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Comparative Antinociceptive Study of Leaf, Bark and Seed Extracts of Neem Collected from Aligarh District

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Abstract

Azadirachta indica (family Meliaceae) is commonly known as neem. The latinized name of neem *A. indica* (in Persian, *Azadi* = free, *diracht* = tree) literally meaning "the free tree of India", is an alliteration for its being intrinsically free from insect and disease problems. The neem tree is considered as a '*sarvaroga nivarini*' (the panacea for all diseases) and has also been hailed as '*heal all*', '*divine tree*', *'village dispensary'* and '*nature's drugstore*'. In an effort to elaborate the pharmacological properties of neem, comparative study of different extracts of neem (leaves, bark and seeds) has been done. The alcoholic extracts of leaf, bark and seed in dose 100 mg/kg showed significant antinociceptive activity. The alcoholic extract of leaves (duration 240 mimutes) showed most significant analgesic activity than other extracts.

Key Words: *Azadirachta indica*, Neem, Antinociceptive activity, Tail flick method

Introduction

Neem has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem [1-5]. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaves. Neem leaves and its constituents have been found to exhibit immunomodulatory, anti-inflammatory [6], antiulcer [7-10], antimalarial [11,12], antifungal [13-17], antibacterial [18], antiviral [19-22], antioxidant, antimutagenic and anticarcinogenic [23-25] properties. Aqueous extract of neem leaves showed both ulcer protective and ulcer healing effect. Nimbolide isolated from the flowers of neem has been found to have antiproliferative activity against cancer cells [26,27]. The feeding of neem effect on helmintic worm in sheep [28], significant antihyperglycaemic activity of neem seeds has been found in diabetes mellitus patients [29]. Medicinal importance of this plant encourages us to carryout the comparative antinociceptive activity of different parts (leaves, bark and seeds) of neem extracts.

Material and Methods

Preparation of Extract

The leaves, bark and seeds of *A. indica* were collected from the Botanical Garden, Aligarh Muslim University, Aligarh, India and identified by Dr. Athar Ali, Reader (taxonomist), Department of Botany, A.M.U, Aligarh, India. The fresh leaves of *A. indica* (250 g) were dried under shade and crushed to make powder. The powdered leaves were defatted with light petroleum (60-80 ^{o}C) and then extracted thoroughly with 95% ethanol in a soxhlet apparatus. The EtOH extract was evaporated to dryness under reduced pressure. The dark green viscous mass (93 g) left behind was subjected to analgesic activity. The same procedure was repeated for bark and seed. The yield of bark and seed extract was found to be 117 g and 82 g respectively.

Antinociceptive Activity

The analgesic activity used in this study was tail flick response in which change in the latency of the tail flick escape from noxious heating of the tail skin was used to asses the antinociceptive effect .The normal reaction time of rats on analgesiometer ranges from 4-5 seconds. The cut off time was obtained after requiring the determination of the reaction time of each untreated rat at 0, 20 and 40 minutes. Rats were selected by preliminary screening. Those showing variation of more than one second between two reaction times at 20 minutes interval or more than 3 seconds from the group mean were discarded. Charles foster rats weighing 150-200 g of either sex were placed in restraining holder so that the tail between the hole and tail tip or single point 3-5 cm from the tip of tail are directly kept over a heated nichrome wire.The reaction due to thermal stimulus in the form of tail flick in normal, untreated rats was adjusted within 4-5 seconds. Since the stimulus capable of producing tissue damage was stated to be about twice that require producing pain; the system was present to automatically cut off time at 7-8 seconds (average multiplied by 1.5) [30]. The rats were housed in standard breeding cages with access to a solid diet (Gold Mohar, Lipton,

India, Ltd) and tap water except during times of experiment. The animal observation room was controlled so that the light dark cycle (light period 6.00 am to 6.00 pm, 12 hours) and temperature (22±2 ^{o}C) were nearly constant [31]. Five groups of eight animals each were taken and grouped as Group 1: Control (double distilled water), Group 2: 100 mg/kg, ethanolic extract of leaves, Group 3: 100 mg/kg ethanolic extract of bark, Group 4: 100 mg/kg ethanolic extract of seeds, and Group 5: 30 mg/kg Standard drug (Pentazocine). Initially basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire of analgesiometer. After administration of drugs, reaction time was noted two hours later in groups 2, 3, 4, and 5 while 20 minutes later in groups 1and 6. All readings were taken in an interval of 20 minutes each.

Results

On comparing all the extracts of neem (Table. 1), alcoholic extract of leaves was found most potent (duration 240 minutes), followed by bark (duration 220 minutes) and seed (duration 180 minutes). The effect of *A. indica* extracts (leaves, bark and seeds) was also compared with standard drug pentazocine, which was given in dose of 30 mg/kg i.p. and was found almost equivalent in efficacy (Fig. 1). The alcoholic extract of leaves (duration 240 minutes) and bark (duration 220 minutes) has been found to be slightly more effective than the standard drug (duration 200 minutes), but the alcoholic extract of seed (duration 180 minutes) is slightly less effective than the standard drug (duration 200 minutes).

Table 1. Antinociceptive Activity of Neem Extracts

Group (n)	Treatment	Onset	Peak	Recovery	Duration
Control (8)	Normal Saline	4.12±0.21	4.23±0.11	4.02±0.22	0 Min
Standard (8)	Pentazocine (30 mg/kg)	6.29±0.58♦ (20 Min)	7.01±0.17* (100 Min)	4.37±0.40@ (220 Min)	200 Min
Test (8)	Ethanolic extract of leaves (100 mg/kg)	4.90±0.30# (20 Min)	6.92±0.50* (160 Min)	4.93±0.10♦ (260 Min)	240 Min
	Ehanolic extract of bark (100 mg/kg)	5.38±0.10* (20 Min)	7.23±0.09* (140 Min)	5.05±0.07* (240 Min)	220 Min
	Ethanolic extract of seeds (100 mg/kg)	4.98±0.09♦ (20 Min)	6.35±0.11* (120 Min)	4.98±0.09* (200 Min)	180 Min

*p < 0.001, ♦p < 0.01, #p < 0.1, @p <0.2

Discussion

Oral administration of ethanolic extracts of the leaves, bark and seeds of *A. indica* at doses of 100 mg/kg p.o. each to Charles foster rats showed significant antinociceptive activity in analgesiometer test (Fig. 1). After two hours of drug administration, the alcoholic extract of leaves at dose of 100 mg/kg in rats caused a gradual increase in reaction time which reached to its maximum values 6.92±0.50 in 160 minutes (Table. 1). The effect gradually declined within next 100 minutes. The administration of the ethanolic extract of bark and seeds in doses of 100 mg/kg p.o. each resulted in gradual increase to its peak value 7.23±0.09 and 6.35±0.11 in 140 and 120 minutes, respectively after drug administration. The effect gradually decreased within next 100 and 80 minutes, respectively (Table 1). The maximum response (duration 240 minutes) was noted with 100 mg/kg of ethanolic extract of leaves, followed by bark and seed extract (duration 220 and 180 minutes, respectively). Peak effect (120 minutes) with ethanolic extract of seed (100 mg/kg) was observed earlier in comparison with bark and leaf extracts.

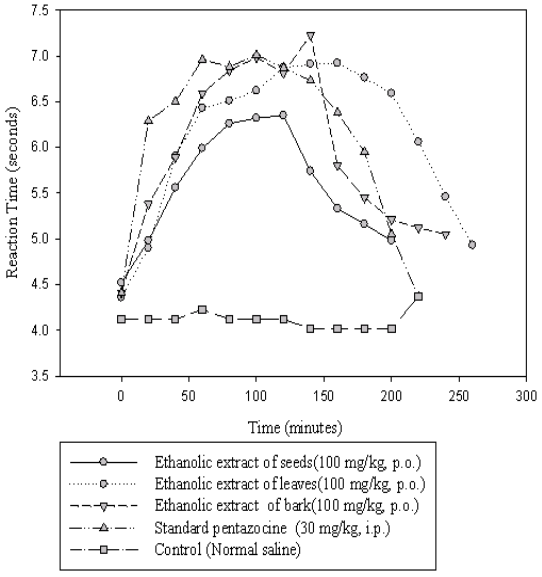

Fig. 1. Time response curve of aggregate mean of reaction time of *A. indica* extracts and pentazocine in analgesiometer.

Conclusion

The significant increase in reaction time of all the extracts suggested that *A. indica* could be one of the alternative analgesic herbal drugs. But leaf extract showed most significant

antinociceptive activity and hence, its use in home remedies as pain killer is justified and may be promoted.

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