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ASSESSMENT OF THE ANTI-ARTHRITIC EFFECTS OF BRASSICA NIGRA SEED EXTRACTS IN EXPERIMENTAL MODELS IN ALBINO RATS

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ABSTRACT

The aim of this study was to assess the anti-arthritic activity of *Brassica Nigra*. Arthritis is induced in the albino rats by inducing the freunds complete adjuvant. The seeds of freunds adjuvant were coarsely powdered and extracted with ethanol (95%) and water using soxhlet. The effect of these plant extracts on arthritic rats were assessed by the various blood parameters and also taking the changes in paw volume. The BN suppressed the anti –arthritic changes induced in rats and results were statically significant.

Keywords: *Brassica Nigra*, Anti-arthritic activity, Seed extract.

INTRODUCTION

Brassica nigra Linn. (Cruciferae) commonly called as black mustard has traditionally used as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulant and vesicant [1]. All the three extracts contain alkaloids, saponins and flavonoids etc. In our laboratory it was observed that crude extract of BN exert anti-arthritic activity in rats. Pharmacological studies reported on the BN are anti-microbial [2], insulinotropic and anti-hyperglycemic [3]. *Brassica nigra* has been traditionally used in India, Sri Lanka, Bangladesh and Pakistan for the treatment of inflammation and rheumatism. However, systematic study of this plant has not been carried out for the anti-arthritic activity.

MATERIALS AND METHODS

The seeds of fresh unadulterated *B.nigra* were collected from the fields of Vikarabad and authenticated by renowed botanist Professor, Vedavyas, L.V.D College, Raichur, Karnataka. The air dried and powdered seeds of *B.nigra* were successively extracted with alcohol in a soxhlet apparatus and macerated with water. The extract obtained was made free of any solvent by rotary evaporator at 40^0 C under reduced pressure, and dried in a vacuum oven.

Test animals

Albino rats of either sex weighing between (150-200 g) were procured from central animal house of N.E.T. Pharmacy College, Raichur for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed *ad libitum* under hygienic conditions. The animals were grouped in cages in an air conditioned room at the temperature of 22 + 1°C with12 h light and dark cycle. The ethical guidelines for the investigation of the animals used in experiment were followed in all the tests.

Acute oral toxicity studies

The acute toxicity of seeds extracts of *Brassica nigra* were determined by using female albino rats of weight between (180-200) g, maintained under standard conditions. The animals were fasted for 12 hr prior to the experiments. Animals were administered with single dose

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of seeds extracts of *Brassica nigra* (Linn.) and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. From the LD_{50} dose $1/5^{th}$ dose was selected and considered as high dose.

Induction of arthritis

Rats were injected with Freunds complete adjuvant, containing 10 mg of heat killed Mycobacterium tuberculosis in 1ml paraffin oil (0.1ml) into the left paw intra dermally. The paw volume was measured using plethysmograph apparatus [4].

Experimental design:

Albino rats of either sex weighing between (150-200g) were divided into following 5 groups of 6 animals each. Group I: Negative control (receives vehicle).Group II: Positive control Group III: Standard Indomethacin (10 mg/kg). Group IV: High dose of alcoholic extract of *Brassica nigra* seeds. Group V: High dose of aqueous extract of *Brassica nigra* seeds. Groups were treated orally with the extracts of *Brassica nigra*, distilled water and standard respectively until 14 days from the day of injection of Freunds Complete Adjuvant. During the 14 days of the treatment, the paw volume, body weight, ankle diameter, rectal temperatures were measured on alternative days. At the end of the 14^{th} day animals were sacrificed by cervical dislocation. The serum was separated and for the further biochemical studies.

Preliminary phytochemical screening:

The extracts were subjected to chemical tests to detect the chemical constituents like saponins, alkaloids, glycosides, proteins flavonoids etc., using standard procedures.

Biochemical assays

Hemoglobin is estimated by using Sahil`s method. Red blood cells and white blood cells were estimated by the according to the method of *chesbrough and Mc Arthur* in an improved Naeubauer chamber. For biochemical determination, blood was collected from the retro-orbital route without any anti-coagulant. After one hour serum was separated by centrifugation and maintained at -4^{o}c until further use. The serum was subjected for determination of Blood Urea Nitrogen (BUN), Total protein, Albumin, and Calcium by using Semi auto-analyzer.

Statistical analysis

It was performed using Student's unpaired ' t ' test and P values less than 0.05were considered significant. Data are represented as mean±SEM

RESULTS

Paw volume:

In arthritic rats, inflammation reached maximum on day 3 and maintained till day 9. Paw maintained its inflammation till day 14. A significant reduction was observed by the standard and extracts treated groups as compared to group II.

Body weight

The changes in the body weights were compared on day1 and the day 14. The mean percentage increase in different groups were calculated and compared with group 2. The changes in body weights for group 1 (14.88%), group 2 (6.91%), group 3 (12.57%), group 4 (16.91%) and group 5 (17.39%).

Rectal temperature

There was no significant variation of rectal temperature from day 1 to day 14 in all the groups they maintained the normal rectal temperature.

Vernier scale

In arthritic rats, ankle diameter reached maximum on day 7. Ankle maintained its inflammation till day 14. A non-significant reduction was observed by the standard and extracts treated groups as compared to group II.

Table 1.

Parameter	Group I	Group II	Group III	Group IV	Group V
RBCx10^6/ mm^3	5.56±0.20	4.30±0.27	5.80±0.28**	5.79±0.29**	6.217±0.26**
WBC x10^3/mm^3	7679±362	12980±616	7726±296***	8431±519***	7516±476***
Hb (cc/100ml)	13.62±0.41	10.87±0.41	13.54±0.48*	11.63±0.88	11.68±0.79
BUN (mg/dl)	23.22±0.10	29.69±0.99	24.03±0.58***	26.85±0.75*	25.18±1.11**
Total protein (g/dl)	5.26±0.41	4.05±0.37	6.69±0.41***	6.42±0.31***	5.88±0.43**
Albumin (g/dl)	3.48±0.40	3.79±0.39	3.15±0.16*	3.31±0.31	3.54±0.15
calcium (mg/dl)	81.03±2.23	145.19±3.19	84.73±1.14***	101.92±2.80***	93.96±0.53***

DISCUSSION

Adjuvant arthritis in rats is considered to be an immunologically mediated and most frequently investigated model of chronic inflammation. This model depicts the very close similarity with the clinical RA. Due to inoculation of CFA, there was an increase in the ankle diameter which signs as an inflammation for the ankle joint. On the 13^{th} day, there was decrease in the ankle joint in both the extracts as well as the standard. Among them, alcoholic extract has given better result than that of the

standard. Adjuvant rats treated with BN and Indomethacin either in prophylactic or therapeutic protocol showed decrease in ankle diameter as compared with that of the toxicant group. Rectal temperature is an index of inflammation which is measured by rectal thermometer on alternate days till 14th day. All the groups excluding Indomethacin group have shown no significant variation in rectal temperature on all the alternate days. Whereas in Indomethacin group there was a little decrease in the rectal temp throughout the experiment because of its cox inhibitory action [5]. Paw volume is an index of paw inflammation which is measured by plethysmograph on alternate days till 14th day. Both the aqueous and the standard decreased the paw volume on day 13. Alcoholic extract produced little effect on paw volume. The ability of BN to reduce a response to AA is consistence with the findings of present study on this plant, as traditionally it was used as an immunosuppressant for arthritis at least in the early phase of disease. The extract treated group and the standard increases their body weight on day 13 as that of the normal animals. Where as in the toxicant group, there is slight increase in body weight. Both the extracts have shown an increase in body weight more than that of the standard and the normal animals signifies its protection from the body weight loss in arthritic condition.

In the present study, the arthritic rats (group II) showed a reduced RBC count and Hb level. All these conditions indicating the anaemic condition and is commonly noted in patients with chronic arthritis. The two most common reasons for anaemia in arthritic patients are gastrointestinal blood loss from arthritic medication and bone-marrow changes in patients with inflammatory arthritis which prevents the release of iron for incorporation into RBCs [6]. Both the extract treated groups and the standard showed a significant recovery from anaemia. The increase in WBC count in arthritic rats is the indication for inflammation which was significantly suppressed in BN extracts treated rats and Indomethacin treated rats. BUN parameter is selected to know about the kidney function. There was increase in the BUN levels in the toxicant group. There was significant decrease in BUN levels in all the extracts and Indomethacin treated group of rats. Among both the extract, the aqueous gave better results than alcohol extract and comparable with the standard Indomethacin. The absence of liver damage induced by AA treated rats was indicated by significant difference in serum total protein and no increase in serum albumin. But there is an increased level of total protein and there is no significant variation in albumin levels indicating no liver damage has occurred in extracts treated groups and Indomethacin. Calcium reabsorbed from the bone, enters the blood stream is enormously excreted through the urine resulting in hypercalemia and hypercalcuria conditions which was in line with previous reports. There was significant decrease in Ca levels in all the extracts and Indomethacin treated group of rats

The phytochemical analysis of the extracts revealed that it contains flavonoids, carbohydrates, glycosides, proteins and alkaloids. Of these flavonoids and alkaloids are well known for there ability to inhibit pain and inflammation. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of chemical mediators of inflammation. Flavonoids are widely utilized in medicine as anti-osteoporotic agents are shown to increase the density of the diaphysis in rats induced with osteoporosis. Experimental studies showed that flavonoids act by inhibiting osteoclastic bone reabsorption with in vitro and in vivo. Flavonoids have been known to be responsible osteoporosis where they increase the bone mineral density

CONCLUSION

This study clearly demonstrates that the anti-arthritic activity of the *B.nigra* seed extracts and the results were comparable to the indomethacin. Further studies are needed for the isolation for the other compounds responsible for the isolation for the anti-arthritic potential.

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