REPORT

Analgesic, anti-inflammatory and anti-pyretic activities of *Thymus linearis*

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Abstract: The present study was aimed to investigate the antipyretic, analgesic and anti-inflammatory activity of aqueous methanolic and n-hexane extract of *Thymus linearis*. For measuring analgesic activity, writhing test, hot plate method and formalin test were performed and abdominal writhing was induced by intra-peritoneal injection of 0.2 ml of 3% acetic acid. While in formalin test, pain was experimentally induced by injecting 25 µl of 2.5% formalin in left hind paw. In hot plate method, pain was induced thermally by keeping the animals on a hot plate with temperature of about 51°C. Anti-inflammatory activity was assessed by carrageenan induced mice paw edema. For determination of antipyretic activity, pyrexia was induced by subcutaneous injection of 15% yeast. The results showed that both the extracts had significant analgesic activity ($p<0.05$); anti-inflammatory activity ($p<0.05$) and anti-pyretic activity ($p<0.05$). Therefore, it was concluded from this study that the extracts of *Thymus linearis* may be used against pain, pyrexia and inflammation.

Keywords: *Thymus linearis*, NSAIDs, carrageenan, yeast, pyrexia, formalin, analgesia

INTRODUCTION

The problem of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid *et al.*, 2009; Hussain *et al.*, 2011) or through nanotechnology (Naz *et al.*, 2012; Ehsan *et al.*, 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011), or synthesis from lactic acid bacteria (Masood *et al.*, 2011), or marine microorganisms (Javed *et al.*, 2011). However, now a day, the trend is also being changed to the use of herbal products or extracts to control the diseases. The plant kingdom still holds many species containing substances of medicinal value which have yet to be discovered: large numbers of plants are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al.*, 2012), hypoglycaemic, amoebicidal, anti-fertility, cytotoxic, antibiotic (Amin *et al.*, 2012), Spasmolytic, bronchodilator, antioxidant (Janbaz *et al.*, 2012) and anti-Parkinsonism properties.

Thymus linearis belonging to family Lamiaceae is known as Thyme in English and Satar Farsi in Urdu. The plant has been proved to have antibacterial (Gilani *et al.*, 2010) antiviral (Hafidh *et al.*, 2009) and anti-cancer activity (Hussain *et al.*, 2012). The folkloric use of *Thymus linearis* in fever, inflammation and pain has been reported (Haq *et al.*, 2011). However, the extracts of plant have not been subjected to pharmacologically screening of the said activities. Therefore, objective of the present study was to evaluate the analgesic, anti-inflammatory, and antipyretic activity of *Thymus linearis* to authenticate its traditional claim.

MATERIALS AND METHODS

Plant collection

Leaves and branches of *Thymus linearis* was used for this study. Plant was collected from DIR Pakistan during the month of July 2012. The plant was identified and authenticated by Dr. Ilyas Iqbal, Assistant Professor of Botany, University of Malakand, Khyber Pakhtunkhwa, Pakistan and submitted to the Herbarium with Voucher Number GC-1670.

Extraction of plant material and sample preparation

The powdered plant was successively extracted by method of cold maceration to prepare n-hexane and aqueous methanolic extracts. 2kg of the plant powder were extracted by using solvents; n-hexane and aqueous methanol (70%) for *Thymus linearis*. For extracting the plant with each solvent, the powdered plant was soaked for 1 week separately with irregular shaking and after each soaked plant material were passed through muslin cloth separately and then filtered out with the help of filter paper. Drying of extracts was done by using the rotary evaporator. The percentage yields for n-hexane and

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aqueous methanolic extracts were 0.2% and 30% respectively. For administration, the extracts of plant were dissolved in normal saline. The n-hexane extract was dissolved in normal saline with small amount of ethanol by using sonicator for 10 minutes.

Analgesic activity

i) Against acetic acid induced writhing

After 30 minutes of treatment (100 mg/kg of the standard and the extracts), mice were injected intraperitoneally 0.2 ml of 3 % acetic acid to induce writhing. Acetic acid causes stretching of hind limb along with abdominal constrictions, which was measured between 5 to 15 minutes after acetic acid administration. After that, the response of different extracts was compared with the responses of animals in control group.

ii) Formalin induced paw licking

2.5% Formalin solution was injected under the surface of hind paw of mice after 1 hour of administration of extracts (100mg/kg). The responses were observed immediately after administration of injection for 30 minutes.

iii) Eddy's hot plate method

After 1 hour of administration of different doses of extracts (100mg/kg), the mice were placed on hot plate. The temperature was kept at 55-56ºC. The reaction time was the time taken by the animal to lick the hind paw or jump out of the place and was measured at 0, 30 and 60 minutes.

Anti-inflammatory activity

Thymus linearis extracts were examined for their anti-inflammatory activities against Carragenan induced paw edema. After 1 hour of treatment (100mg/kg of the standard and the extracts), 0.1 ml of freshly prepared Carragenan suspension (1%) was injected into the sub plantar surface of hind paw. This produced inflammation. The circumference was calculated at 0, 1, 2 and 3 hours after administration of injection with the help of Vernier caliper.

Anti-pyretic activity

Thymus linearis extracts were also examined for their anti-pyretic activities against yeast induced pyrexia. Pyrexia was induced by subcutaneous injection of 20% w/v aqueous suspension of Brewer's yeast 2 ml/kg. After 24 hours, rectal temperatures were noted (pre-treatment values). The rectal temperature for all the groups was taken at 1 hour interval after the treatment (100 mg/kg of the standard and the extracts).

Acute toxicity testing

Acute toxicity was experienced in mice having weight of about 15 to 30 gm. The animals received plants extracts in doses of 500, 1000, 1500 and 2000 mg/kg body weight and normal saline by the intra gastric route and measured the mortality for 2 days, and weight was daily monitored for 1 week (Garba *et al.*, 2009).

Statistical analysis

Values were given as mean ±SEM and the statistical analysis used was analysis of variance (ANNOVA). $p<0.05$ was considered significant.

RESULTS

Analgesic activity of *Thymus linearis* is given in table 1. Both the aqueous methanolic (20±0.37) and n-hexane (21±0.32) extracts of *Thymus linearis* significantly ($p<0.05$) reduced the acetic acid induced writhing as compared to control group (23 ± 0.51). Similarly, both the aqueous methanolic (280±3.54) and n-hexane (292±2.21) extracts of *Thymus linearis* significantly ($p<0.05$) reduced formalin induced paw licking as compared to control group (308±5.23). In hot plate method, after 60 minutes, the aqueous methanolic extract showed reaction time 2.53±0.072 ($p<0.05$) while the n-hexane extract showed 2.30±0.033 ($p<0.05$) as compared to control (1.92±0.108). All these results had non-significant difference from the standard aspirin. Both the extracts also produced a significant anti-inflammatory effect ($p<0.05$) between 2-3 hours of post inflammation induction (table 2). Moreover, both the extracts were found to be effective ($p<0.05$) against yeast induced pyrexia (table 3). For measuring the acute toxicity, animals treated with up to 2000 mg/kg of the extracts showed no change in behavior or weight for 1 week, indicating low toxicity of the extract.

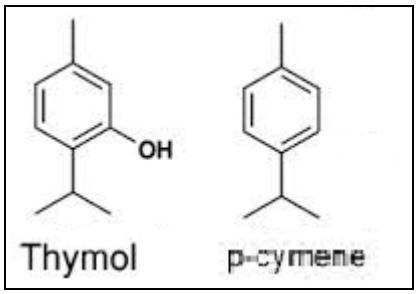

Fig. 1: Essential oil components that may be responsible for analgesic, anti-inflammatory and antipyretic activities of *Thymus linearis.*

DISCUSSION

Thymus linearis contains thymol and p-cymene as major constituents (Verma *et al.*, 2010). Both the constituents have already been reported to have analgesic, anti-inflammatory and antipyretic activities by inhibition of COX enzyme (Veras *et al.*, 2012) by a mechanism same like non-steroidal anti-inflammatory drugs. Therefore, the analgesic, anti-inflammatory and antipyretic activities of *Thymus linearis* might be due to these compounds.

Table 1: Analgesic activity of *Thymus linearis*

Treatment/dose	No. of Writhing (Mean ±SEM)	No. of Paw lickings (Mean ±SEM)	Reaction time in sec (Mean ±SEM)		
			0 min	30 min	60 min
Control (Normal saline 2 ml/kg)	23±0.51	308±5.23	2.04±0.168	1.976±0.164	1.92±0.108
Standard (Aspirin 100 mg/kg)	17*±0.51	265*±5.00	2.58±0.141	3.03±0.045	3.14*±0.022
Aqueous methanolic extract of *Thymus linearis* (100 mg/kg)	20*±0.37	280*±3.54	2.13±0.0474	2.3±0.0329	2.53*±0.072
n-Hexane extract of *Thymus linearis* (100 mg/kg)	21*±0.32	292*±2.21	2.12±0.0252	2.22±0.0355	2.30*±0.033

Table 2: Anti-inflammatory activity of *Thymus linearis* on carrageenan induced edema in mice

Treatment/dose	Level of Inflammation (Mean ±SEM)			
	0 hr	1 hr	2 hr	3 hr
Control (Normal saline 2 ml/kg)	0.52±.09	0.74±0.05	0.80±0.03	0.78±0.04
Standard (Aspirin 100 mg/kg)	0.38±0.04	0.54*±0.04	0.66*±0.06	0.52*±0.03
Aqueous methanolic extract of *Thymus linearis* (100 mg/kg)	0.42±0.04	0.58*±0.06	0.68*±0.07	0.58*±0.08
n-Hexane extract of *Thymus linearis* (100 mg/kg)	0.46±0.05	0.64*±0.05	0.70*±0.03	0.68*±0.04

Table 3: Anti-pyretic activity of *Thymus linearis* against yeast induced pyrexia in mice

Treatment /dose	Rectal Temp (^{o}C) after 18 hrs of Yeast Injection (Mean ±SEM)			
	0 hr	1 hr	2 hrs	3 hrs
Control (Normal saline 2 ml/kg)	41.31±0.18	40.91±0.02	40.47±0.15	39.38±0.16
Standard (Aspirin 100 mg/kg)	40.73±0.22	38.32±0.09	37.67*±0.12	37.44±0.08
Aqueous methanolic extract of *Thymus linearis* (100 mg/kg)	40.78±0.03	39.8±0.20	38.43*±0.20	38.26±0.10
n-Hexane extract of *Thymus linearis* (100 mg/kg)	41.03±0.11	39.04±0.06	39.19±0.09	37.80*±0.13

*p<0.05

CONCLUSION

It was concluded from the present study that aqueous methanolic and n-hexane extracts of *Thymus linearis* have analgesic, anti-inflammatory and antipyretic activities. Therefore, the extracts may be used for the treatment of fever, pain, and inflammation.

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