

## 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  $\text{CCl}_4$  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  $\text{CCl}_4$  on the 10<sup>th</sup> 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  $\text{CCl}_4$  had significant ( $P < 0.05$ ) increase in serum ALT, AST and ALP, total bilirubin, cholesterol and triacylglycerols 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 triacylglycerols. The effect of ethanol leaf extract of *Vernonia ambigua* (*V. ambigua*) on  $\text{CCl}_4$ -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  $\text{CCl}_4$ -induced liver damage in rats.

**Keywords:** carbon tetrachloride, hepatoprotective. *Vernonia ambigua*, silymarin

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### 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 antiparasmodial 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 ( $\text{CCl}_4$ )-induced hepatotoxicity model was employed to assess the effect ethanol extract of *Vernonia ambigua* on liver damage.  $\text{CCl}_4$ , a proven experimental agent for inducing acute liver injury (Reink et al., 2008), is biotransformed by hepatic microsomal cytochrome P<sub>450</sub> to trichloromethyl-free radical ( $\text{CCl}_3$  or  $\text{CCl}_3\text{OO}$ ). These metabolites react with antioxidant enzymes such as catalase and superoxide dismutase (SOD) and lead to lipid peroxidation and liver damage

## 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 herbarium 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  $\text{CCl}_4$  (1ml/kg) on the tenth day, vitamin C (100 mg/kg) for nine (9) days plus  $\text{CCl}_4$  (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  $\text{CCl}_4$  (1ml/kg) on the tenth day respectively. The mls 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  $\pm$ SD of five replicates and were subjected to one way ANOVA followed by Duncan multiple range test to determine significant differences in all parameters using SPSS for windows version 20. Values were considered statistically significant at  $p < 0.05$ . (Hinkelmann and Kempthorne, 2012).

## V. Results

The result of this study showed that albino rats treated with  $\text{CCl}_4$  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  $\text{CCl}_4$  effectively attenuated the  $\text{CCl}_4$ -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  $\text{CCl}_4$  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  $\text{CCl}_4$ -treated albino rats was observed to be dose-independent as shown in (figure 1). The effect of silymarin on  $\text{CCl}_4$ -treated albino rats was also comparable to the control. The serum ALT of the group treated with  $\text{CCl}_4$  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<sub>4</sub> showed significant (P<0.05) increase of cholesterol concentration. There was no significant difference between the control group and the group treated with silymarin.

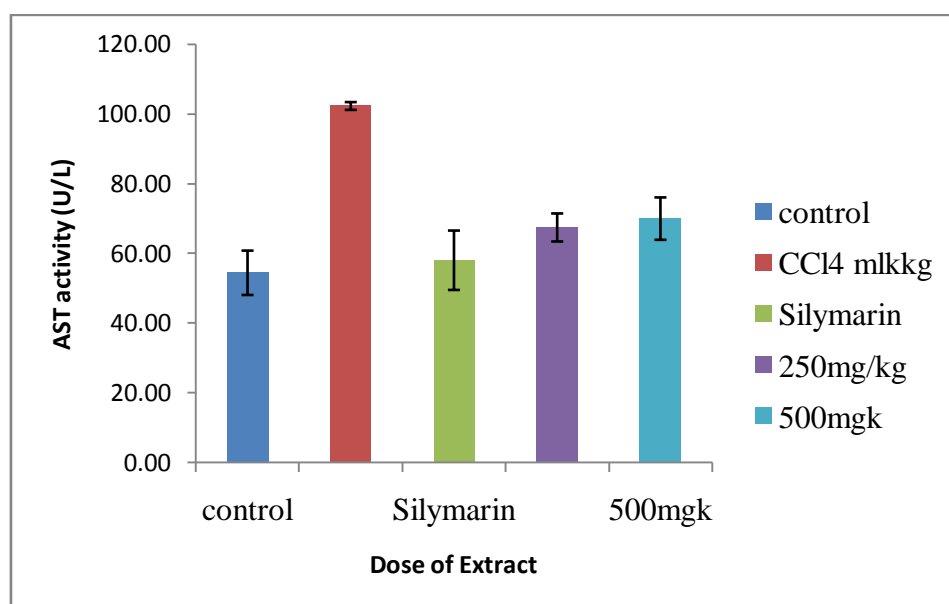


Figure 1. Effect of ethanol extract of *V. ambigua* leaves on AST activity of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of the rat. Bars with different letter are significant (P < 0.05).

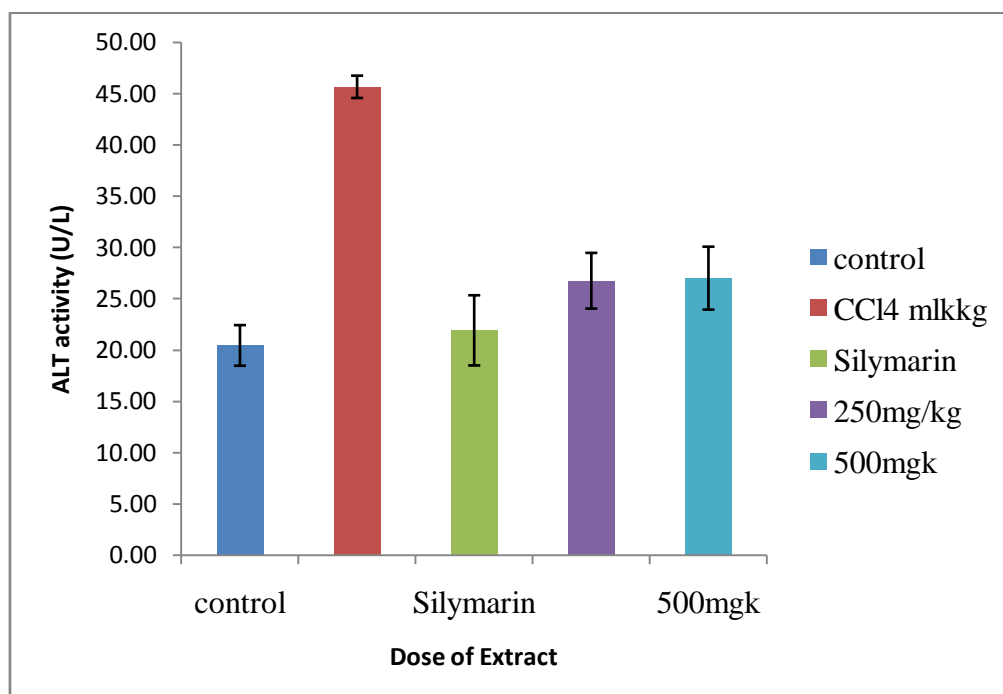


Figure 2. Effect of ethanol extract of *V. ambigua* leaves on ALT activity of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of rats. Bars with different letter are significant (P < 0.05).

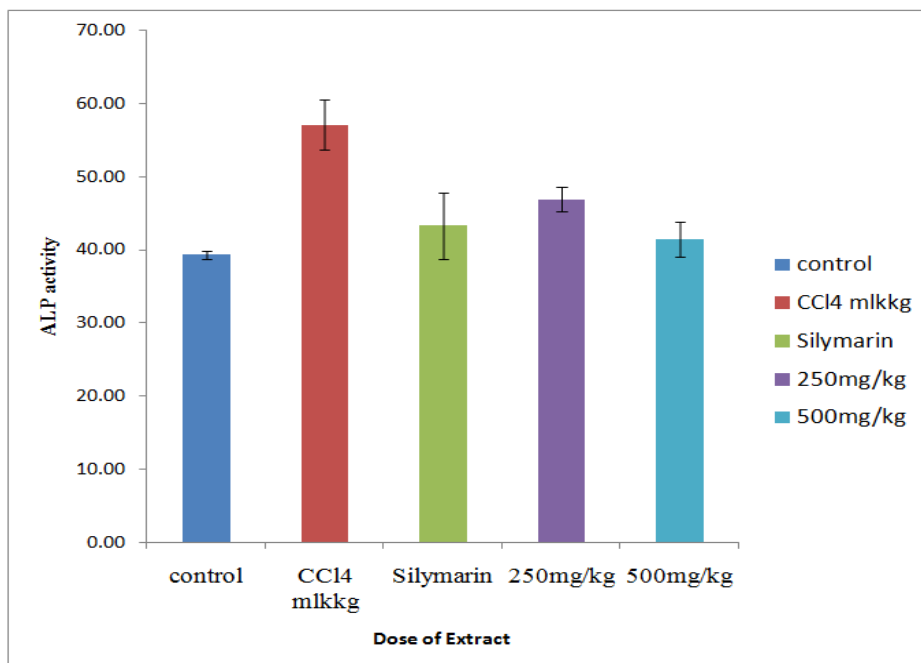


Figure 3. Effect of ethanol extract of *V. ambigua* leaves on ALP activity of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of 5 rats. Bars with different letter are significant ( $P < 0.05$ ).

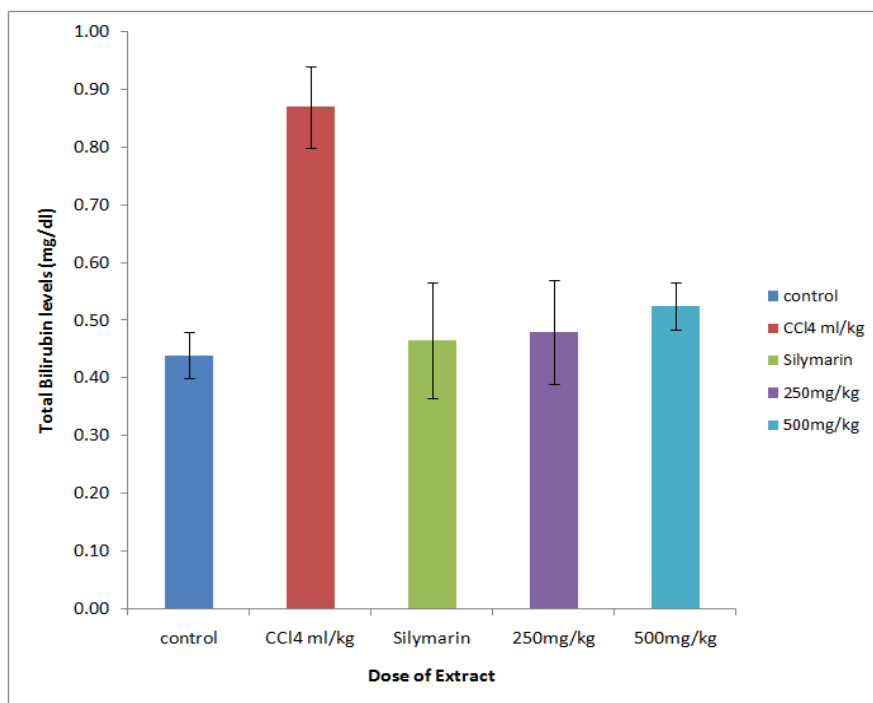


Figure 4. Effect of ethanol extract of *V. ambigua* leaves on total bilirubin levels of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of 5 rats. Bars with different letter are significant ( $P < 0.05$ ).

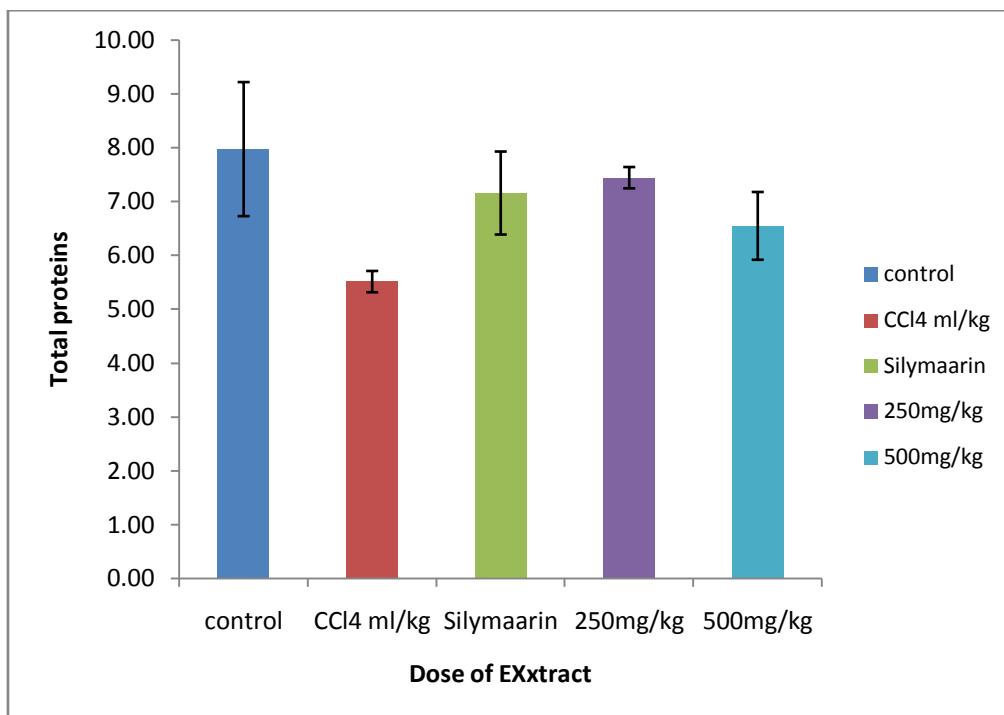


Figure 5. Effect of ethanol extract of *V. ambigua* leaves on total proteins levels of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of 5 rats. Bars with different letter are significant ( $P < 0.05$ ).

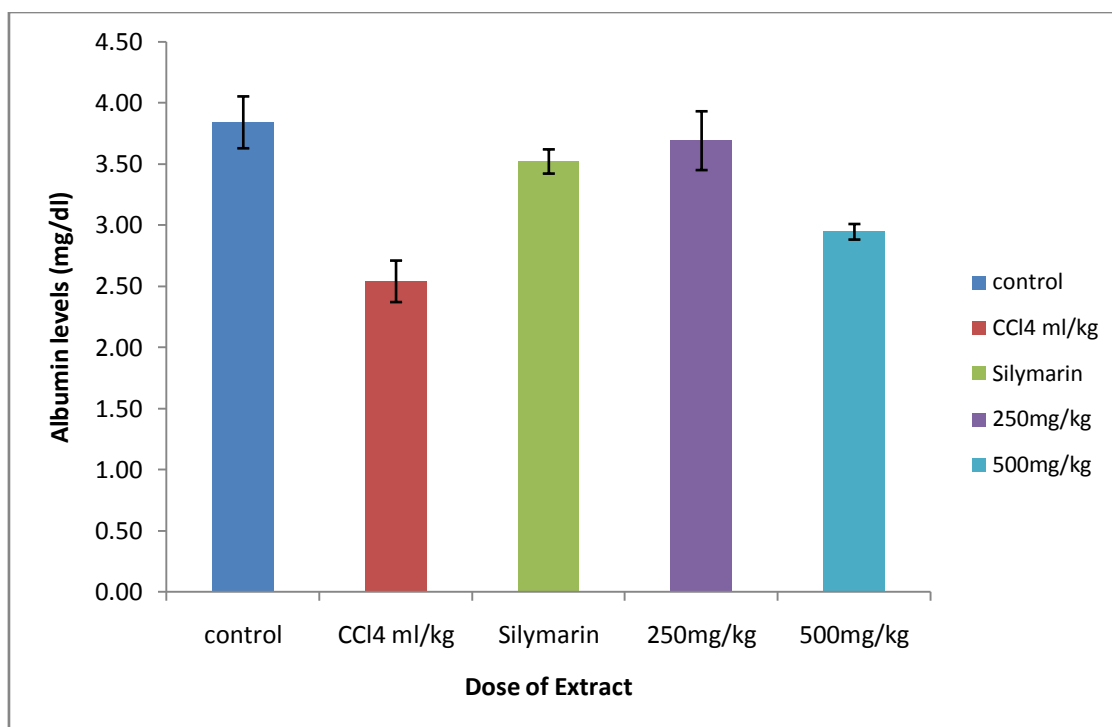


Figure 6. Effect of ethanol extract of *V. ambigua* leaves on albumin levels of CCl<sub>4</sub>-induced hepatotoxic albino rat. Data are presented as mean  $\pm$  SD of 5 rats. Bars with different letter are significant ( $P < 0.05$ ).

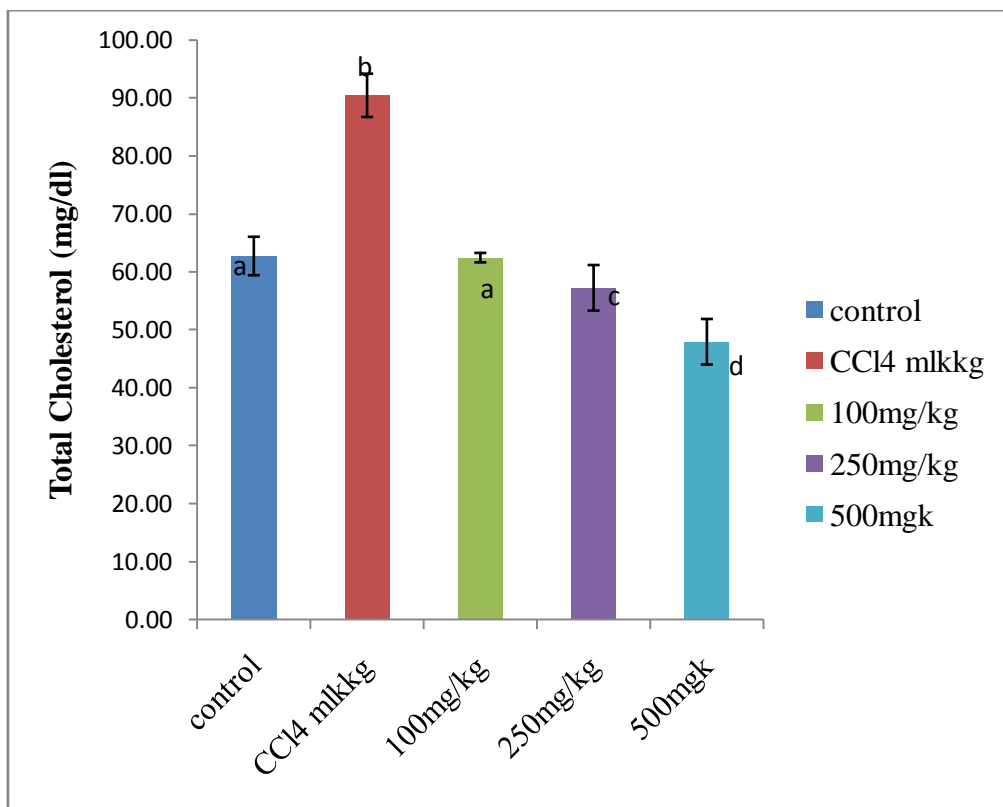


Figure 7: Effect of ethanol extract of Vernonia ambigua on total cholesterol level of the rat. Data are presented as mean  $\pm$  SD of the rat. Bars with different letter are significant ( $P < 0.05$ ).

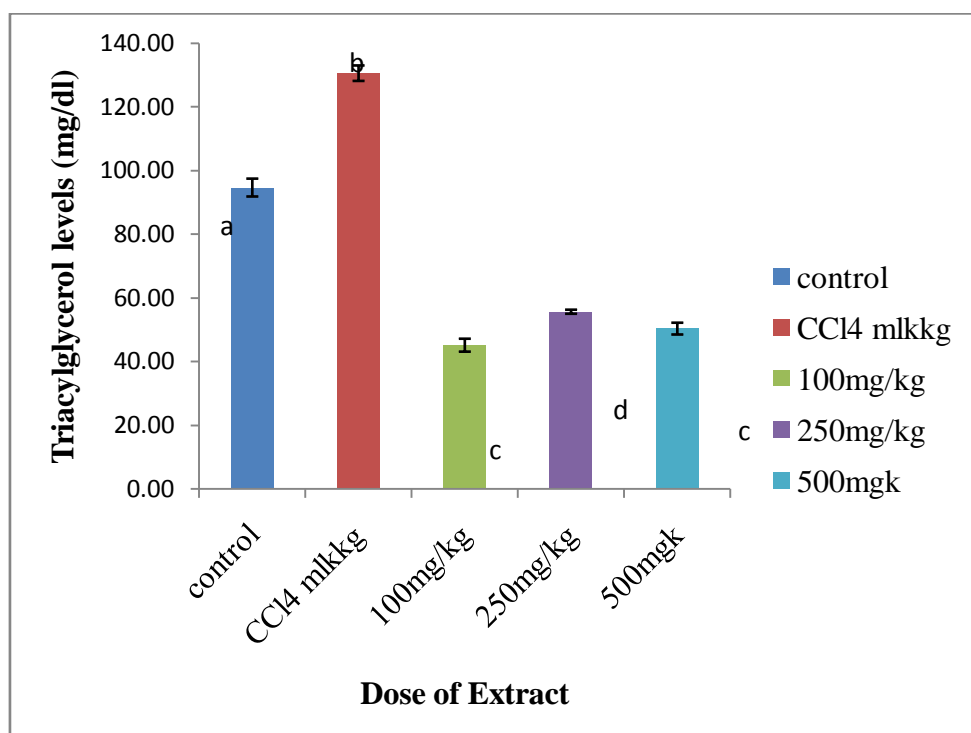


Figure 8: Effect of ethanolic extract of Vernonia ambigua on total triacylglycerol level of the rat. Data are presented as mean  $\pm$  SD of the rat. Bars with different letter are significant.

## 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 (CCl<sub>4</sub>) 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). CCl<sub>4</sub> treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. A primary indication of hepatic damage induced by CCl<sub>4</sub> 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 CCl<sub>4</sub>, 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 CCl<sub>4</sub> -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 (Ramanathan et al., 1989) and are effective scavengers of superoxide anions (Robak and Gygkwski, 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 ethanol 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

- [1]. Akah, P., Njoku, O., Nwanguma, A., and Akunyili, D. (2004). Effect of aqueous leaf extract of *Vernonia amygdalina* on blood glucose and triglyceride levels of alloxan-induced diabetic rats (*Rattus rattus*). *Animal Research International*, **1**: 90-94.
- [2]. Alawa, I., Adamu, M., Gefu, O., Ajanusi, J., Abdu, A., Chiezey, P., Alawa, N. and Bowman, D. (2003). In vitro screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. *Parasitology*, **113**: 73-81.
- [3]. Amole, O., Izegebu, C., Onakoya, A. and Dada, O. (2006). Toxicity studies of the aqueous extract of *Vernonia amygdalina*. *Biomedical Research*, **17**: 39-40.
- [4]. Ekpo, A., Eseyin, A., Ikpeme, O., and Edoho, J. (2007). Studies on some biochemical effects of *Vernonia amygdalina* in rats. *Journal of Biochemistry*, **2**: 193-197.
- [5]. Erasto, P., Grierson, S., and Afolayan, J. (2006). Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *Journal of Ethnopharmacology*, **106**: 117-120.
- [6]. Fadhel, A., and Amran, S. (2002). Effects of black tea extract on carbon tetrachloride induced lipid peroxidation in liver, kidneys, and testes of rats. *Phytotherapy Research*, **16**: S28-S32.
- [7]. Huffman, M.A., Seifu, M. (2012) Observations of the illness and composition of a medicinal plant *vernonia ambigua* by wild chimpanzee in the mohale mountains, Tanzania primates. *Biology* **13**(4): 214-219.
- [8]. Izerbigbe, E.B. (2003). Discovery of water soluble anticancer agents (edo tides) from a vegetable found in Benin city, Nigeria. *Exp. Biol. Med* **228**: 293-298.
- [9]. Jain, A., Soni, M., Deb, L., Rout, P., Gupta, B., and Krishna, L. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. *Journal of Ethnopharmacology* **115**: 61-66.
- [10]. Leonard, S., Karen, L., Bruce, B., Thomas, K., and Jay, H. (2002). Complementary and alternative medicine in chronic liver disease. *Hepatology*, **34**: 595-603.

- [11]. Mandlekar, S. and Kong A.N. (2001). Mechanisms of famoxiten Apoptoses Apoptosis 6:469-477.
- [12]. Manna, P., Sinha, M., and Sil, C. (2006). Aqueous extract of Terminalia arjuna prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complement Alternative Medicine, **6**:33.
- [13]. Nyblom, H., Björnsson, E., Simrén, M., Aldenborg, F., Almer, S., and Olsson, R. (2006). The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. Liver Int. **26** (7): 840-845.
- [14]. Obi, F.O. Usenu, I.A. and Osayande, J.O. (1998): Prevention of CCl<sub>4</sub>-induced hepatotoxicity in the rat by H. rosasinensis anthocyanin extracts administered in ethanol. Toxicology, **131**:93-98.
- [15]. Ramanathan, R. Luu K.K. and Das, N.P. (2002): Anti peroxidative action of flavonoids and related products in ground port (Abstracts) Proceedings of III Int. Symp. On flavonoids in Biology and Medicine Singapore: pp. 56.
- [16]. Rec. GSCC (1999): Optimised Standard Colorimetric Methods. Journal of Clinical chemistry and clinical biochemistry **10**:182.
- [17]. Reink, L.A., Lai, E.K., McCay, P.B., (2008). Ethanol feeding stimulates trichloronethyl radical formation from carbon tetrachloride in liver. Xenobiotica **18**:1311-1318.
- [18]. Reitman, S. and Frankel, A.S. (2007). A colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases American Journal of Clinical Pathology, **28**:53-63.
- [19]. Robark, K. and Gryglewski, R.J. (2008). Flavonoid are scavengers of superoxide anions. Biochem. Pharmacology, **37**:837-841.
- [20]. Somasundaram, A., Karthikeyan, R., Velmurugan, V., Dhandapani, B., Raja, M. (2010). Evaluation of hepatoprotective activity of Kyllinga nemoralis (Hutch & Dalz) rhizomes. Journal of Ethnopharmacology, 127:555-557.
- [21]. Uhegbu, O. and Ogbuehi, J. (2004). Effect of aqueous extract (crude) of leaves of vernonia ambigua (Del) on blood glucose, serum albumin and cholesterol levels in diabetic albino rats. Global Journal of Pure and Applied sciences **10** (1):189-194.
- [22]. Ulicna, O. Greksak, M., Vancova, O., Zlatos, L., Galbavy, S., Bozek, P., Nakamo, M. (2003): Hepatoprotective effect of Rooibos Tea (Aspalathus linearig) on CCl<sub>4</sub>-induced liver damage in rats. Physiology Research **52**:461-466.
- [23]. Yan-Jun, L., Jie ping, Y., Zhao-Hong, J. and Wang, L. (2004): Ginkgo Biloba extract reverses CCl<sub>4</sub>-induced liver fibrosis in rats. World Journal of Gastroenterology **10**:1037-1042.
- [24]. Yiniger, H., Kelbessa, E., Bekele, T., and Lulekal, E. (2007). Ethnoveterinary medicinal plants at Bale Mountains National Park, Ethiopia. Journal of Ethnopharmacology, **112**: 55-70.