

ANTI- INFLAMMATORY AND ANALGESIC ACTIVITY OF TAMARIX GALLICA

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ABSTRACT

The methanolic extract from aerial parts of *Tamarix gallica* was investigated for anti-inflammatory and analgesic properties in animal models. The acute anti-inflammatory study was done using carrageenan and histamine hind paw edema method. The extract was tested at doses between 100 – 300 mg/kg body weight of rats to observe % inhibition of paw edema. The extract administered orally at doses of 100, 200 and 300 mg/kg p.o produced a significant ($P < 0.05$) dose dependent inhibition of edema formation due to carrageenan and histamine. The observed activity was found comparable to that of 10 mg/kg diclofenac that was used as a reference drug in the study. The Preliminary phytochemical screening conducted on *Tamarix gallica* confirmed the presence of tannins, flavonoids, alkaloid and saponins.

The central and peripheral analgesic activity of *Tamarix gallica* was also investigated by tail flick, hot plate and acetic acid induced writhing method on swiss albino mice. The analgesic activity of *Tamarix gallica* was detected by increase in the reaction time and by reduction in number of writhes.

The methanol extract at the dose of 300 mg/kg/p.o showed 58.82 % inhibition against carrageenan induced rat paw edema while the standard drug Diclofenac showed 64.26% inhibition after 4 hrs of carrageenan injection.

Tamarix gallica methanolic extract supports the folkloric use for management of inflammation and pain.

Keywords: *Tamarix gallica*, Anti-inflammatory activity, Carrageenan, Histamine, Tail flick, Hot plate and Acetic acid, Diclofenac.

INTRODUCTION

The term inflammation is derived from the Latin word “*inflammō*”, meaning ignite, set alight or burn. Inflammation is a physiopathological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells¹. Any form of injury to the human body can elicit a series of chemical changes in the injured area. Earlier inflammation was contemplated as a single disease caused by disturbances of body fluids. However, according to the modern concept, inflammation is a healthy process resulting from some disturbance or disease. The cardinal signs of inflammation are heat, redness, swelling, pain and loss of function. Inflammation usually involves a sequence of events which can be categorized under three phases viz. acute transient phase, delayed sub-acute phase and chronic proliferate phase. In the first phase, inflammatory exudates develop due to enhanced vascular permeability leading to local oedema followed by the migration of leukocytes and phagocytes from blood to vascular tissues in the second phase. The third phase of inflammation is marked by tissue degradation followed by fibrosis.

For treating inflammatory diseases, an array of analgesic and anti-inflammatory drugs are available in the market but only very few are free from toxicity. Hence use of plants stages a comeback and herbal ‘renaissance’ all over the globe. Purified natural compounds from plants serve as template for the synthesis of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value. The herbal products hence symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment.

The *Tamarix gallica* L. belongs to the family Tamaricaceae. The *Tamarix gallica* are deep-rooted, gregarious bushy ornamental shrubs or trees that are native to the Mediterranean region and northern China. About 125 species of the plant have been reported from different regions of Europe, USA, Asia and Africa². The plant grows in saline or water logged soil up to 17 to 19 feet. As it ages the smooth, reddish-brown bark of younger plants turns brownish-purple, ridged, and furrowed. Feathery, needle-like leaves, often encrusted with salt secretions, grow on its thin branches. Masses of small, pink flowers blossom on the ends of its branches from June to August. The principle constituent in the plant is tamarixin along with polyphenolic compounds such as flavonoids, phenolic acids, tannins and coumarins^{3, 4}. The Quercetin glycosides are found abundant in

plants and their 3-O-methyl (isorhamnetin) derivatives are not uncommon. However, the 4-O-methyl Quercetin (*Tamarixetin*) glycosides are rare in nature.

Tamarix species are employed in traditional medicine as astringent, aperitif, stimulus of perspiration and diuretic⁵. The branchlets and the leaves are astringent and diuretic. An external compress of the leaves helps to stop the wound bleeding. The plants have been reported to be useful in leucoderma, spleen trouble and eye diseases⁶. Several researches have proved antioxidant and antimicrobial activities of *Tamarix* species such as *T. ramosissima* and *T. hispida*⁷. In Algeria and surrounding areas the plant has been used medicinally for rheumatism, diarrhoea, and other maladies. Vegetable and fruit peels of the plant are used as a novel source of antioxidants⁸. Antimicrobial activity of *Tamarix gallica* has also been reported⁹.

MATERIALS AND METHODS

Plant Material and Preparation of Methanolic extract

The aerial parts of *Tamarix gallica* for the study were procured from Nirankar Herbs; 2211/4344/Aggarwal market, Tilak Bazar, kharibawri market, Delhi-6 (India) locally in the month of February 2009. The authentication of leaves was done by Dr. H.B. Singh, Head, Raw material, Herbarium and Museum division, National Institute of Science Communication and Information Resources (NISCAIR), PUSA New Delhi.

The air-dried aerial parts were made into a coarse powder and macerated with 900ml of methanol for 48 hrs. Extraction was done through Soxhlet apparatus. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to give a semisolid brown syrupy consistency residue of 200gm, which was stored in a closed bottle and refrigerated at temperature below 4°C until tested.

Preliminary Phytochemical Analysis

Preliminary phyto-chemical analysis of extract was performed for analysis of alkaloids, carbohydrates, tannins, saponins, terpenes, coumarins, anthraquinones according to Khandelwal, 2006¹⁰. The extract was solubilized in normal saline (0.9% w/v sodium chloride) for use in in-vivo experimental animals. Results are given in the [Table 1] and [Table 2].

Table 1: Phyto-chemical analysis of Tamarix gallica

	Steroid	Alkaloid	Tannin	Carbohydrate	Phenol	Flavonoid	Saponin
Tamarix Gallica	-	+	+	-	+	+	+

(+) Methanolic extract of TG present; (-) Methanolic extract of TG absent

Table 2: Physicochemical parameters of Tamarix gallica

Parameter	Result (% w/w)
Loss on drying	9.23
Total Ash	13.6
Acid Ash insoluble	1.64
Water extractable matter	23.06
Alcohol extractable matter	10.81

Animals used for the test

Swiss albino mice (25-30g) of either sex were used for screening of analgesic activity by writhing test, tail flick and hot plate method. Wistar albino rats of (150-200g) were used for the anti-inflammatory activity by paw edema method. All experimental protocols were approved by the Institutional animal ethics committee, according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Animals were procured from the central animal house of the Delhi institute of Pharmaceutical Sciences and Research, New Delhi, India.

Test animals were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 2^\circ\text{C}$; relative humidity 60-70%) in a 12 hr. light-dark cycle. The animals were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 hr. before and during the experimental hours.

Drugs used for the test

The analytical grade drugs used for the test were normal saline, acetic acid (CDH), Aspirin (CDH), Carageenan (Sigma Aldrich), Diclofenac Sodium (Voveran, Novartis India Ltd.), and Histamine (Himedia Laboratories Pvt Ltd).

Acute toxicity study

Overnight-fasted Swiss albino mice (17-23g) of either sex were used for the study. The animals were divided into six groups of five animals each. Groups A to D received 100, 200, 400, 800, 1600 and 3200 mg/kg orally of the extract, respectively, while the control (group E), received distilled water (3 mL/kg) by the same route.

General symptoms of toxicity and mortality in each group were observed within 24 hr. and the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxia, diarrhoea, or increased diuresis. Animals that survived after 24 hr. were observed for any signs of delayed toxicity for two weeks¹¹.

Anti-inflammatory activity of Tamarix gallica on Carageenan induced rat edema

Twenty rats were used in this study and they were divided into five groups of four per group. Each group received one of the following treatments: plant extract, (100, 200, 300 mg/kg body weight, p.o), Diclofenac (10mg/kg body weight) or control normal saline (0.9%, 0.1ml in 3% Tween 80) administered orally. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contained Tween 80 in the right paw of rats¹². The paw volume was measured at 0, 1, 2, 3 and 4 hr. after carrageenan injection using a Plethysmometer. Anti-inflammatory activity was measured as the percentage reduction in oedema level when drug was present, relative to control¹³.

The percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = \frac{D_0 - D_t}{D_0} \times 100$$

Where

D_0 - Average inflammation (hind paw oedema) of the control group of rats at a given time

D_t - Average inflammation of the drug treated (i.e. extracts or reference diclofenac) rats at the same time¹⁴.

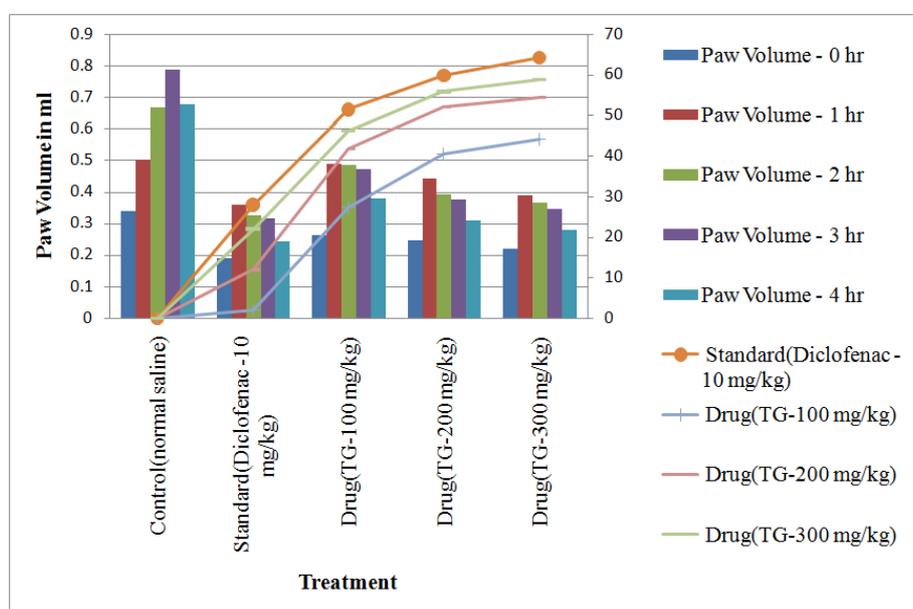


Fig. 1: Anti-inflammatory activity of Tamarix gallica on Carageenan induced rat edema

Histamine induced rat paw oedema

The rats were divided into five groups of four per group. 0.1 ml of 1.0% histamine sulphate in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw¹⁵. Each group received one of the following treatments. Group I (control) received 10ml/kg, p.o of normal saline(0.9% w/v, 0.1ml), Group II received standard drug diclofenac sodium 10 mg/kg, p.o, Group III received Tamarix gallica 100 mg/kg, p.o, Group IV received Tamarix gallica 200 mg/kg, p.o and Group V received Tamarix gallica 300 mg/kg, p.o. The Tamarix gallica was administered to the rats 30 min before histamine injection. The percentage inflammation was calculated from the formula-

$$\% \text{ inhibition} = \frac{D_0 - D_t}{D_0} \times 100$$

Where, D₀- Average inflammation (hind paw oedema) of the control group of rats at a given time

D_t- Average inflammation of the drug treated (i.e. extracts or reference diclofenac) rats at the same time. (Measured initially and at 1, 2, 3 and 4 hr. after histamine injection, using Plethysmograph)

Antinociceptive Activity

The writhing test in mice was carried out using the method of Seigmund, E et.al, 1957¹⁶. The writhes were induced by intraperitoneal injection of 0.06% acetic acid (v/v) that was administered orally to groups of six animals each, 30 min before chemical stimulus. The three different doses 100, 200, 300 mg/kg of methanolic extracts of Tamarix gallica and reference substance (aspirin) or solvent was administered 30 min. before the intra peritoneal administration of acetic acid. Control animals received 0.9%w/v NaCl solution under the same experimental conditions. Number of writhes per animal was counted during a 20 min. series beginning 5min after the injection of acetic acid. The number of writhing's and stretching's were recorded and permitted to express the percentage of protection using the formula ratio¹⁷.

$$\text{Percent protection} = (1 - V_c/V_t) \times 100$$

Where, V_t = Mean number of writhing in test animals

V_c = Mean number of writhing in control

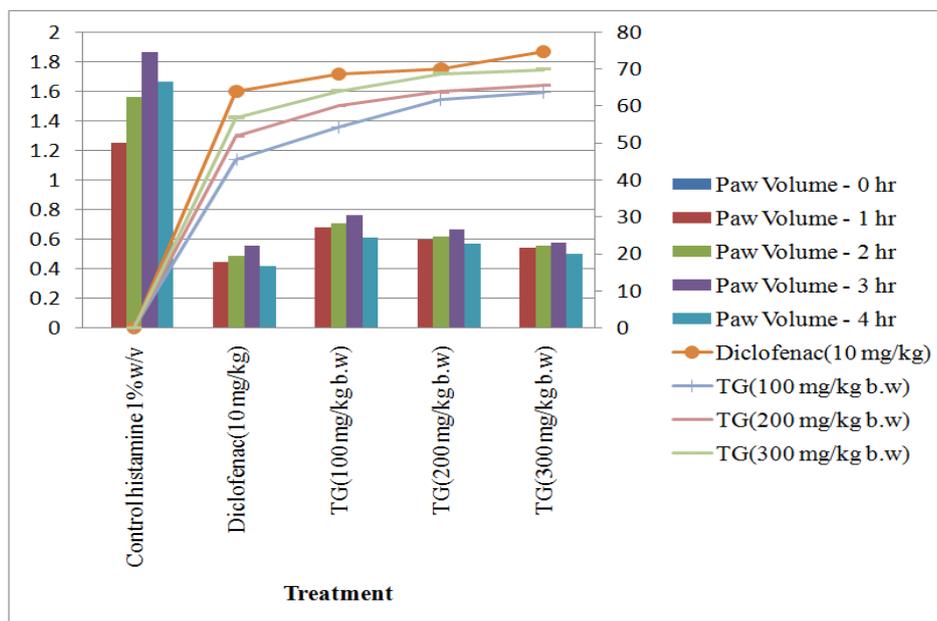


Fig. 2: Anti-inflammatory effect of Tamarix gallica on histamine induced rat edema

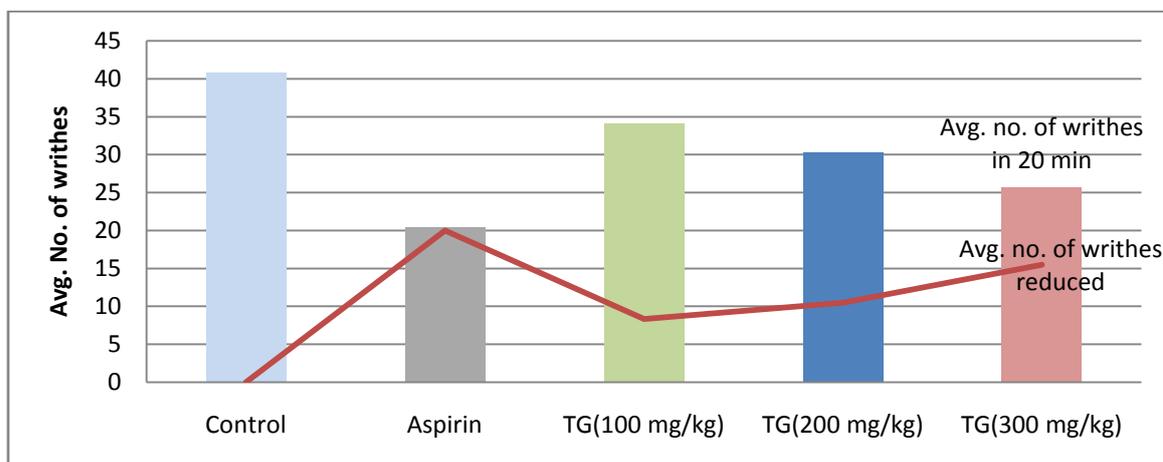


Fig. 3: Effect of Tamarix gallica extract on acetic acid induced writhing in mice

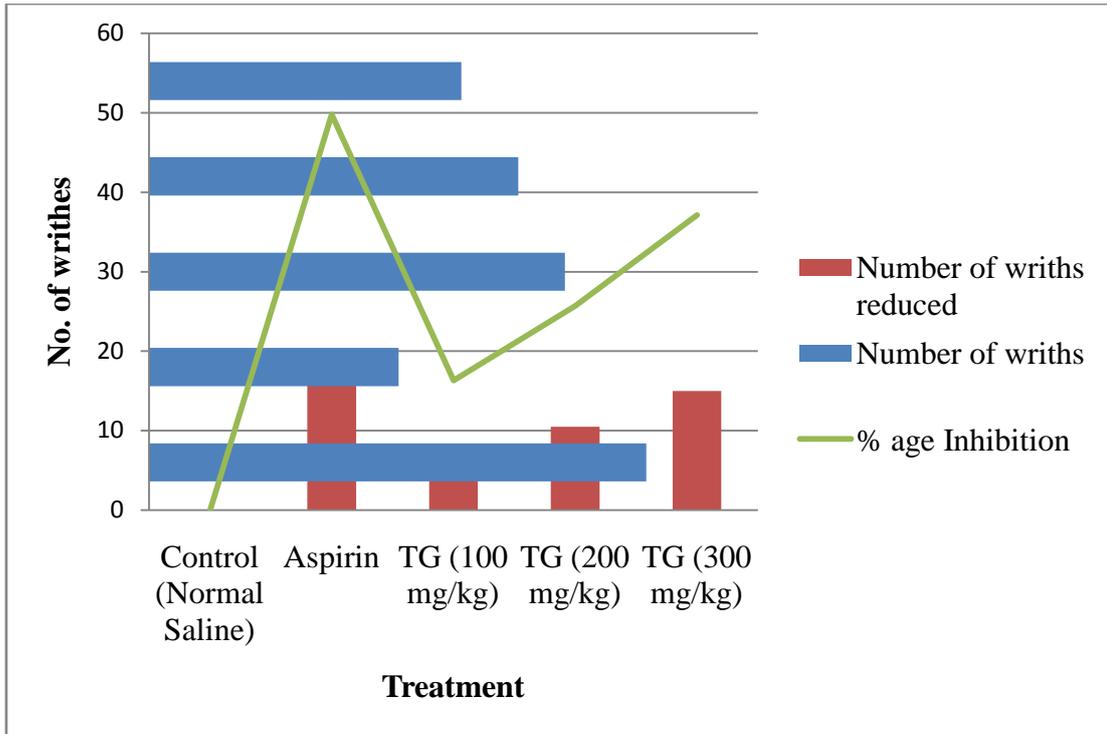


Fig. 4: Results of acetic acid induced writhing in terms of % age inhibition, writhes and writhes reduced

Central Analgesic Activity

The central analgesic activity was determined by radiant heat tail-flick model in mice using analgesiometer (Inco, India) ^{18, 19}. Experimental animals of either sex were randomly selected and divided into five groups designated as group-I, group-II, group-III, group-IV and group-V consisting of six mice in each group. Each group received a particular treatment i.e. control (Normal Saline 0.9%w/v, 10 ml/kg), positive control (Aspirin 100 mg/kg, p.o) and the test sample (methanolic extract of 100mg/kg, p.o & 200 mg/kg, p.o, 300mg/kg p.o respectively). The tail flick latency was obtained thrice before drug administration and mean was used as

pre drug latency. A cut off time of 10 sec was observed to prevent any tissue damage to the animal. The animal which failed to withdraw its tail in 3-5 sec was rejected from the study. The instrument's nichrome wire was heated to the required temperature and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The mice were kept in a holder with only the tail portion protruding. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting.

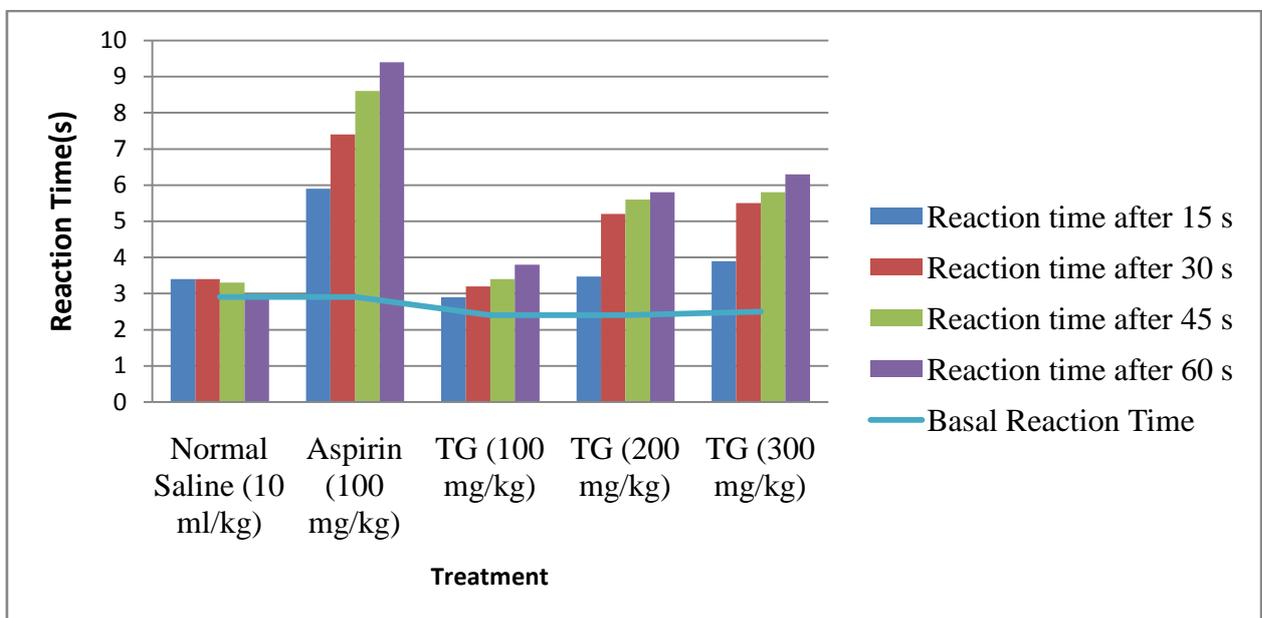


Fig. 5: Analgesic Effect by Tail Flick Method

Analgesic Effect by Hot Plate Method

The hot plate test in mice was performed by Eddy's hot Plate method²⁰. Animals were individually placed on a hot plate maintained at a constant temperature (55°C) and the reaction of animals, such as paw licking or jump response was taken at the end point. Experimental animals of either sex were randomly selected and divided into five groups designated as group-I, group-II, group-III, group-IV and group-V consisting of six mice in each group for

control, positive control and test samples. Each group received a particular treatment i.e. control, positive control. (Aspirin 100 mg/kg, p.o) and the test sample (methanolic extract of 100mg/kg, p.o & 200 mg/kg, p.o, 300mg/kg.p.o respectively).

The animals were positioned on Eddy's hot Plate kept at a temperature of 55±0.5 °C. A cut off period of 15 s was observed to avoid damage to the paw. The reaction time in control and treated animals was recorded at 0, 30, 60 min after the treatment²¹.

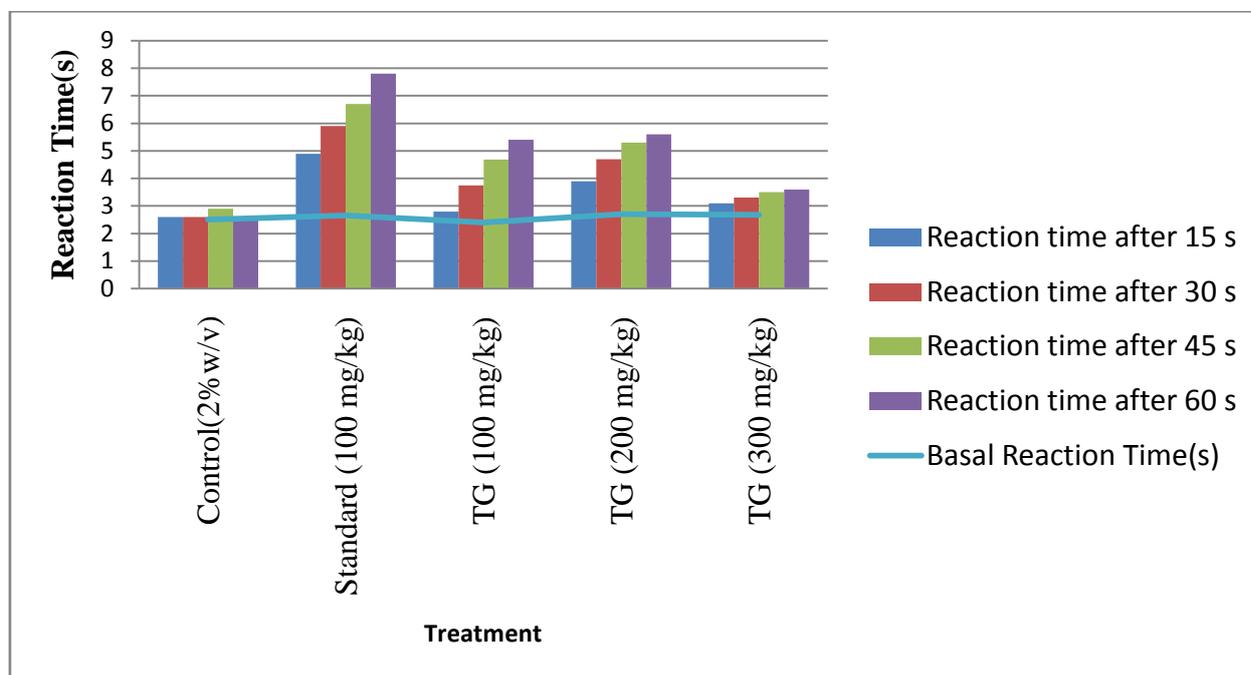


Fig. 6: Effect of Tamarix gallica extract on latency in hotplate test on mice

Statistical Analysis

The results were presented as mean± SEM. "One- way ANOVA" with Dunnett's post-test was performed using Graph Pad Prism version 3.00 for windows. (Graph pad software, San Diego California, USA), P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The present study was conducted to investigate the possible anti-inflammatory and analgesic effects of Tamarix gallica methanolic extract in animal models. It is known that the Carrageenan is a sulfated polysaccharide derived from certain species of algae. Inflammation produced by carrageenan is a triphasic response²². Carrageenan induced hind paw oedema is a standard experimental model of acute inflammation sensitive to cyclooxygenase (COX inhibitors) to evaluate the effect of non-steroidal anti-inflammatory agents. These agents mainly act by inhibiting cyclooxygenase involved in prostaglandin synthesis leading to the exudation of plasmatic liquid and proteins mainly migration of neutrophils²³. Activated neutrophils adhere to each other and to the endothelium. This results in capillary leakage, release of toxic oxygen species and lytic proteins, and recruitment of other inflammatory cells to the site of inflammation. Neutrophilic granules contain enzymes such as superoxide dismutase and myeloperoxidase which form toxic oxygen radicals O₂⁻, H₂O₂ and OH⁻ in the extracellular medium which promote the intensification and amplification of the inflammatory process. During this event, there is the sequential release of several inflammatory mediators, such as histamine, 5-hydroxytryptamine, bradykinin and finally prostaglandins²⁴. Scavenging of free radicals generated by neutrophils in inflammatory processes may be an important mechanism of the anti-inflammatory effect of Tamarix gallica.

Tamarix gallica at 100, 200 and 300 mg/kg administered 30 min before the injection of carrageenan inhibited the formation of edema by 44.11%, 54.41 %, 58.82% respectively 4 hr. after injection of the inflammatory stimulus. The effect of diclofenac (10 mg/kg) on carrageenan induced paw oedema was found to be most pronounced at 4 hr. after carrageenan injection and inhibited the edema by 64.26 %. A dose response correlation was observed for the tested concentrations in the paw edema test. The result was quite similar to the one observed for diclofenac at 10mg/kg. The results were found statistically significant. The extract also caused pronounced reduction in the oedema produced by histamine. This result tends to suggest that the anti-inflammatory activity of the extract is possibly supported by its anti-histamine property. The effect of the extract (100, 200 and 300 mg/kg) and the reference drug on histamine-induced paw oedema was most pronounced at 4 hr. After histamine injection. The pain in the late phase may be attributed to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinin. Since the extract effectively suppressed the oedema produced by histamine, it can be concluded that that the extract exhibits dose-dependent anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

Carrageenan induced edema had no adverse effect on the behavioural responses of the tested mice up to 14 days of observation. There was no mortality observed at all by the tested doses. None of the used doses affected the weight of the mice.

From the study conducted it is also established that Tamarix gallica produces antinociceptive effects when assessed by chemical and thermal methods. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests were found most sensitive to centrally acting

analgesics. In the Central Analgesic test, Tamarix gallica (100mg/kg, 200 mg/kg, 300 mg/kg,) significantly increased the reaction time suggesting towards its analgesic activity. Also, the results of hot-plate and tail-flick model in mice showed that the methanolic extract of Tamarix gallica and aspirin, dose-dependently increased the pain threshold. This indicates that the extract has analgesic property similar to aspirin, a standard NSAID.

The phytochemical screening confirmed the presence of flavonoids, tannins and saponins in the methanolic extract of aerial parts of Tamarix gallica. Flavonoids have been reported to play role in analgesic activity by targeting prostaglandins and are known to inhibit prostaglandin synthetase, more specifically the endoperoxidase^{25, 26}. The role of tannins in anti-nociceptive activity has also been reported²⁷. Besides alkaloids are well known for their ability to inhibit pain perception²⁸. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity of Tamarix gallica.

CONCLUSION

From the current study, it is concluded that Tamarix gallica possesses both anti-inflammatory and analgesic effects comparable to that of non-steroidal drugs such as diclofenac and Aspirin respectively. A significant % inhibition of paw edema suggests usefulness of Tamarix gallica as an anti-inflammatory agent. Since the extract effectively suppressed the oedema produced by histamine and carrageenan and increased the pain threshold, it can be concluded that the extract exhibits anti-inflammatory and analgesic action by inhibiting the synthesis and release of inflammatory mediators such as histamine, serotonin and prostaglandins.

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