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ANTIHYPERTENSIVE AND TOXICITY STUDIES OF AQUEOUS METHANOLIC EXTRACT OF *MENTHA LONGIFOLIA* L.

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ABSTRACT

The present study was conducted to investigate the effect of the aqueous methanolic extract of *Mentha longifolia,* L on blood pressure of both normotensive and hypertensive (egg- feed and glucose- induced) rats. The effect of the extract on systolic, diastolic, mean blood pressures and heart rate were evaluated by using non-invasive blood pressure measurement apparatus (NIBP). The extract at doses of 500 and 1000 mg/kg (p.o) exhibited a significant decrease in blood pressure and heart rate of normotensive rats. While at the dose of 100mg/kg (p.o) it did not produce any significant effect. The 1000mg/kg of the extract produced a highly significant effect was selected for antihypertensive effect in egg feed and glucose treated hypertensive rats. A significant antihypertensive and negative chronotropic effects were observed at 1000 mg/kg (p.o) in both hypertensive models. Acute and sub-chronic studies were carried out and median lethal dose (LD_{50}) of the extract was found to be 3.75 g/kg in mice. The extract did not produce any mortality or signs of toxicity in mice and rats. In addition, a non-significant decrease in ALT, AST, and ALP but significant decrease in total cholesterol, triglycerides, LDL and increase in HDL levels were observed in the serum of the extract treated animals. It is conceivable, therefore, that the aqueous: methanolic extract of *Mentha longifolia,* L possesses safer active principles which exert both hypotensive and antihypertensive effects in normal and hypertensive animal models respectively.

Key words: *Mentha longifolia*, L.; aqueous: methanolic extract; non-invasive; antihypertensive.

INTRODUCTION

Hypertension has become a major problem and is increasing rapidly throughout the world (Lopez and Murray, 1998). High blood pressure has been found to be associated with many chronic conditions such as insulin resistance, obesity, concomitance, atherosclerosis and cardiovascular diseases. Most of these hypertension associated disorders are prevalent in developing countries because of poor lifestyle and insufficient health care system. Hypertension has become a common problem in the affluent population of the developing countries (Trivedi and Nehra, 2004). In Pakistan, the condition is quite similar to other developing countries and it has been reported that one in every four middle aged adults has high blood pressure (Jafer *et al*., 2005). At present, despite abundance of synthetic drugs, a significant proportion of the population of developing countries depends on traditional medicines for their health care needs. This is due to the fact that the efficacy of the modern synthetic drugs has only been 40%-60%, and usually a combination of these drugs is needed to achieve optimal outcomes. Moreover, side effects from these medications have also become a serious medical concern (Du and Chen, 2005). According to a survey by World Health Organization, it has been estimated that about 60% of the world's population rely on traditional herbal medicines, and 80% of the population in developing countries depends entirely on traditional practices for their primary health needs (WHO, 2000).

Traditional medicines have been used for a number of diseases worldwide. Despite the fact that traditional medicines are regaining importance in the modern world; a very little attention has been given to determine their possible mode(s) of action, side effects, toxicities and interactions. Hence biological evaluation of most of these traditional medicines remains yet to be elucidated (Tomassoni and Simone, 2001). Furthermore, there is a need to determine the safety and efficacy of traditional herbal medicines.

Mentha longifolia, L. (Family: Lamiaceae) commonly known as Filil is an herbaceous perennial plant found mostly in Northern regions of Pakistan, Gilgit and Baltistan. Traditionally, this plant has been used for the treatment of diarrhea, dysentery and stomachache and cardiac diseases too (Haq *et al*., 2011). It has been allegedly used to treat high blood pressure in that region (Khan *et al*., 2011). Therefore, a study was conducted to evaluate the hypotensive, antihypertensive and

toxicological effects of aqueous: methanolic extract of *Mentha longifolia,* L.

MATERIALS AND METHODS

Chemicals and Drugs: Glucose (CAS # 50-99-7) and methanol (CAS # 67-56-1) were purchased from Sigma Chemicals Co. All the chemicals and drugs used in the experiments were of standard quality.

Animals: Sprague Dawley rats and Swiss mice weighing 200-300g and 25-35g respectively were used. All the animals were housed in controlled environment (23-25°C) and received human care in accordance with National Institute of Health, Bethesda, Maryland guidelines. The study protocol was approved by the local ethical committee, Faculty of Pharmacy, University of Sargodha.

Plant material and extraction: The aerial parts of *Mentha longifolia* L were collected from a village in district Gilgit, Pakistan, Pakistan during the month of June, 2011 and was identified and authenticated by Dr. Fateh Muhammad, Professor of Botany, Government Postgraduate College, Jauharabad. The voucher specimen has been deposited in the herbarium, Faculty of Pharmacy, University of Sargodha. Aqueous: methanolic (70:30) extract of *Mentha longifolia,* L was prepared by using the cold maceration process. The grounded plant material (3 kg) was soaked in 7 liters of an aqueous: methanolic mixture (70:30) for 72 hours at room temperature. After three days of occasional shaking, whole material was filtered and filtrate was evaporated under reduced pressure using rotary evaporator. The crude extract was then air-dried to obtain a solid mass.

Biochemical parameters: For the estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, triglycerides, low-density lipoprotein (LDL) and high density lipoprotein (HDL) blood sample was collected in clot activator gel tubes. The serum was separated by centrifuging the blood samples at 2000 rev/min for 10 minutes. The separated serum was then used for the measurement of these biochemical parameters by using commercially available reagent kits (Abbott Laboratories, USA) (Biswas *et al*., 2010).

Determination of hypotensive effect of aqueous-methanolic extract of *Mentha longifolia* L in normotensive rats: Normotensive rats of either sex were randomly assigned into three groups (n = 5). Group 1 received 100 mg/kg of the aqueous methanolic extract of *Mentha longifolia* while animals in group 2 and group 3 received 500 mg/kg and 1000 mg/kg of the extract respectively. After intra-peritoneal administration of various doses of the extracts, blood pressure and heart rate of each of these groups was determined from the tails of rats at 0 hour, 2 hour, 4 hour and 6 hours by using non-invasive blood pressure (NIBP) measuring apparatus (ML125, AD instruments). Briefly, the animal was placed in the NIBP restrainer and an appropriate cuff with sensor was then mounted on its tail and warmed to about 33–35°C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure i.e. 250 mm Hg and slowly released during which the pulse was recorded by using Power Lab data acquisition system and computer running Lab chart 5.0 software. Systolic blood pressure (SBP), Mean blood pressure (MBP) and heart rate were measured directly using pulse tracing while the diastolic blood pressure (DBP) was calculated from SBP and MBP using the equation: DBP = (3MBP-SBP) /2 (Ayele *et al*., 2010).

Determination of the antihypertensive effect of the aqueous - methanolic extract of *Mentha longifolia* L in hypertensive rats

Egg feed-induced hypertensive rats: Sprague Dawley rats of either sex were divided into two groups (n = 5). Group 1 was treated with a specially prepared egg feed diet (p.o) for 21 consecutive days in order to produce cholesterol-induced hypertension. Animals in group 2 were treated with (p.o) egg feed diet and an aqueous methanolic extract of *Mentha longifolia* (1000 mg/kg) for the same period. Animals in both groups were given normal saline instead of tap water *ad libitum.* Blood pressure and heart rate of each of these groups were measured at week 0, week 1, week 2, week 3 using NIBP (Saleem *et al*., 2005).

Glucose-induced hypertensive rats: Rats of either sex were randomly divided into two groups (n = 5). Group 1 received 10 % glucose solution (p.o) instead of tap water for 21 consecutive days. Animals in group 2 were given orally 10 % glucose solution and an aqueous methanolic extract of *Mentha longifolia* (1000 mg/kg) for the same time period. Animals were fed on standard diet *ad libitum*. Blood pressure and heart rate of these groups were measured at week 0, week 1, week 2, and week 3 using NIBP (Saleem *et al*., 2010).

Acute Toxicity Study: Albino mice of either sex were randomly divided into five groups (n=2). Group 1 served as control and received normal saline (5 ml/kg p.o) while other groups (Group 2, Group 3, Group 4 and Group 5) were administered (p.o) different doses of the extract in an increasing order i.e. 500, 1000, 1500 and 2000 mg/kg respectively. The mortality rate was observed for 24 hours. Since no mortality occurred so another five groups of mice were taken. They were again treated with the various doses of crude extract in an ascending order i.e. 2500, 3000, 3500, 4000 mg/kg respectively. All the doses were administered by intra-peritoneal route.

The highest dose, which did not kill any animal, and the lowest dose, which killed only one animal, was noted. LD_{50} was calculated from the geometric mean of these two doses (Shetty *et al*., 2007).

Sub-Chronic Toxicity: Albino mice and Sprague Dawley rats of either sex were randomly divided into three groups (n = 6). The first group received normal saline (5 ml/kg body weight p.o.) and the animals in groups 2 and 3 received oral doses of 500 and 1000 mg/kg (p.o) body weight of the extract daily for 28 days. General behavior, Food and water intake of animals were observed during this period. The body weights of mice and rats were determined at 28th day after the administration of the extract. At 29th day, blood was collected from overnight fasted mice and rats of each group by cardiac puncture for the determination of serum biochemical parameters. Then these animals were sacrificed for the study of various organs (Heart, liver and kidney) weights (Biswas *et al*., 2010).

Statistical analysis: The results were expressed as means ± standard error of mean (SEM) and statistical analysis was carried out as one way ANOVA followed by post-hoc Dunnett test for all the experiments of the present investigation except sub chronic studies test for which two way ANOVA followed by post-hoc Bonferroni's test has been applied. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Hypotensive activity in normotensive rats: The aqueous-methanolic extract of *Mentha longifolia*, L showed a significant decrease in the SBP, MBP and DBP and heart rate of normotensive rats at the doses of 500 mg/kg and 1000 mg/kg. However, there was no significant reduction in heart rate and blood pressure at 100 mg/kg. The maximum effect in all the parameters was observed at 1000 mg/kg (Table1).

Table 1. Effect of *Mentha longifolia* on blood pressure and heart rate of normotensive rats

Time (h)	Doses											
	100 mg/kg				500 mg/kg				1000 mg/kg			
	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR
0 h	128 ± 1.91	105 ± 0.62	94.9 ± 0.90	395 ± 11.0	129.0 ± 2.05	106.0 ± 0.74	95.0 ± 2.09	363.2 ± 12.5	125.0 ± 1.28	105.1 ± 0.91	95.1 ± 1.88	389.2 ± 18.3
2 h	126 ± 2.74	104 ± 1.44	96.2 ± 2.44	384 ± 17.7	119.3 ± 2.54^{b}	97.0 ± 2.13^{a}	85.9 ± 3.00^{c}	345.0 ± 7.16	114.3 ± 3.23^{c}	98.56 ± 1.77^{d}	88.5 ± 3.23	364.4 ± 6.37
4 h	129 ± 1.75	104 ± 1.40	95.4 ± 2.85	379 ± 17.5	117.9 ± 2.10^{b}	94.0 ± 1.68^{a}	82.0 ± 2.22^{b}	329.8 ± 8.13^{c}	108.4 ± 2.54^{a}	92.4 ± 3.60^{a}	82.2 ± 3.86^{c}	351.0 ± 4.61^{c}
6 h	128 ± 2.60	104 ± 1.23	92.9 ± 1.20	389 ± 3.48	113.2 ± 1.84^{a}	87.2 ± 2.03^{a}	74.1 ± 3.16^{a}	340.8 ± 4.53^{d}	102.4 ± 3.08^{a}	82.6 ± 2.05^{a}	71.7 ± 2.86^{a}	323.4 ± 15.3^{b}

Values are expressed in means ± SEM (n=6). One way ANOVA followed by post-hoc Dennett Where a = ($P < 0.0001$), b = ($P < 0.001$), c = ($P < 0.01$) and d = ($P < 0.05$) vs. control (0 hour).

Hypertensive rats

Egg-feed induced hypertensive rats: The extract at the dose of 1000 mg/kg significantly prevented the increase in blood pressure and heart rate of treated animals as compared to control. Similarly, a reduction in heart rate was observed after the 1st week of treatment. At week 2 and week 3, the extract exhibited a more significant decrease in heart rate (Table 2).

Table 2. Effect of *Mentha longifolia* on blood pressure and heart rate of egg feed induced hypertensive rats.

Weeks	SBP (mm Hg)		DBP (mm Hg)		MBP (mm Hg)		Heart rate (Beats/min)	
	Control	Treated 1000mg/kg	Control	Treated 1000mg/kg	Control	Treated 1000 mg/kg	Control	Treated 1000 mg/kg
Week 0	121.6±0.96	123.5±1.47	103.7±1.21	105.2±0.54	94.7±1.78	96.0±1.00	380.8±6.24	384.2 ± 6.19
Week 1	131.0±1.67b	115.6±2.16	109.2±2.25c	102.3±0.98	98.3±1.63	95.7±1.26	391.0±4.61	372.8 ± 5.43
Week 2	141.8±2.54^{a}	107.5±5.75^{b}	111.3±1.78^{b}	100.6±0.48	103.3±2.45^{b}	90.8±2.40	429.6±12.1	357.2±10.51^{c}
Week 3	163.5±2.24^{a}	96.5±6.00^{a}	124.0±1.39^{a}	83.3±4.63^{a}	105.6±1.69^{a}	76.8±4.42^{a}	469.8±29.6^{a}	350.0 ± 6.55^{a}

Values are expressed in means ± SEM (n=6). One way ANOVA followed by post-hoc Dennett Where a = ($P < 0.0001$), b = ($P < 0.001$) and c = ($P < 0.01$) vs. control (Week 0).

Glucose induced hypertensive rats: Aqueous-methanolic extract of *Mentha longifolia*, L significantly prevented the increase in SBP, MBP, DBP and heart rate of glucose induced hypertensive rats (Table 3).

Table 3. Effect of *Mentha longifolia* on blood pressure and heart rate of glucose induced hypertensive rats

Weeks	SBP (mm Hg)		DBP (mm Hg)		MBP (mm Hg)		Heart rate (Beats/min)	
	Control	Treated 1000mg/kg	Control	Treated 1000mg/kg	Control	Treated 1000 mg/kg	Control	Treated 1000 mg/kg
Week 0	123.3±0.68	123.6±0.95	103.3±0.43	104.0±0.80	93.3±0.81	94.2±0.89	381.6±10.6	382.1±6.92
Week 1	135.5±4.71	103.4±4.36[a]	114.9±2.04[a]	97.3±2.44[c]	104.7±5.00[c]	94.1±2.85	399.4±7.85	381.4±12.0
Week 2	139.8±4.76[c]	99.0±3.67[a]	120.1±0.84[a]	94.0±2.72[b]	110.3±2.13[a]	86.5±3.65[c]	403.6±8.50[c]	353.4±8.90[a]
Week 3	144.5±3.41[a]	95.0±3.65[a]	128.1±1.40[a]	92.2±1.83[a]	117.7±2.10[a]	84.2±2.78[b]	449.0±17.9[a]	348.2±3.96[a]

Values are expressed in means ± SEM (n=6). One way ANOVA followed by post-hoc Dennett Where a = ($P < 0.0001$), b = ($P < 0.001$) and c = ($P < 0.01$) vs. control (Week 0).

This decrease in blood pressure and heart rate by aqueous methanolic extract of *Mentha longifolia* L in both normotensive and hypertensive rats could be linked to a number of mechanisms. Experimental studies demonstrated that glucose and fructose contributes to the rise in blood pressure (Hwang *et al*., 1987; Midaoui and Champlain, 2002) There is also an evidence that especially prepared egg feed which is rich in cholesterol also induced hypertension in rats (Saleem *et al*., 2005)**.** It is well reputed that one of the reason for glucose-induced hypertension is increase in sympathetic activity. Increase in sympathetic activity by any mean usually contributes to increase in heart rate and blood pressure**.** In the present investigation, the extract tested was found to significantly decrease the heart rate could be a strong reason of its antihypertensive effect in both normotensive and hypertensive rats. Previously it has been documented that one of the factors of hypertension is dyslipidemia which is associated with high glucose intake (Reaven and Ho, 1991). High cholesterol diet such as egg-feed diet is also associated with dyslipidemia as well as hypertension (Saleem *et al*., 2005). In the present study the extract significantly reduced cholesterol level in rats which further justifies its antihypertensive effect. Endothelial dysfunction and oxidative stress are the important factors which lead to hypertension (Tomiyama *et al*., 2000; Cai and Harisson, 2000). It is also well-established that high sugar consumption is associated to increased tissue production of reactive forms of oxygen (Midaoui and Champlain, 2002). Moreover, in hypertensive patients, lower concentrations of antioxidants have been documented (Rodrigo *et al*., 2007). Furthermore, an increased glucose level has also been involved in a reduction in nitric oxide levels ultimately resulting in an increased blood pressure.

It has been reported that plants rich with polyphonies having an antioxidant effect which improves endothelial dysfunction through increase NO formation, Decrease LDL formation, increase prostacyclin formation, increase EDHF mediated vasorelaxation and decrease Endothelin-1 production (Jean-Claude *et al*., 2004). It has also been reported that *Mentha longifolia*, L. is rich with polyphenoles (Krzyzanowska *et al*., 2011). Moreover, *Mentha longifolia* L has been reported to have anti-oxidant effect (Mkaddem *et al*., 2009). Thus the antihypertensive effect of *Mentha longifolia* could be due to the antioxidant effect of polyphenoles. Calcium channel blockers are an important family of drugs currently used in hypertension treatment. In a previous study it has been reported that *Mentha longifolia* have calcium channel blocking activity (Shah *et al*., 2010), this mechanism can be a good explanation of its blood pressure lowering effect.

Acute and Sub-chronic toxicity studies: In albino mice, the median lethal dose (LD_{50}) of the aqueous methanolic extract of *Mentha longifolia* was found to be 3750 mg/kg. In sub-chronic toxicity studies, the results showed that the extract was safe in both rats and mice and did not produce any mortality. No significant signs of toxicity such as decrease in food and water intake, body organs weights of mice and rats were found during the 28 days of treatment with the extract. Similarly, biochemical parameters related to hepatic functions such as ALT, AST and ALP were non-significantly reduced in rats at 500mg/kg when compared to control. However at 1000mg/kg, the extract significantly decreased ALP when compared to control. Previously, it has been reported that the serum levels of ALT, AST and ALP are raised in case of liver and heart damage (Wasan *et al*., 2001). AST is an enzyme found in the cytoplasm and mitochondria in different tissues, chiefly in the heart and skeletal muscles, liver, kidneys, pancreas, and erythrocytes (Aniagu *et al*., 2004). The reduction of these enzymes particularly AST and ALP indicated that the extract did not cause any toxic effects on skeletal muscles, liver, kidneys, pancreas, and erythrocytes. In albino rats, the extract at doses of 500 and 100mg/kg demonstrated a significant decrease in total cholesterol, triglycerides, LDL levels while a prominent increase in HDL levels was observed (Tables 4 and 5). These effects are quite similar to lipid lowering drugs like statins (Barnett and Gara, 2003). This lipid lowering effect in rats could be due to its antioxidant effect.

Table 4: Effect of aqueous-methanolic extract of *Mentha longifolia* on body organs weights of rats and mice

Groups	Rats			Mice		
	LWt (g)	KWt (g)	HWt (g)	LWt (g)	KWt (g)	HWt (g)
Control	5.83 ± 0.13	1.39 ± 0.01	1.48 ± 0.05	2.0 ± 1.33	0.49 ± 0.98	0.19 ± 0.13
500 mg/kg	5.85 ± 0.17	1.38 ± 0.07	1.45 ± 0.04	1.98 ± 1.37	0.47 ± 0.70	0.17 ± 0.13
1000 mg/kg	5.83 ± 0.40	1.36 ± 0.04	1.44 ± 0.07	1.97 ± 1.07	0.48 ± 0.81	0.18 ± 0.19

Values are expressed in means ± SEM (n=6). Two way ANOVA followed by Bonferonis test LWt=Liver weight; KWt= Kidney Weight; HWt=Heart weight

Table 5. Effect of aqueous-methanolic extract of *Mentha longifolia* on various biochemical parameters of rats

Parameters	Control	Extract 500 mg/kg	Extract 1000 mg/kg
ALT (U/L)	40.7 ± 2.79	38.2 ± 1.89	36.7 ± 1.35
AST(U/L)	90.7 ± 1.86	85.2 ± 2.03	83.0 ± 1.73
ALP(U/L)	70.0 ± 2.54	68.2 ± 2.34	58.2 ± 3.24[a]
Triglyceride (mg/dl)	89.2 ± 2.52	75.5 ± 2.54[a]	65.7 ± 2.04[a]
Cholesterol (mg/dl)	60.5 ± 1.96	50.0 ± 2.02[b]	40.0 ± 1.41[a]
LDL (mg/dl)	20.25 ± 2.27	10.10 ± 1.09[b]	8.23 ± 0.36[a]
HDL (mg/dl)	32.3 ± 1.98	50.5 ± 2.12[a]	59.7 ± 2.12[a]
VLDL(mg/dl)	15.2 ± 0.47	10.3 ± 0.39	8.85 ± 0.44

Values are expressed in means ± SEM (n=6), Two way ANOVA followed by Bonferonis test where a = $P < 0.001$, b = $P < 0.01$ vs. control.

Conclusion: It is concluded that the aqueous-methanolic extract of *Mentha longifolia* contains some active principles which exert antihypertensive effect in experiment models of rats. Moreover, the present study shows that *Mentha longifolia* is safe for use and these findings justify the folkloric claim. However, detailed studies are required to isolate the active constituent(s) and validate its exact mechanism of antihypertensive effect.

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