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Research article

TO EVALUATE THE ANALGESIC ACTIVITY OF *Prosopis juliflora* ETHANOLIC EXTRACT IN ACETIC ACID INDUCED WRITHING TEST IN MICE

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ABSTRACT

The investigation has been initiated for natural product from plant sources as potential analgesic activity. In the current investigation, ethanol extract of *Prosopis juliflora* was analysed for presence of major bio-active compounds to evaluate analgesic activity in acetic acid induced Writhing test in mice. It has been found the plants is rich in alkaloid, flavonoids, tannins, anthroquinones and quinone and are responsible for inhibition of H^+, K^+ ATPase. Twenty four male mice between 25-30g were equally divided into four groups and food was deprived off for 24hrs and water provided ad libitum, 0.2 mLf 0.7% acetic acid was administered through intraperitoneal as per time chart. Group 1 was treated with water for injection (10ml/kg), group 2 was treated with acetyl salicylic acid (25mg/kg p.o dissolved in water for injection), significantly reduced the Writhings by 77.07%. Group 3 and 4 were treated with ethanol extract of Prosopis juliflora (250 and 500mg/kg p.o respectively). Significantly reduced the Writhings by 75.16 and 78.34 respectively.

Key words: Ethanol extract, *Prosopis juliflora*, Analgesic activity, Acetic acid, Intraperitoneal.

INTRODUCTION

An analgesic, is any member of the group of drugs used to achieve analgesia i.e. relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems.The primary classes of analgesics are the narcotics, including additional agents that are chemically based on the morphine molecule but have minimal abuse potential; nonsteroidal anti-inflammatory drugs (NSAIDs) including the salicylates; and acetaminophen.

Other drugs, notably the tricyclic antidepressants and anti-epileptic agents such as gabapentin, have been used to relieve pain, particularly neurologic pain, but are not routinely classified as analgesics. Prosopis is a genus of trees and shrubs in the legume family. The products obtained from *Prosopis juliflora* have been used for human consumption in bread, biscuits, sweets, syrup and liquors. Extracts of *Prosopis juliflora* seeds and leaves have several in vitro pharmacological effects such as antibacterial, antifungal and anti-inflammatory properties. These properties have been attributed to piperidine alkaloids.

A number of compounds have also been reported from this plant, the most common of these being steroids, tannins, leucoanthocyanidin and ellagic acid glycosides. A new monocyclic diketone, prosopidione, and two alkaloids, namely, juliprosinene and juliflorinine, have been isolated from the leaves **(Ahmad A *et al*., 1986).** Extracts of *Prosopis juliflora* seeds and leaves have several in vitro pharmacological effects such as antibacterial, antifungal, anti-inflammatory properties.

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MATERIALS & METHODS (Nwafor PA *et al*, 2007)

1. Food was deprived off for all mice for overnight by, but water was provided ad libitum.
2. Group 1 was treated with Water for Injection (10 ml/kg).Group 2 was treated with Acetyl salicylic acid (25 mg/kg p.o dissolved in WFI).
3. An ethanol extract of *Prosopis juliflora* was administered to groups G3 and G4 at a dose of 250 and 500 mg/kg respectively through oral gavage.
4. After 30 minutes Vehicle/Test Compound Administration, each mouse was challenged with 0.2 ml of 0.7% acetic acid was administered through intraperitoneally as per time chart.
5. The number of writhing movements (contraction of abdominal muscle together with a stretching of hind limbs) was counted for 30 minutes
6. Antinociception has expressed as the reduction of the number of abdominal constrictions in comparison with control animals and treated groups by using the following formula.

Husbandry Conditions

Temperature: 23±3°C
Humidity: 30-70%
Light: 12 hours light and 12 hours dark cycle
Air changes: 12-15 changes per hour

RESULTS AND DISCUSSION

Twenty four male Balb/c mice between 25 -30 g were equally divided into four groups (G1, G2, G3 and G4). Food was deprived off for all mice for overnight, but water was provided ad libitum.

The study design of the Analgesic activity of *juliflora* extract in Acetic acid induced writhing test in mice shown in Table 1 and Formulation Details of dose concentration is shown in Table 2. Group 1 was treated with Water for Injection (WFI) at the dose volume of 10 ml/kg. Group 2 was treated with Acetyl salicylic acid (25 mg/kg p.o dissolved in WFI). An ethanol extract of *Prosopis juliflora* was administered to groups G3 and G4 at a dose of 250 and 500 mg/kg respectively through oral gavage. After 30 minutes of Vehicle/Positive Control/Test Compound Administration, each mouse was challenged with 0.2 ml of 0.7% acetic acid was administered through intraperitoneal as per time chart. The number of writhing movements (contraction of abdominal muscle together with a stretching of hind limbs) was counted for 30 minutes. Ant nociception has expressed as the reduction of the number of abdominal constrictions in comparison with control animals and treated groups by using the following formula: % of Writhing Inhibition = **(No.of Writhes of Control)-no.of Writhes of Treadted)/ (No. of Writhes of Control.**

The Analgesic activity of *Prosopis juliflora* extract in acetic acid induced test animals were made into 4 groups of 6 animals in each group and the treatment involves water for injection for group 1 animals.For group 2 acetyl salicylic acid 25mg/kg as a dose. For group 3,Ethanol extract of Prosopis juliflora 250mg/kg was the dose and group 4 ethanol extract of *Prosopis juliflora* with little higher concentration 500mg/kg dose was taken against test animals indicated in table 1. The formulation details of the *Prosopis juliflora* extract in acetic acid induced Writhing test in experimental animals were water for injection for group 1 animals. For G2 group of animals acetyl salicylic acid with a concentration of 2.5 mg/mL and 50 mg was the weight of test and for group 3 and gropu 4 animals were treated with ethanol extract of *Prosopis juliflora* was 25 and 50 mg/mL dose concentration and 500mg/mL and 1000mg/mL weight of dose respectively.The volume of vehicle 20mL for all dose concentration mentioned in table 2.

The volume of 0.2 ml 0.7% acetic acid successfully induced the writhings (26.17 ± 1.35) in mice. Percentage of inhibition is presented in Table 3 and Graph 1. The animals in Group 2 treated with Acetyl Salicylic acid dose 25 mg/kg significantly reduced the writhings by 77.07% of control Group 1 and also Ethanol extract of *Prosopis juliflora*at tested doses 250 and 500 mg/kg significantly reduced the writhings by 75.16 and 78.34 respectively. And individual animal writhing movement counts of groups (G1, G2, G3, G4) are shown in Table 4.The data were analyzed using one way ANOVA followed by the Dunnett T method as a post - hoc test. All values will be reported as Mean ± SEM. Statistical significance will set at $p \leq 0.001$.

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Table 1: Study design of the Analgesic activity of *Prosopis juliflora* extract in Acetic acid induced writhing test in mice.

Groups	Treatment	Dose(mg/kg)	No. of Animals	Animal No.
G1	Water for Injection(WFI)	0(10ml/kg)	6	1-6
G2	Acetyl Salicylic Acid	25	6	7-12
G3	Ethanol extract of *Prosopis juliflora*	250	6	13-18
G4	Ethanol extract of *Prosopis juliflora*	500	6	19-24

Table 2: Formulation details of the Analgesic activity of *Prosopis juliflora* extract in Acetic acid induced writhing test in mice.

Group	Treatment	Dose concentration (mg/ml)	Weight of test/reference item (mg)	Volume of Vehicle (ml)
G1	Water for Injection (WFI)	0	0	20
G2	Acetyl Salicylic acid	2.5	50	20
G3	Ethanol extract of *Prosopis juliflora*	25	500	20
G4	Ethanol extract of *Prosopis juliflora*	50	1000	20

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Table 3: Effect of *Prosopis juliflora* ethanol extract on number of writhings in acetic acid induced writhings in mice.

Treatment groups	Dose (mg/kg rat b.wt.)	Body weight (g)	No. of writhings	% of inhibition
G1 Water for Injection	0 (10ml/kg)	27.43±0.58	26.17±1.35	0
G2 Acetyl Salicylic Acid	25	27.93 ± 0.43	6.00 ± 0.93***	77.07
G3 Ethanol extract of Prosopis juliflora	250	27 ± 0.44	6.50 ± 1.18***	75.16
G4 Ethanol extract of Prosopis juliflora	500	27.33 ± 0.47	5.67 ± 0.84***	78.35

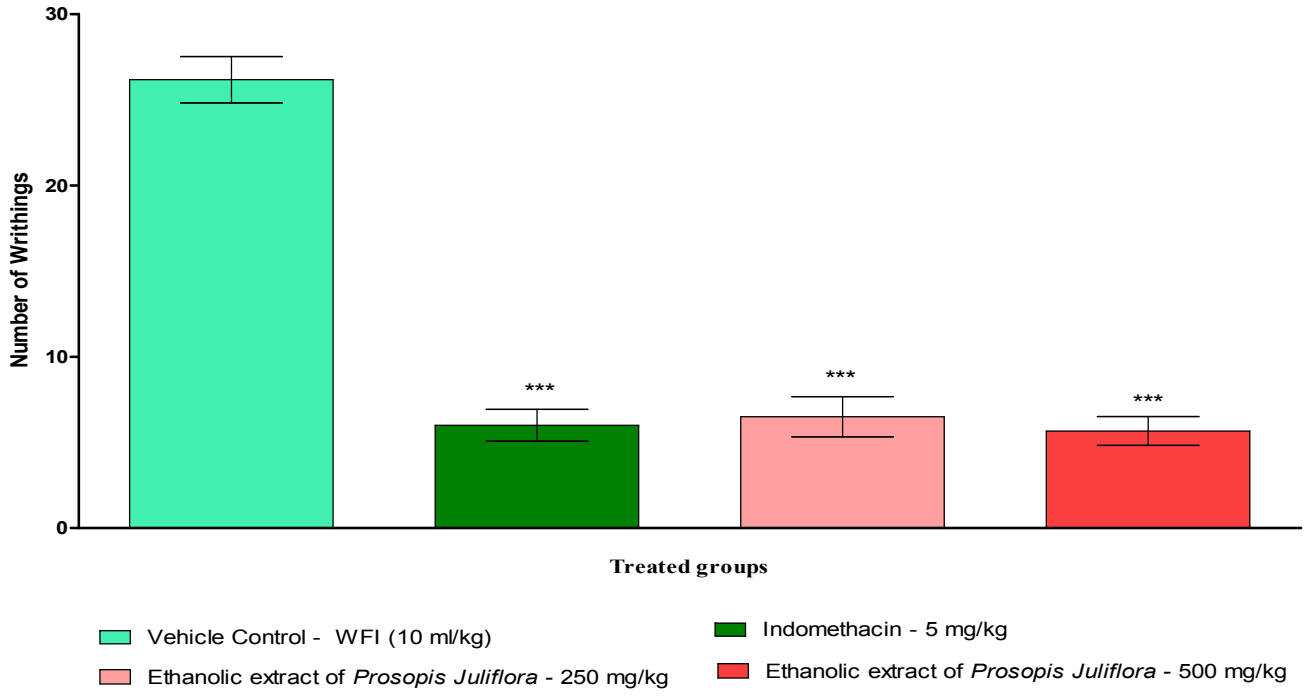
Values are expressed as mean ± SEM; n= 6

* - Statistically significant when compared to control group ($p<0.05$)

** - Statistically significant when compared to control group ($p<0.01$)

*** - Statistically significant when compared to control group ($p<0.001$)

Graph.: 1 Effect of *Prosopis juliflora* ethanolic extract on number of writhings in acetic acid induced writhings in mice



* - Statistically significant than the control group ($p<0.05$)

** - Statistically significant than the control group ($p<0.01$)

*** - Statistically significant than the control group ($p<0.001$)

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Table 4: Individual Animal Writhing Movement Counts.

Group	Dose (mg/kg rat b.wt.)	Animal No.	Body weight (g)	No. of Writhings
G1 Water for Injection	0 (10ml/kg)	1	25.97	22
		2	28.55	25
		3	28.40	29
		4	26.73	31
		5	25.82	26
		6	29.09	24
G2 Acetyl Salicylic Acid	25	7	27.45	5
		8	28.20	4
		9	26.66	3
		10	27.58	8
		11	29.79	8
		12	27.87	8
G3 Ethanol extract of *Prosupus juliflora*	250	13	28.86	5
		14	26.78	4
		15	25.85	6
		16	27.64	11
		17	26.37	4
		18	26.49	9
G4 Ethanol extract of *Prosupus juliflora*	500	19	26.84	8
		20	27.01	8
		21	25.75	5
		22	27.09	6
		23	28.95	3
		24	28.36	4

CONCLUSION

Based on current finding the results reveals that acetic acid induced Writhing test in mice using 0.7% acetic acid in controlled conditions, the ethanol extract of Prosopis juliflora in the range of 250 and 500mg/kg p.o significantly reduced the Writhings by 75.16 and 78.34. The potential drug may be extracted from *Prosopis juliflora* for analgesic activity.

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