

NATURAL DRUGS

EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF *THYMUS SERPHYLLUM* LINN. IN MICE

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Abstract: The present study was conducted to evaluate the analgesic, anti-inflammatory and antipyretic activities of *Thymus serphyllum* Linn. in mice. Anti-inflammatory activity was evaluated by carrageenan and egg albumin induced paw edema in mice, while analgesic activity was assessed using formalin induced paw licking and acetic acid induced abdominal writhing in mice. For determination of antipyretic activity, pyrexia was induced by subcutaneous injection of 20% yeast. All the extracts produced significant anti-inflammatory effect however, ether extract produced maximum effect 34% inhibition ($p < 0.001$) against carrageenan and 22% ($p < 0.01$) inhibition against egg albumin induced paw edema in mice at the end of 3 h. Ether extract produced prominent analgesic effect 77% ($p < 0.001$) inhibition in acetic acid induced abdominal writhing and 59% inhibition in formalin induced paw licking model in mice, respectively. Ether extract also demonstrated significant ($p < 0.001$) antipyretic activity against yeast induced pyrexia. The plant showed no sign of toxicity up to the dose of 2000 mg/kg in mice. This study supports the use of *Thymus serphyllum* in traditional medicine for inflammation accompanied by pain and fever.

Keywords: analgesic, anti-inflammatory, antipyretic, *Thymus serphyllum* Linn.

Inflammation is considered as a primary physiological defense mechanism and is associated with the protection of the body against burns, infections, toxic chemicals, allergens and other harmful stimuli. If the inflammation is left uncontrolled, it would act as an etiologic factor for a number of other chronic diseases (1). It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (2). Earlier, inflammation was considered as a single disease caused by disturbances of body fluids, however, in the modern medical science, inflammation is considered as a healthy process resulting from some disturbances or diseases (3). Currently, inflammatory conditions and accompanied pain and fever are managed by either narcotics e.g., opioids or non-narcotics e.g., salicylates and corticosteroids e.g., hydrocortisone. These synthetic drugs are associated with serious side effects (4). Moreover, these

conventional drugs are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the globe. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary. Drugs of plant origin have been used since long time and have comparatively less adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Many plants had been used to ameliorate the inflammatory conditions and associated pain and fever on the basis of folklore claim. Purified natural compounds from plants can serve as a template for the synthesis of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value (3). Many medicinal plants have been used in developing countries for the management of pain and inflammatory conditions. *Thymus serphyllum* Linn. is a widely growing herbal plant in

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the northern hilly area, Gilgit-Baltistan. *Thymus serpyllum* is commonly used by local people for various purposes such as antiseptic, anthelmintic, carminative and expectorant (5). Its analgesic effect has been reported in Chinese literature. It has also been reported to have antipyretic properties (6). The present study was conducted to ascertain the traditional use of *Thymus serpyllum* for inflammation and associated pain and fever.

MATERIAL AND METHODS

Drugs and reagents

Carrageenan, fresh egg-albumin, formalin, acetic acid, Brewer's yeast, Tween-80 and aspirin.

Plant material

Aerial parts of *Thymus serpyllum* were used in this study. The plant was collected from the village Shikyote, District Gilgit, Gilgit-Baltistan, Pakistan during the month of July 2010. The plant was identified and authenticated by Dr. Sher Wali, Assistant Professor of Botany, Karakoram International University, Gilgit-Baltistan. A voucher specimen has been kept for future reference in the Herbarium of Faculty of Pharmacy, Department of Pharmacognosy with the number of TS-1150. It was shade dried and then, powdered using grinder.

Preparation of extracts

The powdered plant was extracted successively by method of cold maceration to prepare ether, ethanolic and aqueous extracts. For extraction with each solvent, the powder was soaked for 48 h with occasional shaking. It was passed through muslin cloth and then filtered through the filter paper. The extracts were dried with the help of rotary evaporator and lyophilized.

Animals used

Adult healthy mice of either sex weighing 25–30 g were used in the study. Animals were housed in the animal house of Faculty of Pharmacy, University of Sargodha under natural light and dark cycles at a temperature of $28 \pm 4^\circ\text{C}$, given a standard pellet diet and water *ad libitum*. Animals received human care. The study protocol was approved by the local Ethical Committee, Faculty of Pharmacy, University of Sargodha. All animals were treated according to the standard procedures guided by NIH (7).

Analgesic activity

Analgesic activity of ether, ethanolic and water extracts of *Thymus serpyllum* against acetic acid

induced writhing (abdominal constrictions and stretching of hind limbs) and formalin induced paw licking in mice was conducted according to methods described earlier with some modifications (8, 9). Briefly; after an overnight fasting, mice were divided into 5 groups of 5 mice each. Group I served as control and was treated with 2 mL/kg body weight of normal saline *i.p.* Group 2, 3 and 4 were given ether, aqueous and ethanolic extracts in a dose of 100 mg/kg *p.o.*, respectively. Group 5 was treated with standard drug, aspirin 100 mg/kg body weight (*i.p.*). After 30 min of treatment, the mice were injected *i.p.* with 0.2 mL of 3% acetic acid solution to induce writhing. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted between 5 and 15 min after acetic acid injection. The same procedure of grouping of mice was adopted in paw licking test, however, this time after 1 h of treatment with drugs, 20 μL of 2.5% formalin solution was injected subcutaneously under the surface of the left hind paw of each mice and responses were observed for 30 min, immediately after injecting formalin. The time spent by mice in licking the injected paw was noted and was indicative of pain.

Anti-inflammatory activity

Anti-inflammatory activity of ether, ethanolic and water extracts against carrageenan and egg-albumin induced paw edema in mice was evaluated according to the methods described earlier with some modifications (10).

Briefly; after an overnight fasting, mice were divided into 5 groups of 5 mice each. Group I served as control and was treated with normal saline 2 mL/kg *p.o.* Group 2, 3 and 4 were given ether, aqueous and ethanolic extracts of *Thymus serpyllum* 100 mg/kg *p.o.* respectively and Group 5 was treated with standard drug, aspirin 100 mg/kg *p.o.* The same group pattern was adopted for egg albumin induced inflammation. After 1 h of treatment, the inflammation in the hind paw was produced by injecting 0.1 mL of freshly prepared carrageenan suspension (1%) in normal saline and fresh egg-albumin into the sub planer-surface of the right hind paw. The linear circumference of the injected paw was measured at 0, 1, 2 and 3 h of the administration of carrageenan and egg-albumin, with the help of a vernier caliper. The increase in paw circumference at 1, 2 and 3 h after administration of carrageenan injection was adopted as the parameter for measurement of inflammation. The ability of the extracts to suppress the paw inflammation was expressed as a percent inhibition of

paw edema (11) and was calculated according to the following equation:

$$\text{Percentage of inhibition (\%)} = 100 \times (1 - x/y)$$

where x = mean increase in paw thickness of treated mice; y = mean increase in paw thickness of control mice.

Antipyretic activity

Antipyretic activity of various ether, water and ethanolic extracts of *Thymus serpyllum* against yeast induced pyrexia in mice was performed according to the methods described earlier with some modifications (12, 13).

Briefly; after an overnight fasting, mice were divided into 5 groups of 5 mice each. Pyrexia was induced by subcutaneous administration of 2

mL/100 g of 20% aqueous suspension of brewer's yeast. Group I served as a control group and was treated with normal saline 2 mL/kg *p.o.* Group 2, 3 and 4 were treated with ether, aqueous and ethanolic extracts of *Thymus serpyllum* 100 mg/kg *p.o.*, respectively, while group 5 was treated with standard drug, aspirin 100 mg/kg *p.o.* After 24 h of induction of pyrexia, rectal temperatures were noted. Then, the animals were treated as described earlier with control, standard and plant extracts. Rectal temperature of animals was taken at 30, 90 and 150 min after drug administration.

Acute toxicity

Acute toxicity was tested in mice of either sex; the animals received plant extract (ether, aqueous

Table 1. Effect of various extracts of *Thymus serpyllum* on writhing induced by acetic acid in mice.

Treatment	No. of writhings	Inhibition [%]
	Mean \pm SEM	
Control (2 mL/kg)	39.2 \pm 1.35	–
Ether extract (100 mg/kg)	16 \pm 0.7 ^d	59
Aqueous extract (100 mg/kg)	30.4 \pm 0.81 ^b	22
Ethanolic extract (100 mg/kg)	21.6 \pm 1 ^c	45
Aspirin (100 mg/kg)	17.2 \pm 0.8 ^d	57

Data are expressed as the mean \pm SEM. (p < 0.05) was considered as significant ^b = (p < 0.01), ^c = (p < 0.001) and ^d = (p < 0.0001), compared to control group. The means sharing the same letters are not significant to each other.

Table 2. Effect of various extracts of *Thymus serpyllum* on formalin induced paw licking in mice.

Treatment	No. of paw lickings	Inhibition [%]
	Mean \pm SEM	
Control (2 mL/kg)	324.6 \pm 10.4	–
Ether extract (100 mg/kg)	74.4 \pm 5.4 ^d	77
Aqueous extract (100 mg/kg)	121 \pm 6.66 ^c	63
Ethanolic extract (100 mg/kg)	182.8 \pm 7.34 ^b	44
Aspirin (100 mg/kg)	109.8 \pm 2.85 ^c	66

Data are expressed as the mean \pm SEM. (p < 0.05) was considered as significant ^b = (p < 0.01), ^c = (p < 0.001) and ^d = (p < 0.0001), compared to control group. The means sharing the same letters are not significant to each other.

and ethanolic) in doses of 500, 1000, 1500, 2000 mg/kg and vehicle – normal saline by the intragastric route. The mortality rate was observed for 24 h (14).

Statistical analysis

Values are presented as the mean \pm SEM and data were analyzed by ANOVA followed by Tukey's test using Mini Tab software. A value of $p < 0.05$ was considered significant.

RESULTS

Analgesic activity

All the three extracts significantly reduced the acetic acid induced writhing and formalin induced paw licking in mice. The analgesic effect of ether extract was highly significant ($p < 0.0001$) in both experimental models (Tables 1, 2).

Anti-inflammatory activity

All the extracts produced a significant anti-inflammatory effect between 2–3 h after induction of inflammation. However, ether extract produced maximum effect 34% inhibition ($p < 0.001$) against carrageenan and 28% ($p < 0.01$) inhibition against egg albumin induced paw edema in mice at the end of 3 h (Tables 3, 4).

Antipyretic activity

All the extracts were found to produce significant effects against yeast induced pyrexia but the ether extract exhibited a highly significant ($p < 0.0001$) antipyretic effect as compared to control group (Table 5).

Acute toxicity

Animals treated up to 2000 mg/kg of ether, aqueous and ethanolic extracts of *Thymus serpyllum*

Table 3. Effect of various extracts of *Thymus serpyllum* on carrageenan induced paw edema in mice.

Treatment	Paw circumference in mm at indicated time*			
	0 h	1 h	2 h	3 h
Control (2 mL/kg)	3.60 \pm 0.10	4.09 \pm 0.06	4.30 \pm 0.10	4.51 \pm 0.09
Ether extract (100 mg/kg)	3.57 \pm 0.25	3.79 \pm 0.22 ^b	3.85 \pm 0.28 ^d	3.85 \pm 0.27 ^c
Aqueous extract (100 mg/kg)	3.64 \pm 0.04	3.54 \pm 0.07 ^b	3.36 \pm 0.07 ^{bc}	3.35 \pm 0.08 ^b
Ethanolic extract (100 mg/kg)	3.88 \pm 0.21	3.15 \pm 0.26 ^b	3.02 \pm 0.28 ^{bc}	2.65 \pm 0.27 ^b
Aspirin (100 mg/kg)	4.03 \pm 0.13	4.35 \pm 0.05 ^b	4.47 \pm 0.07 ^b	4.55 \pm 0.07 ^b

* Data are expressed as the mean \pm SEM; $p < 0.05$ was considered as significant ^b = $p < 0.01$, ^c = $p < 0.001$ and ^d = $p < 0.0001$ compared to control group.

Table 4. Effect of various extracts of *Thymus serpyllum* on paw edema induced by egg albumin in mice.

Treatment	Paw circumference in mm at indicated time*			
	0 h	1 h	2 h	3 h
Control (2 mL/kg)	3.56 \pm 0.14	3.57 \pm 0.15 ^a	3.71 \pm 0.15 ^a	3.92 \pm 0.14 ^a
Ether extract (100 mg/kg)	4.48 \pm 0.13	4.29 \pm 0.10 ^b	4.12 \pm 0.06 ^{ab}	3.80 \pm 0.06 ^b
Aqueous extract (100 mg/kg)	4.02 \pm 0.15	4.00 \pm 0.13 ^{ab}	4.04 \pm 0.14 ^{ab}	3.80 \pm 0.12 ^{ab b}
Ethanolic extract (100 mg/kg)	3.95 \pm 0.13	4.01 \pm 0.22 ^{ab}	4.02 \pm 0.34 ^{ab}	3.70 \pm 0.31 ^b
Aspirin (100 mg/kg)	3.87 \pm 0.10	3.64 \pm 0.08 ^b	3.54 \pm 0.09 ^b	3.40 \pm 0.11 ^b

* Data are expressed as the mean \pm SEM; $p < 0.05$ was considered as significant ^a = $p < 0.05$, ^b = $p < 0.01$, ^c = $p < 0.001$ and ^d = $p < 0.0001$ compared to control group.

Table 5. Effect of various extracts of *Thymus serpyllum* on Brewer's yeast-induced pyrexia in mice.

Treatments	Temperature at 0 min [°]	Temperature at 30 min [°]	Temperature at 90 min [°]	Temperature at 150 min [°]
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control (2 mL/kg)	36.82 ± 0.23	37.10 ± 0.24 ^a	37.26 ± 0.14 ^a	37.42 ± 0.19 ^a
Ether extract (100 mg/kg)	36.68 ± 0.31	36.24 ± 0.39 ^a	36.00 ± 0.23 ^b	35.90 ± 0.4 ^c
Aqueous extract (100 mg/kg)	36.40 ± 0.46	36.22 ± 0.22 ^a	36.40 ± 0.35 ^b	36.42 ± 0.23 ^b
Ethanol extract (100 mg/kg)	36.90 ± 0.19	36.16 ± 0.15 ^b	36.90 ± 0.29 ^a	37.22 ± 0.25 ^b
Aspirin (100 mg/kg)	36.70 ± 0.39	37.54 ± 0.41 ^b	36.66 ± 0.47 ^b	36.74 ± 0.38 ^a

Data are expressed as the mean ± SEM. ($p < 0.05$) was considered as significant ^a = $p < 0.05$, ^b = $p < 0.01$, ^c = $p < 0.001$ and ^d = $p < 0.0001$ compared to control group. The means sharing the same letters are not significant to each other.

lum produced no death and changes in behavior, a fact indicating low toxicity of the extract.

DISCUSSION

The folkloric use of *Thymus serpyllum* Linn. in fever, inflammation and pain has been reported in literature along with several other traditional claims. Hence, it was thought that investigations of these medicinal properties should be scientifically authenticated to validate the traditional claims. Moreover, this plant has not been subjected to the above mentioned pharmacological screening so far. The results obtained after experimental work indicated that all of the three extracts exhibited analgesic activity by reducing both the acetic acid induced writhing and formalin induced paw licking in mice. Acetic acid causes inflammatory pain by inducing capillary permeability, while formalin exhibits neurogenic and inflammatory pain (15). In acetic acid induced models of pain, analgesic activities were supposed to be present, due to some sterols. Plants possessing some organic acid, and flavonoid also showed significant analgesic activities (16). These results of ether extract exhibited a mode of action similar to those drugs which act through inhibiting Cox-3, enzyme like paracetamol, which has been reported to lack or possess a weak anti-inflammatory activity but possesses a strong analgesic and antipyretic activities and was reported to inhibit centrally the synthesis of prostaglandin in the brain (17).

However, these drugs do not inhibit peripheral biosynthesis of prostaglandin and therefore lacks peripheral anti-inflammatory effect. Ether extract

has also been reported to inhibit acetic acid induced writhing. The central action of the extracts is further supported by its ability to inhibit both phases of formalin induced paw licking, which is the characteristic of drugs (as narcotic) that act centrally (18). Although all the three extracts exhibited analgesic activity, the ether extract showed more pronounced ($p < 0.0001$) analgesic activity even compared to standard drug, aspirin.

In the study of anti-inflammatory activity, we evaluated the extracts against egg-albumin and carrageenan induced paw edema. Synthesis or release of mediators of the injured site has been reported to occur. Prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin are included in these mediators, all of which also cause pain and fever (19). Inflammation, and other symptoms are normally ameliorated by inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects.

Development of carrageenan induced paw edema is commonly correlated with early inflammatory oxidative stage. Carrageenan edema is a multi-mediated phenomenon. It is believed to be biphasic; in the first phase (1 h) serotonin and histamine are released while in the second phase (over 1 h) prostaglandins, cyclooxygenase products are mediated and kinins provide the continuity between the two phases (20). Thus, the carrageenan induced inflammation model is a significant predictive test for inflammation. The anti-inflammatory activity of various extracts of *Thymus serpyllum* against carrageenan induced inflammation in mice was observed but the ethanolic extract at the dose of 100

mg/kg significantly ($p < 0.05 - p < 0.0001$) inhibited the carrageenan induced mice paw edema. The anti-inflammatory activity of ether, aqueous and ethanolic extracts of *Thymus serpyllum* was observed in mice against egg-albumin induced paw edema. Histamine is an important mediator of inflammation, a potent vasodilator and is also involved in increasing the vascular permeability (21). As the plant extracts effectively suppressed the edema produced by egg-albumin, it showed that the extract might exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

In this study, antipyretic effect was evaluated and the ether extract produced a prominent effect ($p < 0.001$) against yeast induced pyrexia. As discussed above, the extracts might exhibit analgesic effects by acting centrally. The extract was likely to reduce pyrexia by reducing the brain concentration of prostaglandin E₂ especially in the hypothalamus through its action on Cox-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine (22).

In acute toxicity testing, animals treated with up to 2000 mg/kg of ether, aqueous and ethanolic extracts of *Thymus serpyllum* produced no death and no changes in behavior of mice that indicates low toxicity of the extracts.

From these investigations, it might be concluded that various (ether, aqueous and ethanolic) extracts of *Thymus serpyllum* exhibited analgesic, anti-inflammatory and antipyretic effects. It justifies the traditional use of this plant in the treatment of various types of pain, fever and inflammation. However, further studies are required to isolate active compounds from the potent extracts and to elucidate their exact mechanism of action.

Acknowledgment

The authors are thankful to Dr. Sher Wali, Assistant Professor of Botany, Karakoram International University, Gilgit-Baltistan for the identification of the plant used in the present study.

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Received: 19. 10. 2013