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Exploration of analgesic and inflammatory prevention potency of *pisonia alba* leaves fractions

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ABSTRACT

Using various pain and inflammation models to examine the analgesic and anti-inflammatory effects of various dried Pisonia alba leaf fractions. Rats and mice were used to assess the analgesic efficacy of P.alba using the tail flick test and acetic acid-induced writhing in mice. The anti-inflammatory efficacy of cotton was evaluated using cotton pellet-granuloma development in rats. P.alba was tested at doses of 20 and 40 mg/kg, p.o. in five different fractions (FRI, FRII, FRIII, FRIV, and FRV). The fractions FRI (40 mg/kg, p.o.) and FRIII (40 mg/kg, p.o.) were shown to be more effective (P0.01) than the other fractions at decreasing cotton pellet granuloma formation and acetic acid-induced writhing. FRI (20 mg/kg, p.o.) and FRIII (20 mg/kg, p.o.) were also discovered to be more effective at lengthening latency time in the tail flick method. Two of the five fractions of P. alba leaves examined—FRI and FRIII—show strong analgesic and anti-inflammatory effects against various forms of pain and inflammation.

Keywords: *Pisonia alba*, tail flick, acetic acid, granuloma formation, carrageenan

INTRODUCTION

Even though medical research has advanced over the past few decades, many major disorders are still difficult to treat. [1] Chronic inflammatory disorders continue to be one of the top causes of death worldwide. In order to treat inflammation, both steroidal anti-inflammatory medications (NSAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are being employed. Although steroids clearly play a role in the treatment of inflammatory disorders, they can only be taken for brief periods of time due to their toxicity, with the exception of really serious situations where the risks are tolerable. Serious side effects, most notably gastrointestinal bleeding, are also linked to long-term usage of NSAIDs.[3],[4] Among the most prevalent medical conditions that are managed with conventional treatments are inflammatory illnesses. Therefore, it is essential to assess the potential of herbal medicines that could act as starting points for the creation of powerful medications. Because they contain different classes of phytochemicals, many Indian medicinal plants are said to have a variety of pharmacological effects.

Pisonia alba, Pisonia alba spanoghe, and Pisonia umbellifera are members of the Nyctaginaceae family. It is a significant component of the habitat, with high biodiversity and a complex food web, and can be found on many of the Seychelles Islands that have undergone habitat restoration. Pisonia was found to be the most frequent nest tree for the Seychelles warbler, an endemic land bird that was brought back from the brink of extinction through careful habitat management and translocation, highlighting the significance of taking into account the entire island ecosystem. It is not as simple as replacing Pisonia with other native tree species. You can consume the leaves. The young leaves are used to make vegetables. Additionally to being used as animal food, leaves are frequently utilised to treat arthritis and rheumatism. In traditional Indian medicine, the leaves are used as an anti-diabetic; naturally, the indigenous people utilise the leaves as cattle feed; they are cooked and eaten for arthritis; the leaves are also carminative; and the leaves are an antidote for snake bites. There is no proof that it has anti-anxiety qualities,

though. This study set out to look into the analgesic and anti-inflammatory properties of various fractions of Pisonia alba root extract. (5-10)

MATERIAL AND METHODS

Collection and Authentication of Plant Materials

The plant was collected in January 2021 from kolli hills,India. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The root were dried in the shade for 10–12 days. After complete drying, the dried root were pulverized to a coarse powder of 40 mesh size in a mechanical grinder. The powdered material was subjected to sohxlet extraction for 18 h at 50–55oC using ethanol and water. The extract was thereafter concentrated under vacuum and air-dried.

Preparation of P.alba Fractions

Petroleum ether and alcohol were used to extract the resin, which was then allowed to gently evaporate in a shallow dish before being discarded. Neutral alumina was first heated to 150 0 C for three hours in an oven to activate it for column chromatography. Slurry was made in benzene, cooled, and then placed in a glass column for two hours. The petroleum ether extract residue was completely combined with neutral alumina after being fully dissolved in a small amount of benzene. It was dried by air before being thrown into the column. Ethyl acetate was used to elute the sample after benzene (FRI) was used initially (FRII). Separating the dried alcoholic extract into its water-soluble and water-insoluble components. When the water-soluble fraction was vigorously shaken with methanol, a gelatinous precipitate was produced (FRIII). By dissolving the water-insoluble component in a small amount of absolute alcohol, column chromatography using benzene (FRIV) and ethyl acetate was performed (FRV).All the fractions were prepared fresh prior to the administration in 0.5% w/v gum acacia.

Experimental Animals

Swiss albino mice (25–30 g) of either sex and albino Wistar rats (150–200 g) were employed throughout the entire investigation. They were housed in typical polypropylene cages and maintained in a controlled indoor environment with a 12-hour light-dark cycle at 24 2°C and 60–70% relative humidity. Water was available at all times, and the animals were fed a conventional laboratory diet. 12 hours prior to and throughout the experimental hours, food was withheld. The Institutional Animal Ethics Committee gave its approval to the experimental protocol.

Phytochemical Investigation

Preliminary phytochemical tests for fractions were performed using specific reagents through standard procedure. [11]

Tail flick latency period in rats.

Male rats weighing between 125 and 150 g were placed into six groups of five each. Each rat tail was placed over a wire heated electrically while an analgesiometer was used to elicit a tail flick reaction (Space Scientific, Nashik, India). Baseline tail flick delay in all animals was altered to have an average duration of 3–4 seconds. The 15-second time limit was set in order to protect the tail. One hour before the test, the doses of the fractions FRI, FRIII, and reference standard ibuprofen were given orally. [12]

Acetic acid induced writhing in mice

Acetic acid (0.1ml of 0.6% solution) was injected intraperitoneally to induce the writhing syndrome, and the number of writhes that occurred for 5 to 20 minutes was noted. [13] 30 minutes before the test, the doses of the fractions FRI, FRIII, and the reference drug ibuprofen were given orally. [14]

Granuloma formation induced by cotton pellet in rats

Male rats weighing between 125 and 150 g were placed into seven groups of five each. The autoclave (Lab Hospital, Mumbai, India) used to sterilise the cotton pellet, which weighed 50 mg, was sterile. Each animal had the pellet placed into its back. Control group was given a car. FRI (20 and 40 mg/kg, p.o.) and FRIII (20 and 40 mg/kg, p.o.) were administered to Groups II, III, IV, and V, while Groups VI and VII were given the reference standard of hydrocortisone (30 mg/kg, p.o.) and ibuprofen (40 mg/kg, p.o.) for a six-day period in a row. [15],[16] On the seventh day, the animals were slaughtered, and cotton pellets and granuloma mass were collected. They were weighed and dried at 60 0 C. The assay's results were computed using the formula 100 (A-B)/A, where A represents the increase in dry weight of the control pellet (mg), and B represents the increase in dry weight of the drug being tested (mg).

Statistical Analysis

Results have been indicated in terms of mean±SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's multiple comparisons test using GraphPad InStat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $P < 0.05$.

RESULTS

Phytochemical investigation

Preliminary phytochemical analysis revealed the presence of different phytochemicals present in different fractions of P.alba plant [Table 1].

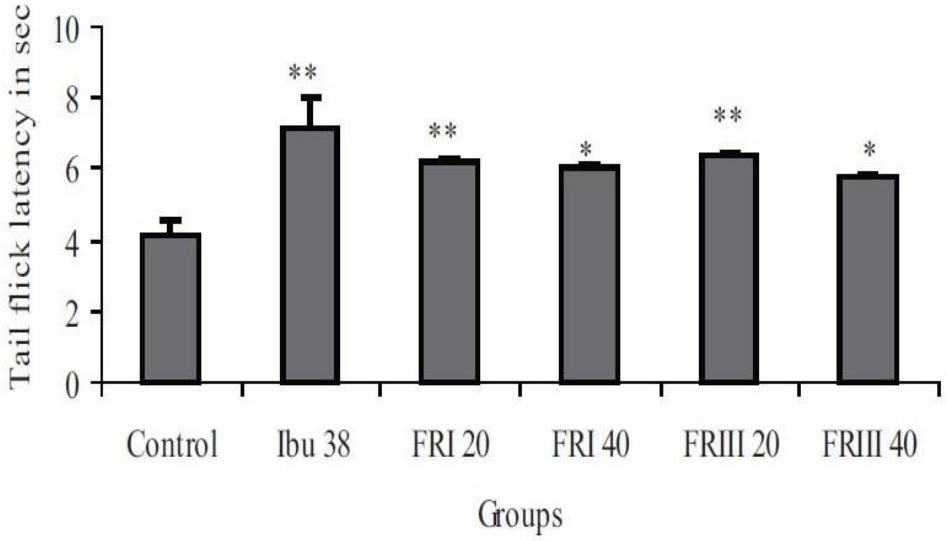
Table 1: Phytochemical analysis of different fractions of *P. alba* +, Positive test; -, Negative test

Test	FR I	FR II	FR III	FR IV	FR V
Carbohydrates	+	+	+	-	-
Proteins	+	-	+	-	-
Amino acids	+	-	+	-	-
Tannins And Phenols	-	+	+	+	+
Glycosides	+	+	+	-	-
Saponins	-	+	+	+	+

Flavanoids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Steroids	+	-	+	+	-

Effect of P. alba fractions on tail flick latency period and acetic acid induced writhing in mice

Treatment of FRI and FRIII (20 mg/kg, p.o.) significantly inhibited nociception in rats by 19.15% and 20.89%, respectively. Whereas, FR I and FRIII (40 mg/kg, p.o) significantly inhibited pain perception by 17.21% and 15.12%, respectively. Ibuprofen treatment (40 mg/kg, p.o) significantly inhibited pain perception by 27.81 % (P<0.01). [Fig 1]



*Data were expressed as mean ± SEM (n=5) and analyzed using one way analysis of variance (ANOVA) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control-0.5% gum acacia FRI-P. alba benzene fraction. FRII- P. alba water soluble alcoholic fraction.*

Fig 1: Effect of *P. alba* fractions on Tail flick latency period

Table 2 shows the effect of different fractions of *P. alba* against acid induced writhing in mice. It was observed that mice treated with FRI 20 (31.78%) and FRIII 20 (36.18%) shows significant (P < 0.01) protection compared to control group, however, FRI 40 (44.08%) and FRIII 40 (46.38%) was found to be more significant (P<0.01) in protecting acetic acid induced writhing compared to control group. Ibuprofen showed 56.09% protection against acetic acid induced writhing in mice.

Table 2: Effect of *P. alba* fractions in acetic acid induced writhing in mice

Treatment (mg/kg)	Number of writhing	% Inhibition
Control	60.1±2.39	--
Ibuprofen (40)	26.3±0.88	56.09
FRI (20)	41±1.14	31.78
FRI (40)	33.5±2.89	44.08
FRII (20)	38.3±1.09	36.18
FRII (40)	32.1±1.88	46.38

Data analyzed using one way analysis of variance (ANOVA) and expressed as mean ± SEM (n=5) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control-0.5% gum acacia FRI-*P. alba* benzene fraction. FRII- *P. alba water soluble alcoholic fraction.*

Effect of P. alba fractions on cotton pellet granuloma formation in rats

Treatment with FRI and FRIII (40 mg/kg, p.o) to rats showed a significant (P<0.01) inhibition in the weight of cotton pellet compared to control group and the percentage inhibition was found to be 38.69 and 40.29, respectively. Treatment with the reference standard i.e. hydrocortisone (30 mg/kg, p.o) and ibuprofen (40 mg/kg, p.o) also showed significant inhibition in cotton pellets granuloma formation as compared to control group [Table 3].

Table 3: Effect of *P. alba* fractions on cotton pellet granuloma formation in rats

Treatment (mg/kg)	Average weight of cotton pellet	Average weight of cotton pellet with granuloma	% Inhibition
Control	50±0.01	127. ±4.89	-
FRI (20)	50±0.01	84±1.59	33.55
FRI (40)	50±0.01	77.4±0.84	38.69
FRII (20)	50±0.01	82±2.55	35.14

FRII (40)	50±0.01	75.3±4.19	40.29
Hydrocortisone (30)	50±0.01	74±01	41.39
Ibuprofen (40)	50±0.01	65.1±6.74	48.27

Data analyzed using one way analysis of variance (ANOVA) and expressed as mean ± SEM (n=5) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control-0.5% gum acacia FRI-*P. alba* benzene fraction. FRII- *P. alba water soluble alcoholic fraction.*

DISCUSSION

Different P. alba fractions were investigated in the current study for their analgesic and anti-inflammatory properties using various experimental models of inflammation and pain. Animal models, including non-narcotic models like acetic acid-induced writhing and narcotic models like the tail flick method, were employed to evaluate analgesic effectiveness. In cases of abdominal constriction brought on by acetic acid, arachidonic acid is released by cyclooxygenase and prostaglandin production, which contributes to the nociceptive mechanism and results in inflammation-related pain. [17-19]

The study's findings showed that when mice were given acetic acid to produce writhing, the treated fractions of P. alba showed stronger antinociception than the control group. The release of endogenous chemicals that stimulate pain nerve endings or a blocking of the effect, similar to how ibuprofen and other NSAIDs work, may be the cause of the analgesic effects of P. alba fractions. The tail flick method is the most widely used narcotics test. This test is based on a highly intense phasic stimulus. The nociceptive experience lasts just a brief period of time, and it is widely acknowledged that agonists of opiate receptors cause analgesia in models of acute pain. [20] As a result, it is thought that opioid receptors are the primary route through which drugs effective in tail flick exert their effects. The P. alba fractions had longer tail flick delay, which suggests that opoid receptors may be partially responsible for the analgesic efficacy. As a result, P. alba fractions that showed analgesic effectiveness in all of the nociception-based animal models employed in this investigation may have had their effects via a variety of mechanisms that may have involved both central and peripheral pathways.

The test fractions demonstrated considerable anti-inflammatory effect in cotton pellet-induced granuloma at doses of 20 and 40 mg/kg, which may be related to the presence of numerous active components in P. alba leaves. Studies on P. alba's phytochemistry have found that it contains a variety of phytoactive substances, including glycosides, sterols, tannins, amino acids, campesterol, isofucosterol, stigmasterol, and lupeol. It has been established that P. alba leaf extract contains significant concentrations of tannins, phenols, triterpenoids, glucosides, and sterols. [21-24], Flavonoids, steroids, and tannins have all been shown to have analgesic and anti-inflammatory properties in the past. [25],[26] Nitric oxide (NO) overproduction has negative effects that have been linked to inflammation and tissue damage. Independent of their antioxidant effects, tannic acid and polyphenols have been found to be powerful NO synthetase inhibitors and NO producers. [27] The analgesic and anti-inflammatory effects noted may therefore be partially attributed to the tannin concentration of this plant. Thus, the analgesic and anti-inflammatory effects produced by these fractions may be attributable to the tannins and steroids either singly or in combination.

CONCLUSION

The findings show that P. alba fractions reduce pain and inflammation and have an intriguing analgesic and anti-inflammatory activity profile that is comparable to other substances of this type that have been previously reported. The findings support P. alba's high value as a source of tannins and polypenol compounds with analgesic and anti-inflammatory activities. P. alba was harvested in Nashik, India.

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