92) compared to the normal control group ($19.66 \pm$

3.21), as well as a non - significant decrease ($P>0.05$) in groups treated with fractions of chloroform (14.25 ± 3.20) and methanol (14.50 ± 1.73); with the residue E treated group (15.50 ± 3.80) having the highest albumin level among all treatment groups compared to the

paracetamol group. Also insignificant decrease ($P>0.05$) was observed in globulin levels in groups treated with ethyl acetate (11.00 ± 2.82), benzene (11.66 ± 1.16) and butanol (11.75 ± 3.59) fractions compared to the paracetamol group.

TABLE 1

Effect of activity directed extracts of *vernonia amygdalina* on serum total protein, albumin and globulin levels during acetaminophen-induced hepatotoxicity in wistar albino rats.

Group	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1	Normal control (water)	45.00 \pm 1.73	25.33 \pm 1.51	19.66 \pm 3.21
2	Paracetamol control	23.66 \pm 0.59 ^a	25.00 \pm 3.36	13.75 \pm 4.92
3	Paracetamol/ benzene	35.32 \pm 0.59 ^a	23.66 \pm 0.59	11.66 \pm 1.16 ^a
4	Paracetamol/ Chloroform	36.00 \pm 3.54 ^{ab}	21.75 \pm 0.96 ^a	14.25 \pm 3.20
5	Paracetamol/ Ethyl acetate	33.75 \pm 2.98 ^b	22.50 \pm 1.29	11.00 \pm 2.82 ^a
6	Paracetamol/ Butanol	32.00 \pm 3.16 ^a	20.25 \pm 2.22 ^b	11.75 \pm 3.59 ^{ab}
7	Paracetamol/ Methanol	32.25 \pm 1.71 ^b	17.75 \pm 0.96 ^{a b}	14.50 \pm 1.73 ^a
8	Paracetamol/ Residue E	36.50 \pm 2.12 ^b	21.00 \pm 1.41 ^a	15.50 \pm 3.80 ^a

Values are expressed as mean \pm SD. Data are statistically significant at $P<0.05$.

n = 6

a = $P<0.05$ compared to normal control group

b = $P<0.05$ compared to paracetamol group

DISCUSSION

Effect of activity directed fractions of *Vernonia amygdalina* on serum total protein, albumin and globulin levels was examined in this study. From the result obtained, there was significant decrease ($P<0.05$) in total protein, and globulin levels in the paracetamol group compared to that of the normal control group. This decrease agrees with George (2009), that decrease in serum protein in hepatotoxicity states simply indicates the presence of para proteins or decreased antibody production.

There was also a significant increase ($P<0.05$) in total protein levels in groups treated with the various fractions of *Vernonia amygdalina* with residue E having the highest protein levels compared to paracetamol group. There was also an insignificant decrease ($P>0.05$) in albumin levels in the remaining groups treated with the various fractions of *Vernonia amygdalina*. The result of the present study strongly suggests that the various fractions of *Vernonia amygdalina* leaf extract have hepatoprotective, which may be mediated by antioxidant activity in rats *in vivo*. That the treatment with activity directed solvent extracts

of *Vernonia amygdalina* were able to increase the levels of serum total protein, and globulin, confirming earlier studies by Archibald (1975).

Vernonia amygdalina is known to have wide therapeutic applications in folk medicine and scientific advancement has provided substantial evidence to support most of its medicinal claims. The present *in vivo* study has further demonstrated the hepatoprotective potential of this plant. In this study hepatocellular damage induced by acetaminophen intoxication in rat was established based on significant decrease ($P < 0.05$) in total protein levels (g/dl) in the paracetamol group (23.66 ± 0.59) compared to the normal control group (45.00 ± 1.73), no significant decrease ($P < 0.05$) in albumin levels (g/dl) in paracetamol group (25.00 ± 3.36) compared to the normal group (25.33 ± 1.51), insignificant decrease ($P > 0.05$), in albumin levels (g/dl) treated with fractions of *Vernonia amygdalina*, and a significant decrease ($P < 0.05$) in globulin level in paracetamol group (13.75 ± 4.92) compared to the normal control group (19.66 ± 3.21) as found by previous workers. The total protein, albumin and globulin level may decrease due to liver dysfunction, malnutrition and malabsorption, diarrhea, nephrosis, alpha-1-antitrypsin deficiency, acute hemolytic anemia, hypogammaglobulinemia / agammaglobulinemia; severe and loss through the urine in severe kidney disease and pregnancy. Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes decrease in the serum levels of total protein, albumin and globulin. Our observed decrease of these parameters due to acetaminophen challenge is in consonance with the findings of Hattori *et al*, (1990) and Emmanuel *et al*, (1995) in which respectively hepatoprotective effect of ajoene from garlic and leaf extract of *Wedelia calendulacca* against acetaminophen-induced hepatic damage were found. The observed dose-dependent reversal of acetaminophen-induced alteration in rat liver total protein levels by pre administration of *Vernonia amygdalina* extract suggest that this plant is hepatoprotective. Our finding appears to validate the earlier observation of Babalola *et al*, (1998) that the terpenoid fraction of *Vernonia amygdalina* leaf extract ameliorates carbon tetrachloride-induced hepatotoxicity in rats. The hepatoprotective ability of *Vernonia amygdalina* may be connected with its sesquiterpene lactones since these phytochemical constituent have been implicated as the hepatoprotective factors in *Cuidium monnieri* and *Zedoariae rhizome* and were also detected in our extract.

Furthermore, a significant reduction in liver total protein level was observed in acetaminophen-challenged rats, and this validates the reported translation inhibition effect of acetaminophen overdose. However, pre administration of *Vernonia amygdalina* resulted in dose-dependent suppression of acetaminophen-induced adverse effects on liver antioxidant status and protein level, thereby suggested that this plant has antioxidant activity based on free radical scavenging or modulation of antioxidant status, tissue regeneration or maintenance of protein metabolism in the liver tissue in rat.

CONCLUSION

The result of the present study strongly suggests that activity directed fractions of *Vernonia amygdalina* have varying hepatoprotective properties in rats *in vivo*. From the result, significantly increased globulin levels in groups treated with residue E, methanol, and chloroform as well as increased total protein levels in residue E, methanol, butanol, ethyl acetate, chloroform, and butanol groups compared to the paracetamol group is an indication that hepatoprotective principles of *vernonia amygdalina* may reside in these fractions.

REFERENCES

- Archibald, P. M., 1975. Association of Clinical Pathologists Symposium on Disorders of Protein Metabolism. Journal of Clinical Pathology Supplement. 4-7.
- Babalola, L., Fashiola, B and Adesina, Y. O., 1998. Phytochemical properties University press Ibadan.
- Bjorston, A. R., Crankshaw, D. P., Morgan, D. J and Prideaux, P. R., 2007. Clinical Chemistry. Department of surgery, Royal Malbourne Hospital, University of Melbourne, Victoria, 3050.
- Chandrase, N. V., Dai, H and Roos, K. L., 2002. Cox-3, A Cyclooxygenase-1 variant inhibited by acetaminophen and other Analgesic/Antipyretic drugs. 13926 - 13931.
- Daly, F. F., Fountain, J. S., Murray, L., Gaudins, A and Buckley, N. A., 2008. A Consensus Statement from Clinical Toxicologists Consulting to the Australasian Poisons. Information Centres. Med. J. Aust. 188, (5): 296-301.
- Emmanuel, J., Kambisi, L and Afolayan, A. J., 1995. An Ethnobotanical Study of Plants Use for the treatment of STD's. Guruye District. Zimbabwe.
- George, R. K., 2009. Biochemistry Laboratory. Philadelphia www.jbc.org.
- Hattori, S. M., Makkai, H. P. S and Becker, K., 1990. Feed Value of Some Browse plants. Central Zone of Delta State, Nigeria; (39): 97-104.
- Igile, G and Oleszek, W., 1995. Vernoniosides D and E, Two Hovel Saponins from *Vernonia Amygdalina*. Journal of Natural Products Loydia 58, (9): 1438-1443.
- Izerbigie, E. B., Bryant, J. L and Walker, A., 2004. Natural Inhibitor of Extracellular Signal-regulated Kinases and Human Breast cancer cells Exp Biol and Medicine 229. 163-169.
- Jisaka, M., 1998. Medicinal herbs. Indian journal of

- Agricultural Chemistry. 60. 14-16.
- Lee, N. W., 2004. Acetaminophen and the U.S. Acute Liver failure Study Group. Hepatology 40, (1): 6-9.
- Ohigashi, H., Jisaka, M., Takagaki, T., Nozaki, H., Tada, T and Nishida, T., 1991. A bitter principle and related steroid glycoside of *Vernonia amygdalina*, a possible medical plant for wild chimpanzee. Agric and Biological Chemistry. 55, (5): 1201-1203.