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ANTI – INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF THE ROOT, BARK AND LEAVES OF AZADIRACHTA INDICA

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ABSTRACT: *Azadirachta indica is a plant of varied uses in Ayurveda since ancient times and is highly extolled by expert physicians and as well as practitioners of folk medicines. Almost every part of the tree has long been used in folklore and traditional systems of medicine for the treatment of a variety of human ailments. The 50% acetone extract of the root, bark and leaves of A. indica sowed marked anti- inflammatory activity in carrageenan induced edema in rats, Antimicrobial activity was also tested.*

INTRODUCTION

A. indica commonly known as "neem" belongs to the family Meliaceae. A variet of biological activities of A. indica and its various preparations are reported in literature. The antifertility (1), antidiabetic (2, 3), analgesic (4), immunomodulatory (5, 6) and metabolic effects have been already reported.

MATERIALS AND METHODS

Plant material

The fresh root, bark and leaves of *A. indica* was collected in the month of Marc in and around Tiruchirappalli. It was confirmed for official monographic specification by t herbarium, Department of Botany, Bishop Heber College, Tiruchirappalli.

Preparation of Extracts

Dried materials (1kg) were extracted with 50% acetone in a soxhlet. The extract was evaporated until a solid residue was obtained. The percentage yield was found to be 0.5.

Animals

Male albino (Swiss) weighing 80 -100g bred in king Institute Guindy, Chennai were selected for studies. The anti-inflammatory activity was studied by carrageenan induced rat hind paw oedema. The animals were kept in Microlon boxes and had access to water ad *libitum*.

Carrageenan induced rat paw oedema

The rats were divided into 11 groups, each consisting of six animals. One group served as a control (received normal saline only), the second group served as a positive control (received the various doses of the extracts suspended in 5% acacia solution and given intrapertioneally.

Group iii, iv, v – Received 150,100,150 mg/kg bark extract of *A. indica*

Group vi, vii, viii – Received 50,100,150 mg/kg bark extract of *A. indica*

Group ix, x, xi – Received 50,100,150 mg/kg bark extract of *A. indica*

The drugs and extracts were administered intraperitoneally.

Anti-inflammatory activity

Edema was produced b the method descried b winter et al (7). The paw volume was measured 0 hr, 1hr, 2hr after the injection of carrageenan (0.1 ml of 1% solution injected in the subplantar region). The apparatus used for the measurement of rat paw volume was that of Buttle et al 1996 modified by singh and gosh (8). This method is able to detect a minimal change of paw volume of 0.02 ml. Drug pretreatment was given 1 hr before the injection of carrageenan. The values are shown in Table 1.

Antimicrobial activity

Antimicrobial activity of the root, bark and leaves of *A. indica* have been evaluated. *Escherichia coli* of the Gram negative group and *Styphyllo aureus* of the gram positive group were chosen as test organisms.

Serial dilution technique

A nutrient broth medium of neutral pH containing peptone 1% (w/v), yeast extract 0.5% (w/v) was prepared in distilled water and sterilized by autoclaving for about 30 min (9). A standard volume (8ml) of nutrient broth medium that would support the growth of the test organisms was added to several labeled, sterile, stoppered and identical assay tubes. Solutions of each test compound at three different concentrations viz 50, 100 and 200 µg/ml and a control containing no drug were also prepared. One loopful of the inoculam (of suitable dilution) of over night broth culture of t test organism was added. All these experimental manipulations were carried out under absolute aseptic conditions, the assay tubes we5re then incubated at 37 ± 1^{o}C for 48 hrs and the resultant turbidities were measured wit Nepheloturbidity meter. The percentage of bacterial growth inhibition produced by a particular growth inhibition produced by a particular concentration of the test compound was calculated from the measure of the turbidity of the control and the turbidity of the specific treatment by employing the following relationship

$$\%\text{Inhibition} = \frac{Tc - Tt}{Tc}$$

Where Tc is the turbidity of the control and Tt is the turbidity after the treatment. The results are listed in Table 2

Statistical analysis

The results were analysed by Analysis of variance (10). The significance of the differences between groups was determined by their P- values calculated by students‘t’ test. Ten values are considered as significantly different from each other only when $P<0.05$.

RESULTS

Table 1 shows the effect of drug treatment on carrageenan induced rat paw edema. Edema suppressant effect of root, bark and leaves extract of *A. indica* was calculated which is lesser tan that of standard drug ketorolac tromethamine (10 mg/kg). Though the extract sowed dose response inhibition of inflammation, it was not significant among all test dose levels. As can be see from Table 2 the extract of root, bark and leaves of *A. indica* are ore active against

Gram negative organism than the Gram positive organism.

DISCUSSION

Carrageenan induced rat paw edema was taken as a prototype of exudative phase of inflammation. The development of edema has been described as biphasic (11). The initial phase is attributable to the release of histamine, serotonin and kinin in the first hour after injection of carrageenan. A more pronounced second phase is related to the release of prostaglandin like substances in 1-2 hours. As far as antimicrobial activity is concern, the selective bactericidal activity of compounds may be attributed among others to the permeability of the dugs trough the thick cell wall of gram negative organisms. The walls of gram positive organisms are thin when compared to that of gram negative organisms.

The anti-inflammatory effect of *A. indica* seems to be related to its histamine, kinin and prostaglandin inhibitory activity (12). Among these root possessed profound activity. The very interesting feature observed in antimicrobial activity was, streptomycin is used as a standard which has gram positive activity, but the extracts have more potent bactericidal activity than streptomycin.

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Table 1
Effect of Extracts on carrageenan induced rat paw Edema

Group	Dose mg/kg BW	0hr		+1hr		+2hr	
		Edema Volume (ml)	% of AIA	Edema Volume (ml)	% of AIA	Edema Volume (ml)	% of AIA
Control	- 50	0.55 0.53 (± 0.004)	- 3.6	0.55 0.53 (± 0.002)	- 3.6	0.56 0.53 (± 0.005)	- 5.3
Bark	100 150 50	0.51 (± 0.002) 0.47 (± 0.002) 0.53 (± 0.001)	7.2 15.0 3.6	0.50 (± 0.002) 0.48 (± 0.004) 0.47 (± 0.004)	9.0 13.0 13.0	0.50 (± 0.002) 0.46 (± 0.002) 0.45 (± 0.002)	10.7 18.0 20.0
Root	100 150	0.52 (± 0.003) 0.50	5.4 9.0	0.47 (± 0.005) 0.42	13.0 23.6	0.43 (± 0.004) 0.42	23.2 25.0

		(± 0.003)		(± 0.003)		(± 0.004)	
	50	0.53 (± 0.003)	3.6	0.49 (± 0.004)	11.0	0.48 (± 0.002)	14.2
Leaves	100	0.52 (± 0.002)	5.4	0.45 (± 0.002)	18.1	0.46 (± 0.001)	18.0
	150	0.51 (± 0.004)	7.2	0.44 (± 0.004)	20.0	0.42 (± 0.003)	25.0
Ketorolac trometha-mine	10	0.49 (± 0.004)	11.0	0.38 (± 0.002)	31.0	0.38 (± 0.001)	32.1

Values in parentheses represent the mean ± SEM of 6 animals

Table 2
Effect of Extracts on the growth of Bacteria

Drug	**Concentration (µg/ml)**	**% Inhibition**	
		E. coli	S. aureus
Bark	50	20	22
	100	28	26
	150	35	37
Root	50	22	20
	100	27	22
	150	37	24
Leaves	50	23	30
	100	30	19
	150	37	19
Streptomycin	50	13	17
	100	22	22
	150	23	40

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