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PHARMACOLOGICAL EVALUATION OF ANTIHYPERTENSIVE EFFECT OF AERIAL PARTS OF *THYMUS LINEARIS* BENTH.

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Abstract: Traditionally *Thymus linearis* Benth. have been used for treatment of various diseases including hypertension. The present study was conducted to evaluate the hypotensive and antihypertensive effect of aqueous methanolic extract of aerial parts of *Thymus linearis* Benth. in normotensive and hypertensive rats. Acute and subchronic studies were also conducted. The aqueous methanolic extract produced a significant decrease in SBP, DBP, MBP and heart rate of both normotensive and hypertensive rats. LD_{50} of the extract was found to be 3000 mg/kg. The extract also exhibited a reduction in serum ALT, AST, ALP, cholesterol, triglycerides and LDL levels, while a significant increase in HDL level was observed. It is conceivable therefore, that *Thymus linearis* Benth. contains certain active compound(s) that are possibly responsible for the observed antihypertensive activity. Moreover, these findings further authenticate the traditional use of this plant in folklore medicine.

Keywords: *Thymus linearis* Benth., antihypertensive, isolated heart, LD_{50}

Cardiovascular diseases (CVDs) have become a major risk and their prevalence has gradually increased throughout the world. CVDs include coronary heart disease, cerebrovascular disease, hypertension, arrhythmias, rheumatic heart disease and heart failure. Hypertension refers to an increased blood pressure and usually 140/90 mm Hg is taken as threshold. It has been estimated that high blood pressure is responsible for 6% of deaths worldwide and remains the prominent risk factor for cardiovascular diseases affecting millions of people in Pakistan (1). According to National Health Survey of Pakistan, about one hundred thousand deaths occur per year in the country due to CVDs. Many synthetic drugs have been commonly used for the treatment of hypertension in developed countries but herbal medicines still remain the popular choice in the developing countries. The abundant use of these herbal medicines has led to an extensive research in this area to determine their potential efficacy and several modern cardiovascular drugs are now available as natural/herbal products. Ethnobotanical surveys in our country have indicated their vast use in the treatments of CVDs. For example, hydro-alcoholic extract of the leaves of *Syzygium guineense,* has produced a decrease in blood pressure of hypertensive rats. Similarly, *Passiflora nepalensis* Wall reported to be effective in hypertension. Moreover, the Chinese have used *Ginko biloba*, *Stephania tetandra* and *Uncaria rhynchophylla* for the treatment of hypertension (2, 3).

Thymus linearis Benth. (Family: Lamiaceae) locally known as "Tumuro " is a perennial plant found mostly in all alpine and subalpine pastures of Pakistan, India, Afghanistan and Nepal (4). Traditionally it has been widely used to treat a num-

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ber of ailments including high blood pressure, toothache, headache, cold, fever as well as skin, eye, and liver diseases (5, 6). In the present study, we have endeavored to evaluate antihypertensive effects of aqueous methanolic extract of *Thymus linearis* Benth. in both normotensive and hypertensive rats.

MATERIALS AND METHODS

Chemicals

Glucose and methanol were purchased from Sigma Chemicals Co. All the chemicals used in the experiments were of the best analytical grade.

Animals

Both male and female Sprague Dawley rats (200–300 g) and, albino mice (20–25 g) were used. The animals were housed in controlled environment (23–25°C) at animal house of University of Sargodha and were handled according to the standard procedures. The study protocol was approved by the local ethical committee Faculty of Pharmacy, University of Sargodha.

Collection and identification of plant material

The aerial parts of plant were collected from a village shikyote district Gilgit, Gilgit Baltistan, Pakistan during the month of July, 2011 and were identified and authenticated by Dr Shair Wali Assistant Professor, Department of Botany, Karakuram International University, Gilgit Baltistan. A voucher specimen with no. T.L.B=57-11 has been deposited in Faculty of Pharmacy, University of Sargodha for future reference.

Preparation of plant extract

Aqueous methanolic (70 : 30, v/v) extract of aerial parts of *Thymus linearis* Benth. was prepared using cold maceration process. The grounded plant material (2 kg) was soaked in 7 liters of an aqueous methanolic mixture (70 : 30) for 72 h at room temperature. After three days of occasional shaking, the whole material was filtered and the filtrate was evaporated under reduced pressure using rotary evaporator and finally dried in lyophilizer.

Effect of extract of *Thymus linearis* Benth. on blood pressure and heart rate of normotensive rats

Sprague Dawley rats of either sex were used for these experiments and were randomly divided into three groups of five rats each. Group I received 100 mg/kg of the extract of *Thymus linearis* Benth. Group II and Group III were given 250 mg/kg and 500 mg/kg of the aqueous methnolic extract of *Thymus linearis* Benth., respectively. Blood pressure and heart rate of each of these groups were determined at 0 hour, then after 2, 4 and 6 hours after administration of extract by using non-invasive blood pressure (NIBP) measuring apparatus (NIBP Controller – AD Instruments) from the tail of rats. Each animal was placed in the restrainer and an appropriate cuff with sensor was mounted on their tails and then warmed to about 33–35°C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure SBP (200 mmHg) and slowly released during which the pulse was recorded by using Power Lab data acquisition system and computer running Lab chart 5.0 software as described by (3). SBP, MBP and heart rate were measured directly using pulse tracing while the diastolic blood pressure was calculated from SBP and MBP using the equation: DBP = (3MBP–SBP)/2.

Effect of aqueous methanolic extract of aerial parts of *Thymus linearis* Benth. on blood pressure and heart rate of cholesterol fed hypertensive rats

Sprague Dawley rats of either sex were randomly assigned into two groups of five rats each. Group I served as control and was treated with a specially prepared egg feed diet in order to produce cholesterol-induced hypertension. The diet was prepared by the addition of yolk of 12 eggs to 500 g standard rat diet. The feed so prepared was dried in the sunlight for 3 days. Animals were then fed on this diet for 21 consecutive days. Group II served as treated group and received egg feed diet and aqueous methanolic extract of *Thymus linearis* Benth. (500 mg/kg) for 21 consecutive days. BP and heart rate of these groups were measured on days: 0, 7, 14 and 21 (7).

Effect of aqueous methanoloic extract of *Thymus linearis* Benth. on blood pressure and heart rate in glucose fed hypertensive rats

Sprague Dawley rats of either sex were divided into two groups of five rats each. Group I served as control and received 10% glucose solution for 21 consecutive days. Animals in group II were given 10% glucose solution and aqueous methanolic extract of aerial parts of *Thymus linearis* Benth. (500 mg/kg) for 21 consecutive days. BP and heart rate of each of these groups were measured at 0, 1, 2, and 3 weeks by placing them in a pre-warmed restrainer. SBP, MBP and heart rate were measured directly using pulse tracing while the diastolic blood pres-

Table 1. Effect of various doses of crude extract of *Thymus linearis* on SBP, MBP, DBP and heart rate of normotensive rats.

Time (h)	Doses											
	100 mg/kg				250 mg/kg				500 mg/kg			
	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR
0	127.5 ± 2.44	104.2 ± 0.78	92.5± 0.90	383 ± 10.0	125.9 ± 0.49	104.8 ± 0.71	94.3 ± 0.68	378 ± 9.80	125.7 ± 1.00	105.2 ± 0.36	95.9 ± 0.84	388 ± 4.56
2	125.3 ± 2.32	100.9 ± 0.79	90.7± 0.68	370 ± 6.62*	122.6 ± 1.62	100.9 ± 0.59	93.5± 0.78	356 ± 7.13*	114.5 ± 3.28*	99.2 ± 2.24	92.0 ± 3.75	360 ± 4.70**
4	120.5 ± 4.69	95.0 ± 2.55*	88.7± 1.08	365 ± 4.68**	116.1 ± 2.48*	95.3 ± 1.28*	90.9 ± 1.11	350 ± 8.65**	104.2 ± 2.76**	90.9 ± 1.48*	85.7 ± 1.51*	340 ± 5.19***
6	118 ± 1.82**	90.0 ± 3.68**	85.5± 0.89	360 ± 8.16**	113.6 ± 2.83**	80.64 ± 2.47**	85.6 ± 0.83*	340 ± 7.25**	100.4 ± 1.6***	86.6 ± 1.60**	77.3 ± 2.75**	320 ± 5.22***

Results are expressed as the mean ± SEM (n = 5), One way ANOVA followed by Dunnett's test have been applied; * = ($p < 0.05$), **= ($p < 0.01$), *** = ($p < 0.001$) *vs.* control 0 h.

Table 2. Effect of *Thymus linearis* on egg feed-induced hypertensive rats.

Days	SBP (mm Hg)		DBP (mm Hg)		MBP (mm Hg)		Heart rate (beats/min)	
	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg
0	121.6 ± 3.49	123.8 ± 1.78	89.60 ± 2.17	100.8 ± 2.34	101.0 ± 1.58	108.6 ± 2.07	361.0 ± 5.98	381.0 ± 5.98
3rd	144.2 ± 3.67	109.2 ± 4.45**	101.4 ± 1.93	90.4 ± 2.45**	114.4 ± 2.48	98.21 ± 2.22*	404.1 ± 3.32	350.1± 6.12***
6th	149.4 ± 3.67	100.8 ± 5.97**	107.4 ± 3.83	85.2 ± 1.84**	120.6 ± 3.60	90.40 ± 3.00**	431.0 ± 4.96	320.2 ± 3.78***
9th	168.0 ± 4.57	95.20 ± 3.88***	110.0 ± 4.28	80.6 ± 2.39***	124.4 ± 2.14	83.60 ± 2.23**	455.0 ± 5.47	300.2 ± 4.93***

Results are expressed as the mean ± SEM (n = 5), One way ANOVA followed by Dunnett's test have been applied; * = ($p < 0.05$), ** = ($p < 0.01$), *** = ($p < 0.001$) *vs.* control day 0.

Table 3. Effect of *Thymus linearis* on glucose induced hypertensive rats.

Weeks	SBP (mm Hg)		DBP (mm Hg)		MBP (mm Hg)		Heart rate (beats/min)	
	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg	Control	Treated 500 mg/kg
0	124.6 ± 2.01	122.6 ± 1.73	87.21 ± 2.79	97.80 ± 2.42	98.01± 1.85	108.2 ± 2.36	383.6 ± 4.85	391.6 ± 2.90
1st	139.6 ± 3.05	102.6 ± 3.50**	93.25 ± 2.62	89.4 ± 2.05^{NS}	109.2± 2.68	95.60± 2.27*	390.4 ± 4.31	371.6 ± 8.18**
2nd	148.4 ± 4.95	99.41 ± 3.94**	102.4 ± 1.71	84.60 ± 2.30*	116.0± 2.40	87.61± 3.03**	406.2 ± 3.99	317.0 ± 9.10***
3rd	166.4 ± 4.94	82.2 ± 2.60***	112.4 ± 2.07	75.60 ± 1.82**	127.1± 2.76	77.00± 3.13***	410.4 ± 3.89	296.6 ± 5.49***

Results are expressed as the mean ± SEM (n = 5), One way ANOVA followed by Dunnett's test have been applied; * = ($p < 0.05$), ** = ($p < 0.01$), *** = ($p < 0.001$) *vs.* control (week 0).

sure was calculated from SBP and MBP using the equation DBP = (3MBP–SBP)/2 (3).

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 (10 mL/kg) while other groups (Groups 2–9) were administered different doses of the extract in an ascending order i.e., 100, 500 1000, 1500, 2000, 2500, 3000, 3500 mg/kg, respectively. The mortality rate was observed for 24 h. All the doses were administered by intraperitoneal 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 (8).

Subchronic toxicity

Sprague Dawley rats of either sex were randomly divided into two groups (n = 6). The first group received normal saline (10 mL/kg body weight *p.o.*) and the animals in group 2 received 500 mg/kg body weight of the extract daily for 28 days. Food and water intake of animals were observed during this period. At 29th day, blood was collected from overnight fasted rats of each group by cardiac puncture for the determination of serum biochemical parameters. Then, the rats were sacrificed by cervical dislocation for the study of various organs (heart, liver and kidney) weights (9).

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 samples were collected in clot activator gel tubes. The serum was separated by centrifuging the blood samples at 2000 r.p.m. for 10 min. Serum biochemical parameters were then measured by using commercially available reagent kits (9).

Statistical analysis

The results were expressed as the means ± SEM. One way ANOVA followed by Dunnett's test have been applied with $p < 0.05$ considered as significant.

RESULTS

Effect of *Thymus linearis* Benth. extract on blood pressure and heart rate of normotensive rats

The extract produced a significant decrease in the SBP, MBP , DBP and heart rate with 100, 250 and 500 mg/kg dose especially after 4 and 6 h of drug administration, however, more pronounced effect was produced by 500 mg/kg, hence, it was selected for further study (Table 1).

Effect of extract *Thymus linearis* Benth on blood pressure and heart rate of cholesterol fed hypertensive rats

The extract of *Thymus linearis* Benth, at a dose of 500 mg/kg produced a highly significant ($p < 0.001$) decrease in SBP, MBP, DBP and heart rate of cholesterol fed hypertensive rats. A highly pronounced effect in all the observed parameters

Table 4. Effect of *Thymus linearis* on body weights, organ weights and various biochemical parameters of rats.

Parameters	Control	Extract 500 mg/kg
Liver (g)	5.86 ± 0.10	5.85 ± 0.12^{NS}
Kidney (g)	1.39 ± 0.14	1.38 ± 0.11^{NS}
Heart (g)	1.47 ± 0.11	1.46 ± 0.12^{NS}
Body weight (g)	280 ± 5.11	281 ± 4.50^{NS}
ALT (IU/L)	0.1 ± 2.29	36.9 ± 1.69*
AST(IU/L)	90.1 ± 1.06	80.2 ± 1.09*
ALP(IU/L)	70.0 ± 2.01	58.2 ± 1.94*
Triglycerides (mg/dL)	89.2 ± 1.92	75.0 ± 1.74*
Cholesterol (mg/dL)	60.5 ± 1.56	45.0 ± 1.02*
LDL (mg/dL)	20.09 ± 2.07	14.20 ± 1.09**
HDL (mg/dl)	33.4 ± 1.18	46.5 ± 2.02*

Values are expressed as the means ± SEM (n = 6); * = $p < 0.05$, ** = $p < 0.01$ and NS = Non-significant as compared to control.

was seen at second and third weeks of treatment (Table 2).

Effect of extract *Thymus linearis* Benth. on blood pressure and heart rate of glucose fed hypertensive rats

The extract of *Thymus linearis* Benth. at a dose of 500 mg/kg produced a highly significant ($p < 0.001$) decrease in SBP, MBP, DBP and heart rate of glucose fed hypertensive rats with more significant effects at week 2 and 3 (Table 3)

Acute and subchronic toxicity studies

The median lethal dose (LD_{50}) of the extract in mice was found to be 3000 mg/kg. In subchronic toxicity studies, the extract did not cause any significant alteration in body weights or organs weights of rats. The results also revealed that the extract (500 mg/kg) produced a significant reduction in serum ALT, AST and ALP levels. The extract exhibited a significant decrease in total cholesterol, triglycerides and LDL levels while increase in HDL levels was observed (Table 4).

DISCUSSION

Plants have been used for the treatment of various diseases since ancient times. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects (10).

In the present investigation in normotensive and hypertensive rats, the extract produced a significant decrease in SBP, MBP and DBP with maximum effect at 500 mg/kg. Similarly, the extract produced a significant decrease in the heart rate of both normotensive and hypertensive rats. It was observed that the degree of antihypertensive response produced by the extract of *Thymus linearis* Benth. was much higher in hypertensive rats than in normotensive rats. This observation was in support of the general finding that hypertensive animals appear to have an exaggerated response to depressor stimuli (11). A decrease in sympathetic activity is involved in decrease in blood pressure (12). The extract also produced a significant decrease in heart rate of normotensive rats possibly due to a reduction in sympathetic activity, which might have also been involved in a decreased blood pressure.

Previous studies demonstrated that atherosclerosis may contribute to an increase in blood pressure (13). In cholesterol fed hypertensive rats, the extract produced a significant decrease in blood pressure and heart rate. Moreover, the extract also cause a decrease in LDL, TG, cholesterol and an increase in HDL level, which could be responsible for its antihypertensive effect in cholesterol-induced hypertensive rats.

It has been reported that a high glucose intake cause an increase in blood pressure through the generation of reactive oxygen species (ROS). These ROS production have been found to be associated with an increased NADPH oxidase activity (14). Moreover, it has also been reported that an increased glucose level is involved in the inhibition of nitric oxide synthase activity, ultimately resulting in decreased nitric oxide levels and an increased blood pressure (15). Thus, the antihypertensive effect of the extract in glucose treated hypertensive rats might be due to an inhibition of NADPH oxidase activity or an increased level of nitric oxide.

During subchronic toxicity studies, the extract was found to be safe and no signs of toxicity were observed. Food and water intake, body weights and organ weights remained unaltered during the 28 days of treatment with the extract. However, biochemical parameters related to hepatic functions, such as ALT, AST and ALP, were significantly decreased when compared to control. The reduction of these enzymes indicated that the extract did not cause any toxic effects on both liver and heart tissues (16). The extract also revealed a significant decrease in serum cholesterol, triglyceride and LDL levels in rats as compared to control. The increase in HDL levels and a decrease in LDL levels by the extract indicated a possible reduction in cardiovascular risk factor that could lead to the death of animals. These effects are quite similar to those of antihyperlipidemic drugs like statins (17). Therefore, the antihypertensive effect of aqueous methanolic extract of *Thymus linearis* could also be associated with its lipid lowering effects.

CONCLUSIONS

It is concluded from this study that the antihypertensive effect of *Thymus linearis* Benth. aerial part might be due to certain biologically active compounds present in the plant extract. However, further studies are needed to isolate the active antihypertensive principle(s) and assess its possible mechanism of action.

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