

REPORT

Analgesic, anti-inflammatory and anti-pyretic activities of aqueous ethanolic extract of *Tamarix aphylla* L. (Saltcedar) in mice

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Abstract: The objective of the study was to investigate the analgesic, anti-inflammatory and anti-pyretic activity of aqueous ethanolic extracts of *Tamarix aphylla*. The powdered plant was extracted by the method of cold maceration using aqueous ethanol (70:30) as solvents. Analgesic activity was assessed by Eddy's hot plate method, formalin-induced paw licking and acetic acid-induced writhing in mice. Anti-inflammatory activity was evaluated by carageenan-induced mice paw edema. The anti-pyretic activity was determined by yeast-induced pyrexia in mice. The aqueous ethanolic extract of *Tamarix aphylla* showed 42% inhibition ($p < 0.005$) of acetic acid-induced writhing, 63% reduction ($p < 0.005$) in formalin-induced paw licking, and 42% increase ($p < 0.05$) in reaction time as compared to normal control. The extract did not show significant anti-inflammatory activity. However, it showed significant antipyretic effect ($p < 0.005$). The results of this study demonstrate that aqueous ethanolic extract of *Tamarix aphylla* exhibit analgesic and antipyretic activity but lacks anti-inflammatory activity.

Keywords: *Tamarix aphylla*, analgesic, antipyretic.

INTRODUCTION

Plants have been proved to be the good source of medicines. *Tamarix aphylla* L. (Family Tamaricaceae) is the most widespread species of *Tamarix*. It has a variety of names including Saltcedar (English), Farash (India), Tamaris (French), Tamariske (German), Taray (Spanish) and Woestyn tamarisk (Afrikaans). Extract from leaves of the plant is used to treat toothache (Kamal *et al.*, 2009). Water-soluble flavonoid glycosides and phenolics have been isolated from the plant (Nawwar *et al.*, 2009). Flavonoid glycosides have shown to possess analgesic and anti-inflammatory activity (Datta *et al.*, 2004). *Tamarix aphylla* is traditionally used for the treatment of rheumatism (Marwat *et al.*, 2009). Hence, the objective of the study was to investigate the analgesic, anti-inflammatory and anti-pyretic activity of aqueous ethanolic extracts of *Tamarix aphylla*.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (20-30g) were used. The temperature and humidity of the animal house was maintained at $22 \pm 2^\circ\text{C}$ and 44-56% respectively. Animals were given standard rodent pellet diet and water *ad libitum*. The protocol was approved by Ethical Review Committee, GC University, Faisalabad, Pakistan.

Plant material

Aerial parts of *Tamarix aphylla* were collected from

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Jhang (Pakistan) in July 2012. The plants were identified by Department of Botany, University of Agriculture, Faisalabad, Pakistan. The powdered plant material was extracted by the method of cold maceration using aqueous ethanol (70: 30) as solvents. After soaking the powdered extract for 48hrs, it was passed through muslin cloth and then through filter paper for filtration. Rotary evaporator was used for concentrating the extracts. The extracts were stored in capped bottles and kept in refrigerators before use. Extracts were dissolved in normal saline before administration.

Analgesic activity

i) Acetic acid-induced writhing

Mice were divided into 3 groups of 6 animals each. Group 1 received normal saline (i.p), group 2 was given aqueous ethanolic extract (100mg/kg) orally while group 3 was treated with Aspirin (100mg/kg) i.p. After 30 minutes of treatment, animals were injected intraperitoneally 1% acetic acid (1ml/100g body weight) i.p. to induce writhing which was measured between 5 to 15 minutes after acetic acid administration. After that, the response of the extract was compared with the responses of animals in control groups.

ii) Eddy's hot plate method

Animals were divided into groups and treated as stated previously. After 1 hour of administration of extracts, the mice were placed on hot plate. The temperature was kept at $55-56^\circ\text{C}$. 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.

iii) Formalin-induced paw licking

Mice were divided into 3 groups of 6 animals each. Group 1 received normal saline (i.p). Group 2 was given aqueous ethanolic extract (100mg/kg) orally while group 3 was treated with Aspirin (100mg/kg) i.p. After 1 hour of administration of extracts/standard, each mouse was given 20 μ L of 5% formalin in 0.9% NaCl, using an injection, to the left paw (sub-plantar). These mice were individually placed in large (2L capacity) beakers for observation. The duration of paw licking was used as an index to measure the painful response during the neurogenic period at 0-5 min (initial phase) and the inflammatory period at 25-30 min (secondary phase) after formalin injection.

Anti-inflammatory activity

The extracts were examined for their anti-inflammatory activities against Carragenan induced paw edema in mice as previously described. After 1 hour of treatment, 0.1ml of freshly prepared Carragenan suspension (1%) was injected into the sub plantar surface of hind paw. This produced inflammation. The paw thickness was calculated at 0, 1, 2 and 3 hours thereafter using of Vernier caliper.

Anti-pyretic activity

Tamarix aphylla extracts were also examined for their anti-pyretic activities against yeast induced pyrexia in mice as previously described. Pyrexia was induced by subcutaneous injection of 20% w/v aqueous suspension of Brewer's yeast 2ml/kg. After 24 hours, rectal temperatures were noted (pre-treatment values) by using clinical thermometer. After treatment, the rectal temperature for all the groups was taken at 1 hour interval for up to 3 hrs.

STATISTICAL ANALYSIS

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

RESULTS

Analgesic activity of aqueous ethanolic extract of *Tamarix aphylla* is given in table 1. The aqueous ethanolic extract of *Tamarix aphylla* showed 42% inhibition ($p < 0.005$) of *acetic acid*- induced writhing as compared to normal control while aspirin showed 57% inhibition ($p < 0.005$). The aqueous ethanolic extract caused 63% reduction ($p < 0.005$) in *formalin-induced paw licking* as compared to normal control while aspirin showed 67% ($p < 0.005$). In *hot plate method*, the aqueous ethanolic extract showed 42% ($p < 0.05$) increase in reaction time as compared to control while aspirin showed 61% ($p < 0.005$).

The aqueous ethanolic extract of *Tamarix aphylla* did not show any anti-inflammatory effect (Table 2). Anti-pyretic

activity of *Tamarix aphylla* extract against yeast induced pyrexia is shown in table 3. The aqueous ethanolic extract showed 39.7 ± 0.4 , 37.9 ± 0.2 , 37.8 ± 0.14 and 37.9 ± 0.3 at 0, 1, 2 and 3 hrs whereas the standard drug aspirin showed 38.5 ± 0.23 , 37.6 ± 0.27 , 37.8 ± 0.19 and 37.4 ± 0.36 at 0, 1, 2 and 3 hours respectively.

DISCUSSION

The emergence 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; Qadir and Malik, 2011; Qadir, 2013), or synthesis from lactic acid bacteria (Masood *et al.*, 2011), or marine microorganisms (Javed *et al.*, 2011). However, now a days, the trend is being changed from synthetic drugs to the natural drugs either from plants or microbes to control the diseases (Iqbal *et al.*, 2014). The natural products are constantly being screened for their possible pharmacological value particularly for their hypotensive (Qadir, 2010), antihyperlipidaemic (Ahmad *et al.*, 2012) hepatoprotective (Ali *et al.*, 2013; Mallhi *et al.*, 2014; Saleem *et al.*, 2014a; Qadir *et al.*, 2014), hypoglycaemic (Nisa *et al.*, 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic (Saleem *et al.*, 2014b), antimicrobial (Amin *et al.*, 2012; Azam *et al.*, 2013; Saleem *et al.*, 2014c), spasmolytic (Janbaz *et al.*, 2014), bronchodilator (Janbaz *et al.*, 2013a), antioxidant (Janbaz *et al.*, 2012), anti-diarrheal (Janbaz *et al.*, 2013b), anti-cancer (Saleem *et al.*, 2013), analgesic (Parveen *et al.*, 2014) and anti-inflammatory (Qadir, 2009) properties. The folkloric claim of *Tamarix aphylla* in pain, fever and inflammation has been authenticated in this study.

Results of the present study showed that aqueous ethanolic extract of *Tamarix aphylla* exhibit analgesic activity by reducing acetic acid induced writhing, mean reaction time on hot plate model and formalin induced paw licking in mice. Formalin test is mostly used for testing the analgesic activity. It produces a distinct two phase effect and various drugs of different nature act in a different way in early and late phases of this test. So, this test can be helpful to investigate the process of nociception of suggested analgesic drugs (Tjolsen *et al.*, 1992). The extracts also showed good results in formalin induced paw licking in mice. The formalin injection when administered produces an intense increase in spontaneous activity of C fiber. That indicates pain in the form of paw licking by different animals. The duration of paw licking during the neurogenic period (0-5 min) and the inflammatory period (25-30 min) are the indication of the pathway through which the extract is acting either

Table 1: Analgesic activity of aqueous ethanolic extract of *Tamarix aphylla*

Treatment/dose	No. of Writhing (Mean $\pm$ SEM)	No. of Paw lickings (Mean $\pm$ SEM)			Reaction time in sec (Mean $\pm$ SEM)		
		0-5 Min	25-30 Min	Total	0 min	30 min	60 min
Control (Normal saline 2 ml/kg)	33 $\pm$ 2.1	85 $\pm$ 6.1	12 $\pm$ 2.3	288 $\pm$ 10.9	5.2 $\pm$ 0.4	4.6 $\pm$ 0.8	5.6 $\pm$ 0.8
Aqueous ethanolic extract of <i>Tamarix aphylla</i> (100mg/kg)	19 $\pm$ 2.4**	33 $\pm$ 4.3**	6 $\pm$ 1.2**	107 $\pm$ 9.6**	4.9 $\pm$ 0.5	11.45 $\pm$ 0.9**	9.65 $\pm$ 1.6*
Standard (Aspirin 100mg/kg)	14 $\pm$ 0.96**	35 $\pm$ 5.5**	3 $\pm$ 0.9**	94 $\pm$ 8.24*	5.3 $\pm$ 0.9	16.8 $\pm$ 1.5**	14.5 $\pm$ 1.4**

Table 2: Anti-inflammatory activity of *Tamarix aphylla* against carrageenan induced paw edema in mice

Treatment	Paw Thickness (cm) (Mean $\pm$ SEM)			
	0hr	1hr	2hr	3hr
Control (Normal saline 2 ml/kg)	0.83 $\pm$ 0.001	1.28 $\pm$ 0.01	1.63 $\pm$ 0.01	1.86 $\pm$ 0.01
Aqueous ethanolic extract of <i>Tamarix aphylla</i> (100 mg/kg)	1.02 $\pm$ 0.01	1.14 $\pm$ 0.02	1.41 $\pm$ 0.04	1.92 $\pm$ 0.07
Standard (Aspirin 100 mg/kg)	0.79 $\pm$ 0.01**	0.86 $\pm$ 0.01**	0.92 $\pm$ 0.01**	1.15 $\pm$ 0.18**

Table 3: Anti-pyretic activity of *Tamarix aphylla* against yeast induced pyrexia in mice

Treatment/Dose	Rectal Temp ($^{\circ}$ C) after 18 hrs of Yeast Injection (Mean $\pm$ SEM)			
	0hr	1hr	2hr	3hr
Control (Normal saline 2ml/kg)	39.2 $\pm$ 0.3	39.5 $\pm$ 0.26	39 $\pm$ 0.4	39.5 $\pm$ 0.22
Aqueous ethanolic extract of <i>Tamarix aphylla</i> (100mg/kg)	39.7 $\pm$ 0.4	37.9 $\pm$ 0.2**	37.8 $\pm$ 0.14*	37.9 $\pm$ 0.3**
Standard (Aspirin 100mg/kg)	38.5 $\pm$ 0.2	37.6 $\pm$ 0.3**	37.8 $\pm$ 0.19*	37.4 $\pm$ 0.4**

* p<0.05, ** p<0.005

peripheral or central pathway. Our extracts showed more paw licking during the nurogenic period than the inflammatory period indicating that the extracts act through peripheral pathway.

Aqueous ethanolic extract of the plant did not show significant anti-inflammatory activity. However, aqueous ethanolic extract of the plant showed significant antipyretic effect in yeast-induced elevation of body temperature in mice.

CONCLUSION

This study has proved that aqueous ethanolic extract of *Tamarix aphylla* exhibit analgesic and antipyretic activity but it lacks anti-inflammatory activity. Hence, it supports the use of these plants in ethno medicine to relieve pain and fever. However, further investigation is required to elucidate cellular mechanism and to establish structural components of active ingredients for standardizing them.

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