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Uric acid, urea and bilirubin levels of albino rats treated with activity directed fractions of *Vernonia Amygdalina* during acetaminophen induced hepatotoxicity

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ABSTRACT

The effect of activity directed fractions of vernonia amygdalina on the levels of urea, uric acid; total and conjugated bilirubin in wistar albino rats during acetaminophen induced hepatotoxicity was investigated in this research. The forty-eight (48) wistar albino rats used were divided into 8 groups of six rats each. Group one, the normal control group received distilled water, group two received only paracetamol throughout the experimental period. Groups three to eight were administered with 171.43mg/kg body weight of paracetamol and treated with 200mg/kg body weight of activity directed fractions of vernonia amygdalina throughout the 14 days of treatment. Result shows significantly lower levels ($p<0.05$) of uric acid in the paracetamol, chloroform, ethyl acetate, butanol, methanol and residue E treated fractions compared to the normal control. However, the benzene fraction had a higher uric acid level ($p<0.05$) compared to the normal control. Urea levels significantly increased ($p<0.05$) in groups treated with fractions of chloroform and residue E while it significantly decreased ($p<0.05$) in the butanol treated group over the normal control group. Total bilirubin levels were significantly higher ($p<0.05$) in the treatment groups compared to the normal control. Whereas, the conjugated bilirubin level was non - significantly higher ($p>0.05$) than the normal control, the levels were significantly higher ($p<0.05$) in groups treated with fractions of benzene and butanol compared with the normal control levels, levels of conjugated bilirubin were non significantly higher ($p>0.05$) in chloroform and residue E treated groups when compared with the normal control. Non - significantly higher levels ($p>0.05$) of conjugated bilirubin were observed in all treated groups except the ethyl acetate fraction group compared to the paracetamol group. Vernonia amygdalina fractions have the ability to protect against acetaminophen induced liver damage coupled with antioxidative potentials. This suggests that vernonia amygdalina is suitable for the treatment of acetaminophen induced hepatotoxicity.

Keywords: *Vernonia amygdalina*, urea, uric acid, bilirubin, acetaminophen, hepatotoxicity

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INTRODUCTION

Acetaminophen, also called paracetamol is a widely used analgesic and antipyretic medication. It is derived from coaltar, it is the active metabolite of phenacetin, but unlike phenacetin, paracetamol has not been shown to be carcinogenic in anyway. Paracetamol is used in the management of more severe pain. (Gormley,1996). It is a drug used to relieve mild headache or muscle and joint pain and to reduce fever. It relieves pain by inhibiting prostaglandin synthesis in the central nervous systems and reduces fever by acting on the temperature regulating centres of the brain. Acetaminophen is commonly liked in multi ingredient preparation for migraine headache with or without caffeine and sometimes, codeine. Acetaminophen is metabolized primarily 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, which is responsible for the toxic effects of acetaminophen due to a minor alkylating metabolite (N-acetyl-P-benzo-quinoneimine). (Borne,1995). The metabolism of acetaminophen is an excellent example of toxication, because the metabolite NAPQI is primarily responsible for toxicity rather than acetaminophen itself (Dong *et al*., 2000).

In recommended doses, acetaminophen does not irritate the lining of the stomach, affect blood coagulation as much as NSAIDS, or affect functions of the kidneys. However, some studies have shown that high dose usage (greater than 2,000mg per day) does increase the risk of upper gastrointestinal complications such as stomach bleeding (Sheen *et al*, 20002). Acetaminophen is safe in pregnancy, and does not effect the closure of the fetal ductus arteriosus as NSAIDS can. Unlike aspirin, it is safe in children, as acetaminophen is not associated with the risk of Reyers syndrome in children with viral illnesses. Acetaminophen particularly in combination with weak opiods, is more likely than NSAIDS to cause rebound headache (medication overuse headache), although less of a risk than ergotamine or triptans used for migraines. (Clarson *et al*, 2005). Excessive use of paracetamol can damage multiple organs, especially the liver and kidney. In both organs, toxicity from paracetamol is not from the drug itself but from one of its metabolites, N-acetyl-P-benzo quinoneimine (NAPQ 1). In the liver, the cytochrome P450 enzymes CYP2EI and CYP3A4 are primarily responsible for the conversion of paracetamol to NAPQI. In the kidneys; cyclooxygenases are the principal route by which paracetamol is converted to NAPQ1 (Borne *et al*., 1995). Paracetamol overdose leads to the accumulation of NAPQ 1, which undergoes conjugation with glutathione. Conjugation depletes glutathione, a natural antioxidant. This in combination with direct cellular injury by NAPQ 1 leads to all damages and death. These injuries are known as paracetamol hepatotoxicity and analgesic nephropathy in the liver and kidney, respectively (Borne, 1995).

Vernonia amygdalina belongs to the plant family compositae. In Nigeria, the Edo people call it Oriwo, Hausa; Chusar doki (a horse tonic food containing the leaves), fate fale/mayemaye (a food prepared from the leaves). Ibibio, etidot, Igbo, anugbu, Tiva Hyuna and Yoruba, ewuro (Ijeh *et al*, 1996). *Vernonia amygdalina* is a shrub or small tree of 2-5m with petiolate leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristics odour and a bitter taste. No seeds are produced and trees have therefore to be distributed through cutting. It grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant. (Bonsi. *et al*, 1995). There are about 200 species of *vernonia amygdalina*. The leaves of *vernonia amygdalina* are very bitter. Bitterness can be due to antinutritional factors such as alkaloids, saponins, tannins and glycosides (Buttler and Bailey, 1973). The leaf is used to make soup after it is washed thoroughly to remove the bitter taste. It can be used to reduce fever, in making beer and also as a local medicine against leech that transmit Bilharzias. It is sometimes applied to wound surfaces to stop the bleeding and is also used in the management of diabetes (Ijhegbu and Ogbuechi, 2004). *Vernonia amygdalina* is a multipurpose shrub as it plays a number of different roles in the treatment of diseases like stomach pains, kidney disorder and hiccups. The leaf also contains saponins, which can create some health hazards such as reduction in red blood cells and white blood cell count (Ogirima *et al*, 2003), it was also discovered that chewing of the bitters pith of the plant by very sick chimpanzees helped to kill intestinal worms (Good all *et al*, 2002). Acetaminophen used for the relief of fever, headaches, other minor aches and pains can also in overdose cause potentially fatal liver damage and in rare individuals a normal dose can do same. (Larson *et al*, 2005).

Vernonia amygdalina which is used in Nigerian folk medicine as a tonic and remedy against constipation, fever, high blood pressure and many infectious diseases has been evaluated to have hepatoprotective effects (Bonsi *et al*, 1995). The study therefore sought to identify which of the fractions of *vernonia amygdalina* exerts the hepatoprotective effect.

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 socked 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.

Animals

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 factions of *vernonia amygdalina*. The 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 which, the rats were fasted over-night i.e twelve hours after last administration, the animals were anesthetized under chloroform vapour, and then dissected and the whole blood was collected from the heart by cardiac puncture using sterile needle. The blood was put into properly labeled plain samples tubes. Sera were obtained from the clotted blood in the plain sample tubes by allowing standing for 2 hours at room temperature to clot before it was centrifuged at 700rpm for 15minutes in a 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.

BIOCHEMICAL ASSAYS:

All Biochemical assays for Urea, billirubin and uric acid were carried out using standard methods.

Uric acid concentration in the serum was estimated by the method of Henry *et al*, (1964), Urea activity is serum was estimated by the calorimetric, endpoint; increasing reaction of Searcy *et al*, 1967, total and conjugated bilirubin in serum was estimated by the method of Jendrassik and Grofmethod.

Statistical Analysis

Data obtained was expressed as Mean ± Standard Deviation and analyzed using the Analysis of Variance 'ANOVA; f-ratio' (Welkowitz 1976) and student 't' test where applicable. Values at $P < 0.05$ were considered significant.

RESULTS

The effect of treatment with activity directed extracts of *vernonia amygdalina* during acetaminophen induced hepatotoxicity on the levels of uric acid, urea, total and conjugated bilirubin was investigated in this research. From table 1, there were significant decreases ($p<0.05$) in all treatment groups except the benzene fraction treated group (1.57± 0.02) compared to the normal control group (1.37± 0.04). Also significant increases ($p<0.05$) were recorded in uric acid levels (mg/dl) in groups treated with fractions of benzene (1.37± 0.02), butanol (0.47± 0.04), methanol (0.33± 0.06) and residue E (0.73± 0.30) compared to the paracetamol group (0.17± 0.01). The ethyl acetate fraction treated group however had a significantly lowered ($p<0.05$) uric acid levels. (0.14± 0.01) compared to the paracetamol group. From the result below there were significant increases ($p<0.05$) in groups treated with fractions of chloroform (4.37± 0.38) and residue E (4.00± 0.67) compared to the normal control (3.73± 0.36), as well as a significant decrease ($p<0.05$) in the butanol fraction treated group (3.13.015±) compared to the normal control group (3.73±0.36). The benzene, ethyl acetate and methanol fraction treated groups however had non-significantly lower

(p>0.05) urea levels compared to the normal control. Also, the benzene, ethyl acetate, butanol and methanol fraction treated groups had non significantly lower (p>0.05) urea levels compared to the paracetamol group (3.68± 0.56). The chloroform and residue E fraction treated groups however, had non-significantly higher urea levels compared to the paracetamol group. From table 1, there were significantly higher (p<0.05) level of total bilirubin in fractions treated with benzene, chloroform, ethyl acetate, butanol and residue E as well as the paracetamol group (4.60± 0.44) compared to the normal control group (3.30± 0.70). There were also significant increases (p<0.05) in groups treated with the fractions of benzene (5.77± 0.44), chloroform (6.40± 0.95) and butanol (7.00 ± 0.10) compared to the paracetamol group (4.60 ± 0.44). From table 1 below, the paracetamol group (2.93± 1.19) had a non-significantly higher (p>0.05) level of conjugated bilirubin (2.93± 1.19) compared to the normal control group (2.17 ± 0.64). Also, there were significant increases (p<0.05) in the levels of groups treated with fractions of benzene (4. 60± 0.10), butanol (4.25± 0.4a) as well as non significant increases (p>0.05) in the chloroform (3.60± 1.11) and residue E (3.73 ± 1.50) treated groups compared to the normal control (2.17± 0.64). Non-significant increases (p>0.05) were observed in all treated groups except the ethylacetate fraction (2.70± 0.36) treated group compared to the paracetamol group (2.93± 1.19).

Table 1 Effect of activity directed extracts of *vernonia amygdalina* on uric acid, urea, total and conjugated bilirubin levels during acetaminophen induced hepatotoxicity in wistar albino rats.

Group	Treatment	Uric acid (mg/dl)	Urea(mg/dl)	Total bilirubin(mg/dl)	Conjugated bilirubin (mg/dl)
1	Normal control (water)	1.37± 0.04	3.73± 0.36	3.30 ± 0.70	2.17 ± 0.64
2	Paracetamol control	0.17 ± 0.01[a]	3.68 ± 0.56	4.60 ± 0.44	2.93 ± 0.19
3	Paracetamol/ benzene	1.57 ± 0.02[a,b]	3.23 ± 0.12	5.77 ± 0.44[a,b]	4.60 ± 0.10[a]
4	Paracetamol/ Chloroform	0.17 ± 0.01[a]	4.37 ± 0.38[a]	6.40 ± 0.95[a,b]	3.60 ± 1.11
5	Paracetamol/ Ethyl acetate	0.14 ± 0.01[a,b]	3.25 ± 0.17	4.85 ± 0.41[a]	2.70 ± 0.36
6	Paracetamol/ Butanol	0.47 ± 0.04[a,b]	3.13 ± 0.16[a]	7.00 ± 0.10[a,b]	4.25 ± 0.49[a]
7	Paracetamol/ Methanol	0.33 ± 0.06[a,b]	3.60 ± 0.56	4.45 ± 1.13	3.45 ± 0.67
8	Paracetamol/ Residue E	0.73 ± 0.30[a,b]	4.00 ± 0.67[a]	4.25 ± 0.68[a]	3.73 ± 1.50

Values are expressed as mean ±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 extracts of *vernonia amygdalina* on uric acid, urea and bilirubin levels was examined in this research. There was significant decrease (p<0.05) in uric acid level in the paracetamol group compared to the normal control group as well as a non-significant decrease (p>0.05) in urea levels in the paracetamol group compared to the normal control group. A non-significant increase (p>0.05) in conjugated bilirubin levels in paracetamol group compared to the normal control group was recorded. There was a significant higher (p>0.05) level of total bilirubin in the paracetamol group compared to the normal control group. These increases in bilirubin levels may be due to acetaminophen challenge and is in consonance with the findings of Hattori *et al* (1990) that prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation in serum bilirubin. The significant decrease in uric acid level conforms with reports of Toncev, (2006) who reported that low levels of uric acid in blood are associated with some kinds of liver or kidney diseases due to exposure to toxic compounds. Uric acid level reduced significantly (P<0.05) in the paracetamol group when compared to the normal control group. However after the administration of extract of *vernonia amygdalina*, the level was significantly increased in the benzene fraction treatment group. Increase levels of serum urea are considered for investigating drug induced nephrotoxicity in animals and man (Toncev, 2006). After the administration with the butanol fraction treatment extract of *vernonia amygdalina*, these levels significantly decreased compared to the paracetamol group.

CONCLUSION

Effect of activity directed fractions of *vernonia amygdalina* on the levels of uric acid, urea and bilirubin (total and conjugated) during acetaminophen overdose was carried out. The results show that the non-significantly lowered urea level, significantly higher total bilirubin and non-significantly higher conjugated bilirubin levels observed in the untreated group were normalised during the 14 days of treatment using activities directed fractions of *vernonia amygdalina*.

Conclusively, the *vernonia amygdalina* fractions have the ability to protect against acetaminophen induced liver damage coupled with antioxidative potentials. This suggests that *vernonia amygdalina* is suitable for the treatment of acetaminophen induced hepatotoxicity.

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