

Analgesic and Antidiarrheal Activities of Leaf of *Podocarpus neriifolius* D. Don

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Abstract

The methanol extract of leaf of *Podocarpus neriifolius* D. Don exhibited *in vivo* peripheral analgesic and antidiarrheal activities in Swiss Albino mice. In the peripheral analgesic activity assay, the methanolic extract showed $50.00 \pm 8.57\%$ and $70.25 \pm 1.18\%$ inhibition of acetic acid-induced writhing at 200 and 400 mg/kg body weight, respectively. In addition, the extract also revealed a dose dependant inhibition of castor oil-induced diarrhea with $43.77 \pm 3.13\%$ and $56.23 \pm 6.49\%$ inhibition of feces at 200 and 400 mg/kg body weight, respectively.

Key words: Analgesic activity, castor oil, diarrhea

Introduction

Podocarpus neriifolius D. Don, also known as brown pine, is a species belonging to the Podocarpaceae family. It grows 10–15 m tall, though very occasionally taller, in tropical and sub-tropical wet closed forests, between 650 m and 1600 m altitudes. Traditionally, it is used to treat joint pain due to rheumatism and arthritis (Abdillahi *et al.*, 2010). Other plants of the *Podocarpus* genus have been reported to be febrifuge, anti-inflammatory and expectorant (Abdillahi *et al.*, 2011). Majority of the people in the developing countries use different types of this herbal concoction in diarrhea and pain. Diarrhea is also considered one of the leading causes of mortality in the developing countries. As part of our ongoing research with medicinal plants of Bangladesh (Tahia *et al.*, 2015, Aktar *et al.*, 2015), the present work has been undertaken to evaluate the analgesic and antidiarrheal potential of a crude methanol extract of the leaves of *P. neriifolius* and we, here in, report the results of our preliminary investigations.

Materials and Methods

Collection and preparation of plant materials: Fresh leaves of *P. neriifolius* were collected from the hill tracts of Chittagong, Bangladesh in September 2013. It was then identified by the taxonomist of Bangladesh National Herbarium, where a voucher specimen (DACB - 39344) has been maintained. The clean leaves were sun dried and ground to a coarse powder. Then 500 gm of this powder was soaked in 3 liters of methanol in a flat bottom flask. The container was sealed with a cotton plug and kept for 7 days with occasional shaking to facilitate the extraction of phytoconstituents. The whole mixture was filtered by cotton plug followed by Whatman number 1 filter paper and the filtrate was evaporated to dryness with a rotary evaporator at reduced temperature and pressure. The concentrated gummy mass was transferred to a clean beaker and stored in refrigerator until used for further experiments.

Drugs and chemicals: Acetic acid, methanol and Tween-80, loperamide (Square Pharmaceutical Ltd., Bagladesh), and diclofenac sodium and normal saline

(Incepta Pharmaceutical Ltd., Bangladesh) were used. Highly purified castor oil was purchased from local market. All other reagents were of analytical grade.

Experimental animals: Swiss Albino mice of both sexes weighing between 25-35 gm and 4-5 weeks old were obtained from the Department of Pharmacy, Jahangirnagar University. The mice were kept in the animal house of the State University of Bangladesh and fed with standard rodent feed. As these animals are sensitive towards environmental change, they were kept for 4 days in the laboratory environment prior to use for the experiments. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental mice.

Grouping of mice: Twenty four Swiss Albino mice were randomly divided into four groups of six animals in each group for each bioassay: positive control, negative control and two test groups receiving methanolic extract at doses of 200-(PN1) and 400-mg/kg of body weight (PN2). **Peripheral analgesic activity:** Analgesic activity was evaluated by acetic acid-induced writhing method as described by Koster *et al.* (1959). In this method, 0.1ml of acetic acid was administered intraperitoneally to the experimental animals to create pain sensation, where, the animals started to squirm their body at regular interval due to pain. This squirm or contraction of the body is termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that exhibits analgesic activity is supposed to lessen the number of writhing in animals within a given time frame with respect to the control group. As positive control, each mouse received 5mg/kg body weight of diclofenac sodium through intraperitoneal route. In the experimental group the crude extract was used for the evaluation of analgesic activity at 200-and 400-mg/kg of body weight and the percentage of writhing inhibition was calculated by using the following equation:

$$\text{Inhibition (\%)} = [(Wc - Wt) \times 100] / Wc$$

Where, Wt is the average number of writhing reflex in the test group and Wc is the average number of writhing reflex in the negative control group.

Antidiarrheal activity: The antidiarrhoeal activity of the methanolic extract of leaves of *P. neriifolius* was evaluated by using castor oil-induced diarrhea in mice, the experimental details of which could be found elsewhere (Shaoba and Thomas, 2001). Here, the animals were divided into negative control, positive control and test groups. Each mouse was fed with 1.0 ml of highly pure analytical grade castor oil. Negative control group received vehicle (1% Tween-80 in water), while the positive control group was administered with loperamide at 50 mg/kg body weight orally. The test groups received methanol extract at 200 and 400 mg/kg body weight orally. The number of fecal stools was recorded for the individual mouse. The results of the experimental groups were compared with that of the control groups to evaluate the antidiarrheal activity. The percentage inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ inhibition} = [(Mo - M) / Mo] \times 100; \text{ where,}$$

Mo = Mean defecation of negative control and M = Mean defecation of test sample or standard (positive control).

Statistical analysis: The values are presented as mean \pm standard error of mean (SEM) and One way ANOVA followed by Dunnett's test were used to determine the significance difference between the control group and experimental groups, the p values < 0.05 were considered to be statistically significant.

Results and Discussion

The effects of methanolic extract of the leaves of *P. neriifolius* in acetic acid-induced writhing are shown in table 1. The number of contraction of abdominal muscle induced by 0.1 ml of acetic acid was effectively reduced by the extract in a dose dependant manner. Statistical evaluation of the data confirmed that the crude methanolic extract of leaves of *P. neriifolius* demonstrated significant peripheral analgesic activity having writhing inhibition of $50 \pm 8.57\%$ and $70.25 \pm 1.18\%$ at 200- and 400-mg/kg of body weight, respectively when compared to that of the standard diclofenac sodium ($77.39 \pm 0.68\%$ inhibition).

The antidiarrheal activity of the methanolic extract of the leaves of *P. neriifolius* is shown in table 2. The extract reduced the castor oil induced diarrheal feces by 43.77 ± 3.13% and 56.23 ± 6.49% (p < 0.05) at the dose of 200-and 400-mg/kg of body weight, respectively.

Table 1. Peripheral analgesic activity of crude methanol extract of *P. neriifolius* leaves.

Animal group	Writhing count			Number of writhing (Mean ± SEM)	% Inhibition of writhing (Mean ± SEM)
	M-1	M-2	M-3		
NC	28	30	26	28 ± 1.15	-
PC	6	6	7	6.33 ± 0.33	77.39 ± 0.68**
PN 1	12	22	8	14 ± 4.16	50 ± 8.57*
PN 2	10	7	8	8.33 ± 0.88	70.25 ± 1.18**

Values are expressed as Mean ± SEM (n=3). **p< 0.01, *p< 0.05 compared to negative control. NC = negative control (1% Tween 80 in water), PC = positive control (Diclofenac sodium at 5mg/kg body weight), PN1 = Methanolic extract of *P. neriifolius* leaves at 200 mg/kg body weight, PN2 = Methanolic extract of *P. neriifolius* leaves at 400 mg/kg body weight. M1, M2, M3 = Mice 1, Mice 2, Mice 3, respectively.

Table 2. Effect of methanol extract of *P. neriifolius* leaves on castor oil-induced diarrhea in mice.

Treatment	Dose	Number of diarrheal feces (Mean ± SEM)	% Reduction of diarrhea (Mean ± SEM)
Negative control (Saline)	10 ml/kg b.w.	10.67 ± 1.33	-
Positive control (Loperamide)	50 mg/kg b.w.	2.33 ± 0.33	78.16 ± 1.78**
PN1	200 mg/kg b.w.	6.00 ± 0.58	43.77 ± 3.13*
PN2	400 mg/kg b.w.	4.67 ± 1.20	56.23 ± 6.49*

Values are expressed as Mean ± SEM (n=3). **p< 0.01, *p< 0.05 compared to control (One way ANOVA followed by Dunnett's test). Positive control = (Loperamide at 50mg/kg body weight), PN1 and PN2 = Methanolic extract of *P. neriifolius* leaves at 200 mg/kg body weight and 400 mg/kg body weight, respectively.

The dose depended reduction of diarrheal feces by the methanolic extract of *P. neriifolius* leaves was found to be significant when compared to the reduction of diarrheal feces by loperamide. Ricinolic acid which is the active metabolite of castor oil stimulates peristaltic activity in the small intestine, leading to changes in electrolyte permeability of the intestinal mucosa (Ammon et al., 1974; Tanko et al., 2012). The liberated ricinoleic acid also causes irritation and inflammation of the intestinal mucosa leading to the release of endogenous prostaglandins. Other mechanisms of castor oil induced diarrhea include inhibition of intestinal Na⁺/K⁺ - ATPase activity, activation of adenylate cyclase or mucosal cAMP mediated active secretion and platelet activating factor (Meite et al., 2009). The decrease in the wetness of feces and the frequency of defecation as well as the

decrease in the intensity and frequency of body squirming observed, proved the potent antidiarrhoeal and analgesic activities of *P. neriifolius* leaves which might be due to the inhibition of prostaglandins biosynthesis. Previous phytochemical screenings of the plant extract revealed the presence of terpenoids, steroids, flavanoids, polyphenolics and fats (Zhen et al., 1993; Rashid et al., 2014). Many plants exhibited analgesic and antidiarrheal activities due to the presence of saponins, terpenoids, steroids and flavonoids (Owleye et al., 2005). It is not unreasonable, therefore to speculate that the terpenoids, steroids and flavonoids present in the plant extract may be responsible for the observed analgesic and antidiarrheal activities.

Conclusion

The results of our preliminary studies indicated potent analgesic and antidiarrheal activities of *P. neriifolius* leaves. Thus, the plant warrants further investigation to isolate the active constituents responsible for these activities and to establish the mechanism(s) of action.

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