INHIBITION OF ADJUVANT INDUCED POLY ARTHRITIS IN RATS BY VARIOUS BARK EXTRACT/FRACTIONS OF SYMPLOCUS RACEMOSA.

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Abstract

The different bark extracts/fractions of Symplocus racemosa were evaluated for inhibition of adjuvant induced poly arthritis in rats. The fractions of the selected plant part i.e. SRAF, SRAF1, SRAF2 were administered at different concentration to the rats. Inhibition of poly arthritis of tested fractions were compared with standard drug Indomethacin. The result which is obtained, support the inhibition of poly arthritis by Symplocus racemosa fractions.

Introduction:

All inflammatory diseases have almost a common pathway of generation of disease which involves generation of various inflammatory mediators at various stages due to initial stimulation by one or various etiological factors which may be an infection, an injury or even an allergic stimulus. The etiological agent causes increased vascular permeability after initial vasodilation and increased blood flow in the area due to release of various substances including Histamine from the mast cells in the areas.

The increase in vascular permeability may be due to formation of endothelial gaps under the influence of Histamine, Leucotrienes, Bradykinins (1) or it may also be because of transcytosis which is due to intacellular formation of vesiculovacular organelles across the endothelial cells under the influence of VEGF and other factors. These vesiculovacular organelles act as channels across the endothelial cells increasing vascular permeability. It may also be because of endothelial retraction or cytoskeletal reorganization which creates gaps in between the endothelial cells under the influence of TNF, IL-1, IFN-γ (2). It may also be because of leucocytes mediated lysosomal and proteolytic injury (3).

Arthritis is a condition of inflammation with pain in one or more joints. Depending on the inflammation and pain pattern and type of joints involved, it is classified into various types like osteoarthritis, rheumatoid arthritis, septic arthritis etc (4). Traditionally lot of plants species mentioned for their different activity. One of them is Symplocus racemosa which is a green tree or shrub about 25 ft height. In North Eastern region of India it grows upto the height 2500 ft. Geographically this plant found in Assam, Bengal, Bihar, Karnataka, Manipur, Meghalaya, Chhota Nagpur, Peru and Berma. In India Kerala is rich in Symplocus racemosa. The stems are up to 6-8.5m high and 15cm in diameter, bark is dark grey in color, thick and also spongy rough, blaze 7.5-13mm, fracture short and fibrous, pale yellow finely mottled, branchlets hairless. Leaves are simple, alternate, ovate, elliptic-oblong 8-15x3.6cm base wedge-shaped to rounded, apex acute to acuminate, margin toothed with rounded to saw like teeth, obscurely recurved, papery hairless, distinctly stalked, lateral nerves 8-11 pairs. Flowers are

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bisexual shortly stalked in 6-17cm long axillary racemes, about 1 cm across, white with pink tinge and fragrant. Pedicels are as long as calyx tubes and stamens are about 100 in number. Fruits are 2 cm in length globose or cylindrical in shape, drupe 1.3 cm long and purplish black in color turning yellow when dry, seeds are 1-3 in number (5, 6). Matured stem barks are found in channel, fracture short and granular, fibrous in inner region, taste, astringent and bitter (7). Transverse section of mature bark shows a wide cork of thin-walled, rectangular cells arranged in radial rows, cork cambium 1-3 layered, secondary cortex consists of thin-walled, oval and tangentially elongated parenchymatous cells towards outer side and rounded cells towards inner side, a number of stone cells, in single or in groups (8). The bark contains mainly alkaloids, loturine 0.06%, coloturine 0.02%, loturidine, clinovinand other glycosides like symplocomoside, symplososide, symploracemoside, benzoyle salireposide, flavonoids and anthocynins, reducing sugars, lignans, phenols, irridoids, steroids, oxalic acid and phytosterol (9).

Ash contains carbonate of soda and large quantity of coloring agents (10). It also inhibit the enteric and dysenteric group of microbes and to reduce the frequency and intensity of the contractions in vitro of both pregnant non pregnant uteri (11).

**Materials and Method:-**

**Plant collection and extraction:**
The barks of *Symplocos racemosa* were collected from herbal garden of BBDNITM, Lucknow, India and were identified in N.B.R.I., Lucknow, India. The dried bark powder was extracted with various solvents and solvents were concentrated separately and dried. The dried extracts were preserved until further use.

**Animals:**
Sprague Dawley rats (120-150 g) were used in the experiments. All animals were fed with a standard diet ad libitum and have free access to drinking water. Animal study was performed in institutional animal house according to CPCSEA norms.

**Inhibition of adjuvant induced poly arthritis in Rats:**
The separated fractions from the plant were screened for their capacity to inhibit adjuvant induced poly arthritis in rats at different doses given intraperitonealy (Administered daily from days 15-22).

**Statistical Analysis:**
The data is presented as mean ± SEM. The data was analyzed by one way ANOVA. Different value of P at different days interval were significant.

**Result:**
Inhibition of adjuvant induced poly arthritis in rats (Administered daily from days 15-22) by various fractions from *Symplocos racemosa*.

| Group | Dose mg/kg | Mean arthritic score ± S.E.M. for days |
|-------|------------|---------------------------------------|
|       |            | 15         | 16          | 17          | 18          | 19          | 20          | 21          | 22          |
| I     | 1 ml i.p.  | 13.2± 1.3  | 13.7± 1.3   | 14.0±1.5    | 14.6±1.3    | 14.8±1.4    | 15.0±1.4    | 15.0±1.4    | 15.0±1.4    |
| II    | 20 mg i.p. | 13.0±1.4   | 10.4±1.4    | 10.0±1.1    | 9.2±1.0*    | 9.0±1.0@    | 8.6±0.9@    | 8.0±0.8#    | 7.4±0.8#    |
| III   | 100 mg i.p.| 13.3 ± 1.3 | 9.2±1.1*    | 9.1±1.0*    | 9.0±1.1@    | 8.4±0.9@    | 8±0.8#      | 7.4±0.8 #   | 7.1±0.9#    |
| IV    | 20 mg i.p. | 13.0±1.4   | 10.4±1.4    | 10.0±1.1    | 9.6±1.0*    | 9.6±1.0*    | 9.4±0.9*    | 9.4±0.8*    | 9.4±0.8*    |
| V     | 100 mg i.p.| 13.3 ± 1.3 | 10±1.1      | 9.6±1.0     | 9.6±1.1*    | 9.6±0.9*    | 9.4±0.8*    | 9.4±0.8*    | 9.4±0.9*    |
| VI    | 20 mg i.p. | 13.0±1.4   | 10±1.4      | 10.0±1.1    | 9.6±1.0     | 9.2±1.0*    | 8.8±0.9*    | 8.4±0.8@    | 7.4±0.8#    |
| VII   | 100 mg i.p.| 13.0±1.4   | 10.4±1.4    | 10.0±1.1    | 9.6±1.0     | 9.2±1.0*    | 8.8±0.9*    | 8.4±0.8@    | 7.4±0.8#    |
| VIII  | 2.5 mg i.p.| 13.3± 1.3  | 9.2±1.1*    | 9.1±1.0*    | 9.0±1.1*    | 8.4±0.9     | 7.8±0.8@    | 7.2±0.8@    | 7.0±0.9#    |

Group I- Arthritic rats treated with saline; Group II - Arthritic rats treated with SRAF 20 mg/kg; Group III - Arthritic rats treated with SRAF 100 mg/kg; Group IV - Arthritic rats treated with SRSF-1 20 mg/kg; Group V - Arthritic rats treated with SRSF-1 100 mg/kg; Group VI - Arthritic rats treated with SRSF-2 20 mg/kg; Group VII - Arthritic rats treated with SRSF-2 100 mg/kg; Group VIII Arthritic rats treated with Indomethacin; *p < 0.05, @p < 0.01, #P <0.001as compared to arthritic control.
**Discussion:**

SRAF showed statistically significant inhibition of arthritic lesions (p<0.05) from day 16, (p<0.01) from day 20 and (p<0.001) from day 21 onwards. The fraction administered in higher doses reduced the lesions to a greater extent showing a dose dependent decrease in lesions. While control animals on average lost 2 gm/rat, during the experimental period, alkaloid treated groups gained in between 4-8 gm per rat, the gain depending on doses.

SRAF showed inhibition of adjuvant edema earlier on from 16th day onwards both for higher nontoxic dose and from 18th day at lower dose of 20 mg/kg and produced more significant inhibition as compared to the lower dose 20 mg/kg (p<0.05 from 19th day, p<0.01 from 22nd day) and higher nontoxic dose (p<0.05 from 18th day, p<0.01 from 21st day) for the SRSF-1 fraction. SRSF-2 fraction was similar in response to SRAF and showed statistically significant inhibition of arthritic lesions (p<0.05) from day 16, (p<0.01) from day 20 and (p<0.001) from day 21 onwards.

**References:**

1. Majno G. and Palade G.E. (1991) “Studies on inflammation I. The effect of Histamine and Serotonin on vascular permeability; an electron microscopical study” J. Biophys. Biochem. Cytol., 11,607.
2. Feng D., (1996) “Vesiculo-vacuolar organelles and the regulation of venule permeability to macromolecules by vascular permeability factors Histamine and Serotonin” J. Exp. Med., 183,1981.
3. Cortan R.S. and Brisco D.M. (1997) “Endothelial cells in Inflammation.” In Kelly W. (eds): Textbook of Rheumatology, 5th edition, W.B. Saunders, Philadelphia, 183-198
4. Hamerman D. (1989) “The biology of Osteoarthritis” N Eng. J.Med.320, 1322-1323
5. The Wealth of India. Raw materials & Industrial Products, Vol.4 (J-Q), Publication and Information Directorate: New Delhi, Council of Scientific & Industrial Research; 1998:95.
6. Kirtikar and Basu, Indian Medicinal Plants, vol – 3, International Book Distributors, Dehradun; 1999:1630.
7. Anonymous, The Ayurvedic Pharmacopoeia of India, vol-1, Government of India Ministry of Family Welfare;2001-c:142-43.
8. Anonymous, The Ayurvedic Pharmacopoeia of India, vol-1, Government of India Ministry of Family Welfare;1998-b:306
9. Abbasi M.A., Planta Medica, vol-70;2004:1189-94
10. Parrat J.R.,West G.B., 5-Hydroxytryptamine and the anaphylacoid reaction in rat,vol-8;Journal of Physol; 2008:27-41
11. Selyse H., Further studies in the participation of the adrenal cortex in the pathogenesis of arthritis. Brid.J of Med; 2001:1129-1135