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Comparative Study of Analgesic Activity of *Diospyros Melanoxylon* (Roxb.) Bark and Root Bark

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Abstract

In order to scientifically appraise some of the ethnobotanical uses of *Diospyros melanoxylon* (Roxb.), the present study was undertaken to examine analgesic properties in the stem bark and root bark by ethanolic extracts in mice. The analgesic effect of ethanolic extracts was evaluated by 'hot plate' and 'acetic acid-induced writhing test' in mice by using pentazocine (5 mg/kg) and asprin (50 mg/kg) simultaneously as reference drugs. *Diospyros melanoxylon* (Roxb.) stem bark's ethanolic extracts possess analgesic activity, but here we also used root bark to check the extent of difference in activity. The ethanolic extract of stem bark (200 mg/kg) possessed significant analgesic activity, as in acetic acid-induced writhing test, the writhing count was reduced significantly compared to standard and root bark ethanolic extracts. In the hot plate method with ethanolic extracts of stem bark (200 mg/kg), the basal reaction time was increased significantly ($p<0.01$) and the percentage increase in threshold to pain was also significant as compared to standard.

Keywords: *Diospyros melanoxylon,* analgesic activity, acetic acid-induced writhing test, hot plate method.

1. Introduction

The plant *Diospyros melanoxylon* (Roxb.) belonging to the family Ebenaceae was selected for our research project on the basis of ethnobotanical information, which reveals its use against post-natal pain by the tribals [1]. This plant is widely distributed in the northern part of India (Bihar, Madhya Pradesh, Chhattisgarh, Himachal Pradesh, West Bengal, Mumbai etc.) and also in Tamil Nadu (Coimbatore, Dharmapuri, Salem etc.) [2, 3, 11]. It is a medium-sized tree, reaching a height of 15 m, and is well known for its bidi-making leaves throughout the world [3]. This plant has been well documented in Ayurveda and Unani texts and also ethanobotonically for its multi-purpose use in different diseases. Stem bark is used in treatment of diarrhoea, dyspepsia, astringent (eye), dysentery, ulceration of cornea and post-natal pain (basis for our present study). Flowers are used in urinary and skin troubles, blood diseases, leucorrhoea, urinary discharge and anaemia and also as a diuretic. Leaves are used as styptic, in the treatment of scabies and old wounds, and as laxative and carminative medicine. Fruits are used in stomach disorders [4–8]. Previous phytochemical studies revealed that stem bark contains tannins (19%), ceryl alcohol, lupeol, betulin, β-sitosterol, sequoyitol, carboxylic acid, diospyric acid and naphthoquinones [9].

2. Materials and Methods

The species for the proposed study is *Diospyros melanoxylon* (Roxb.) stem bark, which were collected initially in the months of June 2005 and analgesic study was conducted successfully. For the present research, we collected the stem and root barks in June 2011 from the forest of Baikunthpur area, District Korea, Chhattisgarh,

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India. Care was taken regarding the age and the health of the plant to obtain a best-condition bark and root bark part. Previously, taxonomically it was identified and authenticated by the botanist of Plant Anatomy Research Center (PARC), Chennai, India, and for recent work it was identified by the botanist of the forest department of Baikunthpur (Chhattisgarh).

2.1 Preparation of the Ethanolic Extracts

The barks collected were washed properly with water to remove the mud or dust if any and were dried under sun for an hour; then they were dried completely in the shade. The dried barks were then powdered by means of a wood grinder, and the powder was sieved (sieve no. 60) to get the coarse powder. This powder was subjected to continuous hot extraction with ethanol. The extracts obtained were dried and kept in a desiccator till experimentation. The yield was found to be 15.04%w/w for stem barks and 16.24%w/w for root barks.

2.2 Selection of Animals

Albino Swiss mice 20–25 g were used. Mice in four groups, six mice in each, were weighed and numbered and kept in a standard polypropylene cage at room temperature of 27±2°C. Relative humidity was kept at 60–70% and the cage was well ventilated. They were kept on a fast initially for 16 hr.

2.3 Acute Toxicity Studies

Toxicity studies were performed for both ethanolic extracts as per OECD guidelines–420, fixed-dose procedure. Fixed-dose levels of extracts starting from 50, 100, 200, 500, 1,000, increasing upto 2,000 mg/kg body weight were given, and signs and symptoms of toxicity were observed for next 48 hr.

2.4 Evaluation of Analgesics Activity of Stem Bark and Root Bark

Both the extracts (prepared as fine suspension in 1% CMC) were evaluated for their analgesic activity by acetic acid-induced writhing method and hot plate method.

In acetic acid-induced writhing method, albino mice were grouped into four different groups (six animals each). Group I served as control, where normal saline (2 ml/kg) was given. Group II served as standard, where asprin (50 mg/kg) was given. Groups III and IV received ethanolic extracts (each 200 mg/kg) orally. The writhing movements were observed and counted after acetic acid administration. [12, 13]

In the hot plate method, albino mice were grouped into four different groups (six animals each). Group I served as control, where normal saline (2 ml/kg) was given. Group II served as standard where, pentazocine (5 mg/kg) was given. Groups III and IV received ethanolic extracts (each 200 mg/kg) orally. Before and after drug administration, the basal reaction time to the heat stimulus was taken by placing the rats on Eddy's hot plate maintained at 55±1°C at 30 min, 60 min, 120 min and 180 min was noted. When the animal licked the fore or hind paws or jumped, it was taken as the end point and was noted [10].

2.5 Statistical Analysis

Results were analysed by student's t-test. The minimum level of significance was fixed at $p<0.01$[14].

3. Results and Discussion

No toxicity or death was observed in the experimental rats when they were subjected to toxicity study. The results showed that ethanolic extracts of stem bark and root bark both possess analgesic activity but the extent of activity was different. The ethanolic extract of stem bark (200 mg/kg) possessed significant analgesic activity in response to acetic acid-induced writhing test, where the writhing count was significantly reduced comparatively (Table 1 (B)). In hot plate method, ethanolic extract of stem bark (200 mg/kg) showed the basal reaction time 10.02 s ($p<0.01$) and percentage increase in threshold to pain was 148.78% as compared to the standard pentazocine, which showed basal reaction time 10.6 s ($p<0.01$) and percentage increase in threshold to pain 152.38%. Whereas the basal reaction time for root bark's ethanolic extract (200 mg/kg) was 9.8 s and percentage increase in threshold to pain was 133.3%, which was comparatively less than stem bark extract (Table 1(A)). Finally, it was concluded that ethanolic extract of stem bark showed more significant activity against acute inflammatory pain as compared to the potent inhibitory

Table 1: Analgesic activity of ethanolic extracts of stem and root bark of *Diospyros melanoxylon* (Roxb.)

A. Eddy's hot plate method

Treatment	Reaction time in s					% increase in threshold to pain
	0 min	30 min	60 min	120 min	180 min	
Control (normal saline) (2 ml/kg)	4.1±0.3	4.0±0.2	4.1±0.08	4.2±0.3	4.1±0.3	0
Standard Pentazocine (5mg/kg)	4.2±0.3	6.9±0.8	8.6±0.6*	10.1±0.4*	10.6±0.4*	152.38*
Ethanolic extract root bark (200 mg/kg)	4.2±0.3	5.5±0.4	7.6±0.2*	9.6±0.5*	9.8±0.5*	133.3*
Ethanolic extract stem bark (200 mg/kg)	4.1±0.80	5.9±0.20	7.8±0.40	9.7±0.7*	10.2±0.3*	148.78*

Data are expressed in mean ± SE, n=6 *p < 0.01 vs Control.

B. Writhing test method

Treatment	No. of writhing (per 30 min)	% of activity
Control (normal saline) (2 ml/kg)	85±0.39	0
Standard Asprin (50 mg/kg)	20±1.6*	75.29
Ethanolic extract root bark (200 mg/kg)	27±1.2*	68.60
Ethanolic extract stem bark (200 mg/kg)	25±0.8*	70.05

Data are expressed in mean ± SE, n=6 *p < 0.01 vs Control

pain, may be by suppressing the formation of pain-inducing substances (prostaglandins and bradykinin) in the peripheral tissues. Therefore, it is likely that *Diospyros melanoxylon* (Roxb.) might suppress the formation of these substances and exerts its analgesic activity in acetic acid-induced writhing test and hot plate test.

4. Conclusion

Analgesic activity of *Diospyros melanoxylon* (Roxb.) barks was studied for its central and peripheral activities. The analgesic activity of both the extracts showed significant analgesic activity as compared to standard drugs. Ethanolic extract of stem bark (200 mg/kg) showed more significant activity by significantly increasing the reaction time in hot plate test, suggesting its central analgesic activity and ability to reduce the writhing counts. The activity might be due to the presence of steroids, tannins, alkaloids and triterpenoids in ethanolic extract.

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