IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
e- ISSN: 2394-0050, P-ISSN: 2394-0042.Volume 10, Issue 4 (July. – August. 2023), PP 01-07www.iosrjournals.org

Biochemical Evaluation Of The Anti-Hepatic Steatosis Activity Of Allium Cepa On Carbon Tetrachloride (Ccl_4)-Induced Wister Rats

Babatunde Peter Femi [1], Olawole Yakubu Adeniran[1],Damilohun Samuel Metibemu[1], Oyewale Mary Bose[1].
[1]*Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo state, Nigeria.*

Abstract:
Background*: Globally, hepatic diseases are known to cause morbidity and mortality. Liver disease accounts for approximately 2 million deaths per year worldwide. In recent years, Non-alcoholic fatty liver disease (NAFLD) has been identified as the most prevalent chronic liver disease in developed nations. The aim of this research was to study the therapeutic effect of onion (Allium cepa) on carbon tetrachloride (CCl_4) induced fatty liver disease.*
Materials and Methods*: Thirty five (35) adult male Wister rats weighing between 150-200 g were uniformly divided into 7 groups. Group 1 was placed on normal feed only, Group 2 was administered intraperitoneally with onion extract 200 mg/kg body weight, Group 3 was administered with CCl_4in olive oil only whileGroups 4-7 were administered with CCl_4 twice a week for five weeks before treatment with onion extract (100 and 200 mg/kg body weight) and raw onion for three weeks. Biochemical assays such as aspartate transaminase, alanine transferase, alkaline phosphatase, cholesterol, triacylglycerides, high density lipoprotein were carried out using standard protocol.*
Results*: CCl_4-induced hepatotoxicity was confirmed by significant ($P < 0.05$) increase in serum biochemical parameters in group 3. Treatment with both onion extract and raw onion showed promising therapeutic tendency with most effective result obtained from 200 mg/kg body weight extract dose.*
Conclusion:*This study showed that Allium cepa exhibited ameliorative potential on the liver damage induced by CCl_4, hence, it can be used as a therapeutic agent in the management of fatty liver disease.*
Key Word*:Allium cepa, Carbon tetrachloride (CCl_4), Therapeutic, Non-alcoholic fatty liver disease,Aspartate transaminase***.**

Date of Submission: 17-07-2023 Date of Acceptance: 27-07-2023

I. Introduction

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma. The recent statistics clearly shows that the global burden of liver disease has risen over time having a huge impact on the overall world population [1,2]. Globally, Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most common causes of chronic liver diseases [4].

Currently, Non-Alcoholic Fatty Liver Disease (NAFLD) is now emerging as a common medical challenge as a result of its high incidence and the difficulty of treatment. The latest epidemiology provided evidence showing that NAFLD has become the second largest liver disease after viral hepatitis, with incidence of 20–30%, and the incidence of obesity in the population is up to 57.74% [3].

The higher incidence rate is observed in the midst of obese and diabetic patients. At present, occurrence of NAFLD is estimated at about 9 % in the developing countries where as 30 % in the developed countries all over the world. NAFLD is estimated to be the most common liver disease in the Western world affecting all racial and ethnic groups irrespective of age or sex [4].

Non-alcoholic fatty liver disease (NAFLD), which is characterized by the presence of fat accumulation (steatosis) in >5% of hepatocyte, can be categorized into Non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) based on histologic findings. Both NAFL and NASH present as hepatic steatosis, but NASH is characterized by inflammation with hepatocyte injury (ballooning) with or without fibrosis, which increases the risk of progression to more advanced liver disease such as cirrhosis, hepatocellular carcinoma, and end-stage liver disease [5].

Onion (*Allium cepa*) is one of such *Allium* genus which is an important source of dietary phytochemicals with proven antioxidant, anti-inflammatory, anti-cholesterol and anticancer properties such as organosulfur compounds, phenolic acids, flavonoids, thiosulfinates, and anthocyanins. It has great health significance and is consumed for its putative nutritional and health benefits for centuries [6,7]. Food-derived flavonoid quercetin, which is a majorly known flavonoid highly distributed in onions is able to inhibit the growth of various cancer cells [6].

Onion consumption combined with healthy diet can be effective in the management of non-alcoholic fatty liver disease [7].

II. Material And Methods

Materials/Chemicals

Carbon tetrachloride (CCl_4), olive oil, methanol, coarse sieve, mechanical blender, filter paper, beaker, distilled water, onion bulb, formalin paraffin wax, Hematoxylin and Eosin (H and E) , microscope.

Extract Preparation.

Several *Allium cepa* L. was obtained from the local market in Akungba, Ondo state. 1490g of onions were cleaned, chopped into small pieces, mashed using mortar and pestle and further shade-dried. They were grounded to powdery form using a mechanical blender and passed through a coarse sieve (0.2mm). The *Allium cepa* L. Powder was further macerated with methanol 96% for 72hrs. The extract was passed through the rotary evaporator and freeze dried. The residue obtained was stored in a refrigerator at -4 ^oC [8].

Animals and experimental design

Thirty five adult male Wister albino rats weighing between 150-200 g was obtained and maintained at the Animal House of Adekunle Ajasin University. They were housed in aluminium cages under the standard environmental conditions of a 12 hour light/dark cycle, and were allowed to have free access to water and standard rodent feed. These animals were maintained under specific pathogen-free conditions so as to avoid contamination and infection. The total experimental period was for a maximum duration of 8 weeks. The experimental protocols were carried out in accordance with the guidelines for the care and use of laboratory animals approved by the Animal Ethics Committee of Adekunle Ajasin University. The guidelines were followed strictly so as to ensure the protection of the animals' welfare for the period of the experiment.

Treatment schedule

The Animals were randomly allocated into seven groups (5 rats each), n = 5.

Group 1: (Normal control group) received water and rat feed

Group 2 : Received onion bulb extract only for 8 weeks

Group 3: Received a single dose of CCl_4 (0.5ml/kg body weight i.p) twice weekly for 8 weeks

Group 4: Received a single dose of CCl_4 (0.5ml/kg body weight, i.p) twice weekly for 5weeks after which onion bulb extract (100 mg/kg body weight/day) was administered orally for 3 weeks.

Group 5: Received a single dose of CCl_4 (0.5ml/kg body weight, i.p) twice weekly for 5 weeks after which onion bulb extract (200 mg/kg body weight /day) was administered orally for 3 weeks.

Group 6: Received a single dose of CCl_4 (0.5ml/kg body weight, i.p) twice weekly for 5 weeks after which raw onion bulb (mixed with feed, 30/70) was administered for 3 weeks.

Group 7: Received a single dose of CCl_4 (0.5ml/kg body weight, i.p) twice weekly for 5 weeks after which raw onion bulb (mixed with feed, 70/30) was administered for 3 weeks.

Serum biochemical analysis

After the duration of the experimental period, the rats were sacrificed through cervical dislocation and blood samples were collected through jugular puncture. Collected blood samples were centrifuged at 4^oC using cooling centrifuge at 3,000 r.p.m for 10 min. The serum obtained after centrifuging were separated for assessment of levels of serum liver function tests. The biochemical markers of hepatic damage including serum AST, ALT, ALP, triaglycerides, cholesterol, HDL and total protein were estimated using available commercial kits following manufacturer's instruction.

Statistical analysis

All values were presented as means ± standard error of the means (SEM). Comparisons between different groups were carried out using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Difference were considered significant when $P<0.05$. GraphPad prism® software version 8.0 for Windows (USA) was used to carry out these statistical tests.

III. Result

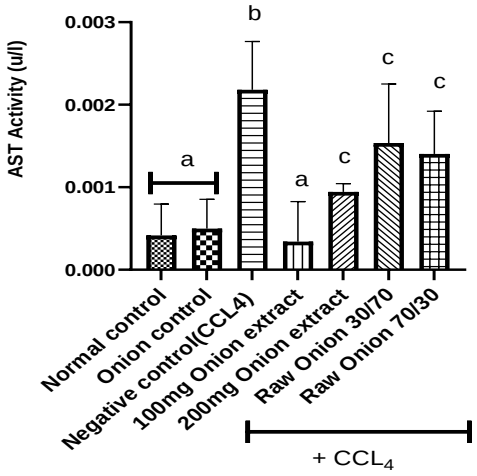

Fig. 1: Effect of *Allium cepa* on liver Aspartate transaminase (AST) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups labelled 'a' and 'b'. ($P < 0.05$). Groups labelled 'c' has no significant difference with group labelled 'b'.

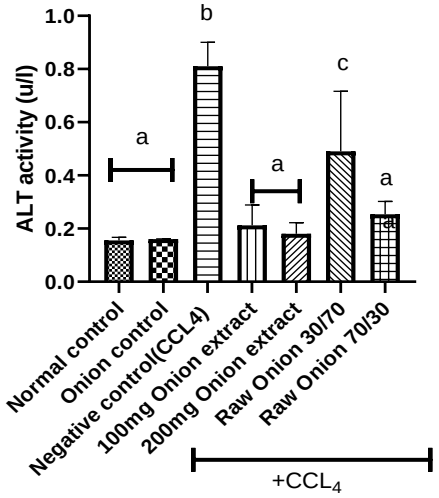

Fig. 2: Effect of *Allium cepa* on liver Alanine transaminase (ALT) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups labelled 'a' and 'b'. ($P < 0.05$). Group labelled 'c' has no significant difference with group labelled 'b'.

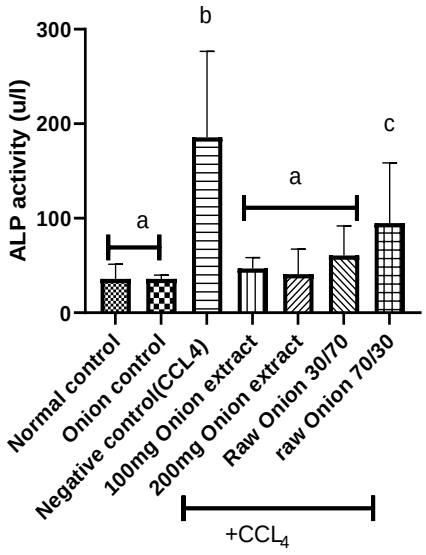

Fig. 3: Effect of *Allium cepa* on liver Alkaline phosphatase (ALP) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups labelled 'a' and 'b'. ($P < 0.05$). Group labelled 'c' has no significant difference with group labelled 'b'.

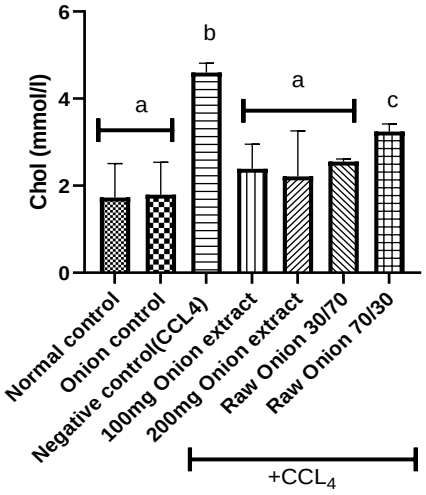

Fig. 4: Effect of *Allium cepa* on liver Cholesterol (CHOL) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups lebelled 'a' and 'b'. ($P < 0.05$). Group labelled 'c' has no significant difference with group labelled 'b'.

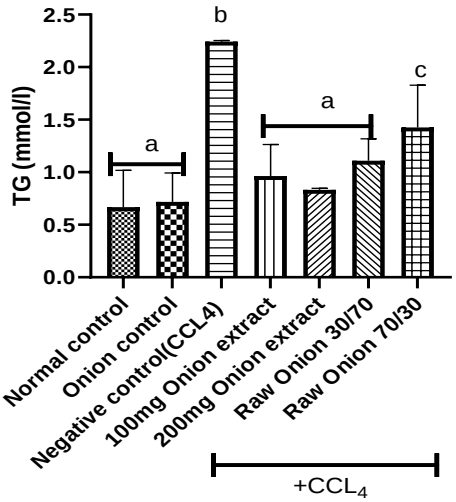

Fig. 5: Effect of *Allium cepa* on liver Triaglycerides (TG) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups lebelled 'a' and 'b'. ($P < 0.05$). Group labelled 'c' has no significant difference with group labelled 'b'.

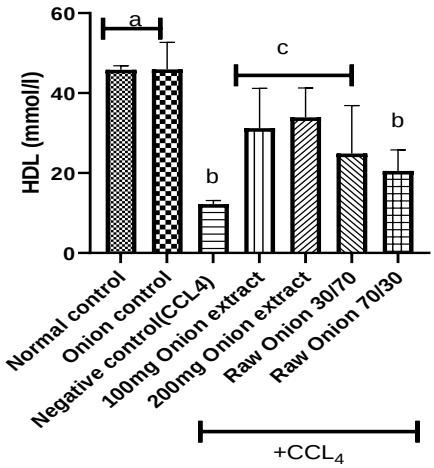

Fig. 6: Effect of *Allium cepa* on liver High Density Lipoprotein-Cholesterol (HDL-C) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups lebelled 'a' and 'b'. ($P < 0.05$). Groups labelled 'c' has no significant difference with groups labelled 'b'.

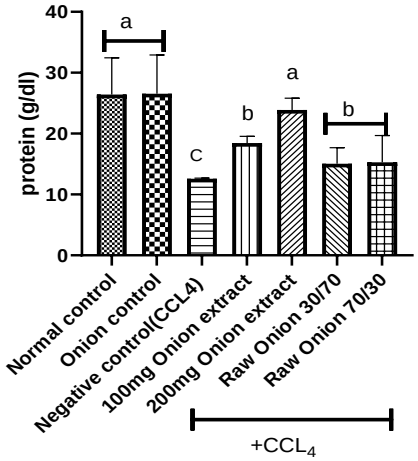

Fig. 7: Effect of *Allium cepa* on liver Total protein concentration (TP) of non-alcoholic fatty liver induced Wister rats

There is statistically significant difference between groups labelled 'a' and 'c'. ($P < 0.05$). There is no significant difference between groups labelled 'b' and 'c'

IV. Discussion

The biotransformation of CCl_4 has been reported as the mechanisms to induce liver injury. The hepatotoxic damage is triggered by the metabolism of CCl_4 in the liver, which is catalyzed by cytochrome P450, mainly CYP2E1 for human beings. CCl_4 is then converted into trichloromethyl radical ($CC1_3$), which directly reacts with oxygen, leading to the formation of trichloromethylperoxyl radical ($OOCC1_3$), a contributor to lipid peroxidation with more chemical reactivity than $CC1_3$. Besides, $CC1_3$ can react with macromolecules like lipids, proteins, and nucleic acids. Futhermore, the permeability of the cellular compartment membranes is lowered, Ca_2+ homeostasis is lost, this also leads to the reduction in protein synthesis [9]. This same mechanism is capable to cause a distruption in the normal function of the hepatocytes which leads to leakage of enzymes from cells, thereby causing an increase in the level of such enzymes.

Elevated serum aminotransferases activities have been reported as biomarkers of liver damage [10,11]. In this study , Administration of CCl_4 induced liver damage and non alcoholic fatty liver in rats as shown by elevation of serum AST, ALT and ALP activities. Serum AST, ALT, and ALP activities were greatly increased ($P<0.05$) in rats administered CCl_4 as compared to normal control which goes in accordance with previous reports [12,13]. The result of the present study shows that groups treated with onion antagonizes these elevated enzymes.

Serum ALT, AST and ALP levels decreased significantly ($P\leq0.05$) in rats treated with onion, (both extract and raw form)than in CCl_4-induced rats only. The result for AST shows significant difference ($P< 0.05$) between Negative control, normal control, onion control and 100 mg treated rats. There was also significant difference ($P<0.05$) between the negative control, normal control and the CCl_4 induced/onion treated rats for ALT activity.

Consequently, the levels of serum cholesterol and triglycerides were elevated in the CCl_4 only treated group compared to the other groups as a result of CCl_4-induced liver damage. NAFLD is considered to be associated with hepatic metabolic disorders, which results in over accumulation of fatty acids, triglycerides and cholesterol [14]. This is in line with previous reports [11]. Rats treated with onion extract resulted in a significant improvement of serum lipid profile (TG and Cholesterol) by lowering their levels.

Mechanisms of action by which onion bioactive compounds carry out their hypolipidemic and hypocholesterolemic activities has been reported to include: inhibition of hepatic lipid/cholesterol biosynthesis by inactivating thiol enzymes (eg. HMGCoA), which promote it, or by reducing the level of NADPH in tissue, thus making them unavailable for cholesterol synthesis and also by enhancement of cholesterol turnover to bile acids thereby promoting its excretion through gastrointestinal tract [15].HDL and Total protein levels were significantly decreased ($P<0.05$) in CCl_4 only treated rats compared to the onion control group and normal control group. They were also significantly higher ($P<0.05$) in CCl_4 induced/onion treated groups. This is in agreement with previous studies [16,17]. Organo-sulphur compounds, flavonoid quercetin and derivatives present in onion have proven to be able to reduce serum concentrations of total cholesterol and to increase serum concentrations of HDL-cholesterol [15].

V. Conclusion

The result obtained showed that *Allium cepa* exhibited ameliorative potential on the hepatocellular damage induced by CCl_4 through maintenance of hepatic membrane integrity as observed from reduction of liver enzymes activities (ALT, AST and ALP), reduction in lipid profile (Cholesterol and TG) with an increase in HDL-Cholesterol and protein concentration.

This results revealed that onion (*Allium cepa*)might be a potential therapeutic agent for the treatment and management of Non-alcoholic fatty liver disease.

References

[1]. Ganesan K, Jayachandran M, Xu B. A Critical Review On Hepatoprotective Effects Of Bioactive Food Components. Critical Reviews In Food Science And Nutrition, 2018; 58(7): 1165-1229. DOI: 10.1080/10408398.2016.1244154

[2]. Sumeet A, Harshad D, John E, Patrick SK. Burden Of Liver Diseases In The World. Journal Of Hepatology,2018; 70(1) 151-171. DOI: 10.1016/J.Jhep. 2018.09.014

[3]. Zhong F, Zhou X, Xu J, GaoL.Rodent Models Of Non-Alcoholic FattyLiver Disease. Digestion,2019, 2020 (101):522–535. DOI: 10.1159/000501851

[4]. Ravisankar P, Pravallika D,AnjaliD, Vidya VS, Anvith PS, Panchumarthy P.Fatty Liver Disease In-Depth Analysis. Indo American Journal Of Pharmaceutical Research, 2015; 5(11): 3622-3642

[5]. DufourJ-F, Scherer R,BalpM-M, Mckenna SJ, Janssens N, Lopez P, Marcos &Pedrosa M. The Global Epidemiology Of Nonalcoholic Steatohepatitis (NASH) AndAssociated Risk Factors–A Targeted Literature Review. Endocrine And Metabolic Science, 2021; 3 (2021):1-9. DOI: 10.1016/J.Endmts.2021.100089

[6]. Upadhyay RK. Nutraceutical, Pharmaceutical And Therapeutic Uses Of Allium Cepa: A Review. International Journal Of Green Pharmacy, 2016; 10 (1): 46-64.

[7]. Zeng Y, Li Y,Yang J, Pu X, Du J, Yang X, Yang T, Yang S.Therapeutic Role Of Functional Components In Alliums For Preventive Chronic Disease In Human Being, Hindawi Evidence-Based Complementary And Alternative Medicine, 2017; 2017 (3): 1-13.DOI:10.1155/2017/9402849

[8]. Fadholly A, Ansori ANM, Jayanti S, Proboningrat A, Kusala MKJ, Putri N, Rantam FA, Sudjarwo SA. Cytotoxic Effect Of Allium Cepa L. Extract On Human Colon Cancer (Widr) Cells: In Vitro Study. Research Journal Of Pharmacy And Technology,2019; 12(7): 3483-3486. DOI: 10.5958/0974-360X.2019.00591.2

[9]. XiaoM, Ya LI, ShaL, Ren-YouG, Hua-BinL.Natural Products For Prevention And TreatmentOf Chemical-Induced Liver Injuries. ComprehensiveReviewsIn Food ScienceAnd Food Safety, 2018; 17 (2018): 1-24. DOI: 10.1111/1541-4337.12335

[10]. Ganaie MA, Khan TH, Siddiqui NA, Ansari MN. AmeliorativeEffectOf Methanol Extract Of Rumex Vesicarius OnCcl4-Induced Liver Damage In Wistar Albino Rats. Pharmaceutical Biology, 2015; 53(8): 1163–1167. DOI: 10.3109/13880209.2014.967782

[11]. Wahid A, Hamed AN, Eltahir HM, Abouzied MM.Hepatoprotective Activity Of EthanolicExtract Of Salix Subserrata Against Ccl4-Induced Chronic Hepatotoxicity In Rats. BMC Complementary And Alternative Medicine, 2016; 16 (263): 1-10. DOI: 10.1186/S12906-016-1238-2

[12]. Mir A, Anjum F, RiazN, Iqbal H, Wahedi HM,KhattakJZK,Khan MA, MalikSA. Carbon Tetrachloride (Ccl_4)Induced Hepatotoxicity InRats: Curative Role Of Solanum Nigrum. Journal Of Medicinal Plants Research, 2010; 4(23): 2525-2532.DOI: 10.5897/JMPR10.482

[13]. Shah GH, PatelBG, Shah GB. Development Of Carbon Tetrachloride-Induced Chronic Hepatotoxicity ModelIn Rats And Its Application InEvaluation Of Hepatoprotective Activity OfSilymarin. Asian Journal Of Pharmaceutical And Clinical Research, 2017;10 (8): 274-278.DOI: 10.22159/Ajpcr.2017.V10i8.18701

[14]. Enjoji M, Yasutake K, Kohjima M, Nakamuta M.Nutrition And Nonalcoholic Fatty Liver Disease:The Significance Of Cholesterol.International Journal Of Hepatology.Review Article, 2012; 2012 (3): 1-6, Article ID 925807. DOI:10.1155/2012/925807
Corzo-Martínez M, Villamiel M. 2012.An Overview On Bioactivity Of Onion (1st Edition) Pp.1-48.Nova Science Publisher, Inc.

[15]. Devi DV, Latha MM, Sumapreethi A, Sharma SSB, Priyanka, M.A Study Of Non- Alcoholic Fatty Liver Disease In PatientsWith MetabolicSyndrome.Journal Of Evolution Of Medical And Dental Sciences, 2013; 2(4):375-382.

[16]. Kara M, Genc H, Tapan S, Meral C, Ercin CN, Erdal M, Dogru T. AlphaFetoprotein Levels And Its RelationshipWith Histopathological Findings In PatientsWith Non-Alcoholic Fatty Liver Disease. European Review For Medical And Pharmacological Sciences, 2013; 17(1) : 1536-1541