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ANTI-ARTHRITIC ACTIVITY OF *PORTULACA OLARACEA L. SATIVA* ON ANIMAL MODELS

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ABSTRACT

The present study was designed to investigate anti-arthritic activity of P*ortulaca olaracea l. sativa leaves in rats.* Arthritis was induced in rats in all groups of animals by injecting 0.05 ml of 0.5(w/v) suspension of killed Mycobacterium tuberculosis in paraffin oil into the left hind limb. Preliminary extraction was subjected to acute oral toxicity study according to the OECD guidelines no 423. Based on that, two dose levels i.e. 200 and 300mg/kg were selected for further study. Oral administration of the extract showed the significant results in Freund's adjuvant induced arthritis in rats. The biochemical study of EEPO (200mg/kg & 300mg/kg) showed the increase WBC count was significantly suppressed and standard diclofenac sodium (P<0.01).The increased lymphocyte count in adjuvant control group was significantly restored back to normal by test and standard drug (P<0.01).There is a decreased protein content in standard and extract drug treatment group as compared to adjuvant control group.

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Introduction

The Indian health care scene has inherited a large number of traditional practices, systems, and medicines as part of its total health care scenario, some of them more than 3000 years old. The earliest mention of the medicinal use of plants is to be found in the Rig-Veda which dates back as early as 3500 BC. It is in the Ayurveda, which is considered as a upaveda (or the supplementary Hymns designed for more detailed instructure of the mankind), is the very strong foundation stone of the ancient medical science of India. From the vast array of the Materia medica of indigenous it is thought that investigation and research on medicinal plants might bring to the scientific world many useful remedies for the alleviation of human sufferings. Inspite of the remarkable achievements of modern medicine and medical research, these ancient systems continue to be a major component, "effectively" used in the control or alleviation of diseases.

Arthritis is a disease that is second only to heart disease when it comes to the cause of disability. In 2005 nearly 46 million adults in the U.S. alone were diagnosed with Arthritis. It can affect not only joints but also other parts of the body. Making these body parts become painful, swollen and causes difficulty in movement. Arthritis is more prevalent in women than in men.

Portulaca oleracea, is an annual succulent in the family Portulacaceae, which can reach 40cm in height .It is a native of India and the Middle East but is naturalized elsewhere and in some regions is considered an invasive weed. It contains Flavonoids, Alkaloids, Saponins, large amounts of l-norepinephrine, and numerous common nutrients, including: Vitamins (A, B_1, B_2, C, niacinamide, nicotinic acid, tocopherol, carotene, etc...), Minerals (especially potassium), Fatty acids, especially Omega -3acids whose concentration in purslane is the highest found in leafy vegetables, glutathione, glutamic acid, and aspartic acid[1] .Other constituents include a mucilage, calcium oxalate, malic acid and citric acid, dopamine, and dopa, coumarins.Traditional uses of *Portulaca oleracea* aphrodisiac, emollient, diuretic, a refreshing agent, antiscobutic, vermifuge

MATERIALS AND METHODS

DRUGS AND CHEMICALS

Freud's adjuvant was obtained from as a gift sample from Post Graduate Institute of Basic Medical Sciences, Taramani, Chennai-113.Mannitol, Potassium chloride, Copper chloride, Bovine Serum Albumin (BSA),Acetic acid, Carbon tetra chloride, Vitamin-E, Urea, All other chemicals and reagents used were of analytical grade.

PLANT MATERIALS

Plant collection and Authentication

The plant material of *Portulaca oleracea* leaf used for the investigation was collected from Chennai in the month of July. The plant was identified and authenticated from Central Research Institute (Siddha), Arumbakkam, Chennai-106.

Extraction

The freshly collected leaf of this plant was shade dried and coarsely powdered. The powder was passed through 40-mesh sieve and was subjected to continuous hot percolation in Soxhlet apparatus with petroleum ether (60%v/v) and the marc left after Petroleum ether extraction were dried and extracted separately with ethanol (50%v/v). The extracts were evaporated under reduced pressure using rota evaporator untill all the solvent had been removed to give an extract sample with the yield of 5.7%w/w and 4.8%w/w for petroleum ether and ethanol respectively. The ethanol extracts were used for preliminary photochemical screening. The ethanolic extract was alone used for the pharmacological studies. The ethanolic extract of *Portulaca olaracea* (EEPO) was administered to the animals by dissolving each time with propylene glycol.

EXPERIMENTAL ANIMALS

Adult Wister rats and mice of both sex weighing 150-175gms were used in the pharmacological and toxicological studies. The

inbred animals were taken from the animal house in Vel's college of pharmacy, Pallavaram, Chennai - 117. The animals were maintained in well-ventilated room temperature with natural 12h ± 1h day-night cycle in the propylene cages .They were fed balanced rodent pellet diet from Poultry Research experimental period . The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature. The experimental protocol was proved by the Institutional Animal Ethics Committee IAEC Ref No: 290/CPCSEA/PHARMACOL-12/06. Dt.18/7/2006.

ASSESSEMENT OF ANTIARTHRITIC ACTIVITY[4]

Arthritis was induced in rats in all groups of animals by injecting 0.05 ml of 0.5(w/v) suspension of killed Mycobacterium tuberculosis in paraffin oil into the left hind limb. Paw value was measured till 12^{th} day by using Plethismometer. Drug treatment was started on the day 13 and terminated on day 21. At 22^{nd} day blood was withdrawn through retro orbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters like total WBC count, lymphocytes, and total protein count were analyzed. The difference in paw volume on day 3 and day 21were considered as edema volume and the percent inhibition of edema was determined.

At the end of the experiment all the animals were killed by decapitation and the liver was isolated in ice cold water and homogenized with phosphate buffer pH 7.8 and this homogenate was used to study Lipid peroxidation and antioxidant enzymes including Super oxide dismutase and Catalase.

ESTIMATION OF LIPID PEROXIDATION[5]:

The reaction mixture containing 0.2 ml of liver homogenate, 1.5 ml of Thiobarbituric acid, 0.2 ml of Sodium Dodecyl Sulphate, 1.5 ml of acetic acid and 0.8 ml of distilled water. The above reaction mixture is kept in boiling water bath at 90^oC for 1 hour and cooled in tap water. After cooling 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm. The percentage inhibition of lipid peroxidation was calculated.

ESTIMATION OF SUPEROXIDE DISMUTASE[6]:

0.05ml of liver homogenate, 1.5ml of the buffer was added. The reaction was initiated by the addition of 0.4ml epinephrine and change in the optical density per min was measured at 480nm.One unit of superoxide dismutase activity is the amount of enzyme required to give 50%inhibition of epinephrine auto-oxidation.

ESTIMATION OF CATALASE[7]:

1.2 ml of phosphate buffer, 0.1 ml of the liver homogenate was added. The enzyme reaction was started by the addition of 1ml of hydrogen peroxide solution. The change in the optical density was measured at 420 nm for 3 min at 30 sec interval. Catalase activity is expressed as n moles of H_2O_2 utilized/min/mg protein in liver homogenate.

RESULTS

PRELIMINARY PHYTOCHEMICAL SCREENING

The ethanolic extract of *Portulaca olracaea* leaves was subjected to preliminary phytochemical screening. The ethanolic extract showed the presence of alkaloids, carbohydrates, proteins, flavonoids, saponins, gums and mucilages. **(Table. 1)**

ANTIARTHRITIS ACTIVITY

The EEPO (200mg/kg & 300mg/kg) showed the significant results in Freund's adjuvant induced arthritis in rats .The standard drug and extract did not suppress the primary lesion on day 3 where as significant inhibition of secondary swelling on day 21 were observed for both extract and diclofenac sodium treated animals. There was an increase in percent inhibition of paw edema with increase in the dose after 21 days where as diclofenac sodium (10mg/kg) produced increase in inhibition of rat paw oedema

after 21 days (P<0.001). Results were shown in **Table 2.**

BIOCHEMICAL PARAMETERS

The biochemical study of EEPO (200mg/kg & 300mg/kg) showed the increase WBC count was significantly suppressed and standard diclofenac sodium (P<0.01).The increased lymphocyte count in adjuvant control group was significantly restored back to normal by test and standard drug (P<0.01).There is a decreased protein content in standard and extract drug treatment group as compared to adjuvant control group. Results were shown in **Table 3.**

Table.1: PRELIMINARY PHYTOCHEMICAL SCREENING

S.No	PHYTOCHEMICAL TESTS	ETHANOLIC EXTRACT
1	Test for Alkaloids	+ ve
2	Test for Carbohydrates	+ve
3	Test for Proteins	+ve
4	Test for Steroids	-ve
5	Test for Sterols	-ve
6	Test for Phenols	-ve
7	Test for Flavonoids	+ve
8	Test for Gums and Mucilage	+ve
9	Test for Glycosides	-ve
10	Test for Saponins	+ve
11	Test for Terpenes	-ve

+ve: indicates the presence of compounds, -ve: indicates the absence of compounds

Table2.

Effect of EEPO (Ethanolic extract of *Portulaca olaracea* L.Sativa) in Freunds adjuvant arthritis paw volume method

S.No	Treatment	Mean Paw Volume			Mean Difference		% Inhibition
		Initial	After 3 days	After 21 days	After 3 days	After 21 days	After 21 days
1	Control(propylene glycol 10mg/kg)	1.123± 0.0234	1.428± 0.0041	1.348± 0.0256	0.305± 0.216	0.225± 0.0294	-
2	Diclofenac sodium 10mg/kg	1.160± 0.0014	1.452± 0.0279	1.263± 0.0047	0.292± 0.027	0.103± 0.0042	54.22*
3	EEPO 200mg/kg	1.303± 0.0225	1.610± 0.02	1.448± 0.0242	0.307± 0.0216	0.145± 0.0035	35.56*
4	EEPO 300mg/kg	1.206± 0.0168	1.518± 0.016	1.359± 0.0198	0.298± 0.020	0.129± 0.0028	32.29*

Standard significance test was done by using Paired Student's "t" test. *P< 0.001(comparison of 1 with 2, 3, and 4). No. of rats =6 per group, tabular value represents mean ±SEM EEPO –Ethanolic Extract of *Portulaca olaracea*.

Table3

Effect of EEPO (Ethanolic extract of *Portulaca olaracea* L.Sativa) on biochemical parameters studied in adjuvant induced arthiritis.

S.No	Parameters	Control(Propylene glycol 10mg/kg)	Standard(Diclofenac sodium 10mg/kg)	EEPO 20mg/kg	EEPO 300mg/kg
1	Total WBC count	10900/cu.mm	8233.33/cu.mm	8783.33/cu.mm	8962.32/cu.mm
2	Lymphocytes	81.16% ±1.1690	52.67%* ±1.9666	55.33%* ±1.6329	58.33%* ±1.732
3	Total proteine count	8.005gm% ±0.0351	5.65gm% *±0.0351	7.358gm%* ±0.1506	7.472gm% ±0.162

Standard significance test was done by using Student's "t" test. *P< 0.001(comparison of all parameters of control group with standard and test group). No of rats =6 per group, tabular value represents mean ±S.D. EEPO –Ethanolic Extract of *Portulaca olaracea*

DISCUSSION

There were few scientific publications on analgesic and anti inflammatory properties of *Portulaca olaracea*. Hence, in our present investigations we have included antiarthritic activity. The EEPO have shown significant and also remarkable antiarthritic activity in adjuvant induced arthritis model. In therapeutic test model of adjuvant arthritis primary lesions are generally seen on day 3 and from day 12 onwards secondary lesions are observed. The right hind paw volume was measured on day 0 and on day 21. Therefore in present study, the difference between the above to values has been considered to be the oedema volume. The standard drug and EEPO did not suppress the primary lesions on day 3 but shown a significant inhibition of secondary swelling in the adjuvant injected and non-injected paw on day 21.T- lymphocytes have been reported to play a central role in the pathogenesis of rheumatoid arthritis. These cells comprise the majority of the lymphoid cell found in the rheumatoid synovium8. The inhibition of secondary inflammation in adjuvant arthritic rats by the EEPO was further strengthened by the biochemical parameters studied. Increased lymphocyte count in arthritic rat was significantly suppressed in the standard and EEPO treated group indicating its immunosuppressant nature. Arthritis condition generally results in accumulation of leucocytes and release of lysosomal enzymes, the main mediators in arthritis9. In present study the migration of leucocytes into the inflamed area is significantly suppressed by the EEPO as seen from the significant decrease in total WBC count. Most of the non- steroidal anti inflammatory agents exert their beneficial effect by inhibiting either release of lysosomal membrane which is responsible for inflammatory process. Possibly EEPO would be acting by the similar mechanism as it reduced the total protein count especially serum albumin content.

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