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# Assessment of the Hepatoprotective activity of the seeds of *Hunteria umbellata* (Hallier F.) on Carbon Tetrachloride (CCl<sub>4</sub>) Induced Liver Damage in Wistar Albino Rats

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**Abstract.** This study was designed to evaluate the hepatoprotective activity of the seeds of *Hunteria umbellata* (HU) on carbon tetrachloride (CCl<sub>4</sub>) induced rats. Rats of groups 1 (normal control), 3 and 5 were not treated with CCl<sub>4</sub> while rats of groups 2 (negative control), 4 and 6 rats were treated with single dose of CCl<sub>4</sub> (2 ml/kg) by intraperitoneal administration. Normal control group 1 rats were given distilled water, groups 3 and 4 rats were given 50 mg/kg of silymarin while groups 5 and 6 rats were given 500 mg/kg of HU. Treatment was administered orally for 28 days and sacrificed on the 29th day after an overnight fast. The weights of the rats were taken before and after the treatment. Blood samples were collected in heparinized tubes and biochemical analysis of liver functions and lipid profile tests were carried out on plasma. There was a significant change ( $p < 0.05$ ) in the levels of alanine aminotransferase, alkaline phosphatase, high density lipoprotein and triglycerides of the CCl<sub>4</sub> induced group treated with HU compared to the CCl<sub>4</sub> untreated group 2 animals. The results obtained showed that the ethanolic extract of HU has hepatoprotective property.

## INTRODUCTION

The liver is a largest glandular organ in the body known for performing important functions such as metabolism, detoxification, digestion, storage and synthesis. Even though, the hepatocytes have a remarkable regenerative capacity, constant exposure to toxicants will eventually lead to hepatotoxicity. Some of the toxicants previously reported are carbon tetrachloride (CCl<sub>4</sub>), acetaminophen, nitrosamines and polycyclic aromatic hydrocarbons [1] CCl<sub>4</sub> is a classic hepatotoxin that causes hepatic failure and commonly used as solvents and most especially in fire extinguishers thus leading to human exposure through inhalation [2]. In CCl<sub>4</sub>-induced hepatotoxicity, liver damage is majorly caused by haloalkylation and lipid peroxidation [3]. These mechanisms involve the production of radicals which covalently bind macromolecules including proteins, nucleic acids and lipids thus leading to changes in the structures of these biomolecules [4]. On activation, CCl<sub>4</sub> affects both synthesis and degradation of lipids leading to accumulation of fat, a major consequence generally seen in CCl<sub>4</sub>-induced hepatotoxicity. CCl<sub>4</sub> inhibits triacylglycerol synthesis and also play a part in impairing the movement of triacylglycerol through very low density lipoprotein (VLDL) [5].

*Hunteria umbellata* from the family Apocynaceae is a plant known for its efficacy in treating various ailments. The small plant thrives well in the tropical regions of West Africa and popularly known as Aadaci by the Hausas, Mkpokiri by Igbos and Abere by Yorubas [6]. Various parts of the plant have been explored for medicinal values and have been reported to be efficient in treating different diseases like diabetes [7]. Seeds of this plant are of high value with

hypolipidemic, weight losing, antihyperlipidemic, hypoglycemic and cardioprotective properties [8, 9, 10]. Even though many studies have shown the efficacy of different parts of this plant, seeds of the plant is yet to be explored for potential benefits. This study is focused on the effects of the seeds of *H. umbellata* on hepatotoxicity.

## MATERIALS AND METHODS

### Plant

Already dried and grounded seeds of *Hunteria umbellata* plant were obtained from previous studies. Botanical identification was done by Dr. O.O. Ogunlana, Covenant University, Ota, Ogun state Nigeria.

### Experimental Animals

Animals of an average weight of 150 g were divided into six experimental groups and housed under standard condition exposing them to 12-hour light and dark cycle. Animals were allowed to acclimatise for two weeks before the commencement of the experiment. All the animal experiment and handling were carried according to standard protocols approved by the animal ethics committee of the department of Biochemistry, Covenant University, Ota, Ogun State Nigeria.

### Experimental Design

Thirty-six (36) experimental animals were divided into six groups of six animals each and were weighed weekly throughout the duration of the study. A modification of the hepatoprotective effect on rats as described by Murugesan et al. [11], Adebayo et al. [12] and Adebayo et al. [13] were adopted in this experimental study.

Animals in groups 1 (Normal control), 3 (Silymarin control) and 5 (AP control) were given through gastric intubation, distil water (1 ml/Kg body weight), silymarin (50 mg/kg body weight) and *H. umbellata* (500 mg/kg body weight) respectively for 28 days and thereafter given intraperitoneal injection of normal saline and olive oil (1 ml/Kg body weight). While animals in groups 2 (Negative control), 4 (silymarin + CCl<sub>4</sub>) and 6 (*H. umbellata* + CCl<sub>4</sub>) were given through oral route, distil water (1 ml/Kg body weight), silymarin (50 mg/kg body weight) and *H. umbellata* (500mg/kg body weight) respectively for 28 days and thereafter followed by a single intraperitoneal injection of CCl<sub>4</sub> in olive oil (2 ml/kg bodyweight). After 24 hours of intraperitoneal injection and overnight fast, all the animals were sacrificed by methods previously described by Ogunlana et al. [14], Ogunlana et al. [15] and Ogunlana et al. [16]

### Biochemical Analysis

After collection of blood in heparinised tubes, plasma was obtained from the blood samples as described in a previous report (Ogunlana et al., 2018). Plasma concentrations of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and cholesterol were assayed using Randox test kit. Total protein was determined using Lowry method described by Noeman et al. [17].

### Statistical Analysis

Data were expressed as mean  $\pm$  standard error of mean (SEM). Statistical analysis was done by one-way analysis of variance (ANOVA) using version 15.0 of Statistical Package for the Social Sciences (SPSS). The means of the groups treated with *H. umbellata* was compared with the least significant difference in the negative and normal control groups with the statistical significance carried out at the 95 % confidence limit.

## RESULTS

In table 2, the high serum concentration of ALP, AST and ALT in the negative control was significantly ( $p < 0.05$ ) reduced when treated with *H. umbellata* and Silymarin with *H. umbellata* treated group showing a similar result to that of normal control group. There was no significant ( $p > 0.05$ ) change in TP concentrations across the groups.

**TABLE 1.** Organ weights

Groups	Kidney	Spleen	Heart	Brain	Intestine	Liver
1	0.86±0.04	0.58±0.04	0.52±0.06	1.32±0.17 <sup>γ</sup>	3.73±0.72	3.49±0.10 <sup>γ</sup>
2	0.84±0.04	0.56±0.07	0.51±0.03	0.93±0.09*	3.97±0.52	4.08±0.17*
3	0.92±0.04	0.60±0.03	0.46±0.03	0.98±0.06*	4.03±0.33	3.41±0.14 <sup>γ</sup>
4	0.92±0.02	0.65±0.03	0.43±0.03	0.94±0.02*	3.84±0.14	3.28±0.06 <sup>γ</sup>
5	0.80±0.02	0.54±0.02	0.49±0.02	1.06±0.07	4.06±0.51	3.17±0.07 <sup>γ</sup>
6	1.07±0.23	0.63±0.04	0.48±0.03	1.05±0.10	4.17±0.17	3.54±0.29 <sup>γ</sup>

Values are represented as mean ± standard error of mean (M±S.E). Values marked with \* shows significant (p < 0.05) difference from the normal group, <sup>γ</sup> shows significant (p < 0.05) difference from the negative group (CCl<sub>4</sub> induced group).

**TABLE 2.** Effects of *H. umbellata* on biochemical parameters

Groups	ALP (U/I)	AST (U/I)	ALT (U/I)	TP (g/dl)
1	703.80 ± 18.51 <sup>γ</sup>	43.87±0.43 <sup>γ</sup>	29.93±0.33 <sup>γ</sup>	7.84±0.07
2	1041.90 ±73.34*	58.01±0.61*	56.72±1.96*	7.59±0.81
3	828.00± 83.25 <sup>γ</sup>	47.25±3 .22 <sup>γ</sup>	20.00±2.28* <sup>γ</sup>	8.55±0.56
4	759±28.73 <sup>γ</sup>	47.43± 0.51 <sup>γ</sup>	26.24±4.61 <sup>γ</sup>	6.60±1.09
5	754.9±61.50 <sup>γ</sup>	43.2±3.66 <sup>γ</sup>	26.9±1.47* <sup>γ</sup>	8.33±0.67
6	678.9±103.9 <sup>γ</sup>	42.4±8.52*	25.7±1.03* <sup>γ</sup>	6.88±0.57

Values are represented as mean ± standard error of mean (M±S.E). ALP, AST, ALT, TP represents alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein. Values marked with \* shows significant (p < 0.05) difference from the normal group, <sup>γ</sup> shows significant (p < 0.05) difference from the negative group (CCl<sub>4</sub> induced group).

Table 3 shows the effects of the plant extract on lipid profile. Concentration of HDL was significantly (p<0.05) reduced in the negative control with the effect reversed in *H. umbellata* and silymarin treated groups comparable to normal control. There was insignificant (p>0.05) increase in the concentrations of cholesterol and LDL in the *H. umbellata* treated groups, this was not comparable to normal control and silymarin treated group. In addition, the significant (p<0.05) increase in triglyceride concentration in negative control was reversed with *H. umbellata* and silymarin treatment.

**TABLE 3.** Effects of *H. umbellata* on lipid profile

Groups	HDL(mmol/l)	LDL(mmol/l)	TRIG(mmol/l)	CHOL(mmol/l)
1	2.6±0.23 <sup>γ</sup>	4.57±1.44	0.43±0.10* <sup>γ</sup>	7.37±1.24
2	0.91±0.25*	7.31±0.66	1.33±0.32	11.8±0.69
3	2.91±0.50 <sup>γ</sup>	5.94±1.09	0.33±0.17 <sup>γ</sup>	9.00±1.41
4	2.53±0.31 <sup>γ</sup>	4.78±1.83	0.47±0.02 <sup>γ</sup>	7.56±1.91
5	3.68±0.69 <sup>γ</sup>	8.95±1.71	0.55±0.21 <sup>γ</sup>	12.9±1.59
6	2.17±0.28 <sup>γ</sup>	11.2±3.23	0.63±0.14 <sup>γ</sup>	13.6±2.96

Values are represented as mean ± standard error of mean (M±S.E). HDL, LDL, TRIG, CHOL represents high density lipoprotein, low density lipoprotein, triglycerides, total cholesterol respectively. Values marked with \* shows significant (p < 0.05) difference from the normal group, <sup>γ</sup> shows significant (p < 0.05) difference from the negative group (CCl<sub>4</sub> induced group).

## DISCUSSION

Increasing concentration of biochemical markers including alkaline phosphatase (ALP), alanine aminotransferases (ALT) and aspartate aminotransferases (AST) is indicative of liver damage caused by the administration of a xenobiotic such as CCl<sub>4</sub>. Transaminases are regarded to as enzymes of the cytoplasm and mitochondrial typically found in important body cells like the hepatocytes [18]. The two most important transaminases include ALT and AST. ALT however is a more specific enzyme to the liver. ALT catalyzes the reaction between L-alanine and α-ketoglutarate giving pyruvate and L-glutamate while AST catalyzes the reaction between L-aspartate and α-ketoglutarate producing oxaloacetate and L-glutamate [19]. Liver damage through necrosis or membrane dysfunction enables the release of liver function enzymes into circulation thus increasing their concentration in the serum and making them a reliable

marker for liver dysfunction [15]. This dysfunction could be as a result of oxidative stress induced by the CCl<sub>4</sub> [20]. This study showed a result in line with the above explanation in which CCl<sub>4</sub> treated groups showed a significant increase in transferases when compared to the normal control [21]. A cell membrane enzyme, ALP generally related to bone and hepatobiliary diseases is known to be found in cases of cholestasis and was also elevated in this study clearly indicating the disruption to the hepatic membrane of the hepatocytes from CCl<sub>4</sub> exposure [22, 23, 24].

Treatment with seeds of *H. umbellata* ameliorated the damage caused by CCl<sub>4</sub> in relation to ALP, AST and ALT which could be attributed to the safety of the plant on the liver as established by [25]. The relationship between these liver function enzymes and lipid metabolism cannot be overemphasized due to the role the liver plays in the latter. Impaired lipid metabolism is an expected consequence of CCl<sub>4</sub> induced hepatotoxicity which is characterised by reduced high density lipoprotein (HDL) and increased low density lipoprotein (LDL), triglyceride (TRIG) and cholesterol (CHOL) levels. From the study, group 2, the CCl<sub>4</sub>-induced group showed a significant decrease in HDL level. However, there was increase in LDL, TRIG and CHOL which correlate with previous reports [26, 27]. This could be explained by an increased influx of acetate to the hepatocytes upon CCl<sub>4</sub> administration thus leading to an increased production of fatty acids and triglycerides [28]. Seeds of *H. umbellata* reversed the effect of lipid metabolism dysfunction by increasing the plasma level of high density lipoprotein (HDL) and reducing the level of triglyceride.

## CONCLUSION

It can be concluded that seeds of *H. umbellata* exhibited a hepatoprotective property due to its efficacy in normalising high density lipoprotein and triglyceride levels, as well as the activities of alkaline phosphatase, aspartate aminotransferase and alanine transaminase in damaged liver. However, its anticholesteremic activity could not be established in this study at the concentration tested.

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