IJMPR 2024, 8(1), 08-11

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

STUDIES ON THE HEPATOPROTECTIVE EFFECTS OF MINT (*MENTHA PIPERITA*) HYDROETHANOLIC LEAF EXTRACTS ON LIVER INJURY INDUCED BY CARBON TETRACHLORIDE (CCL_4) IN RATS.

[1*]Said S. S., [1]Abdullahi M. A. and [2]Dambazau S. M.

[1]Department of Biochemistry and Molecular Biology, Faculty of Life Science, Federal University, Dutsin-Ma, Nigeria.
[2]Department of Biochemistry, Federal University Dutse, Jigawa, Nigeria.

Article Received on: 12/11/2023
Article Revised on: 01/12/2023
Article Accepted on: 22/12/2023

***Corresponding Author**
Said S. S.
Department of Biochemistry and Molecular Biology, Faculty of Life Science, Federal University, Dutsin-Ma, Nigeria.

ABSTRACT

The value of medicinal plants to mankind is very well proven and forms the basis of health care worldwide, having significant role in international trade. This study was carried out to evaluate the hepatoprotective effects of the hydroethanolic leaf extract of *Mentha piperita* on liver injury induced by carbon tetrachloride (CCl4) in rat. Twenty-four albino rats were used in the study, and were divided into four (4) groups, six (6) rats each in group I, II, III and IV. The rats in the first group were not induced, they served as positive control, whereas rats in groups II, III, and IV were induced with liver injury using 120 mg/kg CCl_4, using standard methodology. Rats in group II were not administered mint leaf extract but were administered 10mg/kg Livolin, and they served as negative control. Rats in groups III and IV were administered with a daily dose of 500mg and 1000mg /kg body weight of mint leaves extract. Two (2) rats were removed from each group after 2, 5 and 8 days of mint leaf extracts treatment respectively and sacrificed. Blood and liver samples were collected for Biochemical and Histopathological studies. The study has shown that mint leaf extracts has hepatocurative effect at the dose of 1000mg/kg in 8 days of administration. Histopathological study showed intense vacuolation, hepatic necrosis and haemorrhage in CCl_4 pretreated group. The study shows that mints extracts have hepatoprotective effects against CCl_4 induced liver injury.

KEYWORDS: *Mentha piperita*, liver injury, Carbon tetrachloride, Hepatoprotective.

INTRODUCTION

The use of herbs to treat disease is almost universal, among non-industrialized societies and in most part of the world 70-90% of the people relies on plants for medication.[1] The value of medicinal plants to mankind is very well proven and forms the basis of health care worldwide, having significant role in international trade.[2] *Mentha piperita*, commonly known as mint is a flowering plant belonging to the *Lamiaceae* family. *Mentha* plants constitute one of the main valuable sources of essential oil used in foods and for medicinal purposes. Several mint hybrids commonly occur in Africa, it is used among communities in making tea, and as medicine among Hausa/ Fulani in Northern Nigeria. Mint has been shown in clinical trials to treat headaches through analgesic properties, reduce painful muscle spasms in patients undergoing endoscopy of the upper and lower gastrointestinal (GI) tract, and reduce abdominal pain and dyspepsia.[3] Mints are known to serve as powerful antioxidants making it very crucial in the treatment of many diseases such as liver disease.[4]

Liver is a discrete largest organ in human body that has many interrelated functions and it may be damaged due to one or more of the following: injury from metabolic disturbances, injury from toxins, drugs, chemicals and poisons, lesion of biliary tract, certain viral infections, hypoxia, and tumors.[5] Carbon tetrachloride (CCl4) induces lipid peroxidation and liver damage and high dose of CCl_4 generates an ideal hepatotoxicity model in organism that allows for evaluating the curative effects rather than reporting natural healing.[6]

The aim of this study was to investigate the hepatoprotective effects of Mint (*Mentha Piperita*) hydroethanolic leaf extracts on Liver injury induced by carbon tetrachloride (CCL_4) In Rats and compare these effects with a standard drug – Livolin.

MATERIALS AND METHODS

Collection of Plant

The Fresh leaves of mints (*Mentha Piperita)* samples were collected from local garden in Katsina state, Nigeria on the 9th of October, 2021.

Preparation of extract

Mentha piperita Leaves were cleaned of dirt and blots, air-dried under shade at the Biochemistry laboratory. The dried mint leaves was pounded using mortar and pestle, and then sieved into powder using a sieve. 200g of the powdered sample were extracted with 1L of hydroethanolic solution 50% (v/v) by maceration. The crude preparation was filtered through Whatman paper n^{o} 1 and concentrated under reduced pressure in a rotary evaporator to produce a crude extract, which was placed in a lyophilizer (4 atm of pressure and temperature of -40^{o}C) for 48 h. The lyophilized extracts were stored in amber flasks at 5^{o}C (freezer).of hydro-ethanol and allowed to stay for 48 hours with periodic stirring. The solution was filtered using whatman filter paper; the filtrate was then placed in the ovum at 80^{0} C for 8 hours for complete drying.

Experimental Animals

The animal used were 24 albino rats of either sex, purchased from Ahmadu Bello University Zaria, Kaduna State, Nigeria on the 13th of January, 2022 and were acclimatized for the period of two weeks given them only feeds and water. The rats were divided into four (4) groups, six (6) rats each in group I, II, III and IV. The rats in group I were not induced liver injury, they served as positive control, whereas rats in groups II, III, and IV were induced liver damage using 120 mg/kg CCl_4, following the methodology of.[6] Rats in group II were not administered mint leaf extracts but were administered orally with 10mg/kg Livolin, and they served as negative control. Rats in groups III and IV were administered with a daily dose of 500mg and 1000mg /kg body weight of mint leaves extract. Two (2) rats were removed from each group after 2, 5 and 8 days respectively and sacrificed.

Blood collection and preparation of tissue sample

Animals were anaesthetized 24hours after the last treatment on the 9th day with diethyl ether prior to dissection and blood samples were collected through cardiac puncture into lithium heparinized bottles for aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and total bilirubin (TB) assays. Serum was obtained by centrifuging the blood at 4000 rpm for 15 minutes into 2ml tubes and stored at -20^{o}C until required for the assays. The liver was excised and washed in normal saline (0.9%NaCl) and a portion fixed in 10%formaldehyde for Histopathological examination.

Histological analysis

Immediately after sacrifice, section of the liver was fixed in 10 % formalin. The fixed liver sections were embedded in paraffin, 5-6µm thick, stained in hematoxylin-eosin. This was then examined under compound microscope for determination of histopathological changes.

Biochemical Analysis

Serum was separated and analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of[7], serum alkaline phosphatase (ALP) activity by the method of[8], total bilirubin (TB)[9] and serum total protein and albumin by the method of.[10]

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. $P < 0.05$ was considered as statistically significant.

RESULTS

Table 1: Serum ALT, AST, ALP, TB, CB, TP and ALB activities after 48hrs of intramuscular administration of CCL4.

Parameters	ALT(U/L)	AST(U/L)	ALP(U/L)	TB(µmol/L)	CB(µmol/L)	TP (g/L)	ALB(g/L)
Normal control	8.27±0.20^{a}	20.30±0.3^{a}	31.5±0.32^{a}	4.15±0.13^{a}	3.26±0.25^{a}	58.43±0.53^{a}	30.7±0.33^{a}
120mg/kg CCl_4	34.70±0.74^{b}	109.8±0.2^{b}	51.1±0.9^{b}	10.71±0.47^{b}	9.09±0.11^{b}	61.58±1.26^{a}	32.4±0.46^{a}

KEY: Values (mean ± standard error of the mean) of 2 determinations (n=2). Values in the same column with different superscripts differ significantly ($p < 0.05$). CCl_4, Carbon tetrachloride; ALT, Alanine transaminase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; TB, Bilirubin; TP, Total protein; ALB, Albumin; CB, Conjugated Bilirubin.

Table 2 Serum ALT, AST, ALP, TB, CB, TP and ALB activities in rats after oral administration of Mint leaves extract for five days.

Parameters	ALT(U/L)	AST(U/L)	ALP(U/L)	TB(µmol/L)	CB(µmol/L)	TP(g/L)	ALB(g/L)
Normal control	8.27 ± 0.75^{a}	3.57±0.41^{a}	30.1±0.35^{a}	4.04±0.40^{a}	3.12±0.02^{a}	57.88±0.62^{a}	30.5±0.47^{a}
10mg/kg LIV	15.28±0.26^{b}	9.05±2.8^{c}	60.1±0.35^{b}	8.88±0.13^{d}	5.47±0.21^{b}	63.17±0.63^{b}	31.1±0.13^{a}
500mg/kg MLE	18.62±0.48^{c}	68.56±0.7^{d}	71.7±0.73^{c}	8.50 ±0.49^{b}	11.15±0.20^{b}	65.52±0.67^{c}	30.8±0.44^{a}
1000mg/kg MLE	15.53±0.28^{b}	32.56±1.2^{b}	59.7±0.80^{b}	7.33±0.53^{c}	11.79±1.21^{b}	61.57±0.72^{b}	30.8±0.72^{a}

KEY: Values (mean ± standard error of the mean) of 2 determinations (n=2). Values in the same column with different superscripts differ significantly ($p < 0.05$). LIV, *Livolin;* MLE, *Mint* leaf extract; CCl_4, Carbon tetrachloride; ALT,

Alanine transaminase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; TB, Bilirubin; TP, Total protein; ALB, Albumin; CB, Conjugated Bilirubin.

Table 3: Serum ALT, AST, ALP, TB, CB, TP and ALB activities in rats after oral administration of Mint leaves extract for eight days.

Parameters	ALT(U/L)	AST(U/L)	ALP(U/L)	TB(µmol/L)	CB(µmol/L)	TP(g/L)	ALB(g/L)
Normal control	8.17± 0.30[a]	2.67±0.54[a]	30.15±0.54[a]	4.25±0.25[a]	3.49±0.38[a]	59.52±0.97[a]	31.06±0.11[a]
10mg/kg LIV	15.04±0.50[b]	25.98±0.81[b]	49.62±0.72[b]	9.48±0.67[b]	11.25±0.67[b]	60.09±0.15[a]	32.01±0.25[a]
500mg/kg MLE	16.21±0.20[b]	28.25±0.26[b]	62.90±0.95[c]	10.14±0.35[b]	10.46±0.43[b]	62.02±1.69[a]	31.39±0.50[a]
1000mg/kg MLE	14.85±0.65[b]	24.16±0.41[c]	46.77±2.19[d]	10.48±0.61[b]	11.88±0.36[b]	50.61±0.53[a]	30.90±0.53[a]

KEY: Values (mean ± standard error of the mean) of 2 determinations (n=2). Values in the same column with different superscripts differ significantly ($p < 0.05$). LIV, *Livolin*; MLE, *Mint* leaf extract; CCl_4, Carbon tetrachloride; ALT, Alanine transaminase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; TB, Bilirubin; TP, Total protein; ALB, Albumin; CB, Conjugated Bilirubin.

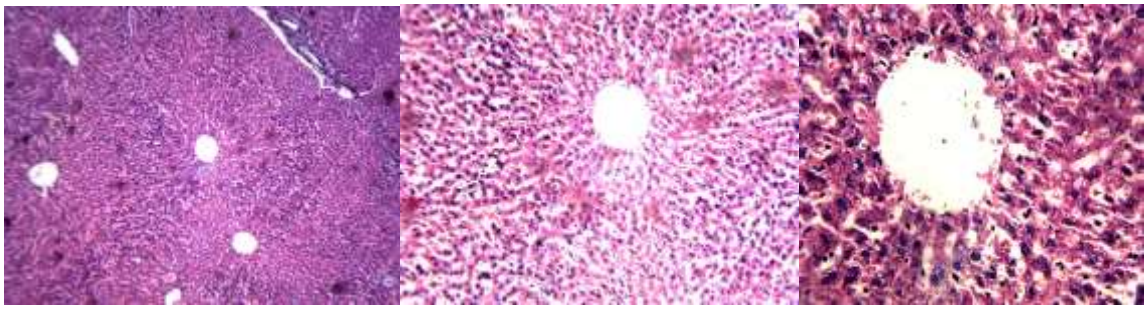

Figure1. Group 1 at x40, 100 and 250 respectively, at all magnifications Normal liver histoarchitectures were seen.

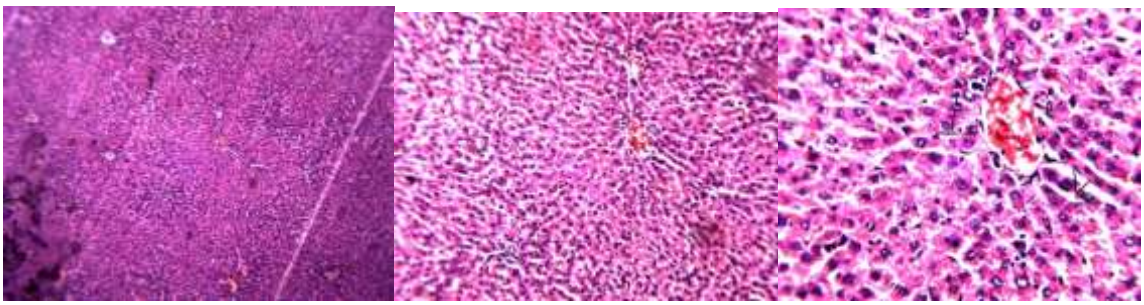

Figure 2: Group 2 at x40, 100 and 250 respectively, at x400 shows damaged hepatocytes.

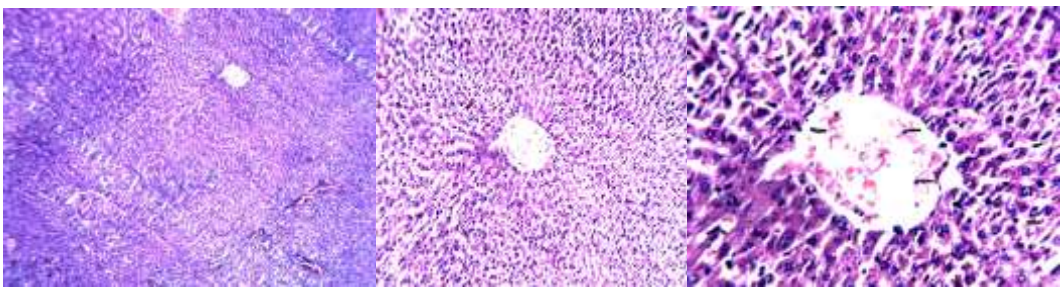

Figure 3: Group 4 at x40, 100 and 250 respectively, revealed the normal architecture of liver section.

DISCUSSION

From the results, Rats injected with 120 mg/kg CCl_4 had significantly higher ($p<0.05$) serum AST, ALT, ALP, TB, CB, TP and ALB than the normal rats (Table 1). In Table 2, rats treated with daily dose of 500mg/kg, mint leaf extracts for 5 days not significantly higher ($p>0.05$) than the control group. This is an indication of possible fibrosis, the initial repair mechanism of liver injury, as indicated by[11] that all cellular damage induces fibrosis as a healing response. However, the healing effect is more pronounced in groups that received daily doses of 1000mg/kg and the negative control group treated with daily doses of 10mg/kg of Livolin for 8 days compared to group III rats which received a daily dose of 500mg/kg; this may be possibly due to low serum bioavailability of the extract. In Table 3, rats in groups IV which received 1000mg/kg daily doses of the mint

leaf extract, for 8 days showed significantly lowered ($p<0.05$) serum levels of ALT, AST, ALP, TP and ALB while there was significantly higher ($p<0.05$) levels of TB and normalised CB compared to the negative control.

Carbon tetrachloride is widely used for the experimental induction of liver damage. CCl_4 at a dose of 120mg/kg body weight (into olive oil 1:1) used in the induction of liver injury in rats showed remarkable elevation of liver enzymes. Histopathological examination showed hepatic necrosis, hemorrhage and intense vacuolation. But administration of the extracts showed its ability to reserve the normal structure of hepatocytes.

The possible hepatocurative effects of mint leaves extract could be attributed to the chemical composition of Mint leaves. *Mentha piperita* is rich in fats, protein, some mineral elements and Vitamins.[12] The presence of Fe and Cu also have roles in hepatic healing being required by lysine and proline hydroxylase respectively, the activities of these enzymes are required for maturation of collagen, this enhances the healing of injured hepatocytes as it has been part of management of hepatitis to reduce fat and protein rich diet efficiency of oxidative phosphorylation in rat liver mitochondria.[13] Cellular respiration, occurring only in the presence of oxygen, results in the breakdown of nutrient molecules to generate ATP. Cells such as the liver and muscle use this ATP for energy to fuel during various processes like stimulating the uptake of nutrients, repair of dead or damaged tissue.[14]

CONLUSION

It can be concluded that *Mentha piperita* leaf extracts possessed a protective effect against CCL_4 induced hepatotoxicity in rats as evidenced by the biochemical, histological parameters. However the hepatoprotective activity of the mint leaves extract is dependent on dosage and period of administration.

REFERENCES

1. Hostettmann, K., Marston, A., Ndjoko, K. and Wolffender, J., (2000). The potential of African plants as a source of drug, Curr. Org. Chem, 4: 973-1010.
2. Ahmed, I., Agil, F. and Owais M., (2006). Modern Phytomedicine, Turning Medicinal Plant into Drugs, John Wiley and Sons, West- Sussex England, 2-24.
3. Abbaszadeh B., Valadabadi S.A., Farahani H.A., Darvishi H.H. (2009). Studying of essential oil variations in leaves of Mentha species. African Journal of Plant Science, 3(10): 217–221.
4. Breckell, C., and T. Cole (2002). The American horticure society; Encyclopedia of pants and flowers. New York, NY; DK publishing. ISBNs 0789489937.
5. MacSwean, R.N.M., (1980). Liver, biliary tract and exocrine pancrease, In: Murs Textbook of Pathology, (Ed J.R. Anderson), Edward Arnold Publishers Ltd., London, pp. 663-654.
6. Alhassan, A.J., Sule, M.S., Hassan, J.A., Baba, B.A. and Aliyu, M.D., (2009b). Ideal hepatotoxicity model in rats using carbon tetrachloride (CCl4), Bayero Journal of Pure and Applied Sciences, 2(2): 185-187.
7. Reitman, S. and Frankel, S., (1957). A colorimetric method for the determination of serum Glutamic Oxaloacetate and Glutamic Pyruvic transaminases, *Amer. J. Clin. Path*, 28: 5661.
8. Rec, G.S.C.C., (1972). Colorimetric method for serum alkaline phosphatase determination, *J. Clin. Biochem*, 10(2): 182.
9. Malloy, H.T. and Evolyn, K.A., (1937). Recommendation on a uniform Bilibirun standard, *J. Biol. Chem*, 119: 481.
10. Chawla, R., (1999). Practical Clinical Biochemistry (Methods and Interpretations), Second Ed., Jaypee Brothers Medical Publishers, New Delhi, India, 106-118.
11. Burtis C.A., Ashwood E.R. and Boder B.G., (2001). Tietz Fundamentals of Clinical Chemistry, 5th Ed., W.B. Saunders Company, 352-770.
12. Lugg , Liang CY, Li WL(2008) Analysis on Chemical Components of Volatile Oil from *Mentha piperita* L. J Anhui Agric Sci, 36: 400, 425.
13. Visser, S.A., (1987). Effect of humic substances on mitochondrial respiration and oxidative phosphorylation, The Science of the Total Environment, 62: 347.
14. Nelson, D. and Cox, M., (2006). Lehninger Principles of Biochemistry, 4nd Edn., Worth Publishers, New York, pp. 481-488.