Original Article

EVALUATION OF AREAL PARTS OF *Pisonia alba* FOR ANTI-INFLAMMATORY AND ANTIULCER ACTIVITIES

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Abstract

Objective of this study is to evaluate the ethanolic and aqueous extracts of aerial parts of *Pisonia alba* for anti-inflammatory and antiulcer activities. Anti-inflammatory action of the ethanolic and aqueous extracts of *P. alba* (100 and 200 mg/kg b.w.) (EEPA and AEPA) were evaluated by cotton pellet granuloma models. Pylorus ligation and aspirin induced ulcer models were employed for evaluating antiulcer activity for both the extracts. Ulcerogenic potential of PA was also evaluated. The ethanolic and aqueous extracts of *P. alba* significantly decreased ($P<0.01$) the granuloma tissue development. EEPA and AEPA at both the doses exhibited significant ($P<0.01$) antiulcer activity by decreasing the ulcer score in both the ulcer models and it was not ulcerogenic. The ethanolic and aqueous extracts of aerial parts of *P. alba* (EEPA and AEPA) possess significant anti-inflammatory and antiulcer activities.

Keywords: Anti-inflammatory, antiulcer, *Pisonia alba*, Pain, Inflammation.

Introduction

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 (1-4). An attempt has been made in the present study to evaluate the anti-inflammatory and antiulcer actions of the ethanolic and aqueous extracts of areal parts of *P. alba* (Linn.) Sw (EEPA and AEPA).

Materials and methods

Collection and extraction of Plant

The fresh aerial parts of *P. alba* (Linn.) Sw. (leaves, stem bark, flowers, buds, and pods) was procured. The authenticated aerial parts were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the aerial part (1 kg) was Soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the processes of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (18.70%) and aqueous (12.51%) extracts.

Phytochemical Test

Phytoconstitutents were identified by qualitative chemical tests on ethanolic and aqueous extracts of aerial parts of *P. alba*.

Animals

Healthy albino Wistar rats of either sex and approximately 12 to 13 weeks of age weighing 150-200 g were included in the study. The animals were acclimatized by keeping in animal house facility for a week. They were housed in polypropylene (32x24x16 cm) cages containing bedding material as husk and maintained under controlled conditions of temperature (23±2 0 C), humidity (55±5%) and 12 h light and 12 h dark cycles. They were fed with commercial pellet rat chow (M/S Gold Mohur foods and feds, Mumbai.) with water ad libitum. The animals were maintained in accordance with the PACSEA guidelines. The research protocol was approved by Institutional Animal Ethical Committee.

Acute Toxicity Studies

Acute oral toxicity study of aqueous and ethanolic extracts of *Pisonia alba* was carried out according to OECD guidelines 423. [5]

Cotton Pellet Granuloma Model [6]

Albino Wistar rats of either sex were randomly divided into six groups of six animals each. Group I served as control, groups II to VI were treated with indomethacin 10 mg/kg b.w., ethanolic extracts (100 mg/kg and 200 mg/kg b.w.) and aqueous extracts (100 mg/kg and 200 mg/kg b.w.) of *P. alba*, respectively. After 30 min, two autoclaved cotton pellets 30±1.0 mg were aseptically implanted subcutaneously in the axillary region of rats anesthetized with diethyl ether. Extracts were administered once daily for the next 7 days. On day 8, animals were anesthetized again and cotton pellets were removed surgically, freed from extraneous tissue and dried in the oven overnight at 60 0 C. The dried pellets were weighed and the mean weight of granuloma tissue around each pellet was determined. The percentage inhibition of granuloma tissue development was calculated using the formula:

(T c -T t)/T c ×100, where T c = weight of granuloma tissue of control groups

T t = weight of granuloma tissue of treated groups.

Aspirin Induced Ulcers in Rats [7]

Albino Wistar rats were randomly divided into six groups of six animals each. Group I served as control. Groups II to VI received EEPA and AEPA at 100 and 200 mg/kg b.w. and ranitidine 20 mg/kg b.w. p.o. respectively for 5 days. On day 5, 1 h after administration of extract/ standard, aspirin 200 mg/kg b.w. was administered orally to

all the animals. After 4 h, animals were sacrificed, stomach was removed and opened along the greater curvature. The intensity of gastric lesions was assessed and ulcer index was calculated.

Pylorus Ligation Induced ulcers in Rats [8]

Albino Wistar rats weighing 250-280 g were randomly divided into six groups of six animals each. Group I served as control. Groups II to VI received EEPA and AEPA at 100 and 200 mg/kg b.w. and ranitidine 20 mg/kg b.w., respectively. One hour after the administration of the drug, pyloric ligation was done under anesthesia. After 4 h, animals were sacrificed, stomach was isolated and, opened along the greater curvature. Gastric fluid was collected separately for measurement of total gastric volume and estimation of free and total acidity. pH of gastric volume was measured, total and free acidity was determined by titrating with 0.1N NaOH using topfers solution and phenolphthalein as indicators, respectively. Intensity of gastric lesions was assessed, the number of ulcers was noted and the severity was recorded with the following scores procedures.

Normal coloration: 0, red coloration: 0.5, spot ulcer: 1.0, hemorrhagic streak: 1.5, ulcer ≥ 3 but ≤5: 2, ulcer >5: 3. [9] Ulcer index was calculated.

Study of Ulcerogenic Potential

Albino Wistar rats were divided into five groups, the animals of groups I to V were treated with EEPA and AEPA 100 and 200 mg/kg and indomethacin 10 mg/kg b.w., respectively. The extracts and standard were administered p.o., once daily for 5 days. At the end of the study, the animals were sacrificed and the stomachs were examined for ulcers and scoring was done. [10]

Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism version 3.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical comparisons between different groups were done using one-way analysis of variance (ANOVA) followed by the Dunnett and Tukey multiple comparison test. $P<0.05$ was considered significant.

Results

Acute Toxicity Study

There was neither change in behavioral pattern nor any sign of toxicity during the observations up to 24 h for mortality. The extracts were safe up to a maximum dose of 2000 mg/kg b.w. The biological evaluation was carried out at doses of 100 and 200 mg/kg b.w.

Results of Preliminary Phytochemical Screening

The phytochemical screening revealed that ethanolic extract contained flavonoids, alkaloids, steroids, tannins, and phenolic compounds, glycosides, saponins, cardiac glycoside like cardenoloids and carbohydrates. The aqueous extract contained flavonoids, alkaloids, tannins, and phenolic compounds, glycosides, saponins, and carbohydrates.

Cotton Pellet Granuloma

All the extracts produced a significant (P<0.01) decrease in the growth of granuloma tissue when compared to the control [Table 1]. AEPA 200 produced a significant (P<0.05) inhibition of granuloma tissue that was better than standard (54.09±2.76% and 53.32±9.00%, respectively). The aqueous extracts were more effective in anti-inflammatory activity as compared to ethanolic extracts.

Table 1: Effect of ethanolic and aqueous extracts of *Pisonia alba* on granuloma tissue formation in rats (n=6)

Groups (Dose mg/kg b.w.)	Dose (mg/kg b.w.)	Weight of granuloma tissue (mg)	% inhibition of granuloma tissue
Control	-	81.85 ± 9.85**a	-
Ranitidine (20)	10	38.76 ± 7.29**a	54.33 ± 9.04
EEPA (100)	100	50.38 ± 6.03**a	39.95 ± 7.42
EEPA (200)	200	46.26± 8.78**a	45.05 ± 10.86
AEPA (100)	100	42.51 ± 6.87**a	49.67 ± 8.50
AEPA (200)	200	38.13 ± 2.24**a	55.09 ± 2.75*b

Values are expressed as mean ± SD; CPE= ethanolic extracts of Caesalpinia pulcherrima; CPA= aqueous extracts Caesalpinia pulcherrima; *P P<0.05, **P<0.01; a'indicates the comparison of both the extracts with control; 'b'indicates the Comparison of extracts with standard indomethacin

Aspirin induced Ulcers in Rats

A significant (P<0.01) decrease in the ulcer score was produced by ranitidine, EEPA, and AEPA as compared to control. 200 mg/kg b.w of EEPA and AEPA was more effective in decreasing the ulcer score. The minimum ulcer index was observed with ranitidine followed by EEPA 200. Percentage protection against ulcers by EEPA 200 was comparable to that of standard drug ranitidine. The maximum protection against ulcers was produced by ranitidine followed by EEPA 200 and AEPA 200 [Table 2].

Table 2: Effect of ethanolic and aqueous extracts of *Pisonia alba* in aspirin induced ulcer model in rats (n=6)

Groups (Dose mg/kg b.w.)	Ulcer score	Ulcer index	Percentage protection against ulcers
Control	5.63±0.56	18.41	-
Ranitidine (20)	0.65±1.14**	0.67	97.21
EEPA (100)	1±11**	2.44	88.87
EEPA (200)	1.15±0.56**	0.84	96.43
AEPA (100)	1.32±1.43**	2.73	86.82
AEPA (200)	1.15±1.05**	1.17	94.89

Values are the mean ±SD; CPE= ethanolic extracts of *Caesalpinia pulcherrima*; CPA= aqueous extracts *Caesalpinia pulcherrima*; ** P < 0.01as compared to control.

Pylorus ligation induced Ulcers in Rats

Pylorus ligation produced an increase in gastric volume, total acidity, free acidity and a decrease in pH in control animals. Ethanolic and aqueous extracts produced a significant (P<0.01) decrease in the ulcer score when compared to control. EEPA 200 produced a decrease in the ulcer score comparable to that of ranitidine. The ulcer index was minimum with ranitidine followed by EEPA 200 (0.62 and 0.87, respectively) [Table 3]. There was a significant (P<0.01) reduction in gastric volume, increase in pH, and decrease in free and total acidity in ranitidine- and extract-treated groups when compared to control. AEPA 200 produced a significant (P<0.05) reduction in gastric volume that was better than ranitidine. Aqueous extracts at both the doses produced a maximum reduction in gastric volume when compared to both the doses of ethanolic extracts. Aqueous extracts at both the doses produced a further increase in pH as compared to the ethanolic extracts. A reduction in free acidity by EEPA 200 was comparable to that of ranitidine.

Table 3: Effect of different extracts of *Pisonia alba* in pylorus ligation induced ulcer model in rats (n=6)

Groups (Dose mg/kg b.w.)	Ulcer score	Ulcer index	Percentage protection against ulcers
Control	5.4±0.57	18	--
Ranitidine (20)	0.61±0.62**	0.61	97.56
EEPA (100)	1±0.86**	1.1	95.75
EEPA (200)	0.86±1**	0.86	96.41
AEPA (100)	1.35±0.8**	1.35	93.82
AEPA (200)	1.11±1.02**	1.13	95.31

Values are the mean ±SD; CPE= ethanolic extracts of Caesalpinia pulcherrima; CPA= aqueous extracts Caesalpinia pulcherrima; ** P < 0.01as compared to control.

Ulcerogenic Potential

Ethanolic and aqueous extracts did not produce any ulceration, whereas indomethacin was found to produce ulcers with an ulcer score of 5. This indicates that *P. alba* does not have ulcerogenic potential, indicating that it is non ulcerogenic.

Discussion

The cotton pellet-induced granuloma formation is a sub-acute inflammatory response that includes transudative and proliferative phase. Monocyte infiltration and fibroblast proliferation are commonly seen in granuloma. Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma by inhibiting granulocyte infiltration, preventing generation of collagen fibres and suppressing mucopolysaccharides. [11] In the present study, both the extracts exhibited a significant anti-inflammatory activity in a cotton pellet granuloma test by the decrease in granuloma tissue development. This may be due to inhibition of fibroblasts and synthesis of collagen

and mucopolysaccharides during granuloma tissue formation by lupeol and quercetin in PA. Flavonoids in general and quercetin in particular are reported to possess analgesic, anti-inflammatory, and antiulcer activities. [12],[13]

Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion, and back diffusion of H + ions. [14] In stomach, prostaglandins play a vital protective role by stimulating secretion of HCO -3 and mucous, maintaining mucosal blood flow and regulating mucosal cell turnover, and repair. Thus the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastro duodenal ulceration. [15] It is also shown that ROS (reactive oxygen species) plays an important role in pathogenesis of mucosal damage caused by aspirin besides inhibition of COX enzymes. The present study observed that ethanolic and aqueous extracts reduced aspirin induced ulcers suggesting possible involvement of prostaglandin and mucus. PA is also reported to possess antioxidant activity that might have also contributed in antiulcer activity exhibited by extracts.

Pylorus ligation induced ulcers are due to auto-digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. [15] An increase in acid-pepsin accumulation due to pylorus obstruction may cause subsequent mucosal digestion. [16] The ethanolic and aqueous extracts of PA at both the doses decreased ulcer score and provided protection against ulcers. Protection against pylorus ligation induced ulcers also indicates the antisecretory activity of PA. These results indicate that *P. alba* is not only non-ulcerogenic but is also ulcer protective. This makes *P. alba* to stand apart from other anti-inflammatory agents.

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