HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF PURPLE ONION BULB (*Allium cepa* Linn) ON CCl_4 INDUCED HEPATOTOXICITY IN WISTAR RATS

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Abstract: Carbon tetrachloride is widely used for experimental induction of liver damage by lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals. Onions are known to contain anthocyanins and the flavonoids, quercetin and kaempferol which have free radical scavenging activity. The study was designed to evaluate the hepatoprotective effect of ethanol extract of purple onion against carbon tetrachloride-induced hepatotoxicity in Wistar rats. Liver damage was induced in experimental animals by administering CCl_4 (2 ml/kg). The ethanolic extract of purple onion (200, 400 and 600 mg/kg) was given for seven days before CCl_4 intoxication. Hepatoprotective effect was studied by assaying the activities of serum marker enzymes like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and other biochemical parameters such as direct and total bilirubin, total protein and albumin and lipid profile. Antioxidant enzymes like catalase and glutathione-S transferase levels were also assayed. The results of the present investigation revealed that the given dose of Carbon tetrachloride produced significant increase in aspartate aminotransferase, alanine amino transferase, and alkaline phosphatase while there was a significant decrease in total protein level in group 2. There was also a significant decrease in the activities of CAT and GST in CCl_4 treated group all indicating impaired liver function. The pre-treatment with ethanol extract of purple onion decreased the CCl_4-induced elevation in liver enzymes and other biochemical parameters. These results show that ethanol extract of purple onion have potential therapeutic effect on hepatotoxicity.

Keywords: Antioxidant, ethanol, extract, liver, hepatoprotective, purple-onion

Introduction

Liver is considered to be the key organ in the metabolism of nutrients (carbohydrates, proteins, and lipids). It also performs the role of excretion of drugs and xenobiotics from the body and this role confers protection on the body against foreign materials by detoxifying and eliminating them (Saleem *et al*., 2010). Thus the liver is prone to injury due to chronic exposure to drugs, environmental toxicants and other xenobiotics (Kumar *et al*., 2013). Certain medicinal agents when taken in overdoses and sometime even when introduced within therapeutic ranges may injure the organ. Other chemicals such as agents used in laboratories and industries, and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injuries are called hepatotoxins (Achiliya *et al*., 2003). Carbon tetrachloride (CCl_4) are widely used for experimental induction of liver damage (Alavian *et al*., 2014). The principle of carbon tetrachloride (CCl_4) induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (Basu, 2011).

Carbon tetrachloride (CCl_4), bromobenzene, choloroform, paracetamol, ethanol and polycyclic aromatic hydrocarbons are activated into their corresponding reactive metabolites through the action of the cytochrome P450 system mainly located in the livers (Sheweita *et al*., 2001).

The hepatotoxicity induced by tetrachloromethane (CCl_4) is due to its metabolite $CCl_3^{\bullet}$, a free radical that binds to nucleic acids, proteins and lipids. This radical is very reactive and it is capable of initiating the process of lipid peroxidation (Basu, 2011). The ability of a hepatoprotective drugs to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons why people in the world including those in developed countries are shifting to complementary and alternative medicine. Many folk remedies from plant origin have been long used for the treatment of hepatotoxicity (Manoj *et al*., 2011). Onion (*Allium cepa* Linn.) is a bulbous herb belonging to the family of *Alliceae* is a widely consumed vegetable (Olayeriju *et al*., 2015). Onion is one of the richest sources of flavonoids and organosulphur compounds. *A. cepa L.* possess high level of antioxidant activity attributed to flavonoids, quercetin and pigments such as anthocynins (Patil, 2007). Many reports revealed that onion was found to have antiviral, antiparasitic, antifungal, antihypertensive, antimicrobial, anti-inflammatory and antihyperlipidemic and antioxidant activities (Kumar *et al*., 2013). In view of the antioxidant property of Onion, this study was designed to evaluate the possible hepatoprotective effect of ethanolic extract of purple *A. cepa* L. on CCl_4 induced hepatotoxity in Wistar rats.

Materials and Methods

Chemicals

Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein and total cholesterol kits were obtained from Randox Laboratory (UK) while Gluthathione, DTNB, Epinephrine, Hydrogen peroxide were gotten from Sigma Chem Co. (St. Louis, MO USA). All other chemicals were of analytical grade.

Plant material

Purple onions (*Allium cepa L.*) were bought from Keffi Market, Nasarawa State. The onions were identified by the Plant Science and Biotechnology Unit of Nasarawa State University, Keffi.

Preparation of ethanol extract of red onion

The onions were washed with tap water and cut into medium pieces. The chopped onions were then blended using HGB550 Waring blender at high speed for 5 min. The blended onion (200 g) was macerated in 2000 ml ethanol and allowed to stand for a period of 48 h. It was filtered using a fine strainer and Whatman filter paper (No. 1) was used to re-filter the filtrate. The filtrate was concentrated in a water bath at 40^0C for complete dryness. Crude extract was obtained for the

analysis after the drying, and stored at 4^0C until required for use.

Phytochemical screening

Phytochemical screening of the ethanol extract of onion was carried out to determine the presence of flavonoids, alkaloids, saponins, tannins, carbohydrates, glycosides, phytosterols, phenols, phenols, proteins and Diterpenes using the method of Trease and Evans (2001).

Animal study

The study was carried out using 30 albino rats (160±20 g), obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. They were acclimatised for 2 weeks at the animal house of the Department of Zoology, Nasarawa State University, Keffi. They were kept under temperature of 25^0C and light and dark cycles of 12:12 h and were fed with commercial pellet diet (Vital Feeds) and water was provided ad libitum. After acclimatization, the animals were weighed and divided into 5 groups with 6 animals per group.

Group I: Normal Control received feeds and water throughout the period of 14 days.

Group II: Received CCl_4 (2 ml/kg) at every 72 h for 14 days.

Group III: Received Purple onion extract 200 mg/kg for 7 days before CCl_4 administration and simultaneously with CCl_4 (2 ml/kg) at every 72 h for another 7 days.

Group IV: Received Purple onion extract 400 mg/kg for 7 days before CCl_4 administration and simultaneously with CCl_4 (2 ml/kg) at every 72 h for another 7 days.

Group V: Received Purple onion extract 600 mg/kg for 7 days before CCl_4 administration and simultaneously with CCl_4 (2 ml/kg) at every 72 h for another 7 days.

Groups III, IV and V were all subjected to oral pre-treatment with the ethanol extract of purple onion (200, 400 and 600 mg/kg/day), respectively for 7 days before CCl_4 was administered and given simultaneously with CCl_4 for another seven days. At the end of the experiment all the animals were sacrificed and blood was collected through cardiac puncture and centrifuged at 3000 rpm for 30 min to obtain serum. The liver was also excised and homogenate prepared for the evaluation of biochemical parameters.

Measurement of serum parameters

The activities of Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, total protein and lipid profile were estimated from the serum using standard kits from Randox Laboratory, UK according to the instruction of the manufacturer.

Determination of the antioxidant status

Catalase activity was determined by the method of Sinha (1971) by following the decomposition of hydrogen peroxide. Glutathione-S-transferase (GST) activity was determined according to Habig *et al*. (1974). Lipid peroxidation was determined by measuring MDA released according to the method of Varshney and Kale (1990).

Protein determination

Protein concentration was measured by the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

Data and statistical analysis

The results were statistically analysed using one way analysis of variance (ANOVA) followed by Student t-test. The values were expressed as mean ± standard deviation. The significant level was set at $p<0.05$.

Results and Discussion

The phytochemical Screening of the purple *A. cepa L.* extract reveals the presence of flavonoids, saponins, phenol, diterpenes, triterpenes, alkaloids, phytosterol and proteins (Table 1). Flavonoids are considered as the most active antioxidant phenolic compounds due to their chemical structure (Kähköne *et al*., 1999). This finding is in agreement with the study of Kumar *et al*. (2013) who found the presence of flavoniods, saponin, phenols in aqueous extract of *A. cepa L*. Although he reported the presence of carbohydrate and tannins which were absent in our own study probably due to the difference in the solvents used for extraction.

Table 1: Phytochemical constituent of the ethanol extract of *Allium cepa.* L

Phytochemicals	Ethanolic Extract
Alkaloids	+
Carbohydrates	-
Glycosides	-
Saponin	+
Phytosterol	+
Phenol	+
Flavonoids	+
Proteins	+
Diterpenes	+

Present (+); Absent (-)

Table 2: Effect of ethanol extract of purple onion (*Allium cepa L.*) on AST, ALT and ALP

Group	Treatment	ALP (U/L)	ALT (U/L)	AST (U/L)
1	PC	82.85 ± 2.85*	20.05 ± 2.26*	27.47 ± 0.80*
2	NC (CCl_4)	138.00 ± 4.24	30.90 ± 0.57	36.88 ± 1.49
3	EP 200 mg/kg + CCl_4	137.00 ± 2.04	15.05 ± 0.35**	24.35 ± 0.61**
4	EP 400 mg/kg + CCl_4	113.00 ± 1.41**	18.40 ± 1.13**	22.69 ± 1.94**
5	EP 600 mg/kg + CCl_4	88.90 ± 1.41**	7.65 ± 0.12**	16.23 ± 1.92**

PC= Positive Control, NC = Negative Control, EP = Ethanolic Purple Onion Extract, CCl_4 = Carbon Tetrachloride

*Statistically significant at ($p< 0.05$) when comparing group 2 with group 1; **Statistically significant at ($p< 0.05$) when comparing groups 3, 4 and 5 with group 2

The result presented in Table 2 shows that the levels of Serum enzymes (ALP, ALT, and AST were significantly increased in Group 2 (Negative control) ($p<0.05$) compared to group 1 (Positive control). Treatment of animals with CCl_4 is known to cause severe hepatic injury (Terblanche, 1991).

These increases indicate that CCl_4 induces hepatocellular damage which in turn alters the structure and function of liver cells (Kumar *et al*., 2013). The ethanol extract of purple *A. cepa L.* at 200, 400 and 600 mg/kg b.w significantly decreased ($p<0.05$) the AST and ALT levels while ALP was significantly decreased ($p<0.05$) at 400 and 600 mg/kg b.w when compared with group 2. These results agree with the study of Kumar *et al.* (2013) which demonstrate that aqueous extract of *A. cepa L.* reduced levels of AST, ALP and ALT which were elevated by ethanol consumption. Riyak Shaik *et al*. (2012) also demonstrated that *A. cepa L.* leaves protects hepatocytes by preventing the release of ALP, AST and ALT. Ogunlade *et al*. (2012) also found out that *A. cepa L.* reduces serum levels of liver biomarker enzymes. The results in Table 2 suggest that the ethanol extract of *A. cepa L.* protects the hepatocytes structural integrity and prevents the leakage of cytosolic enzyme into the bloodstream.

According to Olaleye and Rocha (2006), effective control of alkaline phosphatase points towards an early improvement in the secretory mechanisms of the hepatic cells. The protective effect may be the result of stabilization of plasma membrane, thereby preserving the structural integrity of cell as well as the repair of hepatic damage caused by CCl_4 (Pari and Murugan, 2004).

The table above showed that there was a significant increase ($p<0.05$) in Total bilirubin level in group 2 when compared with group 1. There was significant decrease ($p<0.05$)

observed in all the treated groups when compared with group 2. Also a significant increase in Direct bilirubin levels ($p<0.05$) was observed in group 2 when compared with group 1. At 400 and 600 mg/kgb.w (group 4 and 5) there was a significant decrease ($p<0.05$) in the direct bilirubin concentration. The concentration of total protein decreased significantly in group 2 when compared with group 1, but increases significantly ($p<0.05$) in all the treated groups. The increase was dose-dependent. Albumin levels in all the treated groups increased significantly ($p< 0.05$) when compared with group 2. Elevated plasma levels of both conjugated and unconjugated bilirubin results from the blockage of uptake at the plasma membrane of the hepatocyte (Kshirsagar, 2008). Both Total and direct bilirubin level significantly reduced in the treated groups. This signifies that *A. cepa L.* alleviates hepatic blockage. Albumin and Total protein were significantly reduced ($p<0.05$) in group 2. This may be as a result of the liver being unable to perform its synthetic function.

Table 3: Effect of ethanol extract of purple onion (*Allium cepa* L.) on biochemical parameters

Groups	Treatment	Total Bilirubin (nmol/L)	Direct Bilirubin (nmol/L)	Total Protein (g/dl)	Albumin (g/dl)
1	PC	2.06 ± 0.08	1.38 ± 0.31	6.69 ± 0.95	4.10 ± 0.23
2	NC (CCl_4)	9.05 ± 0.64*	5.98 ± 0.95*	2.39 ± 0.20*	1.57 ± 0.24*
3	200 mg/kg + CCl_4	3.80 ± 0.14**	2.85 ± 1.64	6.58 ± 0.76**	5.86 ± 2.34**
4	400 mg/kg + CCl_4	2.34 ± 0.08**	1.14 ± 0.11**	8.10 ± 1.14**	4.14 ± 0.37**
5	600 mg/kg + CCl_4	3.45 ± 0.22**	0.51 ± 0.42**	8.45 ± 0.29**	4.26 ± 1.20**

PC= positive Control, NC = Negative Control, EP = Ethanolic Purple onion extract, CCl_4 = Carbon tetrachloride
*Statistically significant at ($p< 0.05$) with group 1; **Statistically significant at ($p< 0.05$) with group 2

Table 4: Effect of ethanol extract of purple onion (*Allium cepa L.*) on lipid profile

Groups	Treatment	TAG (mg/dL)	CHOL(mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	PC	11.0 ± 0.10	19.50 ± 1.00	18.20± 0.05	1.38 ± 0.09
2	NC (CCl_4)	8.0 ± 0.14*	15.97 ± 0.1*	14.45 ± 1.01*	0.48 ± 0.42*
3	200 mg/kg + CCl_4	16.0 ± 0.25**	16.20 ± 0.33	14.22 ± 0.94	0.56 ± 1.01
4	400 mg/kg + CCl_4	14.20 ± 0.1**	21.10 ± 0.23**	15.98 ± 1.61**	1.37 ± 0.37**
5	600 mg/kg + CCl_4	18.12 ± 0.41**	19.20 ± 1.20**	15.43 ± 2.60**	2.76 ± 0.06**

PC= positive Control, NC = Negative Control, EP = Ethanolic Purple onion extract, CCl_4 = Carbon tetrachloride
* Statistically significant at ($p< 0.05$) with group 1; **Statistically significant at ($p< 0.05$) with group 2

The result of the lipid profile is presented in Table 4. There was a significant decrease ($p<0.05$) in triglyceride (TAG) in group 2 but a significant increase ($p<0.05$) was observed in groups 3, 4 and 5, respectively. Total cholesterol decreased significantly ($p<0.05$) in group 2 when compared with the group 1. Treatment with the extract increases the concentration of cholesterol significantly ($p<0.05$) in groups 4 and 5 as observed in Table 4. There is a significant decrease ($p<0.05$) in LDL level in group 2 when compared with the group 1. Treatment with the extract increases the LDL levels significantly ($p<0.05$) in groups 4 and 5 in comparison with group 2. A significant decrease ($p<0.05$) in HDL level was observed in group 2 when compared with group1 and a significant increase ($p<0.05$) was observed in groups 4 and 5 treated with 400 and 600 mg/kg b.w of the extract. The result shows that Triacylglyceride (TAG), Cholesterol (CHOL), Low density lipoprotein (LDL) and High density Lipoprotein (HDL) were significantly decreased in group 2 (negative control) This agrees with the study of Ghadir *et al*. (2010), who found that lower lipid levels are found in patients with liver diseases, and all four studied variables (HDL, LDL, total cholesterol and TG) were significantly lower in cirrhotic patients than in the comparison normal group. The plasma triglyceride and cholesterol reduced in chronic liver disease has been said to be due to the lower biosynthetic capacity of lipoprotein (Mandal *et al*., 2012).

Arain *et al.* (2017) discovered that serum lipids level, total cholesterol, triglycerides HDL-C, LDL-C, VLDL-C and total lipids are significantly lower in HBV-cirrhosis patients which indicate hypolipidemia in patients. Major injuries to hepatocytes, such as those caused by alcohol consumption, chronic viral hepatitis or cirrhosis of the liver, might produce abnormal liver function and a moderate decrease in levels of total cholesterol and HDL-C. Treatment with *A.cepa L.* brought about an increase in all the parameters especially at 400 and 600 mg/kg b.w.

Table 5: Effect of ethanolic extract of purple onion (*Allium cepa L.*) on liver antioxidant parameter

Group	Treatment	CAT (U/L)	GST (U/L	MDA (nm/mgprotein)
1	PC	3.42 ± 1.23	1.19 ± 0.40	0.34 ± 0.21
2	NC (CCl_4)	1.12 ± 0.82*	0.67 ± 0.13*	0.84 ± 0.24*
3	200 mg/kg + CCl_4	1.24 ± 0.22	1.25 ± 0.50**	0.36 ± 0.20**
4	400 mg/kg + CCl_4	1.88 ± 0.29**	1.52±1.25**	0.22 ± 0.12**
5	600 mg/kg + CCl_4	2.12 ± 0.37**	1.28 ± 0.01 **	0.37 ± 0.10**

*Statistically significant at ($p< 0.05$) with group 1; **Statistically significant at ($p< 0.05$) with group 2

Results of the present study revealed that exposure of rats to CCl_4 resulted in depletion of antioxidant enzyme activities (Table 5), Lipid peroxidation products are formed when reactive oxygen species attack polyunsaturated fatty acids leading to membrane structural and functional damage (Yoshida *et al*., 2013). Markers of Lipid peroxidation have been found to be high in liver fibrosis induced by CCl_4 (Al-Sayed *et al*., 2014). MDA is a commonly used marker for the assessment of lipid peroxidation (Thanh, 2015). From Table 5, the activities of Catalase, and GST were significantly decreased ($p<0.05$) in group 2 (Negative control) compared with group 1 while the activities in the groups treated with the ethanol extract significantly increased. MDA significantly increased ($p<0.05$) in group 2 compared with group 1 but treatment with *A. cepa L.* at 200, 400 and 600 mg/kg b.w (groups 3, 4 and 5) shows a significant decrease ($p<0.05$) in MDA. This shows that the ethanol extract of *A. cepa L.* is able

to offer protection against lipid peroxidation in the hepatocyte membrane.

Conclusion

The ethanol extract of *Allium cepa L.* when administered orally to rats showed a significant dose dependent hepatoprotective activity at 200, 400 and 600 mg/kg. This present study reveals that pre-treatment with ethanolic extract of *Allium cepa L*. (Purple onion) might confer protection on the hepatocytes against CCl_4-induced liver injury.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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