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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF ROOTS OF *RUMEX OBTUSIFOLIUS*

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ABSTRACT: *Rumex obtusifolius* belong to the family Polygonaceae, it is a common wayside weed and it has a good medicinal value. In the present study, the methanolic extract (sample named as R2) of roots of *R. obtusifolius* was used to examine the *in-vitro* and *in-vivo* anti-inflammatory activity using human red blood cell membrane stabilization method to measure the potential of the extract on human red blood cell membrane. Diclofenac sodium was used as a standard drug. The percentage of membrane stabilization for methanolic extract of roots of *R. obtusifolius* was done at concentration 200µg/ml. It was concluded that methanolic extract of *R. obtusifolius* showed a good anti-inflammatory effect against the standard drug. This study states that examination of the anti-inflammatory effect of methanolic extract of *R. obtusifolius* for the improvement of medicinal uses.

INTRODUCTION: The *Rumex* species, belonging to the Polygonaceae family, comprise about 200 species widely distributed around the world. The name *Rumex* originated from the Latin word for a dart, alluding to the shape of the leaves¹. Traditional names for several species used as food reflect their gustatory characteristics, taste, and aroma, e.g. sour weed in the case of *Rumex*. The roots of many species belonging to the *Rumex* genus have been used in medicine from ancient times because of their gentle laxative effect. *R. acetosa* is officially listed in the Korean Food Code (Korea Food & Drug Administration) as one of the main food materials and has been used in folk medicine as a mild purgative and also for the treatment of cutaneous diseases².

Some of the species are cultivated, e.g. *R. acetosa* and *R. vesicarius*³. On the other hand, the members of this genus include many invasive weeds (e.g. *R. obtusifolius* and *R. crispus*)⁴. Plants belonging to the genus *Rumex* are annuals, biennials or perennials, mainly herbs, rarely shrubs. usually, they have long, stout roots, sometimes the roots are rhizomatous. Leaves are alternate, sometimes hastate or sagittate and subgenera *Acetosella* and *Acetosa* are acid tastings.

Flowers are hermaphrodite or unisexual, arranged in whorls on simple or branched inflorescences. *Rumex obtusifolius* L. (Polygonaceae), commonly called 'broad leaf dock', is one of the most common wayside weeds, and it also occurs in silage fields, on river banks, in ditches, and on waste grounds. This perennial plant has long been used in folklore medicine. The ethnobotanical uses of this species include its use as an antidote to nettle, depurative, astringent, laxative, and tonic, and in the treatment of sores, blisters, burns, cancer, and tumors⁵.

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The name *Rumex* L. originated from rums (to suck) alluding to the habit of Romans sucking the leaves to allay thirst⁶. *R. obtusifolius* is a chilling-resistant plant, and it is important to elucidate the apparent correlation between the glucosylceramide composition and chilling sensitivity⁷. *R. obtusifolius* among the *Rumex* species is the most tolerant to low pH and aluminum ion stress in the rhizosphere. The leaves of *Rumex* species use to make sarma, a traditional Middle-Eastern and South-Eastern food (it roll around a filling made of rice, bulgar or minced meat and gently cooked)⁸.

In Albania, one of the most commonly quoted and used wild food plants are *Rumex spp.* Which are used as vegetables mainly cooked with dairy products and rice or, more often, as filling for homemade savory pies⁹. The decoction of the seeds of *R. obtusifolius* is used against coughs of all kinds, colds and bronchitis¹⁰. In Ireland, *R. obtusifolius* is used as astringent, laxative, tonic, an antidote to nettle, and for the treatment of sores, blisters, burns and cancer¹¹. In Australia *Rumex spp.* are used for the treatment of stings¹².

MATERIALS AND METHOD:

Sample Collection: *Rumex obtusifolius* roots were collected from the Joshimath block, District Chamoli, Uttarakhand during the month of September-October. The collected plant samples were packed in polyethylene bags and stored until further process. The plant was identified by Department of Botany, H. N. B. Garhwal University, Srinagar Garhwal, Uttarakhand.

Preparation of Sample: The whole plant material was washed first with tap water and then rinsed with distilled water to remove dust and other foreign materials and kept under shade for drying. The air-dried plant material was pulverized into the powdered form using heavy duty blender. The powdered form of plant material was used for the preparation of extracts with different solvents.

Preparation of Extracts: The powder samples (150 gm) were extracted with methanol solvent (1000 ml) by using Soxhlet extractor for 72 h. After completion of the extraction process, the methanol solvent was evaporated by using Rotatory evaporator under reduced pressure to obtain a methanolic crude extract (7.3 gm). The obtained crude extract was stored for further biological

assays like antimicrobial, anti-inflammatory, anticancer, antioxidant activities and phytochemical screening.

Anti-Inflammatory Activity: Male wister albino rats weighing in the range of 180 - 200 g were used for the study of anti-inflammatory activity of plant extract. They were maintained in a well-ventilated room with a 12 h light/dark circles at 22 ± 1 °C. The experimental protocol was approved by the institutional animal ethical committee (IARC) of National centre of Fungal Taxonomy (NCFT), New Delhi. The internal ethical committee certificate number for the approval of the study was NCFT/EC/17/2314.

RESULTS AND DISCUSSION:

Membrane Stabilization: The HRBC membrane stabilization has been used as a method to study the *in-vitro* anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane.

Heat Induced Haemolysis: The extract was effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that R2 at concentration 200 and 120 µg/ml protect significantly ($p < 0.05$) the erythrocyte membrane against lysis induced by heat.

(**Table 1** - diclofenac sodium 120 µg/ml offered a significant ($p < 0.01$) protection against damaging effect of heat solution).

Inhibition of Albumin Denaturation: Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt,

an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied.

It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition was observed at 200 $\mu\text{g/ml}$. Diclofenac sodium, a standard antiinflammation drug showed the maximum inhibition at the concentration of 120 $\mu\text{g/ml}$ compared with control **Table 1**.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF SAMPLES (IN-VITRO)

Samples / Positive control	Percent protection of HRBC membrane	Percent inhibition of Albumen denaturation	Percent protection of heat induced hemolysis
R2 @200 $\mu\text{g/ml}$	54.23 \pm 0.04	51.23 \pm 0.023	56.45 \pm 0.025
Diclofenac sodium @ 120 $\mu\text{g/ml}$	96.56 \pm 0.042	95.43 \pm 0.034	88.45 \pm 0.031

Each value represents the mean \pm SD. N = 3, Experimental group were compared with control **p<0.01, considered extremely significant R

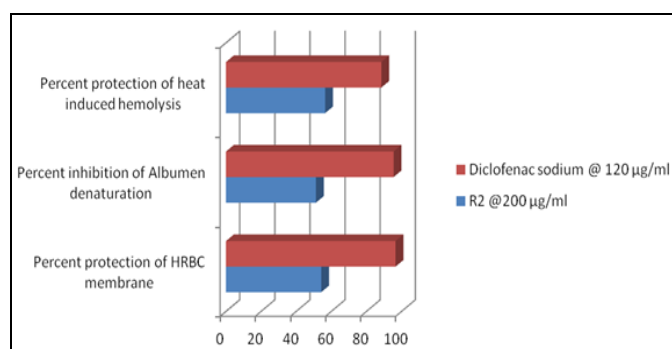


FIG. 1: ANTI-INFLAMMATORY ACTIVITY OF SAMPLES (IN-VITRO)

CONCLUSION: In the present study, results indicate that the methanol extracts of R2 possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation. Purification of each bioactive compound is necessary and this purified form of the compound can be used which may show increased activity.

This study gives on idea that the compound of the plant R2 can be used as lead compound for designing a potent anti-inflammatory drug which

can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation

Anti-Inflammatory Activity (in-vivo): The anti-inflammatory activities of the solvent extracts of the bark of the plant, R2 were determined by carrageenan induced albino rats model. The anti-inflammatory activities of the extract were found to have effect in dose-dependent manner. Administration of Carrageenan (0.1 ml of 1% N-saline) in the sub plantar region of the left hind paw of each rat caused a significant increase in the paw volume of control group animals after 2 h of administration.

Methanol extract at dose level of 200 $\mu\text{g/kg}$ showed reduction in paw oedema which was significant ($p<0.05$) as compared to control. Standard drug Diclofenac sodium (120 mg/Kg) showed % reduction in paw oedema. The values of reduction in paw volume at 4 h after carrageenan administration *viz.* 0.16 \pm 0.045 were found significantly of methanol extract of R2 Diclofenac sodium (120 $\mu\text{g/kg}$) showed 0.13 \pm 0.043 reduction in paw volume at 4 h after carrageenan administration. The results are shown in **Table 2**.

TABLE 2: ANTI-INFLAMMATORY ACTIVITY OF SAMPLES (IN-VIVO)

Experiment	Negative Control Group (10 ml/kg) (N-saline) Paw volume (ml)	Positive Control group (Diclofenac) (120 $\mu\text{g/kg}$ orally) Paw volume (ml)	R2 (200 $\mu\text{g/kg}$) Paw volume (ml)
1 h after treatment	0.25 \pm 0.025	0.17 \pm 0.045	0.21 \pm 0.043
2 h after treatment	0.25 \pm 0.034	0.13 \pm 0.043	0.24 \pm 0.035
4 h after treatment	0.25 \pm 0.05	0.09 \pm 0.042	0.16 \pm 0.045

\pm , S.D, Standard Deviation

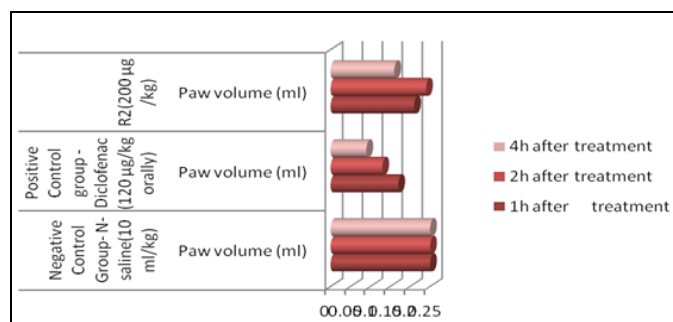


FIG. 2: ANTI-INFLAMMATORY ACTIVITY OF SAMPLES (IN-VIVO)

CONCLUSION: The values of reduction in paw volume at 4 h after carrageenan administration *viz.* 0.16 ± 0.45 were found significantly of methanolic extract of R2 Diclofenac sodium ($120 \mu\text{g/kg}$) showed 0.13 ± 0.043 reduction in paw volume at 4h after carrageenan administration which was significant ($p < 0.05$) as compared to control.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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