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Modulatory Effects of *Bryophyllum Pinnatum* Leaves Extract on Peroxidation Indices of CCl_4 Induced-Hepatotoxicity in Wistar Albino Rats

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Abstract: This research investigated the modulatory effect of methanol extract of *Bryophyllum pinnatum* leaves on peroxidation indices of CCl_4-induced hepatotoxicity. Thirty rats of average weight 150g divided into six groups of 5 rats each were used for the study. Group one (control) received water and paraffin for ten days. Group two (treatment control) received water only, group 3 (standard control), received a 100 mg/kg b.w of ascorbic acid, groups 4, 5 and 6 received 200, 400 and 600 mg/kg b.w of *Bryophyllum pinnatum* extract, respectively. Hepatotoxicity was induced by CCl_4 administration in groups 2 to 6 on the 7^{th} and 8^{th} days. There was significant increase in the level of high density lipoprotein (HDL) and non-significant decreases in total cholesterol and triacylglycerol in the extract treated groups (4-6) compared to group two (CCl_4 untreated). There was a significant increase in the level of low density lipoprotein (LDL) in the extract treated groups (4-6) compared to group one. Animals in groups 4-6 showed a significant decrease in MDA concentration compared to that of group one. Report from this study shows that *Bryophyllum pinnatum* extract has modulatory effect on the lipid profile of Wistar rats intoxicated with CCL_4.

Keywords: *Bryophyllum Pinnatum*, Peroxidation Indices, Hepatotoxicity, Modulatory Effects, Carbon Tetrachloride

Introduction

The liver is a large organ in vertebrate internal anatomy responsible for detoxifying the poisonous substances within the body by remodeling and removing cyanogenic wastes (Sulekha *et al*., 2016). The liver serves varied functions; the foremost of which is metabolism (Ngobidi *et al*., 2016). Some chemicals perceived as toxic can cause injuries to the liver (Gurusamy *et al*., 2010). Exposure to varied environmental pollutants can cause cellular damages via metabolic activation of such reactive substances (Kang and Koppula, 2014). Carbon Tetrachloride (CCl_4) is a well studied hepatic-toxin, whose exposure causes oxidative stress and liver injury via formation of free radicals (Gnanaprakash *et al*., 2010). Studies have shown that CCl_4 is bio-transformed by the Cyt p450 system to trichloromethyl radical. This radical reacts with oxygen to form a trichloromethyl peroxyl radical that can attack lipids on the membrane of endoplasmic reticulum, resulting in lipid peroxidation (Sulekha *et al*., 2016). Plant-derived products such as flavonoids, terpenoids and steroids have been reported to possess hepatoprotective activities. Medicinal herbs are vital sources of pharmaceutical drugs and recent researches have shown increasing interest in phyto-drugs (Ngobidi *et al*., 2016). *Bryophyllum pinnatum* (Crassulaceae) commonly known within the Eastern part of Nigeria as oda opue (Sulekha *et al*., 2016) is a crassulacean herb that grows up to one metre tall, with hairless leaves. It grows mainly in the rain forest regions (Sampathkumar *et al*., 2011). The leaves and bark of *B. pinnatum* are bitter to taste, astringent, analgesic and carminative. They are used ethno-pharmacologically for the treatment of diarrhoea, vomiting, ear ache, burns, abscesses, internal organ ulcers and insect bites (Sule *et al*., 2016). The plant is accepted as seasoning remedy in some parts of the world (Ghasi *et al*., 2011). The juices from the leaves are used for the treatment of small pox, otitis,

cough, asthma, palpitations, headache, convulsion and general unfitness. The plant has been used for treating oedema in different parts of the body (Sule *et al*., 2016). The leaf juice is also used for the treatment of coughs, cartilaginous tube infections, blood infectious diseases, jaundice and arthritis (Muhammad *et al*., 2012). This research was aimed at determining the effect of the methanol extract of *B. pinnatum* against (CCl_4)-induced liver injury in wistar rats.

Materials and Methods

Plant Materials

Bryophyllum pinnatum leaves were collected from the envrons of University of Nigeria, Nsukka in Enugu-Nigeria. They were identified by Mr. Alfred Ozioko of the Department of Botany, University of Nigeria, Nsukka.

Extraction

The leaves of *Bryophyllum pinnatum* were shade dried for one week and pulverized. The pulverized plant (2000 g) was soaked for 24 h in 2.5 litres of methanol, filtered and concentrated with a rotary evaporator.

Experimental Animals

Thirty adult Wistar rats of average weight (120g) were purchased from the Veterinary breeding centre, Department of Veterinary medicine, University of Nigeria, Nsukka. The rats were housed in metal cages and sustained at light and dark cycle at 28°C in a well ventilated animal house. They were acclimatized for seven days and fed with normal pelleted diet and water.

Induction of Hepatic-Toxicity in Rats

Hepatic injury was induced in the rats via intra-peritoneal injection of 1.5 ml/kg b.w CCl_4 in liquid paraffin (1:1 v/v). The experiment lasted for ten days. CCl_4 (1.5 ml/kg b.w) was injected on the seventh and eighth day.

Experimental Design

Group one rats (vehicle control), received distilled water for 10 days, however, on the seventh and eighth days, they were administered with paraffin orally.

Groups two (treatment control), and three (standard control) received distilled water and ascorbic acid respectively for ten days. On the seventh and eighth days, they were challenged with 1.5 ml/kg b.w CCl_4 in liquid paraffin.

Groups 4, 5 and 6 received 200, 400 and 600 mg/kg b w of the extract respectively, for ten days. On the seventh and eighth day, they were injected with 1.5 ml/kg b.w CCl_4 dissolved in liquid paraffin and were sacrificed on the tenth day, after eighteen hour fasting.

Serum Sample Collection

The rats were sacrificed via cervical decapitation in mild chloroform. Blood was collected and serum prepared for biochemical assays.

Estimation of Biochemical Parameters

Randox analytical kit was used to assay for total cholesterol according to the method of Paglia and Valentine (1967), low density lipoprotein, high density lipoprotein and triacylglycerol were by Allain *et al*. (1976) while lipid peroxidation (MDA) was by Wallin *et al*. (1993).

Statistical Analysis

Data obtained were expressed as mean + SD and were analyzed using one way ANOVA. Differences between means were expressed using Duncan's new multiple ranges. The acceptance level was $p<0.05$ degree of confidence.

Results

Acute Toxicity Test

In the acute toxicity (LD_{50}) test of *Bryophyllum pinnatum* extract, a significant toxicity was observed at 5000 mg/kg where a mouse died after 24 hrs.

Effect of Methanol Extract of Bryophyllum Pinnatum Leaves on High Density lipoprotein of control and CCl_4 treated Rats

Result in Fig. 1 showed significant ($p>0.05$) reduction in High Density Lipoprotein (HDL) of group 2 rats administered with CCl_4 only compared to the normal control (group one). A significant increase ($p<0.05$) in HDL comparable to that of group one, was observed in animals in groups 4 to 6 treated with the extract when compared to those in group 2.

Effect of Methanol Extract of Bryophyllum Pinnatum Leaves on Cholesterol Level of Control and CCl_4 Treated Rats

Results in Fig. 2 showed significant increase ($p<0.05$) in the total cholesterol level in group 2 administered with CCl_4 only and groups 4 to 6 treated with the extract after CCl_4 administration when compared to group one. Significant decrease ($p<0.05$) in cholesterol level compared to that of group one was observed in the group 3 animals treated Vitamin C after CCl_4 administration.

Effect of Methanol Extract of Bryophyllum Pinnatum Leaves on Low Density lipoprotein of Control and CCl_4 Treated Rats

There was a significant increase ($p<0.05$) in the level of Low Density Lipoprotein (LDL) in group 2 (CCl_4

untreated) and in groups 4 to 6 treated with 200, 400 and 600 mg/kg of the extract after CCl_4 administration when compared to the control (group one) animals (Fig. 3). Group 3 animals treated with vitamin C showed significant decrease ($p<0.05$) in LDL compared to group 2. LDL level of group 3 animals was comparable to that obtained for the normal control (group one).

Effect of Methanol Extract of Bryophyllum Pinnatum Leaves on Triacylglycerol level of Control and CCl_4 Treated Rats

Significant increase ($p<0.05$) in the level of triacylglycerol (TAG) was observed in groups 2 to 6 administered with CCl_4 compared to that of group one (normal control) (Fig. 4).

Effect of Methanol Extract of Bryophyllum Pinnatum Leaves on Malondialdehyde (MDA) of Control and CCl_4 Treated Rats

Result in Fig. 5 showed significant ($p<0.05$) increase in the level of malondialdehyde (MDA) (lipid peroxidation product) in the CCl_4 untreated (group 2) compared to the normal control (group one). Groups 3, 4, 5 and 6 treated vitamin C and graded doses (200, 400 and 600 mg/kg) of the extract respectively, showed significant ($p<0.05$) decrease in MDA concentration compared to group 2.

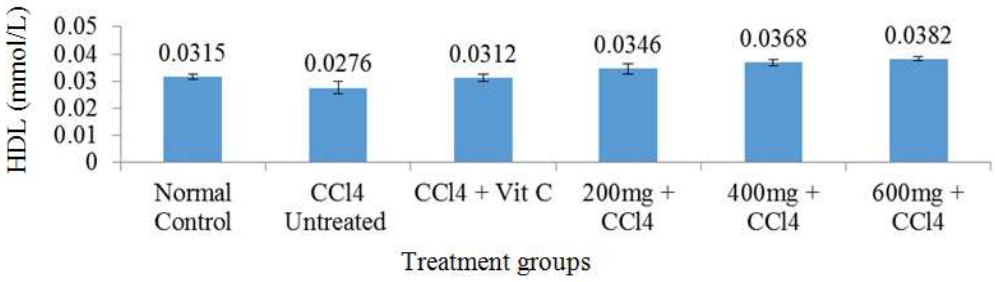

Fig. 1: Effect of methanol extract of *Bryophyllum pinnatum* on high density lipoprotein of normal and CCl_4-treated rats

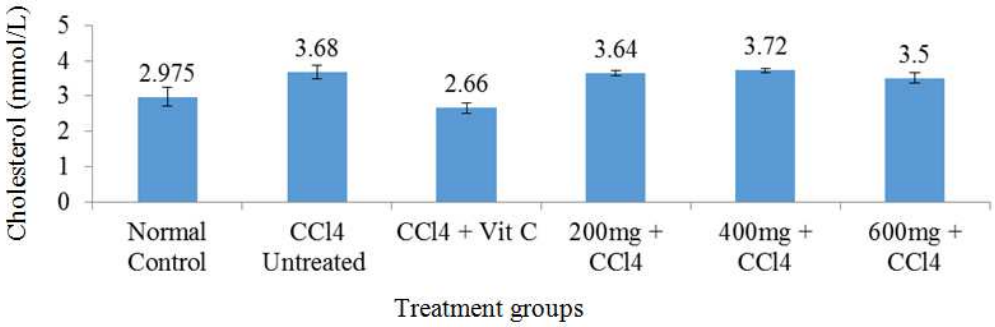

Fig. 2: Effect of methanol extract of *Bryophyllum pinnatum* on cholesterol level of normal and CCl_4-treated rats

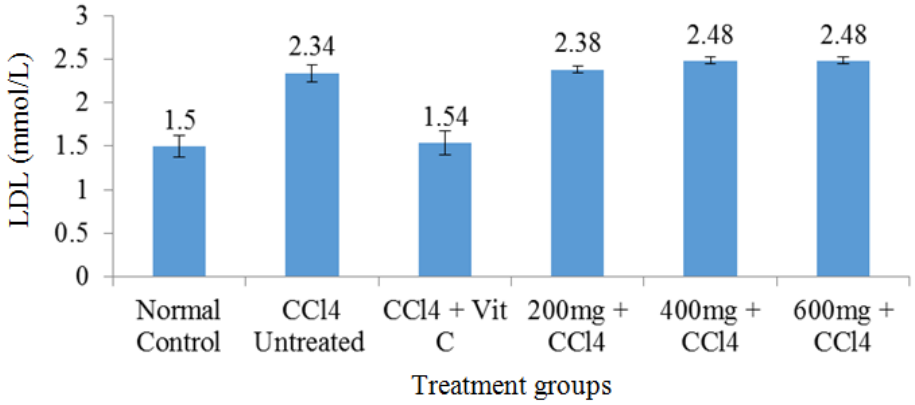

Fig. 3: Effect of methanol extract of *Bryophyllum pinnatum* on low density lipoprotein of normal and CCl_4-treated rats

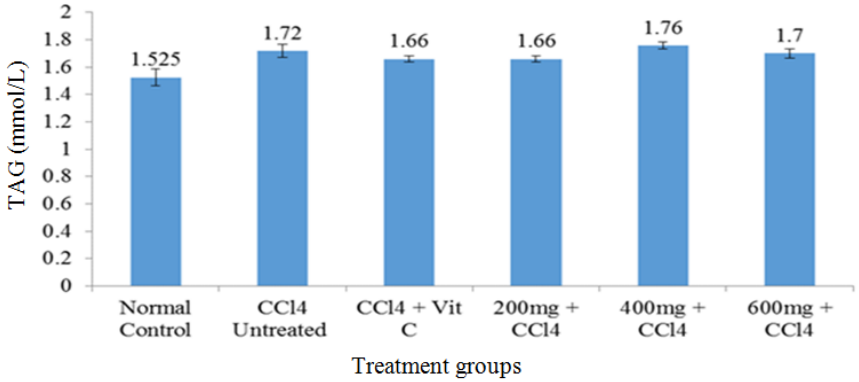

Fig. 4: Effect of methanol extract of *Bryophyllum pinnatum* on triacylglycerol level of normal and CCl_4-treated rats

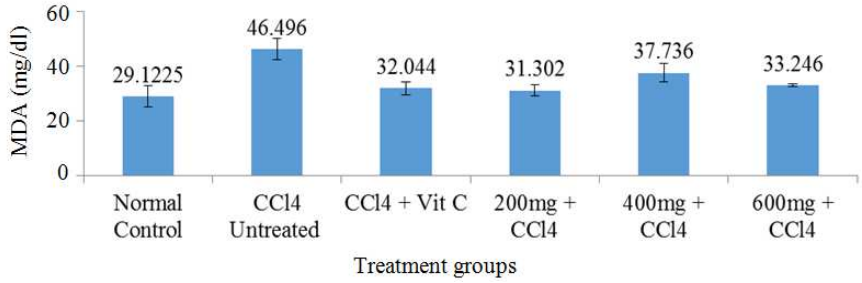

Fig. 5: Effect of methanol extract of *Bryophyllum pinnatum* on malondialdehyde concentration of normal and CCl_4-treated rats

Discussion

Exposure to Carbon tetrachloride has been reported to cause injury in tissues such as liver, heart, lung, testis and brain (Ahmed *et al*., 1987). The acute toxicity of CCl_4 in the liver has been investigated by oral administration of CCl_4 (Hanan, 2016). Carbon tetrachloride is lipophilic and diffuses easily across the cell membrane into the tissues (Ritesh *et al*., 2015). In CCl_4-administered rats, injury to the endoplasmic reticulum (ER) and lipid accumulation occurs within the hepatic cells (Joen *et al*., 2003). Boll *et al*. (2001) reported that increase in the esterification of free fatty acids, triglycerides and phospholipids occurred due to induction of CCl_4, thus causing a disturbance in the normal lipid homeostasis and consequent increase in cholesterol biosynthesis.

Results from this research showed significant increase in triacylglycerol, total cholesterol and LDL levels and a decrease in HDL level in the groups administered with CCl_4 when compared to the control group. These findings were in agreement with that of Hanan, (2016) who reported hypercholesterolemia in CCl_4-administered rats. This condition was due to injury of the hepatic parenchyma cells that led to the disturbance of total lipid metabolism in the liver (Raju and Binda 2005). The elevated levels of triacylglycerol, total cholesterol and LDL induced by the administration of CCl_4 were ameliorated by pre-treatment with *B. pinnatum* extract. The plant is known to be a rich source of lipids, which accounts for the high level of total cholesterol, TAG, and LDL in the extract-treated groups compared to the normal control. *Bryophylum pinnatum* extract also caused increase in the level of high-density lipoprotein which carries cholesterol in the blood and transports it back to the liver. This eliminates cholesterol from the body and reduces the level of LDL, and consequently, reduces the incidence of atherosclerosis and cardiovascular diseases caused by LDL oxidation.

Carbon tetrachloride is metabolized in the liver by cytochrome P450, thus generating the highly reactive free radical trichloromethyl (CCl_3). This product initiates the progression of lipid peroxidation of endoplasmic reticulum cell membrane, thus leading to the formation of Malondialdehyde (MDA); a harmful substance that causes injury to many organs (Hanan, 2016). The increase in MDA level in the blood results in failure of the antioxidant mechanisms to combat the generation of excessive free radicals (Hanan, 2016). From this study, the groups treated with *B. pinnatum* extract after the administration of CCl_4 showed significant decrease ($p<0.05$) in lipid peroxidation compared to group two rats that were administered with CCl_4 without any treatment. This result agrees with the work of Abraham *et al*. (1999), who reported that exposure to CCl_4 caused elevated lipid peroxidation and

consequent injury to the lungs and kidney of rats. The increased lipid peroxidation level obtained in CCl_4-administered but untreated group could also be as a result of the activation of CCl_4 resulting in the formation of CCl_3 and peroxy radicals. Thus, the decrease in MDA in *B. pinnatum* extract-treated groups compared to group two (CCl_4 untreated group), indicates the ability of the extract to protect cell membrane against free radical attack.

Conclusion

In this study, methanol extract of *Bryophyllum pinnatum* leaves showed great potential for the treatment of hepatotoxicity in Wistar rats. This validates the claim that *Bryophyllum pinnatum* leaves are potent for the management of liver disorders.

Recommendation

Studies on the effect of extracts of *Bryophyllum pinnatum* leaves on cellular organells such as mitochondria, endoplasmic reticulum, and nucleus through the activation and/or inhibition of signal kinases, transcription factors and gene-expression profiles are recommended.

Author's Contributions

Chioma Assumpta Anosike: Designed and supervised the research work and also edited the manuscript.

Sophia Uyo Mokwunye: Carried out the experimental and animal studies and analysed the results.

Victor Eshu Okpashi: Prepared the manuscript for publication.

Obiorah Abonyi: Co-supervised the experimental and laboratory work and drafted the manuscript.

Ethics

The authors declare no conflict of interest in the work.

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