Effect of phenolic compoundsof leaves extracts from Mentha longifolia and Mentha spicata on somebiochemical parameters of female rats

Khalid G. Al-Fartosi

Eman A. Al-Rekabi

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

Abstract:

The objective of this study was performed toextract the phenolic compounds from *Mentha* longifoliaand Mentha spicata, and to shed a light on their activity against oxidant- antioxidant status and their effect on lipid profile in female rats. Two doses (200 and 400 mg/kg) from each plant were used and the animals were injected intraperitonially for 30 days as one dose/day. The results showed a significant decrease in the MDA and CP of the female rats treated with phenolic extracts of *M. longifolia* at dose (200-400) mg/kg and phenolic extracts of *M. spicata* at dose 200 mg/kg, and a significant decrease in the serum level of cholesterol and TG of the female rats treated with phenolic extracts of*M. longifolia* at dose (200-400) mg/kg and phenolic extracts of *M. spicata* at dose 200 mg/kg, and The results showed a significant increase in the HDL and a significant decrease in the LDL with phenolic extracts of *M. longifolia* at dose (200-400) mg/kg when compared with control group, which treated with normal saline. While the female rats treated with phenolic extracts of *M. longifolia* and *M. spicata* at dose (200,400) mg/kg showed non significantdecrease in plasma VLDL, when compared with control group.

Key Words: *Mentha spicata, Mentha longifolia*, phenolic compounds, MDA, CP, HDL, LDL, VLDL.

تأثير المركبات الفينولية المستخلصه من اوراق نباتي *Mentha longifolia* البطنج في بعض المعايير الكيموحيوية لإناث الجرذان المختبرية والنعناع *spicata*

خالد گاطع الفرطوسي

إيمان علي الركابي

قسم علوم الحياة-كلية العلوم-جامعة ذي قار

الخلاصة:

هدفت الدراسة الحالية لاستخلاص المركبات الفينولية من نباتي البطنج والنعناع البحث في فعاليتها كمضادات أكسده وتأثيرها على دهون الدم لإناث الجرذان المختبرية.

ملغم/كغم من المستخلصات الفينولية للنباتين وحقنت الحيوانات المختبرية تحت) 400,200(أستعملت الجرعتان 30 يوم بواقع جرعة واحدة يوميا. أظهرت النتائج أنخفاضا معنويا في مستوى المالون ثنائي البريتون لمدة الإلديهايد والسيرولوبلازمين لإناث الجرذان المعاملة بالمستخلصات الفينولية لنبات البطنج عند التركيزين (400,200) ملغم/كغم ونبات النعناع عند التركيز(200) ملغم/كغم ، وانخفاضا معنويا في مستوى الكولستيرول والكليسريدات الثلاثية لإناث الجرذان المعامله بالمستخلصات الفينولية لنبات البطنج عند التركيزين (400,200) ملغم/كغم ونبات النعناع عند التركيز(200) ملغم/كغم, كما أظهرت النتائج ارتفاعا معنويا في مستوى البروتينات الدهنية عالية الكثافة وانخفاضا معنويا في مستوى البروتينات الدهنيه واطئة الكثافة بالمستخلصات الفينولية لنبات البطنج عند الجرعتين(200 ,400) ملغم/كغم عند المقارنة مع مجموعة السيطرة المعاملة بالمحلول الفسلجي بينما أظهرت الإناث المعاملة بالمستخلصات الفينولية للنباتين وبكلا التركيزين (400,200) ملغم/كغم انخفاضا لم يصل الى مستوى المعنوية في مستوى البروتينات الدهنية واطئة الكثافة جدا عند المقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: النعناع، البطنج، المركبات الفينولية، مضادات الأكسدة، دهون الدم.

Introduction:

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 (Tan *et al*., 2004). which can be classified into fifteen groups according to the basic part of their molecule, as for example phenols, phenolic acid, flavonoids, anthocyanins, quinones, catechins and tannins, just to name a few (Đilas *et al.,* 2002). In recent years, many polyphenolic compounds have attracted scientists involved in food medicine and chemistry, because of their antioxidant, anti-inflammatory, antimutagenic, anticancer, antibacterial, antiviral and antiproliferative properties, as well as their ability to change the function of some basic cell enzymes (Ho *et al.,* 1992; Mattila *et al.,* 2006). They occur as universal plant components bonded to lignins by ester bonds and play the role of inhibitors of cellulase secreted by pathogens across the membrane of cells and prevent the penetration of pathogens across the cell membrane. Phenolic compounds have been reported to accumulate in parts of plants infected by fungi (Moon&Terao,1998)and have been shown to inhibit the In vitro oxidation of human low-density lipoprotein (Frankel *et al.,* 1995). The role of the phenolics and flavonoids as natural antioxidants and free radical scavengers has attracted considerable interest (Chen & Ho, 1997; Pekkarinen *et al.,* 1999) due to their pharmacological behavior (Rosenberg Zand *et al.,* 2002).

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 (Richardson, 1992; Zegorka & Glowniak, 2001). *Mentha* has a large number of species that differ widely in their characteristics and polyploidy level. It is known to comprise about forty recognizable species, among them spearmint (*Mentha spicata* L.), a hybrid of *M. longifolia* and *M. rotundifolia*. As a hybrid, spearmint is rarely propagated by seeds and as a consequence, is exclusively propagated from its vegetative parts and by micropropagation. Spearmint has many uses in foodstuff, flavours, beverages, cosmetics and folks medicine (Li, *et al.,* 1999; Kanatt *et al.,* 2007).

The present study aimed to investigate the effect of phenolic compounds from *Mentha longifolia* and *Mentha spicata* on MDA and CP and lipid profile level which including total cholesterol, TG, HDL, LDL and VLDL in female rats.

Materials and Methods:

Plant material and powder preparation:

Fresh, spermint and horsmint were obtained from the local market in Thi-Qar province, Iraq. Each plant leaves were cleaned and dried In 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.

Phenolic compounds reagents:

General phenols wereIsolated according to (Jaffer *et al.*,1983).

Extraction of crude phenolic compounds:

Crude Phenolic compounds were extracted according to (Ribereau-Gayon, 1972) .

Experimental design:

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

1- the first group(control group) was injected by (0.5ml/animal) from normal physiological saline (0.9% NaCl).

2- the second group was injected by (0.5ml/animal) of (200mg/kg) of *M. longifolia* phenolic extract.

3- the third group was injected by (0.5ml/animal) of (400mg/kg) of *M. longifolia* phenolic extract.

4- the fourth group was injected by (0.5ml/animal) of (200mg/kg) of *M. spicata* phenolic extract.

5- the five group was injected by (0.5ml/animal) of (400mg/kg) of *M. spicata* phenolic extract.

the animals were injected intraperitonially for 30 dayes as one dose daily.

Blood collection:

After thirty 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, centrifugedat 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20ºC until the time of assay.

Measuring of serum malondialdehide (MDA) and ceroloplasmine (CP) level

According to Muslih *et al*. (2002) the level of MDA was determined by a modified procedure described by Guidet&Shah, (1989**)** . while serum Cp concentration was measured by the method of Menden *et al.* (1977).

Measurement of serum lipid profile :

The used reagents were supplied by Biolabo (France), and Serum total cholesterol was measured according to (Allan and Dawson ,1979**)**. and Serum TG was measured according to(Tietz *et al*., 1994,1999). while serum HDL was measured according to (Lopes-Virella, 1977). and measurement of LDL and VLDL according to (Friedwald *et al*.,1972) , LDL and VLDL concentration was measured as follows :

LDL = total cholesterol – (HDL + VLDL)

VLDL = serum TG /5

Statistical analysis:

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ($P<0.05$) was considered to be statistically significant. and used to calculate least significant difference (LSD) values for the comparison of means following.

Results and Discussion:

The obtained results revealed a significant decrease ($p<0.05$) in serum MDA level of the female rats treated withphenolic extractes of *M. longifolia*at dose (200,400) mg/kg *and M. spicata* at dose (200,400) mg/kg when compared with control group, The results showed a significant decrease ($p<0.05$) in plasma CPlevel of the female rats treated with phenolic extractes of *M. longifolia* at dose (200,400) mg/kg *and M. spicata* at dose 200 mg/kg, while the rats treated with phenolic extractes of*M. spicata*at dose 400 mg/kg showed non significant decrease in plasma CPwhen compared with control group(table1).

Table 1 : Effect of phenolic compounds of *Mentha spicata* and *Mentha longifolia*on malondialdehyde & ceroloplasmine level of female rats.

Animal groups	MDA (nmol/Ml) Mean ± S.E	CP (g/L) Mean ± S.E
First group	55.80± 5.24[a]	0.75± 0.03[a]
Second group	39.52± 2.49[b]	0.48± 0.03[c]
Third group	39.18± 2.27[b]	0.71± 0.04[b]
Fourth group	22.96± 1.95[c]	0.71± 0.03[b]
Fifth group	29.49± 3.69[c]	0.76± 0.03[a]
LSD	9.77	0.03

Values are means ± S.E.

Different letters refer to significant differences ($p<0.05$).

Same letters refer to No significant differences ($p<0.05$).

The decrease of MDA levels as reported in this study was compatible with finding of Djeridane *et al*, (2006), who reported that phenolic compounds act in the scavenging of free radical and in the inhibition of lipid peroxidation, especially the flavonoids. The decreases MDA and CP levels in animal groups after treatment with phenolic extracts this result agreed with the result ofAL-Gazi,*et al*, (2010) which reported that the phenolic extract of *Camellia sinensis, Vitis vinifera*, *and Punica granatum* have potent antioxidative and radical-scavenging and the result agreed withPakdeechote

et al. (2011) which reported that the *Mentha cordifolia* extract induced hypertensive rats and decrease MDA level. 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(2006). 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 (lipogenese, myeloperoxidase, xanthine-oxidase, NAD(P)H oxidase, cytochrome enzymes P-450) (Rice-Evans *et al.,* 1996; Amorati *et al.,* 2006).

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 peroxyl radicals (Ćetković, 2008).

The results showed a significant decrease ($p<0.05$) in the serum level of cholesterol and TG of the female rats treated with phenolic extractes of *M. longifolia* at dose (200,400) mg/kg *and M. spicata* at dose 200 mg/kg, while the rats treated with phenolic extractes of *M. spicata* at dose 400 mg/kg showed non significant decrease in plasma cholesterol and TGwhen compared with control group (table2).

The results showed a significant increase ($p<0.05$) in the serum level of HDL of the female rats treated with phenolic extractes of *M. longifolia* at dose (200,400) mg/kg *and M. spicata* at dose 400 mg/kg, while the rats treated with phenolic extractes of *M. spicata* at dose 200 mg/kg showed non significant increase in plasma HDLwhen compared with control group (table2).

Female rats treated with phenolic extractes of *M. longifolia* at dose (200,400) mg/kg and *M. spicata* at dose (400) mg/kg showeddecrease in plasma LDL, non significant when compared with control group, while the rats treated with phenolic extractes of *M. spicata* at dose (200) mg/kg showed a significant decrease ($p<0.05$) in plasma LDL when compared with control group (table 2).

While the female rats treated with phenolic extractes of *M. longifolia* at dose (200,400) mg/kg and *M. spicata* at dose (200,400) mg/kg showeddecrease in plasma VLDL, non significant when compared with control group (table 2).

Table 2 : Effect of phenolic compounds of *Mentha spicata* and *Mentha longifolia*on lipid profile levels of female rats.

Animal groups	Cholesterol Mg/dl Mean ± S.E	T.G Mg/dl Mean ± S.E	HDL Mg/dl Mean ± S.E	LDL Mg/dl Mean ± S.E	VLDL Mg/dl Mean ± S.E
First group	90.16±2.32^{a}	27.62±1.47^{a}	32.16 ± 2.42^{c}	34.38 ± 1.98^{a}	9.76± 0.76^{a}
Second group	68.66±2.47^{dc}	13.50 ± 2.15^{d}	39.50± 0.88^{a}	32.40± 1.70^{ab}	9.43 ± 0.55^{a}
Third group	71.83±2.12^{c}	15.63± 1.23^{c}	35.00± 1.87$^{a b}$	29.36± 2.13^{ab}	9. 73± 1.54^{a}
Fourth group	81.33±1.83^{b}	19.49±1.24^{b}	33.00± 1.21$^{b c}$	28.76± 1.82^{b}	8.20± 0.72^{a}
Fifth group	85.66±1.72^{ab}	28.03±2.30^{a}	38.33± 2.61^{ab}	32.69± 1.58^{ab}	9.03± 0.35^{a}
LSD	6.16	0.74	5.6	5.41	2.6

Values are means ± S.E.

Different letters refer to significant differences ($p<0.05$).

Same letters refer to No significant differences ($p<0.05$).

this result agreed with the Barbalho *et al.* (2011) which reported that offspring from diabetic dams treated with peppermint showed significantly reduced levels of glucose, cholesterol, LDL-c, and triglycerides and significant increase in HDL-c levels. The use of the *M. piperita* juice has potential as culturally appropriate strategy to aid in the prevention of Diabetes mellitus (DM), dyslipidemia, and its complications. Samarth and Samarth (2009) showed that M. piperita leaf extract possesses high amount of phenolic content, flavonoids content, and flavonols. They also observed that this plant has radioprotective effects possibly because of the amount of phenolic compounds, flavonoids, and flavonols due to their antioxidant and radical scavenging activity.

The plant contain both ascorbic acid and flavonoids that could have contributed to an increase in HDL cholesterol concentrations in treated dyslipidaemic animals (Maurya *et al*., 2009). The mechanisms by which flavonoids elevate plasma HDL-cholesterol concentrations remains unclear. One hypothesis is that increase the

expression and production of apolipoproteinA1, the major protein component of HDL, has a role in increasing HDL cholesterol (Baba *et al*., 2007).

Flavonoids may augment the activity of lecithin acyl transferase (LCAT) which plays an important role in the incorporation of free cholesterol into HDL, causing an increase in the serum HDL concentration (Ghule , 2006). Flavonoids may act in a number of different ways on the various components of the blood, such as lipids (Doyama *et al.,* 2005). Literature data may not be sufficient to explain the effects on plasma lipids, but phenolic acids, flavonoids and terpenoids, besides their antioxidant effects, may also be associated with benefits for triacylglycerol and HDL-c, as found by Choudhury *et al.,* (2006) in their study using *Mentha spicata* leaves.

Refrence:

-**AL-Gazi, S.; Al-Salih, R. and Al-Fartosi, K. (2010).** Study of some plant polyphenolic extracts as ahypoglycemic and antioxidant agents. Master, thesis.collage of science, Thi-Qar university.

-Allan, C. and Dawson, J.G. (1979) : Enzymatic assay of total cholesterol involving chemical or enzymatic hydrolysis-a comparison of methods. Clin.Chem. ; 25 (6) : 976-984.

- Amorati, R.; Pedulli, G.F. ; Cabrini, L.; Zambonin, L. and Landi, L.(2006). J. Agric. Food Chem. 54 ,2932-2937.

-Baba, S. ; Osakabe, N. ; Kato, Y. ; Natsume, M. ; Yasuda, A. ; Kido, T.; Fukuda, K. ; Muto, Y. and Kondo, K. (2007) : Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humansAm J. Clin Nutr;85:709–717.

-Barbalho, S.; Damasceno, D.; Spada, A.; Silva, V.; Martuchi, K.; Oshiiwa, M.; Machado, F. and Mendes, C. (2011). Metabolic Profile of Offspring from Diabetic *Wistar* Rats Treated with *Mentha piperita* (Peppermint). Evid Based Complement Alternat Med: 430237.

-Ćetković, G. (2008). The chemistry of natural products, Faculty of Technology, University of Novi Sad, in Serbian.

-Chen, J.H. and Ho, C. T.(1997). J. Agric. Food Chem. 45, 2374.

-Choudhury, R. P.; Kumar, A. and Garg, A. N. (2006). Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behaviour. J *Pharm Biomed Anal*; **41**(3), 825-832.

-Đilas, S.; Ĉanadanović-Brunet, J. and Ćetković, G.(2002). Chem. Ind. 56 ,105-112.

-Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P. and Vidal, N. (2006). Food Chemistry97.654.

-Doyama, J. T.; Rodrigues, H. G.; Novelli, E. L. B.; Cereda, E. and Vilegas, W. (2005), Chemical investigation and effects of the tea of Passiflora alata on biochemical parameters in rats. *J. Ethnopharmacol;* **96**, 371-374.

-Frankel, E.N. ; Waterhouse, A.L. and Teissedre, P.L.(1995). J. Agric. Food Chem. 43, 890.

-Friedwald, W.T. ;Levy, R.I. and Fredrickson, D.S. (1972) : Estimation of concentration of LDL-C in plasma without the use of preparative ultracentrifuge .Clinical Chemistry .Chapter18 : 499-502.

-Ghule, B.V. ; Ghante, M.H. ; Saoji, A.N. and Yeole ,P.G. (2006) : Hypolipidemic and antihyperlipidemic effect of Lagenaria siceraria fruit extract **.** Indian journal of of experimental biology; 44 : 905-909.

-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.

-Ho, C.T. ; Lee, C.Y. and Huang, M.T.(1992). (Eds). Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, and Chemistry. ACS Symposium Series 506, American Chemical Society: Washington, D.C.

-Jaffer, H.J.; Mahmod, M.J.; Jawad, A.M.; Najis, A. and AL-Naib, A. (1983). Phytochemical and Fitoterapia LIX,229.

-Kanatt, S.R.; Chander, R. and Sharma, A.(2007). Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem , 100: 451-8.

-Li, X.; Niu, X.; Bressan, R.A.; Weller, S.C. and Hasegawa, P.M.(1999). Efficient plant regeneration of native spearmint (*Mentha spicata* L.). *In vitro* Cell Dev Biol Plant , 35: 333-8.

-Lopes-Virella, M.F. (1977) : Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem. ; 23(25) : 882-884.

-Mattila, P. ; Hellström, J. and Törrönen, R.(2006). J. Agric. Food Chem. 54, 7193-7199

-Maurya, S.K. ; Raj, K. a Srivastava, A.K. (2009) : Antidyslipidemic activity of glycyrrhiza glabra in high fructose diet induced dyslipidemic Syrian golden hamsters .Indian Journal of Clinical Biochemistry ;24 (4): 404-409.

-Menden, C.; Boian, J.; Murthy, L. and Petering, H.G. and Anal, L. (1977). Plasma antioxidant. Anal Lett, .10: 197.

-Moon, J. H. and Terao, J.(1998). J. Agric. Food Chem. 46, 5062.

-Muslih , R.K.; Al nimr, M.S. ; Mizi'l , O. and Al – Zamely ,Y. (2002) : The level of malondialdehyde after activation with (H2O2 and CuSO4) and inhibition by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infarction . national journal of chemistry ; 5 : 139-148.

-Pakdeechote, P.; Kukongviriyapan, U.; Berkban, W.; Prachaney,P.; Kukongviriyapan,V. and Nakmareong, S.(2011). *Mentha cordifolia* extract inhibits the development of hypertension in L-NAME-induced hypertensive rats. Journal of Medicinal Plants Research Vol. 5(7), pp. 1175-1183.

-Pekkarinen, S.S. ; Stockmann, H. ; Schwarz, K. ; Heinonen, I.M. and Hopia, A.I.(1999). J. Agric. Food Chem. 47, 3036.

-Riberean-Gayon, P. (1972). Plant phenolics Oliver and body. U.S.A. PP 254.

- Rice-Evans, C. ; Miller, N. and Paganga, G.(1996). Free Radical Bio.Med. 20,933-956.

-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.

-Rosenberg Zand, R.S. ; Jenkins, D.J.A. and Diamandis, E.P.(2002). J. Chromatogr. B 777 , 219.

-Samarth, R. and Samarth, M.(2009). Protection against radiation-induced testicular damage in Swiss Albino Mice by *Mentha piperita* (Linn.) *Basic and Clinical Pharmacology and Toxicology*;104(4):329–334.

-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.

-Tietz, N.W. ; Burtis, C.A. , Ashwood, E.R. and Saunder, W.B. (1994) : Text book of clinical chemistry , 2nd Ed. : 1030-1058 et 1073-1080.

-Tietz, N.W. ; Burtis, C.A. ; Ashwood, E.R. and Saunder ,W.B. (1999) : Text book of clinical chemistry, 3rd Ed. : 809-857.

-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.