Hepatoprotective Activity of Ethanol Extract of Vernonia ambigua Against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats.


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Abstract: This research aimed at evaluating the hepatoprotective activity of the ethanol extract of Vernonia ambigua in CCl₄ induced liver damage in albino rat. A total of 25 wistar albino rats were randomly divided into control and experimental four groups designated as group A-E. Group A serves as the control and were given only distilled water for ten days. Group B, C, D and E were treated with distilled water, silymarin (100mg/kg), 250mg and 500mg/kg of ethanol extract of V. ambigua for nine days. Group B-E also received 3ml/kg of CCl₄ on the 10th day of treatment. After 24 hours, the blood samples were collected through the jugular vein for biochemical analyses. The result showed that albino rats treated with CCl₄ had significant (P<0.05) increase in serum ALT, AST and ALP, total bilirubin, cholesterol and triglycerides levels and significant decrease (P<0.05) in the levels of total proteins and albumin. Pretreatment with ethanol leaf extract of V. ambigua significantly (P<0.05) decreased the liver marker enzymes, bilirubin, total cholesterol and triglycerides. The effect of ethanol leaf extract of Vernonia ambigua (V. ambigua) on CCl₄-treated rats albino rats was observed to be dose-dependent. The present investigation suggests that ethanol leaf extract of V. ambigua has a potent hepatoprotective action against CCl₄-induced liver damage in rats.

Keywords: carbon tetrachloride, hepatoprotective. Vernonia ambigua, silymarin

I. Introduction

The liver is an organ of paramount importance, which plays an essential role in the metabolism of foreign compounds entering the body (Johnston, 1999). Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. Hepatic diseases represent a serious health problem for which modern medicine offers few effective treatments apart from traditional herbal formulations (Nyblom et al., 2006). It is therefore necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

In Nigeria, V. ambigua is used for gastrointestinal disorders, as a general tonic and appetite stimulant, for skin diseases and as a medication for fever, dysentery, malaria, diabetics and constipation (Amole et al., 2006). Plants of the Vernonia genus produce characteristic compounds such as sesquiterpene lactones, with several reported biological activities, such as fungistatic (Mandlekar and Kong, 2001), and cytotoxic activities (Ekpo et al., 2007). Some other compounds have been isolated from Vernonia, including flavonoids, steroids and polysaccharides (Leonard et al., 2002).

Vernonia ambigua has been reported for its use by wild chimpanzee for the treatment of parasite related diseases in Tanzania (Huffman and Seifu, 1999). Philipson et al., (1993) reported the antiplasmodial effects of sesquiterpene and steroidal constituents of V. ambigua and some were also effective against plasmodium falciparum in vitro. Uhegbu and Ogbuehi (2004) reported the antidiabetic effects of aqueous extract of leaves of Vernonia ambigua.

Recently, Izevbigie (2011) isolated some peptides (edotides) from the aqueous extract of V. ambigua. The peptides were shown to be potent inhibitor of nitrogen activated protein kinases (NAPK) which are crucial for breast tumour growth and also represent a key regulatory point for tumour growth. The anti-estrogen breast cancer drug (Tamoxiten) has also been shown to modulate MAPK activity (Atanaskova et al., 2002; Mandlekar and Kong, 2001), this indicates that edotides from V. ambigua may be considered as alternative to tamoxiten.

In this present study, the carbon tetrachloride (CCl₄)-induced hepatotoxicity model was employed to assess the effect ethanol extract of Vernonia ambigua on liver damage. CCl₄, a proven experimental agent for inducing acute liver injury (Reink et al., 2008), is biotransformed by hepatic microsomal cytochrome P₄₅₀ to trichloromethyl-free radical (CCl₃ or CCl₃OO). These metabolites react with antioxidant enzymes such as catalase and superoxide dismutase (SOD) and lead to lipid peroxidation and liver damage.

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II. Material And Methods

Biological Materials
Twenty five (25) albino rats were collected from animal house in the university of Nigeria Nsukka (UNN) Nigeria. Fresh leaves of Vernonia ambigua were collected on November from Onueke, Ezza south L.G.A of Ebonyi State South-East region of Nigeria and were identified by Dr. (Mrs) C. V. Nnamani, taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A part was also deposited in the hebarium for reference purposes.

III. Methods

Extraction Of Plant Material
The leaves were washed and sun-dried before being grinded with blender into powdered form. The 300 g of the powdered leaves was soaked in 1000 ml of ethanol and left for 24 hours. As muslim cloth was then to filter out the liquid part of the Vernonia ambigua paste to get the ethanol extract of Vernonia ambigua leaves. The ethanol was allowed to evaporate to obtain the sticky extract of Vernonia ambigua leaves.

Experimental Design
Twenty five (25) albino rats were randomly separated into five (5) groups: A, B, C, D and E with each group containing five rats. Group A (control) received only water for ten consecutive days; while groups B to E were treated with: distilled water for nine (9) days plus CCl4 (1ml/kg) on the tenth day, vitamin C (100 mg/kg) for nine (9) days plus CCl4 (1ml/kg) on the tenth day, 250 mg/kg and 500 mg/kg of ethanolic leaf extract of Irvingia gabonensis for nine (9) days plus CCl4 (1ml/kg) on the tenth day respectively. The ml's were given in respect to the kg body weight of the rats. Throughout the period of administration, the rats were fed with rat feed bought from livestock feeds LTD, Abakaliki, Nigeria.

Collection of Blood Samples
The blood samples of the animals were collected by cutting the jugular veins. Sterile and plain sample collection bottle was used to collect whole blood. The content of each tube was rocked to mix and thereafter centrifuged at 5000r/min for 10 minutes to separate the serum which was used for the analysis.

Biochemical Assays
Aspartate amino transferase (AST, U/L), alanine amino transferase (ALT, U/L), alkaline phosphates (ALP, U/L), total cholesterol (mg/dL), triacylglycerols (mg/dL) were determined using assay kits supplied by Randox Laboratories Limited, BT29 4QY, United Kingdom.

IV. Statistical Analysis

The basic statistics, means, standard deviation and ranges of the measured parameters were estimated. Data were expressed as mean ±SD of five replicates and were subjected to one way ANOVA followed by Ducan multiple range test to determine significant differences in all parameters using SPSS for windows version 20. Values were considered statistically significant at p< 0.05. (Hinkelman and Kempthorpe, 2012).

V. Results

The result of this study showed that albino rats treated with CCl4 had significant increase in serum ALT, AST and ALP, total bilirubin, cholesterol and triacylglycerols levels, whereas total proteins and albumin levels reduced significantly (P<0.05). Treatment of the rats with doses of ethanol leaves extract of Vernonia ambigua (250 and 500 mg/kg) before administration of CCl4 effectively attenuated the CCl4-induced biochemical alterations in serum marker enzymes, bilirubin, cholesterol, triacylglycerols, total proteins and albumin similar to silymarin (100mg/kg, po) treated groups. The result of this study showed that albino rats treated with CCl4 had significant increase in serum AST level (from 54.50 – 102.35) but when treated with 250 and 500 mg/kg ethanol leaf extract of Vernonia ambigua decreased to 67.50, 70.04 u/i respectively. The results of the effect of ethanol leaf extract of Vernonia ambigua on CCl4-treated albino rats was observed to be dose-independent as shown in (figure 1). The effect of silymarin on CCl4-treated albino rats was also comparable to the control. The serum ALT of the group treated with CCl4 increased from 20.44 (control) – 45.66 U/L. After treatment with silymarin (100mg/kg) and ethanol leaf extract of Vernonia ambigua (250 and 500mg/kg) it decreased to 21.91, 26.75, and 27.00 respectively as shown in (Figure 2). Similarly, the serum ALP activity increased from 39.33 (control) to 57.13. After treatment with the standard drug (silymarin 100mg/kg) and ethanol leaf extract of Vernonia ambigua of different doses (250 and 500mg/kg) it decreased to 43.33, 46.92 and 41.41 respectively as shown in (Figure 3).

The effects of the ethanolic extract of Vernonia ambigua leaves on total cholesterol level and triacylglycerol are presented in figures 4 and 5. There was a dose dependent significant (P<0.05) decrease of
total cholesterol levels in the extract treated groups. The group treated with CCl$_4$ showed significant (P<0.05) increase of cholesterol concentration. There was no significant difference between the control group and the group treated with silymarin.

Figure 1. Effect of ethanol extract of V. ambigua leaves on AST activity of CCl$_4$-induced hepatotoxic albino rat. Data are presented as mean ± SD of the rat. Bars with different letter are significant (P < 0.05).

Figure 2. Effect of ethanol extract of V. ambigua leaves on ALT activity of CCl$_4$-induced hepatotoxic albino rat. Data are presented as mean ± SD of rats. Bars with different letter are significant (P < 0.05).
Figure 3. Effect of ethanol extract of V. ambigua leaves on ALP activity of CCl\(_4\)-induced hepatotoxic albino rat. Data are presented as mean ± SD of 5 rats. Bars with different letter are significant (P < 0.05).

Figure 4. Effect of ethanol extract of V. ambigua leaves on total bilirubin levels of CCl\(_4\)-induced hepatotoxic albino rat. Data are presented as mean ± SD of 5 rats. Bars with different letter are significant (P < 0.05).
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Figure 5. Effect of ethanol extract of V. ambigua leaves on total proteins levels of CCl₄-induced hepatotoxic albino rat. Data are presented as mean ± SD of 5 rats. Bars with different letter are significant (P < 0.05).

Figure 6. Effect of ethanol extract of V. ambigua leaves on albumin levels of CCl₄-induced hepatotoxic albino rat. Data are presented as mean ± SD of 5 rats. Bars with different letter are significant (P < 0.05).
Figure 7: Effect of ethanol extract of Vernonia ambigua on total cholesterol level of the rat. Data are presented as mean ± SD of the rat. Bars with different letter are significant (P < 0.05).

Figure 8: Effect of ethanolic extract of Vernonia ambigua on total triacylglycerol level of the rat. Data are presented as mean ± SD of the rat. Bars with different letter are significant.
VI. Discussion

In developing countries, the indigenous population largely depend on traditional systems of medicine (Leonard et al., 2002; Erasto et al., 2006). Plants have long been used for therapeutic purposes, and many of the currently available drugs are directly or indirectly derived from plants (Akah et al., 2004).

Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into plasma indicates the sign of hepatic tissue damage (Jain et al., 2008; Somasundaram et al., 2010). Generally measurement of ALT, AST and ALP are used as important diagnostic markers to indicate liver injury due to hepatotoxins (Jain et al., 2008).

Carbon tetrachloride (CCl₄) is a common hepatotoxin used in the experimental study of liver diseases (Obi et al., 1998; Ulicina et al., 2003; Yan-Jun et al., 2004). CCl₄ treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. A primary indication of hepatic damage induced by CCl₄ was obtained by the evaluation of hepatic enzymatic markers of injury such as AST, ALT and ALP. The levels of these enzymes, 24 hrs after the administration of CCl₄, were significantly elevated relative to the control group. These enzymes enter the circulatory system due to altered permeability of membranes and their increased levels reflected severe damage to the structural integrity of the liver (Ulicina et al., 2003; Erasto et al., 2007). Administration of V. ambigua significantly attenuated CCl₄-induced elevation of AST and ALT, ALP, total bilirubin, cholesterol and triacylglycerols indicating its hepatoprotective activity (Erasto et al., 2007). Amadi et al., (2010) reported an increase in ALP activity when they studied on the protective ability of Gmelina aboea and explained that the serum levels of transaminases returns to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. In this view, the increased activity of ALP is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage as reported by Thabrew et al., (2009). This proved that the integrity of the liver cells was preserved from leakage as ALT enzymes were reduced in blood stream. These results proved that V. ambigua extracts significantly decreased liver toxicity due to its nontoxic nature and tissue protective nature against various toxic metabolites (Manna et al., 2006).

Fadhel and Amran, (2002) reported that the leaves of V. ambigua contain saponins, sesquiterpenes, lactone, steroid glycosides, alkaloids, tannins and flavonoids. Flavonoids are reported to exhibit antioxidant activity (Ramathan et al., 1989) and are effective scavengers of superoxide anions (Robak and Gygkewski, 1988). The ethanol leaf extract of V. ambigua may have exhibited hepatoprotective activity due to its antioxidant property attributable to flavonoids (Fadhel and Amran, 2002).

V. ambigua extracts have strong antioxidant properties due to the various bioactive constituents found in different solvent extracts (Yineger et al., 2009). These antioxidant bioactive compounds effectively prevent liver damage from hepatotoxin-induced toxicity.

VII. Conclusion

The results of this study showed that ethanolic leaf extract of V. ambigua might have a potent hepatoprotective action similar to that produced by silymarin, a well known hepatoprotective agents.

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References


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