



## EVALUATION OF HEPATOPROTECTIVE AND LIPOTROPIC EFFECT OF *MENTHA PIPERITA* LEAF AGAINST CARBONTETRACHLORIDE-INDUCED HEPATIC DAMAGE IN RATS

Rajagopalan Prabu<sup>\*1,2</sup>, Sunhare Raksha<sup>3</sup>, Niranjana Suralikerimath<sup>2</sup>, KR. Nagulendran<sup>1,4</sup>

<sup>1</sup> Department of Biochemistry, Ponnaiyah Ramajayam College, Thanjavur, Tamilnadu, India.

<sup>2</sup>Hindustan Petroleum Green R&D Centre, KIADB Industrial Area, Tarabanahalli, Devenagundi, Hoskote, Bangalore, India.

<sup>3</sup>Genetic Medicine Research Centre, Clinical Genetics Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

<sup>4</sup>Gerophyta micro algal Biotechnological Research and Training Centre, Illupur, Pudukottai, Tamil Nadu, 622102, India.

### ABSTRACT

Herbal and plant origin crude drugs with anti-lipid peroxidative activity had become a central focus for study of hepatoprotection due to its safety, efficacy and cost effectiveness. The aim of present study is to analyse the hepatoprotective and lipotropic effect of *Mentha piperita* leaf in CCl<sub>4</sub> induced free radical mediated oxidative stress rats. In the present study, an attempt has been made to evaluate the antilipid peroxidative and indirect antioxidant of crude powder of *Mentha piperita* against CCl<sub>4</sub> induced free radical mediated oxidative stress. Hepatic enzyme marker such as SGPT, SGOT and MDA, glutathione, blood serum calcium, iron and lipid profile have used to evaluate hepatoprotective and lipotropic effect. The result exhibits the significant amount of hepatic cell regeneration processes though the reduction of SGPT, SGOT and MDA as well as increased in the level of glutathione towards the normal value. Similarly, lipid profile, serum calcium and iron attained towards normal of CCl<sub>4</sub> induced oxidative stress rats after treated with the crude *Mentha piperita* extract. In conclusion, *Mentha piperita* leaf extracted may play a protective role against liver dysfunction and toxicity with the enhancement of hepatic cell regeneration processes.

**Keywords:** Herbal drugs, *Mentha piperita*, Oxidative stress, Hepatoprotection.

### INTRODUCTION

The liver is the major and complex organ that maintained internal environment of the body through its multiple and diverse functions. It plays an important role in handling the metabolism and excretion of drugs, and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying those [1]. Hence, any injury or impairment of hepatic cell has grave implication for the health of the affected person. Beside the viral infection, xenobiotics, excessive drug therapy, environmental pollutants and chronic alcohol ingestion can also cause severe hepatic injury; since it plays a central role in processing, metabolizing and disposition of foreign chemicals.

So far, we do not have satisfactory liver protective drugs in allopathic medical practice for serious hepatic disorders. Herbal drugs have become increasingly popular in the management of critical liver disorders by speed up the natural healing processes of the liver [2]. The Indian traditional medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Now a day, Herbal drugs have gained popularity due the efficiency and cost effectiveness. The hepatotoxic effect of CCl<sub>4</sub> is largely due to its active metabolite \*CCl<sub>3</sub> (Trichloro methyl radical), \*CCl<sub>3</sub> O<sub>2</sub> (Trichloro methyl peroxy radical). \*CCl<sub>3</sub> O<sub>2</sub> is one of the most reactive species and causes damage to biological macromolecules by combining with other macromolecules by causing the

covalent modification and it leads to lipid peroxidation and oxidative damage through free radical generation revealed to cell death [3].

Many plant and plant product protect the cell and organ system by exerting their antioxidant effect against deleterious effect of free radical mediated hepatotoxicity [4]. Currently, search for the suitable crude drugs of plant origin with anti-lipid peroxidative activity has become a central focus for the study of hepatoprotection. In the present study, an attempt has made to evaluate the anti-lipid peroxidative and hepatoprotective activity of *Mentha piperita* leaf against oxidative stress rats induced by CCl<sub>4</sub>.

## MATERIALS AND METHODS

### Animals

Adult albino wistar rats (100-130 gm) were obtained from the Indian Institute of Science, Bangalore. The animals housed in polypropylene cages and maintained in controlled temperature with alternate light and dark were fed with standard rat chow. Food and water were provided *ad libitum*. Rats were incubated a week to acclimate to the laboratory conditions. The experimental protocol was approved by the Animal Ethics Committee of the PG & Research Department of Biochemistry, Ponnaiyah Ramajayam College, Thanjavur.

### Plant material and Drug preparation

Fresh matured leaves of *Mentha piperita* were collected locally in the month of January from herbal garden, Ponnaiyah Ramajayam College, Thanjavur. The leaves were shade dried and powdered. The powder was extracted with methanol according to the maceration method and the extract was filtered by Whatman no. 1 filter paper. The filtrate was concentrated in a rotary evaporator at 40°C and concentrated by oven dried at 40°C for 3 days. The freeze dried (48 h) extracts was stored at -20°C and used as crude *Mentha piperita* (CMP) drug.

### Induction of oxidative stress and hepatic damage

Liver damage was induced in rats by administrating CCl<sub>4</sub> intraperitoneally in suspension of liquid paraffin (LP) 1:2 V/V at the dose of 1ml CCl<sub>4</sub>/Kg body weight of each animal. CCl<sub>4</sub> was administered on every first and fourth day of the week for a period of 14 days. Body weights of animals were recorded and they were divided into five groups of 8 animals each as follows.

**Group 1:** Control animals received liquid paraffin only at the dose of 3 ml/kg body weight along with standard feed and water *ad libitum*.

**Group 2:** Hepato toxic group received intraperitoneal administration of LP+CCl<sub>4</sub> twice a week for 14 days.

**Group 3:** Herb treated animals received LP+CCl<sub>4</sub> as mentioned above, and simultaneously received CMP by oral intubation in a suspension of 1ml water at the dose of 80 mg/kg body weight daily for 14 days.

**Group 4:** Drug positive control animals received CMP alone as mentioned above.

**Group 5:** Standard drug treated animals received LP+CCl<sub>4</sub> as mentioned above and simultaneously receive standard drug Silymarin by oral intubation in a suspension of 1ml water at the dose of 20 mg/kg body weight.

After the completion of experimental regimen, the rats were fasted overnight and blood samples were collected by the puncturing the retro orbital plexus under light ether anaesthesia. Subsequently animals were sacrificed with overdose ether and the liver was excised immediately for analysis.

### Homogenate preparation

The liver samples were homogenized in phosphate buffer solution (0.1 M, pH 7.4, 4 (C) for the production of 10% homogenate in cold condition. The total protein was measured according to the Bradford [5] method using Bovine serum albumin (BSA; Sigma, USA) stock solution (1 mg/mL) as a standard. Then 10-20 µl of crude samples was mixed with the Bradford dye and the absorbance was measured at 595 nm.

### Analytical methods

Blood was collected and allowed to clot at room temperature. Subsequently, serum was separated by centrifugation at 2500 rpm for 10-13 min at 4°C and used for biochemical parameters estimation to determine the functional state of the liver. Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel [6]. Malondialdehyde (MDA) [7], reduced glutathione [8], serum iron [9], serum calcium [10], triglycerides [11], High-density lipoprotein (HDL) [12] and total serum cholesterol [13] were calculated.

## RESULTS

The present study was carried out to evaluate the antioxidant property of CMP against induced oxidative stress. The observations made on different groups of experimental animals and control animals were compared in Table 1. CCl<sub>4</sub> intoxicated rats showed a significant decrease in the levels of TGL, VLDL, HDL, total cholesterol and serum calcium to compare with non-toxicated rats. However, CCl<sub>4</sub> toxicated along with CMP treated rats showing significant elevation in the levels of TGL, VLDL, HDL, total cholesterol and serum calcium. Similarly, the drug alone treated rats showed changes in serum HDL level, which is higher than standard drug Silymarin.

CCl<sub>4</sub> toxicated animal shows higher iron content in serum as compared with control rats and, were recovered after CMP treatment. This result closely associated with standard drug and non-toxicated rats. Same time, liver marker enzymes SGOT, SGPT and MDA level

elevation and depletion of glutathione in serum showed hepatic cell harm by the presence of CCl<sub>4</sub>. The damaged rats hepatic cell were showing significant improvement after treated with CMP drug. In addition, positive drug administrated animals showed reverse action in the liver

marker enzyme level, MDA and glutathione in serum, revealed that hepatic cells are in healthy condition as compared with control rats as well as rats treated with Silymarin.

**Table 1. Effects of CMP treatment on different biochemical parameters in the serum of rats**

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	CCl <sub>4</sub> treated	CCl <sub>4</sub> + Drug	Drug Positive	Standard Drug Silymarin
Triglycerides (mg/dl)	101.52 ± 4.8	43.23 ± 2.0	61.23 ± 2.9	85.23 ± 4.1	71.41 ± 3.4
VLDL (mg/dl)	27.01 ± 1.2	19.24 ± 0.9	24.05 ± 1.2	26.37 ± 1.3	28.28 ± 1.4
Total cholesterol (mg/dl)	64.09 ± 3.5	54.21 ± 5.5	66.03 ± 3.2	57.04 ± 0.4	59.12 ± 02.8
HDL (mg/dl)	54.09 ± 3.7	32.23 ± 1.5	49.10 ± 4.5	60.03 ± 3.3	57.02 ± 6.1
Calcium (mg/dl)	9.6 ± 0.5	6.4 ± 0.4	8.5 ± 0.5	9.3 ± 1.2	9.7 ± 0.8
Iron (µg/dl)	148.23 ± 7.1	270.07 ± 12.9	182.31 ± 9.5	151.13 ± 8.6	149.08 ± 8.1
MDA (nm/mg of protein)	1.23 ± 0.1	4.21 ± 0.2	2.73 ± 0.1	1.02 ± 0.1	1.81 ± 0.1
Glutathione (mg/100gm of tissue)	130.12 ± 7.3	97.47 ± 6.1	127.25 ± 7.1	137.41 ± 6.9	128.29 ± 6.6
SGOT (U/mL)	46± 1.6	116± 3.1	70± 2.6	35± 1.1	38± 2.1
SGPT (U/mL)	33± 1.1	125± 2.1	65± 2.1	32± 1.1	34± 1.1

## DISCUSSION AND CONCLUSION

The present study evaluates the antilipid peroxidative and indirect antioxidant of crude powder of *Mentha piperita* against CCl<sub>4</sub> induced free radical mediated oxidative stress. Standard drug Silymarin was used to compare the result of *Mentha piperita* drug treated animals. CCl<sub>4</sub> induces fatty liver cell and necrosis, and plays a significant role in inducing triglyceride accumulation in parenchyma cells, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzymes Ca<sup>2+</sup> homeostasis alteration by causing hepatic cell injury or death [14]. It is well established that CCl<sub>4</sub> induce the hepatotoxicity through free radicals (\*CCl<sub>3</sub> and \*Cl) production, which disrupts the structure and function of membrane macromolecule (lipid and protein) of the cell organelles [15,16].

Fatty liver is the result of an imbalance between the synthesized and utilized hepatic triglycerides with a deficiency of lipotropic factors. Triglycerides secretion combined with glycoprotein moiety along with VLDL-C, thus help to transport of hepatic triglycerides to extrahepatic tissue [17]. Significant decrease observed in the level of triglycerides in serum in CCl<sub>4</sub> intoxicated rats showed the excessive accumulation of triglycerides in hepatic parenchyma cell [18]. Accumulation of triglycerides in hepatic cell and the blemish the synthesis of VLDL-C were revived in CCl<sub>4</sub> intoxicated rats co-administered with *Mentha piperita* drug which confirms the *Mentha piperita* lipotropic effect. The alteration in total cholesterol and HDL-C cholesterol was also found to be normalized in serum of CCl<sub>4</sub> intoxicated rats co-administered with *Mentha piperita*. CCl<sub>4</sub> free radicals bind to protein, hepatocyte membrane and elicit lipid

peroxidation, and disturb the Ca<sup>2+</sup> homeostasis which damage to plasma membrane [19]. Depletion in serum calcium level of CCl<sub>4</sub> intoxicated rats clearly presented that the calcium influx into hepatocyte and cell necrosis. On the other hand, co-administration of *Mentha piperita* maintains Ca<sup>2+</sup> homeostasis in serum of CCl<sub>4</sub> intoxicated rats and thereby stabilize the integrity of plasma membrane damage by CCl<sub>4</sub>. Iron is also an essential element for maintaining proper cell function. Conversely, iron overload may lead in deleterious reactions such as degradation of protein, nucleic acid and peroxidation of PUFA [20]. CCl<sub>4</sub> intoxicated rats co-administered with the crude drug of *Mentha piperita* enhance the level of serum iron to near normal and thereby reduce oxidative stress associated with Fenton reaction mediated free iron overload, due to the *Mentha piperita* chelating property.

The liver is viewed as a glutathione generating factor, which supplies glutathione re-synthesis constituents to kidney and intestine [21]. Reduced glutathione is the most abundant in mammalian tissues, which involved in the protection of the cell against damage from electrophiles free radicals and reactive oxygen species (ROS) formed during xenobiotic metabolism [22]. To prevent the lipid peroxidation by CCl<sub>4</sub>, reduced glutathione acts as a hydrogen donor instead of abstracting the hydrogen from methylene hydrogen of the membrane polyunsaturated fatty acids and the free radicals of CCl<sub>4</sub> abstract the hydrogen from SH group of reduced glutathione. Hepatic cells are normally protected from injury by the conjugation of toxic metabolites in the presence of hepatocyte glutathione content available for detoxification. When glutathione is exhausted, the hepatocyte becomes vulnerable to the noxious effect of the metabolite, resulting in necrosis. Decline in the hepatic glutathione content of

CCl<sub>4</sub> intoxicated rats observed in the present study is due to its increased utilization in protecting SH group containing protein from the activation of <sup>\*</sup>CCl<sub>3</sub> and O<sub>2</sub><sup>\*</sup> radical produced from CCl<sub>4</sub> by the cytochrome p450 system.

The study of different enzyme activities such as SGOT, SGPT, and total protein have been found to be of significant value in the assessment of clinical and experimental liver damage. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids. In the present study, it was observed that the animals treated with CCl<sub>4</sub> resulted in significant hepatic damage as shown by the elevated levels of serum markers. Reduction

in the levels of SGPT, SGOT and MDA as well as increased in the level of glutathione towards the normal value is an indication of the regeneration process. The above result concluded that *Mentha piperita* leaf extracted may play a protective role against liver dysfunction and toxicity.

#### ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support from the department of Biochemistry, Ponniya Ramajayam College, Tamilnadu, India. Technical help received from Dr. Ramesh Thiyaghu, M. Suman and Sharmila Srinivasan are gratefully acknowledged.

#### REFERENCES

1. Saleem TSM, Chetty CM, Ramkanth S, Alagusundaram M, Gnanaprakash K, Thiruvengada Rajan VS, Angalaparameswari S. Hepatoprotective Herbs. *Int J Res Pharm Sci*, 1, 2010, 1-5.
2. Debasish D, Sourav G, Subhasish M, Rabiul H, Samir S. Study of hepatoprotective effect of *Hygrophila difformis* L.f. Blume (Acanthaceae) roots against paracetamol induced hepatotoxicity in rats. *Int J Ph Sci*, 1, 2009, 323-329.
3. Aksenova MV, Michael Y. Aksenov Mactutus CF, Booze RM. Cell culture models of oxidative stress and injury in the central nervous system. *Curr Neurovasc Res*, 2, 2005, 73-89.
4. Nishigaki I, Kuttan R, Oku H, Ashoori F, Abe H, Yagi K. Suppressive effect of curcumin on lipid peroxidation induced in rats by CCl<sub>4</sub> or cobalt-60 irradiation. *J Clin Biochem Nutr*, 13, 1992, 23-30.
5. Bradford MM. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem*, 227, 1976, 680-685.
6. Reitman S, Frankel AS. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol*, 28, 1957, 53-56.
7. Nichans WG, Samuelson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem*, 6, 1968, 126-130.
8. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82, 1959, 70-77.
9. Silva LFD, Oliveira JEDD, Marchin JS. Serum iron analysis of adults receiving three different iron compounds. *Nutr Res*, 24, 2004, 603-611.
10. Clark and Collip. *Hawk's Physiological Chemistry*. McGraw-Hill Publisher, London, 1925.
11. Rice EW. Triglycerides (neutral fat) in serum. *Stand Methods Clin Chem*, 6, 1970, 215-219.
12. Gidez LI, Miller GJ, Burstein M Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res*, 23, 1982, 1206-1210.
13. Zak B, Boyle AJ, Zlatkis A. A method for the determination of cholesterol *J Lab Clin Med*, 41, 1953, 486-492.
14. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. *J Ethnopharmacol*, 90, 2004, 229-232.
15. Hodgson E. A textbook of modern toxicology. 3rd edn. John Wiley & Sons, New Jersey, 2004, 269-270.
16. Rajendran R, Hemalatha S, Akasakala, K. Hepatoprotective activity of *Mimosa pudica* leaves against Carbontetrachloride induced toxicity. *J Nat Prod*, 2, 2009, 116-122.
17. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Illustrated Biochemistry, 26 edn. The McGraw-Hill Companies, USA, 26, 2000, 205-218.
18. Khalaf AAA, Mekawy, MEM, Moawad, MS, Ahmed AM. Comparative study on the protective effect of some antioxidant against CCL4 hepatotoxicity in rats. *Egy J Nat Toxins*, 6, 2009, 59-82.
19. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanism of carbon tetrachloride toxicity. *Pharmacol Therapeut*, 43, 1989, 139-54.
20. Reifen R, Matas Z, Zeidel L, Berkovitch Z, Bujanover Y. Iron supplementation may aggravate inflammatory status of colitis in a rat model. *Dig Dis Sci*, 45, 2000, 394-397.
21. Rana SV, Allen T, Singh R. Inevitable glutathione, then and now. *Indian J Exp Biol*, 40, 2002, 706-16.
22. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal, Applications in research and therapy. *Pharmacol Therapeut*, 51, 1991, 155-194.