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Evaluation of Anti-inflammatory activity of *Nyctanthes arbor-tristis* and *Onosma echioides*.

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ABSTRACT - Anti-inflammatory activity of the ethanolic extract of the orange tubular calyx of *N.arbor-tristis* and pet.ether extract of root bark of *O.echioides* was studied in albino rats of Wistar strain using the carrageenan induced paw edema model. The ethanolic extract of *N.arbor-tristis* (200 mg/kg, i.p.), isolated carotenoid from the extract *N.arbor-tristis* (200mg/Kg, i.p.) and pet.ether extract of *O.echioides* (5mg/Kg, i.p.) inhibited carrageenan induced rat paw edema. The results indicated that all the extract produced significant (p < 0.05) anti-inflammatory activity when compared with the standard drug (diclofenac sodium) and untreated control.

KEYWORDS - Anti-inflammatory, *Nycthanthes arbor-tristis*, carotenoid, *Onosma echioides*

INTRODUCTION

Scientific interest in medicinal plants has burgeoned in recent times due to increased efficiency of new plant-derived drugs and rising concerns about the side-effects of conventional medicine. Inflammation is seen in conditions such as Alzheimer's disease, cancer, irritable bowel syndrome and hepatic diseases. It is believed that controlling inflammation may help to alleviate these conditions or even prevent them. Thus the present investigation was carried out to evaluate the anti-inflammatory potential of *Nyctanthes arbor-tristis* and *Onosma echioides*.

Nyctanthes arbortristis Linn. z(*Oleaceae*) commonly known as Parijatak (Marathi) and Night Jasmine (English) is cultivated in gardens almost throughout India and in many other tropical countries (1). The leaves of *Nyctanthes arbor-tristis* Linn. are used extensively in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism and internal worm infections and as a laxative, diaphoretic and diuretic (2,3).

Onosma echioides (*Boraginaceae*), known as Ratanjot, Laljari is a perennial herb growing to 0.3m. The leaves are made into a powder and given to children as a purgative. The flowers are used as a cordial and stimulant in the treatment of rheumatism and palpitations of the heart. The root is bruised and used as an external application to skin eruptions. A red dye obtained from the root, is used as an alkanna substitute (4).

Lack of scientific data with respect to the pharmacological properties of the flowers of *Nyctanthes arbor-tristis* and root bark of *Onosma echioides* encouraged for the evaluation of *N.arbor-tristis* and *O.echioides* for anti-inflammatory activity. The effect of the extract was also compared with the standard drug viz. diclofenac sodium.

MATERIALS AND METHODS

The fresh flowers of *N. arbor-tristis* were collected early in the morning from wildly grown trees from Dombivli, District Thane, Maharashtra, India and were authenticated at St.Xavier's College, Mumbai. The dried *O. echioides* roots were procured from the local market (Princess Street) of Mumbai and were authenticated by Dr. A.M. Mujumdar, Head, Plant Sciences Division, Agharkar Research Institute, Pune. The orange coloured tubular calyx was separated from corolla and was dried in vacuum oven at temperature not exceeding 50°C. The dried root bark (outer covering of root) was separated from dried roots of *O. echioides*, dried at 100°C for 2 hours and coarsely powdered.

Preparation of extract

The dried tubular calyx of *N.arbor-tristis* was macerated with ethanol for 6-8 hours at room temperature, filtered and filtrate was concentrated under reduced pressure of 15mmHg at 40°C. Preliminary phytochemical screening of the extracts revealed presence of sugars and carotenoids. The extract was chromatographed on Silica gel (60-120 mesh) column eluted with mobile phase of ethyl acetate: isopropanol: water (65:25:10) to isolate a major carotenoid molecule corresponding a R_f of 0.4. The isolated carotenoid molecule was also evaluated for anti-inflammatory activity. The dried powdered material of *O. echioides* was extracted using Soxhlet

apparatus with petroleum ether (60 - 80°C). The extract was concentrated under vacuum to obtain semi-solid mass and qualitative phytochemical evaluation indicated presence of naphthoquinones. All the extracts were suspended in 5%w/v gum acacia (LOBA Chemie) for the present study.

Animals

Albino rats of Wistar strain of the either sex (150 – 200g), maintained under standard environmental conditions ($27^0 \pm 2^0$C, relative humidity 60±5% light-dark cycle of 12h) and fed with standard pellet diet and water ad libitum, were used for the present study. All the experimental protocols were approved by Institutional Animal Ethics Committee.

Acute Toxicity study

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (5%w/v gum acacia) whereas the test group consisting of ethanolic extract of *N.arbor-tristis* and isolated carotenoid received increasing doses of 250, 500, 1000, 1500 (mg/Kg b.w.i.p.) whereas the test group consisting of pet.ether extract of *O.echioides* received increasing doses of 25, 50, 75(mg/Kg b.w.i.p.) and were observed for mortality till 48 hours and LD_{50} was calculated (5).

Anti-inflammatory activity

The rats were divided into 4 groups where six animals in each group were used for study. Acute inflammation was produced by the sub–plantar administration of 0.1ml of 1% w/v carrageenan (Sd fine – Chem Ltd.) in 5% w/v gum acacia in the right hind paw of rats (6). The Group I was treated with ethanol extract of *N.arbortristis* (200mg/Kg i.p.), Group II with isolated carotenoid (200mg/Kg i.p.), Group III with pet.ether extract of *O.echioides* (5mg/Kg i.p.), Group IV with diclofenac sodium (Sun Pharmaceuticals) (10mg/Kg i.p.) and Group V as Control (5%w/v gum acacia) was administered intraperitonially. The paw volume was measured before the injection and then at intervals of 30 min. for a period of 2 h after carrageenan injection using plethysmometer. The animals were pretreated with the extract 30min. before the administration of carrageenan.

% inhibition of inflammation was calculated using the formula,

where % inhibition = $100[V_c - V_t/V_c]$

'V_c' represents oedema volume in control and 'V_t' oedema volume in treated with test extracts.

Statistical Analysis

Results are expressed as mean±s.d. The statistical analysis was performed by using unpaired Student's t-test for comparing test group with control group. P values less than 0.05 were considered statistically significant.

RESULTS

The orange tubular calyx of *N.arbor-tristis* macerated with ethanol. The average percentage yield of ethanolic extract of *N.arbor-tristis* was found to be 8.7 w/w. On preliminary phytochemical screening of *N.arbor-tristis* revealed the presence of glycosides, carotenoids. The LD_{50} was found to be 1500 mg/Kg i.p. for ethanol extract of *N.arbor-tristis*.

The root bark of *O.echioides* oven-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of pet.ether extract of *O.echioides* was found to be 5.7 w/w. On preliminary phytochemical screening of the root bark of *O.echioides* revealed the presence of napthoquinones. The LD_{50} was found to be 50 mg/Kg i.p.for pet.ether extract of *O.echioides*.

The ethanolic extract of *N.arbor-tristis*, isolated carotenoid of *N.arbor-tristis* and the petroleum ether (60–80°C) extract of *O.echioides* exhibited significant ($p<0.05$) anti-inflammatory activity at doses of 200, 200 and 5 (mg/Kg b.w. i.p.) respectively.

As shown in fig.1 ethanolic extract of *N.arbor-tristis*, isolated carotenoid and petroleum ether extract of *O.echioides* exhibited inhibition in rat paw oedema, 67.4, 70.0 and 85.76 % respectively whereas standard drug showed 96.7% of inhibition of inflammation.

DISCUSSION

Indigenous drug systems can be source of variety of new drugs which can provide relief in inflammation. The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent(5). This edema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins (7). The development of edema in the paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase, observed around 1 h, is attributed to the release of histamine and serotonin; the second, accelerating phase of swelling is due to the release of prostaglandin-like substances.

It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents (8). Significant anti-inflammatory activity was observed for ethanolic extract of *N.arbor-tristis*, isolated carotenoid of

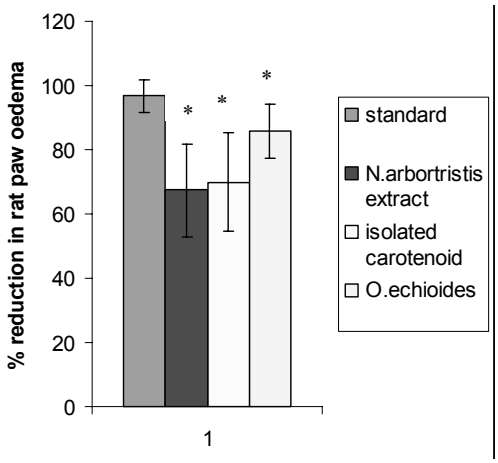

Fig. 1 Anti-inflammatory activity of ethanolic extract N.arbor-tristis, isolated carotenoid, pet.ether extract O.echioides as compared against standard drug.

N.arbor-tristis and petroleum ether extract of *O.echioides* in carrageenan induced oedema model. The red dye obtained from *O.echioides* is used as a substitute for *Alkanna tinctoria*. *Alkanna tinctoria* contains the napthoquinones, alkannin and shikonin which exhibit anti-inflammatory activity (9). Hence the anti-inflammatory activity in *O.echioides* can be attributed to the presence of napthoquinones as the pet. ether extract of *O.echioides* showed positive test for napthoquinones.

The study indicates the potential of these herbal drugs as anti-inflammatory drugs. Such drugs can be explored in various inflammatory diseases. The activity may be attributed to the inhibition of the COX-2 enzyme or inhibition of the activation of transcription factors.

It can be concluded that all the extracts have potential to be explored as anti-inflammatory agents. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory activities of *N.arbor-tristis* and *O.echioides*.

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