

## ANTI-OXIDANT AND HEPATOPROTECTIVE ACTIVITY OF PHENOLIC COMPOUNDS OF LEAVES EXTRACTS FROM *MENTHA LONGIFOLIA* AND *MENTHA SPICATA* IN DIABETIC MALE RATS

Eman A. Al-Rekabi\*

Department of Biology, College of Science, University of Thi-Qar, Iraq.

Article Received on  
27 March 2015,

Revised on 19 April 2015,  
Accepted on 11 May 2015

\*Correspondence for

Author

Dr. Eman A. Al-Rekabi

Department of Biology,  
College of Science,  
University of Thi-Qar,  
Iraq.

### ABSTRACT

Diabetes mellitus is one of the most widely distributed metabolic disorders and occurs in almost all populations of the world at a variable prevalence. The present study was conducted to investigate the effect of phenolic compounds extracted from *Mentha longifolia* and *Mentha spicata* on serum malondialdehyde (MDA), ceroloplasmine (CP), body weight, liver enzymes Alanine Transaminase (ALT) and Aspartate Transaminase (AST) level of diabetic male rats. One dose (200 mg/kg) from each plant were used and the animals were injected intraperitoneally for 14 days as one dose/day. The results showed a significant decrease ( $P < 0.01$ ) in the serum (MDA) and (CP) level and a significant increase ( $P < 0.01$ ) in the body weight of the male rats

treated with phenolic extractes of *M. longifolia* and *M. spicata* at dose 200 mg/kg. and the results showed a significant decrease ( $P < 0.01$ ) in the serum (AST) and (ALT) level of the male rats treated with phenolic extractes of *M. longifolia* and *M. spicata* at dose 200 mg/kg.

**KEYWORDS:** *Mentha spicata*, *Mentha longifolia*, MDA, CP, AST, ALT, diabetes mellitus, phenolic compounds.

### 1. INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion or insulin action, or both.<sup>[1]</sup> It is well documented that chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually the failure of organs especially the eyes, kidneys, nerves, heart and blood vessels.<sup>[2,3]</sup> Many herbs have been shown to have antidiabetic action in both human and animals.<sup>[4]</sup> Medicinal plants are of great importance to the health of individuals. The medicinal value of these plants

lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.<sup>[5]</sup> The herbs have been used as antiphlogistic, diuretic, expectorant and antidiabetic in traditional Chinese medicine.<sup>[6]</sup> The phenolic or polyphenols embraces a wide range of plant substances possessing in common an aromatic ring with one or more hydroxyl groups. These compounds, located in the vacuole tend to be water-soluble as they occur in combined forms with sugars as heterosides. Furthermore, the phenolic have the advantage of being the most widely distributed than other secondary metabolites. They are widespread in the plant kingdom.<sup>[7]</sup>

*Mentha* is a genus of aromatic perennial herbs belonging to the family of Lamiaceae. It is distributed mainly in the temperate and sub-temperate regions of the world. This family contains a wide range of compounds such as terpenoids, iridiods, phenolic compounds and flavonoides have been reported from the members of the family.<sup>[8,9]</sup> The present study aimed to investigate the effect of phenolic compounds from *Mentha longifolia* and *Mentha spicata* in the serum (MDA), (CP), liver enzymes Alanine Transaminase (ALT), Aspartate Transaminase (AST) level and the body weight in diabetic male rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant material and powder preparation

Fresh, spearmint and horsmint were obtained from the local market in Thi-Qar province, Iraq. Each plant leaves were cleaned and dried under shade at room temperature (25°C), samples were ground to a powder form using electrical grinder, and the powder was kept in refrigerator in clean container until using.

### 2.2 Phenolic compounds reagents

General phenols were detected according to Jaffer *et al.*<sup>[10]</sup>

### 2.3 Extraction of crude phenolic compounds

Crude Phenolic compounds were extracted according to Riberean-Gayon,<sup>[11]</sup>

### 2.4 Induction of diabetes mellitus

The animals were fasted for 12 hr and diabetes was induced by a single intraperitoneal (IP) injection of alloxan monohydrated (BDH, England) dissolved in D.W at a dose of 125 mg.kg<sup>-1</sup> body weight in a volume of 0.5 ml. The diabetic state was confirmed 7 day after alloxan

injection by the blood serum. Sugar value was greater than 200 mg/dl (hyperglycemia). Survived rats with a fasting blood glucose level higher than 200 ml /dl were included in the study.<sup>[12]</sup>

## 2.5 Experimental design

The study was carried out on twenty four mature male rats (*Rattus norvegicus*), age as 10-12 weeks and weighing between 180 – 200 gm were procured from Department of Biology, College of Science, University of Thi Qar, Iraq. The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. The animals were divided into four equal groups, each group consist of (6) rats.

- 1- The first group(control group) was injected by (0.5ml/animal/day) from normal physiological saline (0.9% NaCl).
- 2- The second group was injected by (0.5ml/animal/day) with alloxane (125mg/kg).
- 3- The third group was injected by (0.5ml / animal/day) with alloxane (125mg/kg) ,after week, this group was injected by (0.5ml/animal/day) of (200mg/kg) of *M. longifolia* phenolic extract.
- 4- The fourth group was injected by (0.5ml / anima/day 1) with alloxane (125mg/kg) ,after week, this group was injected by (0.5ml/animal/day) of (200mg/kg) of *M. spicata* phenolic extract.

The animals were injected intraperitoneally for 14 days as one dose daily. The animals weight was measured at the end of experiment by using Animals balance.

## 2.6 Blood collection

After fourteen days of treatment, the animals were sacrificed. Subsequently, the blood samples were collected by cardiac puncture, 5mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20°C until the time of assay.

## 2.7 Measuring of serum MDA, CP, ALT and AST level

According to Muslih *et al.*<sup>[13]</sup> The level of MDA was determined by a modified procedure described by Guidet&Shah,<sup>[14]</sup> while serum Cp concentration was measured by the method of Menden *et al*<sup>[15]</sup> And serum Aspartate transaminase(AST), serum Alanine transaminase (ALT) were determined by enzymatic colorimetric methods using Atlas Medical(UK).

## 2.8 Statistical analysis

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ( $P < 0.01$ ) was considered to be statistically significant. And used to calculate least significant difference (LSD) values for the comparison of means following.

## 3. RESULTS AND DISCUSSION

The obtained results revealed a significant decrease ( $p < 0.01$ ) in body weight of the diabetic male rats compared with control group. while, the diabetic male rats treated with phenolic extractes of *M. longifolia* and *M. spicata* at dose 200 mg/kg showed a significant increase ( $p < 0.01$ ) in body weight when compared with diabetic rats and showed non-significant differences ( $p < 0.01$ ) when compared with control group (table 1).

The results showed a significant increase ( $p < 0.01$ ) in the serum level of MDA and CP of the diabetic male rats compared with control group. while, the diabetic male rats treated with phenolic extractes of *M. longifolia* and *M. spicata* at dose 200 mg/kg showed a significant decrease ( $p < 0.01$ ) in MDA and CP when compared with diabetic rats and showed non-significant differences ( $p < 0.01$ ) when compared with control group (table 1).

The results showed a significant increase ( $p < 0.01$ ) in the serum level of AST and ALT of the diabetic male rats compared with control group. while, the diabetic male rats treated with phenolic extractes of *M. longifolia* and *M. spicata* at dose 200 mg/kg showed a significant decrease ( $p < 0.01$ ) in AST and ALT when compared with diabetic rats and showed non-significant differences ( $p < 0.01$ ) when compared with control group (table 1).

**Table 1: Effect of phenolic compounds of *Mentha spicata* and *Mentha longifolia* on body weight, MDA,CP,AST & ALT level of Male rats.**

Animal groups	Body weight(g) Mean $\pm$ S.E	MDA (nmol/ml) Mean $\pm$ S.E	CP g/L)( Mean $\pm$ S.E	AST (UL) Mean $\pm$ S.E	ALT (UL) Mean $\pm$ S.E
First group	52.71 $\pm$ 8.36 <sup>a</sup>	87.42 $\pm$ 4.23 <sup>b</sup>	0.92 $\pm$ 0.03 <sup>b</sup>	42.00 $\pm$ 1.54 <sup>b</sup>	5.66 $\pm$ 0.22 <sup>b</sup>
Second group	32.14 $\pm$ 4.35 <sup>b</sup>	113.53 $\pm$ 2.38 <sup>a</sup>	1.48 $\pm$ 0.03 <sup>a</sup>	52.64 $\pm$ 2.30 <sup>a</sup>	8.13 $\pm$ 0.46 <sup>a</sup>
Third group	46.55 $\pm$ 5.47 <sup>a</sup>	86.18 $\pm$ 1.26 <sup>b</sup>	0.95 $\pm$ 0.04 <sup>b</sup>	39.50 $\pm$ 2.13 <sup>b</sup>	6.00 $\pm$ 0.41 <sup>b</sup>
Fourth group	43.54 $\pm$ 8.74 <sup>a</sup>	92.96 $\pm$ 1.69 <sup>b</sup>	0.97 $\pm$ 0.03 <sup>b</sup>	40.56 $\pm$ 2.37 <sup>b</sup>	6.66 $\pm$ 0.56 <sup>b</sup>
LSD	10.54	8.64	0.32	7.42	1.84

Values are means  $\pm$  S.E.

Different letters refer to significant differences ( $p < 0.01$ ).

Same letters refer to No significant differences ( $p < 0.01$ ).

The results revealed decrease in body weight of diabetic rats after 14 days of alloxane injection, this result is in agreement with Al-Dujaily.<sup>[16]</sup> who observed that there was a decrease in body weight of alloxane-induced diabetic rabbits. consumption of diabetic rats a larger quantity of food (hyperphagia), and the decrease in their body weight was perhaps due to the mobilization of protein and fat store which may be responsible for the body weight loss noticed in diabetic rats. Since food conversion efficiency of a diabetic animal is lower than that of non-diabetic animals.<sup>[17]</sup> This in turn results in inflammation and exacerbated oxidative stress at the whole body level, and malfunction in several organs including pancreas, liver, muscle and adipose tissue.<sup>[18]</sup> The phenolic phytochemicals in maintenance of glucose and energy homeostasis and in suppression of metabolic syndrome and diabetes as evidenced by rapidly expanding literature. However, the antioxidant role of these compounds in metabolic syndrome, extensively reviewed recently.<sup>[19,20]</sup>

The increase of MDA and CP levels of the diabetic male rats as reported in the present study; this agrees with the results of Cole *et al.*,<sup>[21]</sup> This rising in MDA level is directly associated with the degree of lipid peroxidation which is one of the most important measurement of oxidative stress in diabetes. The decreases MDA levels after treatment this result agreed with the result of Djeridane *et al.*,<sup>[22]</sup> who reported that phenolic compounds act in the scavenging of free radical and in the inhibition of lipid peroxidation, especially the flavonoids. And the result agreed with the result of AL-Gazi, *et al.*,<sup>[23]</sup> which reported that the phenolic extract of *Camellia sinensis*, *Vitis vinifera*, and *Punica granatum* have potent antioxidative and radical-scavenging. The antioxidant activity of flavonoids is of extreme importance. These polyphenolic compounds inhibit the oxidation of lipids, inhibit some of the enzyme systems, have an influence on the formation and transformation of peroxy radicals.<sup>[24]</sup> many polyphenolic compounds have attracted scientists involved in food medicine and chemistry, because of their antioxidant, and antiproliferative properties, as well as their ability to change the function of some basic cell enzymes. It has been claimed that polyphenolic compounds show their antioxidant activity in the following ways: by giving out an H-atom, by directly connecting free oxygen and nitrogen radicals, by chelating prooxidant metal ions (Fe, Cu) and by the inhibition of prooxidant enzymes (lipogenesis, myeloperoxidase, xanthine-oxidase, NAD(P)H oxidase, cytochrome enzymes P-450).<sup>[25,26]</sup>

The antioxidant activity of flavonoids is of extreme importance. These polyphenolic compounds inhibit the oxidation of lipids, inhibit some of the enzyme systems, have an influence on the formation and transformation of peroxy radicals.<sup>[24]</sup>

The increase in the activities of plasma AST and ALT of the diabetic male rats as reported in the present study indicated that diabetes may be induced hepatic dysfunction. Supporting our finding it has been found by Larcan *et al.*,<sup>[27]</sup> that liver was necrotized in diabetic patients. Therefore, the increment of the activities of AST and ALT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream<sup>[28]</sup>, which gives an indication on the hepatotoxic effect of alloxan. On the other hand, treatment of the diabetic rats with phenolic compound caused reduction in the activity of these enzymes in plasma compared to the mean values of diabetic group. These results are in agreement with those obtained by Ohaeri.<sup>[29]</sup> in rats. The reduction in liver enzyme activities (Table 1) is mainly due to leakage of these enzymes into the blood stream as a result of alloxan toxicity which leads to the liver damage. However, treatment of alloxan diabetic groups with phenolic compounds could restore the activities of the above enzymes to their normal levels. and liver is that these treatments may inhibit the liver damage induced by alloxan. It is frequently used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents.<sup>[30]</sup> Enzymes from diseased or damaged tissues may become recognizable in the serum presumably by leakage through altered cell membrane of the rat organs.<sup>[31]</sup> The result of this study indicated a significant decrease on serum AST and ALT which suggest that the extract has hepato-cellular function enhancing effect. Both plant extracts when combined not only show their abilities in lowering the levels of oxidative stress enzymes (ALT and AST) in serum, it is reliable marker of liver integrity.

## CONCLUSION

In conclusion, we demonstrate that phenolic compounds of leaves extracts from *Mentha longifolia* and *Mentha spicata*, enhanced the antioxidant defense system in an experimental diabetic model. These effects highlighted once again the phenolic compounds byproduct as a source of antioxidants able to reduce the frequency of oxidative stress-related metabolic diseases such as diabetes. In the absence of reliable liver-protective drugs in medical practice, herbs have become reliable substitutes and have so far played significant role in the management of various liver disorders and the accompanying oxidative stress.

## REFERENCES

1. Prajapati DD, Patel NM, Savadi RV, Akki KS and Mruthunjaya K. (2008). Alleviation of alloxan –induced diabetes and its complications in rats by *Actinodaphne hookeri* leaf extract., 3: 102-106.
2. Zimmet P, Alberti KGMM and Shaw J. (2001). Global and Societal implication of the diabetes epidemic. *Nature.*, 414: 782-787.
3. Susheela T, Balaravi P, Theophilus J, Reddy TN and Reddy PUM. (2008). Evaluation of hypoglycemic and antidiabetic effect of *Melia dubia* CAV fruits in mice. *Current Science.*, 94: 1191-1195.
4. Bhattaram VA, Ceraefe M, Kohlest C, Vest M and Deundorf H. (2002). Pharmacokinetics and bioavailability of herbal medicinal products. *Phytomed.*, 9: 1-36.
5. Shylaja, H.; Lakshman, N. and Vikas, G. (2008). Analgesic and anti-inflammatory activity of topical preparation of lantana camara leaves. *Pharmacology online.*, 1: 90-96.
6. Attaur, N.; Rahman, T. and Choudhary, M. (2001). Shairinginnovative Experiences. Page 8.
7. Tan, J.; Schneider, B.; Svatos, A.; Bednarek, P.; Liu, J. and Hahlbrock, K. (2004). Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves. *Phytochemistry.*, 65: 691-699.
8. Richardson, P.(1992). The chemistry of the Labiatae: An introduction and overview. In: Harley RM and Reynolds T. (Eds.) *Advances in Labiatae Science*. Botanical Garden Kew, 291- 297.
9. Zegorka, G. and Glowniak, K.(2001). Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *J. Pharm. Biomed. Anal.*, 26: 179-187.
10. Jaffer, H.J.; Mahmood, M.J.; Jawad, A.M.; Najis, A. and AL-Naib, A. (1983). *Phytochemical and Fitoterapia* LIX, 229.
11. Riberean-Gayon, P. (1972). Plant phenolics Oliver and body. U.S.A. PP 254.
12. Alarcon-Aguilara, F. J.; Romas, R.; Perez-Gutierrez, S. ; Aguilar-Contreras, A.; Contreras-Weber, C.C. and Flores-Saenz, J. L. (2002). Study of antihyperglycemic effect of plant used of antidiabetic. *J. Ethnopharmacol*, 61(2): 101 – 110.
13. Muslih , R.K.; Al nimr, M.S. ; Mizi'l , O. and Al – Zamely ,Y. (2002) : The level of malondialdehyde after activation with (H<sub>2</sub>O<sub>2</sub> and CuSO<sub>4</sub>) and inhibition by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infarction . *national journal of chemistry.*, 5: 139-148.

14. Guidet, B. R. and Shah, S. V. (1989): In vivo generation of hydrogen peroxide by rat kidney cortex and glomeruli. *Am. J. Physiol.* 256 (Renal Fluid Electrolyte Physiol., 25: F158-F164.
15. Menden, C.; Boian, J.; Murthy, L. and Petering, H.G. and Anal, L. (1977). Plasma antioxidant. *Anal Lett*, 10: 197.
16. Al-Dujaily, S.S.M. (1989). Cardiac Structural Functional Aspects in Experimentally Induced Diabetes Mellitus. M. Sc. Thesis, University of Baghdad, College of Veterinary Medicine., 59.
17. Kechrid, Z. and Bouzerna, N. (2004). Effect of zinc deficiency and experimental diabetes on glutamate oxaloacetate, glutamate pyruvate aminotransferases and alkaline phosphatase activities in rats. *Int. J. Diabetes and Metabolism.*, 11: 14-18.
18. Lann, D.; Gallagher, E. and Leroith, D.(2008). Insulin resistance and the metabolic syndrome. *Minerva Med.* 99: 253–262.
19. Maritim, A.C.; Sanders, R.A. and Watkins, J.B., III.(2003). Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.*, 17: 24–38.
20. Vincent, H.K.; Innes, K.E. and Vincent, K.R.(2007). Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes. Metab*, 9: 813–839.
21. Cole, G. p.; lim, F.; Yang, B.; Teter, A.; Begum, Q.; Harris, W. and Frautschy, P. (2005). Prevention of Alzheimer Oega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol. Aging*, 26: S133-S136.
22. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P. and Vidal, N. (2006). *Food Chemistry.*, 97: 654.
23. AL-Gazi, S.; Al-Salih, R. and Al-Fartosi, K. (2010). Study of some plant polyphenolic extracts as a hypoglycemic and antioxidant agents. Master, thesis collage of science, Thi-Qar university.
24. Ćetković, G. (2008). The chemistry of natural products, Faculty of Technology, University of Novi Sad, in Serbian.
25. Rice-Evans, C.; Miller, N. and Paganga, G.(1996). *Free Radical Bio. Med.*, 20: 933-956.
26. Amorati, R.; Pedulli, G.F. ; Cabrini, L.; Zambonin, L. and Landi, L.(2006). *J. Agric. Food Chem.*, 54: 2932-2937.



27. Larcán, A., Lambert, H., Laprevote-Heully, M.C and Delorme, N., (1979). Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients. *Diabetes Metabolism.*, 5: 103–112.
28. Navarro, C.M., Montilla, P.M., Martín, A., Jiménez, J .and Utrilla, P.M., (1993). Free radicals scavenger and antihepatotoxic activity of Rosmarinus. *Plant Medicine.*, 59: 312–314.
29. Ohaeri, O.C., (2001). Effect of garlic oil on the levels of various enzyme in the serum and tissue of streptozotocin diabetic rats. *Bioscience and Reproduction.*, 21: 19–24.
30. Akanji, M. A.; Olagoke, O. A & Oloyede, O. B (1993). Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. *Toxicol.*, 81: 173-179.
31. Akanji, M. A & Ngaha, E. O. (1989). Effect of repeated administration of berenil on urinary excretion with corresponding tissue pattern in rats. *Pharmacol. Toxicol.*, 64: 272-275.