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Anti-Inflammatory, Analgesic, Antipyretic and the Membrane-Stabilizing Effects of *Tamarix aphylla* Ethanolic Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOB and AHM designed the study; author MAAD performed the experiments, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MFL managed the analyses of the study, the literature searches and revise the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: 1) To screen *Tamarix aphylla* for phytochemical constituents. 2) To study the anti-inflammatory effect, analgesic and antipyretic activity of the ethanolic extract of the plant, and 3) to assess membrane-stabilizing activity of the plant extract as a mode of its actions.
Place and Duration of Study: The study was conducted in the laboratories of pharmacology and phytochemistry of the medicinal and aromatic plant research institute, national center for research, Khartoum, Sudan during a six Month period.
Methodology: Standard methods from the laboratory sheet were used to detect the phyto-

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conistituents of the plant. The pharmacological activities; the edema inhibition percentage (EI%), the granuloma tissue-formation inhibition percentage, the antipyretic, analgesic and membrane-stabilizing ability were determined using animal models as described in standard methods.
Results: *Tamarix aphylla* phytochemical screening showed that it contains flavonoids, saponin, cumarins, and tannins and traces of triterpenes and alkaloids.
Tamarix aphylla at a dose of 200 mg/kg achieved highest EI% 4 hours after oral dosing of the extract suspended in distilled water. *Tamarix aphylla* at a dose of 200 mg/kg and 100 mg/kg caused 71.86% and 67.05% inhibition of the granuloma tissue formation respectively; which were significantly more compared to indomethacin (32.25%, P< 0.05). *Tamarix aphylla* at a dose of 200 mg/kg and/or 100 mg/kg significantly increases the response time of the rats and reduces rats' body temperature compared to acetylsalicylic acid (P< 0.05). It also showed significant inhibition of RBCs hemolysis by heat or hypotonic solution compared to acetylsalicylic acid (P< 0.05), indicating membrane-stabilizing ability.
Conclusion: The present results indicates that the ethanolic extract of *Tamarix aphylla* possesses anti-inflammatory, anti-pyretic, analgesic activity and membrane-stabilizing ability.

Keywords: Tamarix aphylla anti-inflammatory; analgesic; antipyretic; membrane stabilizing ability.

1. INTRODUCTION

Tamarix aphylla; (local Altarfa or Athil) is a member of Tamaricaceae; a family that is widely distributed particularly in semi-arid regions of the tropics and subtropics. In Sudan it is found in Northern Sudan on sites adjoining rivers or seasonal water courses and in the main Nile, Blue Nile between Sinnar and Singa and Rahad and upper Atbara, Gash Delta, Khor Baraka and KhorArbaat [1].

Inflammation is a universal host defense mechanism involving a complex network of cell-cell, cell-mediated and tissue interactions. It occurs in response to a variety of harmful stimuli, physical, chemical, traumatic, antigen challenge, infections and ionization, etc. Immune system gets activated, communication and coordination occurs between different classes as well as actions of immune cells to produce inflammation [2]. Researches in the last few decades have shown that inflammation is regulated by a large number of pro and inflammatory mediators [3,4].

In a developing country as Sudan, traditional herb medicines serve as the major source of treatment for people and animals as well. Hundreds of plants were used in the treatment of several diseases and conditions. *Tamarx aphylla* is one of these plants although it did not receive any scientific attention.

Mohsin et al. [5], reported that *Tamarix nilotica* ethanolic extract has significant analgesic and antipyretic activities and contains alkaloids, flavonoids, cyanogenic glycosides, tannins. In Sudan Folk medicine, the decoct of bark is used for the treatment of various conditions, including fever, joint pain and edema; also it is used to treat filariasis and jaundice. However, it lacks any scientific proofs for its benefits. The aims of this study were; phytochemical screening of *Tamarix aphylla,* to evaluate its anti- inflammatory activity, to determine its antipyretic and analgesic effects. The membrane-stabilizing ability of *Tamarix aphylla* was studied as a possible mechanism of action.

2. EXPERIMENTAL

2.1 Collection and Extraction of Plant Materials

The plant was authentificated by taxonomists of Medicinal and Aromatic Plants Research Institute (MAPRI) – Sudan, a sample was deposited at the herbarium in the institute (Code number: MAPRI H.W.S.44-95). The stem bark of the plant was collected and dried at room temperature, coarsely powdered and extracted using the soxhlet apparatus, by petroleum ether, then by chloroform and finally by 70% ethanol. The ethanolic extract was evaporated to dryness under reduced pressure and kept into a refrigerator to be used for the different tests.

2.2 Animals

Adult Wistar albino rats (both sexes) weighing 90-200 g (a total of 230 rats), were purchased at the time of each experiment, from the animal center of MAPRI - National Center for Research – Khartoum - Sudan. In each experiment a suitable animals weights were used. All animals were left free to access food and water, at room temperature 25±1ºC; day and night was maintained at 12/12cycle.Animals were fasted

before conducting the experiments for at least 12 hours. An ethical consent of using the animals in the experiments were obtained from the Collage of Veterinary Medicine Research Board, Sudan University of science and technology, and The Medicinal and Aromatic Plant Research Institute Research Committee.

2.2.1 Screening for phytoconistituents

Standard methods adopted by the department of Chemistry, Medicinal and Aromatic Plants Research institute, for the phytochemical screening of the plants, were used to detect the phytoconistituents as follows:

I. Unsaturated sterols and triterpenes: To 0.5 g of the ethanolic extract, one ml chloroform was added, and then 0.5 ml of acetic acid anhydride was added followed by 2 drops of concentrated sulphuric acid. The gradual appearance of green, blue, pink to purple colors, was considered as an evidence of the presence of sterols (green to blue) and triterpenes (pink to purple).
II. Alkaloids: To 0.5 g of the extract five ml of 2N hydrochloric acid were added and heated with stirring in a water bath for 10 minutes. The mixture was cooled, filtered and divided into two test tubes. Few drops of Mayer's reagent were added to one test tube. Turbidity or heavy precipitate in either tube was considered presumptive evidence for the presence of alkaloids.
III. Flavonoids: half g of the ethanolic extract of the plant was dissolved in 1 ml ethanol and then 1 ml of 1% KOH was added. Dark yellow color indicates the presence of flavonoids. To confirm the result, 1 ml of aluminum chloride was added to the extract. A change of the mixture to yellow color confirms the results.
IV. Saponin: one ml of distilled water was added to the extract in a test tube and was shaken. Foams Formation was indicative for the presence of saponin.
V. Cumarins: half g of the extract was boiled in 20 ml of distilled water; a filter paper was attached to the test tube to be saturated with the vapor then a spot of 0.5 N KOH was put on it. The filter paper was inspected under ultraviolet light. Adsorption of ultraviolet light was taken as positive for cumarins.
VI. Anthraquinon: half g of the extract was boiled in 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was shaken with 5 ml benzene and allowed to separate into two layers, and then 3 ml of 10% ammonium hydroxide solution were added. The presence of anthraquinones was indicated if the alkaline layer was changed to pink or red color.
VII. Tannins: 1 g of the extract was cooled after the addition of 10 ml of hot normal saline, and then to 5 ml of the mixture, gelatin salt reagent was added. Immediate precipitation was considered positive for the presence of tannins. In addition, To the other 5 ml of the mixture ferric chloride test reagent was added, blue, black or green colors were considered positive for the presence of tannins.

2.3 Anti-Inflammatory Activity

2.3.1 Induced paw edema model

The anti-inflammatory activity of ethanolic extract was studied using rat paw formalin edema method as described by Ramadan et al. [6] as a modification of the method described by Domenjoz et al. [7]. Twenty rats (both sexes of 150-180 g body weight) were divided into 4 groups, 5 rats in each. At the beginning of the experiment the thickness of the left paw was measured in mm. Thereafter the first group was kept as a non treated control and the second one was orally dosed with indomethacin (in Ramadan et al. [6] phenylbutazone was used instead and formalin is used in this study instead of carrageenan, [8]), at a dose rate of 5 mg/kg. Group 3 and 4 were orally given the extract in doses of 200 and 100mg/kg. One hour later .1ml of 1% formalin was injected in the sub-planter region of the left paw of the rat. The rat's paw thickness were measured before the formalin injection and then 1,2,3,4,6, and 24 hours after oral dosing of the drug and the extract. The inflammatory response to formalin was calculated by comparing the mean paw thickness (MPT) in mm to the mean of the increase in paw thickness after injection of formalin. EI% was calculated by the formula:

Edema inhibition percentage (EI (% [6] calculated based on edema formation percentage as follows:

- Edema formation percentage (EF %) = (Tt-To)/ To × 100.

- Edema inhibition percentage (EI %) = (EFc -EFt)/EFt × 100.

Where

- To = the paw thickness before formalin injection (mm).
- Tt = the paw thickness after t hours of formalin (mm).
- EFc = edema formation rate of the control group
- EFt = edema formation rate of the treated group at t hours time.

Analysis of variance and Dennett's tests as part of one- and two-way ANOVA and multiple comparisons between the observations of the extract at two doses, indomethacin and normal saline were conducted via SPSS program version 18 [9,10].

2.3.2. Cotton pellet granuloma model

Cotton pellet induced granuloma: Granuloma-formation inhibition was studied using the method described by [8,11]. Twenty rats were divided into 4groups (N = 5 rats of 180-200g body weight), then sterile cotton pellet of half g was implanted in the subcutaneous tissue, in the groin region under light anesthesia in each rat. A stitch was made to prevent the leakage of the exudates. Oral treatment of the rat in the different groups as follows: group 1 *Tamarix aphylla* 200 mg, group 2 *Tamarix aphylla* 100 mg, group 3 indomethacin 5 mg/kg, and group 4 normal saline once a day for 5 days was carried. Rats were sacrificed under anesthesia and the cotton pellets were removed cleaned from extraneous materials, dried in an oven at 60°C overnight, then weight. The increase in weight from the original weight was considered as the granuloma tissue and was expressed as means ±SEM. Percentage from the means of the extract and indomethacin was calculated considering the mean increase of the group treated with normal saline as 100%. [8,11]

The GTI% was calculated using the following formula:

Granuloma tissue-formation inhibition percentage (GTI %) = (C0-Ct)/Ct ×100

Where

- C0= the mean of the differences in cotton weight of the control group
- Ct= the mean of the differences in cotton weight of the treated group.

ANOVA test followed by Dennett's tests as part of one- and two-way ANOVA and multiple comparison of the result of the different groups was used via SPSS software, Version 18.

2.4 Evaluation of Analgesic Activity

The hot-plate method [12], was used to study the analgesic activity of the ethanolic extract of *Tamarix aphylla*. Twenty rats of both sexes of 100-120 g body weight were divided into 4 groups (N=5 rats). Oral dosing of the groups was carried as follows: group 1 was given aqueous suspension of the ethanolic extract of *Tamarix aphylla* at 200 mg/kg, group 2 was given aquous suspension of the ethanolic extract of *Tamarix aphylla* at 100 mg/kg, group 3 was given acetylsalicylic acid (Bayer Company - Germany) at 100 mg/kg, the fourth group was given normal saline as a control. The response time of the rat towards a hot plate maintained at 55±0.5ºC was recorded 10 minutes, and 5 minutes before the treatment; and the 60, 90 and 150 minutes after the treatment. The response time was described as the interval from rat reaches the hot plate till it start liking its feet or jumping outside the jar(using the Hot plate model 39 - Wagtech International Ltd- England).

Observations were subjected to statistical analysis by SPSS software version 18 as ANOVA and Dunnett's test for multiple comparisons were calculated.

2.5 Evaluation of Antipyretic Activity

Antipyretic activity was studied by the induction of hyperpyrexia by subcutaneous injection of 20% yeast aqueous extract at a dose of 20ml/kg [6,12]. Twenty rats (both sexes), were selected on the base of elevated body temperature, and were divided into 4 groups(N = 5 rats, of 90-110g body weight). Each group of the rat was orally dosed as follows; group 1 was given normal saline as a control group, group 2 was given acetylsalicylic acid at a dose of 100 mg/kg, group 3 was given *Tamarix aphylla* at 100 mg/kg, and group 4 was given *Tamarix aphylla* at 200 mg/kg.

Body temperatures were measured at 5 minutes before the treatment and the 1,2, and 4 hours after the treatment [6,12], by Them alert model No.TH5 (Physitemp -U.S.A). Data were subjected to statistical analysis using SPSS software version 18 to calculate ANOVA and

Dennett's test for multiple comparisons between the standard drug, the extract, and normal saline.

2.6 Evaluation of Membrane Stabilizing Ability

The membrane stabilizing activity of *Tamarix aphylla* was evaluated according to Shinde et al. [13], and Abe et al. [14]. Erythrocytes were separated from rat's blood and suspended in 40% 10 mM NaPO4 and was used to determine the membrane-stabilizing ability against heat-induced and hypotonic solution-induced hemolysis. *Tamarix aphylla* ethanolic extract at 50, 100, and 200 µg/ml in isotonic buffer of 10 mM sodium phosphate were poured in duplicated test tubes with 2 test tubes containing only the isotonic buffer as a control and a pair containing acetylsalicylic acid as a reference drug at a concentration of 200 µg/ml. To each test tube, erythrocyte 5 ml suspension was added (30µl), and mixed gently. One of the pairs was kept at 0-5°C while the others were incubated at 54°C. The tubes were then centrifuged, the supernatant was removed to measure its optical density at 540 nm by UV-160A.Hypotonic solution(154mM Na Cl), containing the extract in 50, 100, and 200 µg/ml concentrations, acetyl salicylic acid (aspirin) 200 µg/ml, and hypotonic solution drug-free, were used in duplicated test tubes .the tubes were left at room temperature for 10 minutes, then centrifuged and the optical densities of the supernatant were recorded as previous [13]. The results were calculated as percentage inhibition using the following formula:

%Acceleration or inhibition of hemolysis= (1-[(OD2-OD) /(OD3−OD1)]) ×100

Where:

- OD1=test sample unheated or in isotonic solution.
- OD2=test sample heated or in hypotonic solution.
- OD3=control sample heated or in hypotonic solution.

Observations were subjected to statistical analysis by SPSS software version 18 to calculate ANOVA, and Dennett's multiple comparison between the standard drug, normal saline and the extract at two doses.

3. RESULTS

The phytochemical screening: (Shown in Table 1) revealed the presence of flavonoids, saponin, cumarins, and tannins in abundance in the ethanolic extract of *Tamarix aphylla*, while there were traces of triterpenes and alkaloids but it is devoted from unsaturated sterols and anthraquinon.

Table 1. Phytochemical screening of *Tamarix aphylla*

Ingredients	Results
Flavonoids , Saponin, Cumarins, &Tannins	present
Triterpenes & Alkaloids	Traces
Anthraquinon & Unsaturated sterols	absent

As shown in Table 2, for both indomethacin at a dose of 5 mg/kg, and *Tamarix aphylla* at a dose of 200 mg/kg the peak EI% were reported after 4 hours of oral dosing. *Tamarix aphylla* at a dose of 100 mg/kg showed a peak EI% after 3 hours (Table 2). The reduction in MPT and EI% following administration of ethanolic extract of *Tamarix aphylla* was dose-dependent.

As shown in Table 3 granuloma tissue-formation inhibition percentage of *Tamarix aphylla* at a dose of 200 mg/kg caused 71.86% and at a dose of 100 mg/kg caused 67.05% which were significantly more compared to indomethacin which caused 32.25% inhibition of the granulation tissue, (P< 0.05).

Table 2. Effects of ethanolic extracts of *Tamarix aphylla* and indomethacin on rat paws' thickness and edema inhibition percentage at studied time intervals

Extract/ drug	Parameter	Time interval						Mean
		1 hour	2hours	3hours	4hours	6hours	24hours	
T. aphylla	EI%	82.1	39.5	51.2	90.6*	85.9	82.7	72.9
200 mg/kg	MPT(mm)	5.14±.4	6.36±.5	5.64±.7	4.83±.2	4.78±.2	4.64±.1	5.13±.7
T. aphylla	EI%	47.8	38	73.5*	61.3	60.4	52	38
100 mg/kg	MPT(mm)	6.88±.1	6.82±.2	5.95±.3	6.08±.2	5.92±.1	5.89±.1	6.07±.6
Indomethacin	EI%	24.2	32.2	50.9	97*	83.4	71.4	64
5 mg/kg	MPT(mm)	8.29±.6	7.59±.4	6.67±.5	5.63±.3	5.91±.3	6.05±.2	6.52±1.1
Normal saline	MPT(mm)	9.03±.7	8.45±.4	7.7±1.4	8.24±.4	7.69±.3	7.21±.2	7.68±1.2

**The highest edema-inhibition percentage*

Table 3. Granuloma tissue formation inhibition percentage for *Tamarix aphylla* ethanolic extracts and Indomethacin

Extract/drug	Granuloma weight (mg) Mean ± SEM	Percent inhibition
T. aphylla 200 mg/kg	33.17±11.01	71.86
T. aphylla 100 mg/kg	38.83±0.6	67.05
Indomethacin 5 mg/kg	79.83±4.46	32.25
Normal saline	117.83±0.6	

The peak rats' response to analgesia was recorded after 60 minutes of 200 mg/kg, and 100 mg/kg. The extract significantly increased the response time compared to acetylsalicylic acid ASA (Table 4).

Tamarix aphylla ethanolic extract at a dose of 200 mg/kg and 100 mg/kg, significantly reduced body temperature of hyper-thermic rats compared to acetylsalicylic acid (Table 5).

As shown in (Table 6), *Tamarix aphylla* ethanolic extracts at a concentration of 50µ/ml, 100µ/ml, 200µ/ml, showed significant inhibition of heat-induced and hypotonic solution-induced red cell hemolysis compared to acetylsalicylic acid at 200µ/ml concentration ($P<0.05$ using Dunnett test).

Table 4. The analgesic activity of *Tamarix aphylla* ethanolic extracts and acetylsalicylic acid (ASA)

Extract/ drug	Body temperature/interval (°C)					Mean±SEM (°C)
	10 min. before Treatment	5min. after treatment	1hour after treatment	90 min. after treatment	150 min. after treatment	
T. aphylla 200 mg/kg	7.02	7.44	24.44	17.72	12.28	13.77±1.23
T. aphylla 100 mg/kg	6.33	7.42	17.53	16.48	12.09	11.97±.88
ASA 100 mg/kg	7.91	10.91	13.42	11.68	9.24	10.63±.57
Normal saline	6.75	7.87	16.88	14.19	10.19	5.49±.19

Table 5. The antipyretic effect of *Tamarix aphylla* ethanolic extracts and acetylsalicylic acid (ASA)

Extract/drug	Body temperature/interval (°C)				Mean±SEM (°C)
	Before treatment	1hour after treatment	2hours after treatment	4hours after treatment	
T. aphylla 200 mg/kg	39.56±.42	36.36±.11	36.38±.11	36.28±.1	37.03±.18
T. aphylla 100 mg/kg	39.52±.29	36.32±.07	36.35±.11	36.31±.06	37.06±.17
ASA 100 mg/kg	39.44±.3	36.58±.27	36.36±.06	36.39±.11	37.32±.17
Normal saline	38.7±.81	38.24±.34	38.9±.44	37.46±2.29	38.46±.14

Values are means of five observations

Table 6. Membrane stabilizing ability percentage inhibition of RBCs hemolysis produced by *Tamarix aphylla*ethanolic extracts and acetylsalicylic acid

Extract/Drug	Concentrations	Heat-induced hemolysis % inhibition Mean ± SEM	Hypotonic solution induced hemolysis% inhibition Mean ± SEM
Tamarix aphylla	50 µg/l	33.56±1.17*	79.32±.31*
	100 µg/l	61.68±.63*	87.33±.49*
	200 µg/l	77.94±1.49*	95.67±.38*
ASA	200µg/l	25.19±.28	76.41±.61

*n=6; *P<0.001 vs. control, Student's t-test values are means of 5 ±SEM*

4. DISCUSSION

The results of the current study represent an earlier report on the possible therapeutic effects of *Tamarix aphylla* and give scientific confirmation for the anti-inflammatory, anti-pyretic and analgesic effects of this herb. These exciting therapeutic benefits are probably attributed to the unique phytoconstituents of *Tamarix aphylla* like triterpenes, alkaloids, flavonoids, saponin, cumarins, and tannins.

Although literature lacks previous researches in pharmacological uses of *Tamarix aphylla*, there are limited studies on comparable herbs. Mohsin et al. [5] studied the anti-inflammatory analgesic and antipyretic activities of a number of plants and reported that *Tamarix nilotica* ethanolic extract had significant analgesic as well as antipyretic activity. The same study confirmed presence of phytoconstituents similar to *Tamarix aphylla* like alkaloids, flavonoids, tannins, saponins and triterpenes. An interesting finding demonstrated is the present study is the ability of *Tamarix aphylla* to inhibit granuloma tissue formation. In the recent years there has been increased focus on the component III of inflammatory response and the leucocyte migration [2]. In the current study, granuloma-tissue formation inhibition method is used to study the proliferative and the leucocyte migration phase of inflammation. The significant reduction in granuloma tissue demonstrated in the present results is attributed by some authors to better maturation of collagen which leads to shrinkage of the granuloma tissue [14-16]. Ahmed et al. attributed the anti-inflammatory, analgesic, and antipyretic activity to flavonoids or glycosides [17,18]. The anti-inflammatory, and analgesic effects may also be due to the presence of sterols, saponins and triterpenes [6]. In the present study *Tamarix aphylla* contains all these phytoconistituents which explain its anti-inflammatory, analgesic, and antipyretic activity as well as its membrane-stabilizing ability. Antipyretic activity of *Tamarix aphylla* is likely to be attributed to saponins as reported by Mohsin et al. [5]. In addition, Abe et al. [14] claimed that saponins have a membrane stabilizing effect, which explains the significant reduction in erythrocytes heat-induced and hypotonic solution-induced hemolysis of the present study. The possible explanation for the membrane-stabilizing activity could be modifying surface area/volume ratio of the cells by interacting with certain cytoskeletal proteins [5,13]. The therapeutic effects of *Tamarix aphylla*seems to be secondary to its membrane stabilizing activity, which minimize the release of inflammatory mediators, and thus fever, pain and other inflammatory signs [5].

5. CONCLUSION

In conclusion, the current study gives evidences for the promising therapeutic benefits of *Tamarix aphylla* in treating inflammation and its consequences like pain and fever. *Tamarix aphylla* can be utilized in countries where governments are unable to sustain a complete coverage with western-type of drugs. However, further phytochemical and toxicological investigations are needed to verify the active ingredients responsible to the therapeutic effects of *Tamarix aphylla* and the potential side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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