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ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF SALVADORA OLEOIDES DECNE.

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ABSTRACT

The plant *Salvadora oleoides* Decne is a small herb, grows particularly along rocks and in the dry mountainous areas of Gujarat. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of glycosides, phytosterols, fixed oils and fats, saponins and triterpenes in the leaf extracts of *Salvadora oleoides*. The anti-inflammatory effect of chloroform, ethyl acetate, alcohol and water extracts of *Salvadora oleoides* leaf were tested on various animal models. All the extracts were tested at the dose of 200 and 400 mg/kg body weight. The chloroform and ethyl acetate extracts of leaves of *Salvadora oleoides,* does not produce significant anti-inflammatory activity, while alcohol and water extracts, at the dose of 400 mg/kg body weight, produced dose dependent and significant inhibition of paw edema induced by carrageenan, histamine and serotonin. The exhibited anti-inflammatory activity was comparable with the standard drug indomethacin.

KEYWORDS: ***Salvadora oleoides*, Leaf extracts, Anti-inflammatory activity and Paw edema.**

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INTRODUCTION

Salvadora oleoides Decne (Salvadoraceae) grows naturally by seed germination and is one of the dominant tree species in the vast area of Gujarat. Only two species of *Salvadora* are found in Gujarat i.e. *Salvadora oleoides* and *Salvadora phlomoides*. It is a small herb, grows particularly along rocks and in the dry mountainous areas of Gujarat. The plant generally grows during the rainy season (June – September). The whole plant is traditionally used in the treatment of various uterine and skin disorders by the local people of the Kachchh region of Gujarat[1-2]. The oil extracted from seeds is applied in rheumatic pain. Decoction of unripe fruits is given to cure enlarged spleen and rheumatic fever. Leaves are used in dry cough and fruits are useful in asthma and digestive disorders[3]. The leaf extract of *Salvadora oleoides* possess antiplasmodial activity with low IC_{50} values of 42 µg dry extract/ml[4]. *Salvadora oleoides* seed oil showed 100% toxicity to *Anopheles stephensi* at 0.01%. Leaves are used to relieve cough, and are given to horses as a purgative. Root bark is used as a vesicant. Fruits are used in the treatment of an enlarged spleen, rheumatism and fever. The seed fat is used in the treatment of rheumatic pains, in preparation of suppositories and as a base for ointments. Unspecified part used to treat throat swelling of domestic animals in India[5]. The plant possesses good medicinal value and is used by the people for the treatment of various diseases. The leaf paste was applied to an open wound and also useful in inflammation of legs. The whole plant is used as cooling herb, anti-inflammatory agent and wound healing herb[1]. A survey of literature revealed that scientific study on the anti-inflammatory activity of the plant *Salvadora oleoides* does not reported to validate the folklore claims. The present studies were carried out to validate the folklore claim.

MATERIALS AND METHODS

Collection and authentication of the plant material

Fresh leaves of *Salvadora oleoides* Decne were collected from the Kachchh district of Gujarat (India) in the month of July. The plant material was identified and authenticated by the Botany Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat (India). The voucher specimen SOPMA-01 was also preserved for future reference. The collected leaves were shade dried for 15 days and size reduced by mechanical grinder into coarse powder. It was then stored in a well closed container free from environmental climatic changes till usage.

Method of extraction[6]

The powder of dried leaves of *Salvadora oleoides* was subjected to continuous extraction with soxhlet extractor using various organic solvents such as petroleum ether (60-80 °C), chloroform, ethyl acetate, ethanol and water respectively. After concentration and drying of each extract, identification of phytoconstituents was carried out by performing different qualitative chemical tests. The colour, consistency and percentage yield of the extracts were also noted.

Preliminary phytochemical screening of various extracts[6-9]

The leaf extracts of *Salvadora oleoides* obtained during the extraction process were subjected to a preliminary phytochemical screening to determine the presence of various phytoconstituents by using reported methods.

Preparation of the plant extracts

After concentration and drying of each extract, chloroform, ethyl acetate, ethanol and water extracts were selected for the biological screening in various animal models. A suspension of the required quantity of each chloroform, ethyl acetate, alcohol and water extract was prepared in normal saline and used for the oral administration to the animals.

Vehicle

All the plant extracts and standard drug indomethacin were suspended in normal saline and administered orally to animals.

Carrageenan, histamine and serotonin diluted separately in normal saline and injected.

Experimental animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g were used for experimental purpose. The animals were kept in polypropylene cages at room temperature and under 12:12 hours light/ dark cycle. The animals had free access to standard rat pellet and water under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize, if any non-specific stress. The animals were divided into groups of six animals each and fasted for 12 hours before the experiment. The study was carried out as per the guidelines of the institutional animal ethical committee.

Acute toxicity studies

An acute toxicity study was conducted for all the extract by the stair-case method[10]. The healthy Wistar rats of either sex were fed with plant extracts in increasing doses of 50, 100, 500, 1000, 2000 and 4000 mg/kg body weight respectively. The toxicity was assessed by mortality and behavior changes of the animals.

Selection of dose

The Safety of the medicinal plants is equally important when they are used clinically. The doses up to 4000 mg/kg body weight did not produce any signs of toxicity and mortality. The animals were physically active and were consuming food and water in a regular way. A five and ten percent of the maximum tolerated dose i.e. 200 and 400 mg/kg body weight was selected for the study. A substance is considered safe if it produces no adverse effect in 10 times of the therapeutic dose[11]. These findings support the observation of safety of the plant extracts.

Anti-inflammatory studies

Carrageenan, histamine and serotonin induced pawedema models[12-16] were used for evaluating potential of *Salvadora oleoides* leaf extracts on inflammation. For each model, rats were divided into six groups (n = 6). All the plant extracts (200 and 400 mg/kg) and indomethacin (10 mg/kg) was administered orally one hour before the subplantar injection of edematogenic agent. The control groups of animals were received vehicle (10 ml/kg) orally. All the treatments were given one hour before the carrageenan injection. The measurement of paw volume was accomplished immediately by displacement technique using plethysmometer before the carrageenan injection and at 1, 2, 4 and 6 hours after the carrageenan injection. Percentage inhibition of paw edema volume was calculated using the formula:

Percentage Inhibition = [1-Vt/Vc] x 100

Where, Vt = difference in paw edema volume of drug treated animals

Vc = difference in paw edema volume of control treated animals

Table 1
Experimental design for anti-inflammatory studies in rat

Group	Treatment	Dose
I -Control	Normal saline	10 ml/kg; *p.o.*
II-Standard	Indomethacin (suspended in normal saline)	10 mg/kg; *p.o.*
III-Test Extract CESO-200	Chloroform extract of *Salvadora oleoides* (suspended in normal saline)	200 mg/kg; *p.o.*
IV-Test Extract CESO-400	Chloroform extract of *Salvadora oleoides* (suspended in normal saline)	400 mg/kg; *p.o.*
V-Test Extract EESO-200	Ethyl acetate extract of *Salvadora oleoides* (suspended in normal saline)	200 mg/kg; *p.o.*
VI-Test Extract EESO-400	Ethyl acetate extract of *Salvadora oleoides* (suspended in normal saline)	400 mg/kg; *p.o.*
VII-Test Extract AESO-200	Alcohol extract of *Salvadora oleoides* (suspended in normal saline)	200 mg/kg; *p.o.*
VIII-Test Extract AESO-400	Alcohol extract of *Salvadora oleoides* (suspended in normal saline)	400 mg/kg; *p.o.*
IX-Test Extract WESO-200	Water extract of *Salvadora oleoides* (suspended in normal saline)	200 mg/kg; *p.o.*
X-Test Extract WESO-400	Water extract of *Salvadora oleoides* (suspended in normal saline)	400 mg/kg; *p.o.*

(i) Carrageenan induced edema in rat

In this method, acute inflammation was produced by the subplantar administration of 0.1 ml of 1% w/v carrageenan in the right paw of the rat. The thickness (mm) of the paw was measured immediately and at 1, 2, 4, and 6 hours of intervals after the administration of the carrageenan[17-18].

(ii) Histamine induced edema in rat

Edema in rats was induced by injecting 0.1 ml of 0.1% w/v histamine in the subplantar region of the right hind paw. The thickness (mm) of the paw was measured immediately and at 1, 2, 4, and 6 hours of intervals after the administration of the histamine[19-20].

(iii) Serotonin induced edema in rat

Edema in rats was induced by injecting 0.1 ml of 0.2% w/v serotonin in subplantar region of the right hind paw. The thickness (mm) of the paw was measured immediately and at 1, 2, 4, and 6 hours intervals after the administration of the serotonin[19-21].

Statistical analysis

All data were expressed as standard error of mean (±SEM) and one-way analysis of variance (ANOVA) was applied to determine the significance difference between the rats treated with test extracts, controls and standards. P value <0.05, 0.01 and 0.001 were considered statistically significant.

RESULTS AND DISCUSSION

Average extractive values (%w/w)

During successive solvent extraction, the percentage yields were determined as petroleum ether 7.97 %, chloroform 4.34 %, ethyl acetate 4,78 %, ethanol 8.66 % and water 11.40 % w/w. The colour and consistency of the each extract were also noted during the extraction process as shown in Table 2.

Table 2
Percentage yield of leaf extracts of Salvadora oleoides

Sr. No.	Solvent used	Colour and consistency	Average percentage yield (% w/w on dry weight basis)
1	Petroleum ether (60-80° C)	Greenish sticky mass	7.97
2	Chloroform	Brown sticky mass	4.34
3	Ethyl acetate	Brown sticky mass	4.78
4	Ethanol	Dark brown mass	8.66
5	Water	Dark brown mass	11.40

Preliminary phytochemical screening of Salvadora oleoides leaves

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of glycosides, phytosterols, fixed oils and fats, saponins, triterpenes were present in the leaf extracts of *Salvadora oleoides*.

Anti-inflammatory activity

(i) Carrageenan induced paw edema

The anti-inflammatory effect of chloroform, ethyl acetate, alcohol and water extracts of *Salvadora oleoides* on carrageenan induced paw edema in rats are shown in Table 3. The result obtained indicates that the chloroform and ethyl acetate extract does not show any significant results, while alcohol and water extracts found to be having significant anti-inflammatory activity in rats. The alcohol extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg, reduced the paw edema induced by carrageenan by 46.70 and 81.70 % respectively after six hours, on oral administration as compared to the untreated control group. The water extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg,

reduced the paw edema induced by carrageenan by 51.16 and 83.30 % respectively after six hours, on oral administration as compared to the untreated control group. Indomethacin at a dose of 10 mg/kg inhibits the paw edema volume by 89.30 %.

Table 3
Percentage inhibition of carragenan induced paw edema in rat.

Group	Dose *mg/kg, p.o.*	Paw Edema Volume in ml (% Inhibition of Paw Edema)				
		0 hr	1 hr	2hr	4 hr	6 hr
Control (N/saline)	10 ml/kg	1.07 ±1.34	1.29 ±1.30	1.61 ±1.15	1.74 ±1.20	1.79 ±1.64
Standard (Indomethacin)	10	1.14 ±1.32	1.30 ±1.45 (27.60)	1.41 ±1.80* (50.80)	1.30 ±1.66** (76.20)	1.22 ±1.73** (89.30)
CESO 200	200	1.16 ±1.31	1.35 ±1.50 (13.20)	1.59 ±1.43 (19.70)	1.67 ±2.32 (23.30)	1.68 ±1.43 (27.60)
CESO 400	400	1.29 ±1.14	1.47 ±1.50 (16.50)	1.70 ±1.27 (23.30)	1.78 ±1.59 (27.10)	1.79 ±1.93* (30.40)
EESO 200	200	1.28 ±1.65	1.48 ±1.23 (11.10)	1.71 ±1.37 (20.80)	1.81 ±1.43 (21.50)	1.82 ±1.36 (25.30)
EESO 400	400	1.03 ±1.44	1.22 ±1.63 (15.40)	1.45 ±1.50 (21.80)	1.51 ±1.22 (28.90)	1.52 ±1.74* (31.30)
AESO 200	200	1.09 ±1.21	1.27 ±1.62 (17.00)	1.49 ±1.03 (25.10)	1.49 ±1.81 (39.60)	1.47 ±1.53* (46.70)
AESO 400	400	1.24 ±1.24	1.41 ±1.19 (20.70)	1.60 ±1.60* (33.50)	1.48 ±1.36** (64.50)	1.37 ±1.61** (81.70)
WESO 200	200	1.22 ±1.82	1.40 ±1.41 (18.70)	1.61 ±1.39 (28.20)	1.58 ±1.47* (46.30)	1.57 ±1.67 (51.16)
WESO 400	400	1.18 ±1.31	1.35 ±1.29 (23.40)	1.51 ±1.14* (38.30)	1.40 ±1.42** (67.40)	1.30 ±2.15** (83.30)

**p<0.05, **p<0.01, ***p<0.001 as compared to control, as per one way analysis of variance (ANOVA).*
Values are expressed as mean ± SEM of n=6 animals in each group.

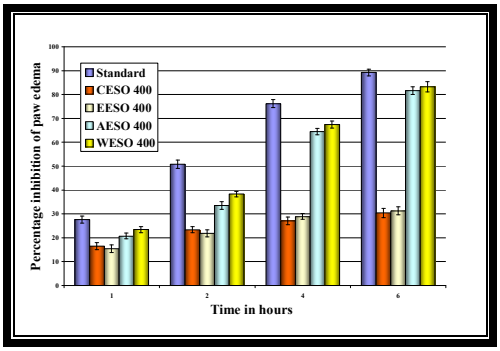

Figure 1
Effect of various extracts of Salvadora oleoides on percentage inhibition of carrageenan induced paw edema in rat.

(ii) Histamine induced paw edema

The anti-inflammatory effect of chloroform, ethyl acetate, alcohol and water extracts of *Salvadora oleoides* on histamine induced paw edema in rats are shown in Table 4. The result obtained indicates that the chloroform and ethyl acetate extract does not show any significant results, while alcohol and water

extracts found to be having significant anti-inflammatory activity in rats. The alcohol extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg, reduced the paw edema induced by histamine by 45.60 and 81.40 % respectively after six hours, on oral administration as compared to the untreated control group. The water extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg, reduced the paw edema induced by carrageenan by 49.60 and 84.40 % respectively after six hours, on oral administration as compared to the untreated control group.

Table 4
Percentage inhibition of histamine induced paw edema in rat.

Group	Dose	Paw Edema Volume in ml (% Inhibition of Paw Edema)				
	mg/kg, p.o.	0 hr	1 hr	2hr	4 hr	6 hr
Control (N/saline)	10 ml/kg	1.07 ±1.34	1.29 ±1.30	1.61 ±1.15	1.74 ±1.20	1.79 ±1.64
Standard (Indomethacin)	10	1.14 ±1.32	1.30 ±1.45 (27.60)	1.41 ±1.80* (50.80)	1.30 ±1.66** (76.20)	1.22 ±1.73** (89.30)
CESO 200	200	1.23 ±1.37	1.42 ±1.34 (13.70)	1.67 ±1.24 (18.80)	1.75 ±1.44 (22.50)	1.75 ±1.82 (27.20)
CESO 400	400	1.21 ±1.54	1.40 ±1.22 (15.20)	1.63 ±1.41 (22.80)	1.70 ±1.28 (27.30)	1.71 ±1.76* (31.10)
EESO 200	200	1.09 ±1.43	1.28 ±1.09 (12.00)	1.52 ±1.38 (21.10)	1.61 ±1.47 (22.10)	1.64 ±1.08 (24.10)
EESO 400	400	1.08 ±1.72	1.27 ±1.63 (14.40)	1.51 ±1.38 (20.80)	1.56 ±1.37 (28.90)	1.45 ±1.39* (32.20)
AESO 200	200	1.33 ±1.47	1.51 ±1.41 (18.40)	1.73 ±1.15 (25.70)	1.73 ±1.71* (40.00)	1.72 ±1.23* (45.60)
AESO 400	400	1.32 ±1.64	1.49 ±1.51 (21.40)	1.68 ±1.08* (34.10)	1.56 ±1.12** (64.60)	1.45 ±1.71** (81.40)
WESO 200	200	1.05 ±1.42	1.23 ±1.31 (16.50)	1.43 ±1.85 (28.80)	1.41 ±1.58* (46.40)	1.41 ±1.72* (49.60)
WESO 400	400	1.15 ±1.44	1.32 ±1.62 (23.80)	1.48 ±1.36* (38.80)	1.36 ±1.63** (68.90)	1.26 ±1.69** (84.40)

**p<0.05, **p<0.01, ***p<0.001 as compared to control, as per one way analysis of variance (ANOVA).*
Values are expressed as mean ± SEM of n=6 animals in each group.

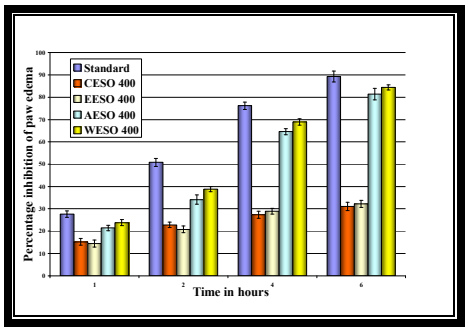

Figure 2
Effect of various extracts of Salvadora oleoides on percentage inhibition of histamine induced paw edema in rat.

(iii) Serotonin induced paw edema

The anti-inflammatory effect of chloroform, ethyl acetate, alcohol and water extracts of *Salvadora oleoides* on serotonin induced paw edema in rats are shown in Table 5. The result obtained indicates that the chloroform and

ethyl acetate extract does not show any significant results, while alcohol and water extracts found to be having significant anti-inflammatory activity in rats. The alcohol extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg, reduced the paw edema induced by serotonin by 48.00 and 80.80 % respectively after six hours, on oral administration as compared to the untreated control group. The water extracts of *Salvadora oleoides* at a doses of 200 and 400 mg/kg, reduced the paw edema induced by serotonin by 51.10 and 82.60 % respectively after six hours, on oral administration as compared to the untreated control group.

Table 5
Percentage inhibition of serotonin induced paw edema in rat.

Group	Dose *mg/kg, p.o.*	Paw Edema Volume in ml (% Inhibition of Paw Edema)				
		0 hr	1 hr	2hr	4 hr	6 hr
Control (N/saline)	10 ml/kg	1.07 ±1.34	1.29 ±1.30	1.61 ±1.15	1.74 ±1.20	1.79 ±1.64
Standard (Indomethacin)	10	1.14 ±1.32	1.30 ±1.45 (27.60)	1.41 ±1.80* (50.80)	1.30 ±1.66** (76.20)	1.22 ±1.73** (89.30)
CESO 200	200	1.21 ±1.43	1.40 ±1.09 (12.30)	1.65 ±1.17 (18.50)	1.72 ±1.72 (24.40)	1.73 ±1.07 (28.20)
CESO 400	400	1.12 ±1.62	1.30 ±1.03 (16.70)	1.53 ±1.72 (23.80)	1.60 ±0.98 (28.20)	1.62 ±1.05* (30.90)
EESO 200	200	1.14 ±1.06	1.34 ±1.83 (10.50)	1.57 ±1.08 (19.50)	1.66 ±1.32 (22.50)	1.66 ±1.17 (27.10)
EESO 400	400	1.17 ±1.13	1.36 ±1.11 (15.20)	1.58 ±1.24 (24.00)	1.63 ±1.26* (32.00)	1.65 ±1.40* (33.20)
AESO 200	200	1.11 ±1.22	1.29 ±1.49 (17.50)	1.51 ±1.58 (26.80)	1.51 ±1.09* (39.70)	1.48 ±1.56* (48.00)
AESO 400	400	1.22 ±1.37	1.39 ±1.47 (21.70)	1.58 ±1.58* (33.20)	1.46 ±1.40** (64.90)	1.36 ±1.87** (80.80)
WESO 200	200	1.20 ±1.36	1.38 ±1.09 (19.20)	1.58 ±1.35* (28.90)	1.56 ±1.16* (46.30)	1.55 ±1.40* (51.10)
WESO 400	400	1.16 ±1.67	1.33 ±1.31 (24.00)	1.50 ±1.43* (37.90)	1.38 ±1.55** (67.60)	1.29 ±1.23** (82.60)

** p<0.05, ** p<0.01, *** p<0.001 as compared to control, as per one way analysis of variance (ANOVA).*
Values are expressed as mean ± SEM of n=6 animals in each group.

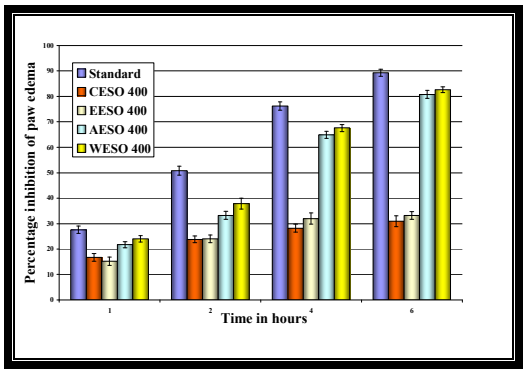

Figure 3
Effect of various extracts of Salvadora oleoides on percentage inhibition of serotonin induced paw edema in rat.

CONCLUSION

The present results indicate the efficacy of alcohol and water extract of leaves of *Salvadora oleoides* as an efficient therapeutic herb in inflammatory conditions. Possibly, the constituents like glycosides, phytosterols, fixed oils and fats, saponins and triterpenes, which may be present in the leaves of *Salvadora oleoides,* may play a major role in the inhibition of inflammatory process.[22] It may be either due to their individual or additive effect, inhibiting the process of inflammation. The present investigation offers scientific evidence to the folkloric accounts of the use of leaf extract of *Salvadora oleoides* in treating inflammations. However, it needs further evaluation in clinical settings before consideration for the treatment of inflammations.

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