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Research article

EVALUATION OF ANTI ARTHRITIC ACTIVITY OF PET – ETHER EXTRACT OF PORTULACA OLERACEA (Linn.)

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ABSTRACT: To investigate the antiarrthritic activity of petroleum-ether extract of Portulaca oleracea. The petroleum-ether extract of Portulaca oleracea was subjected to preliminary phytochemical screening. Acute toxicity studies were carried out in Male Wistar rats and anti-arthritic activity by Fruends adjuant arthritis model. Phytochemical evaluation revealed the presence of alkaloids, tannins, flavonoids, saponins and triterpenoids. Acute toxicity studies showed that the extract was non-toxic upto a maximum dose of 2000 mg/kg body weight. Petroleum-ether extract exhibited significant anti-arthritic activity. The present study indicates that the petroleum-ether extract of Portulaca oleracea has a potential anti-arthritic activity can be used as anti-arthritic drug.

Keywords: Portulaca oleracea (Linn.), petroleum-ether extract, arthritis, adjuant, indomethacin.

INTRODUCTION

Rheumatoid arthritis is a systemic autoimmune disorder characterized by polyarticular symmetrical arthritis. Various inflammatory mediators produce joint inflammation with pain, function loss, joint destruction and permanent deformity after certain time if remained untreated. This disease has a worldwide distribution but its pathogenesis is not clearly understood (Harris EDJR, et al., 1960) although there are few anti-rheumatic drugs showing effectiveness on the treatment of rheumatoid arthritis, the side effect and toxicity call for new and more effective natural drugs (Scott DL, et al., 1998).

Portulaca oleracea (P. oleracea) belonging to the family "Portulacaceae" is an herbaceous plant widely distributed throughout the world. It contains many biologically active compounds and is a source of many nutrients like free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, protein, (Ezekwe MO, et al., 1999) a-linolenic acid and b-carotene (Liu LX, et al., 2000, Barbosa – Filho JM, et al., 2008) mono terpene glycoside (Sakai NK, et al., 1996) N-trans-feruloyltyramine (Mizutani M, et al., 1998). It was also found to contain vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, 0a-tocopherol and glutathione (Chatterjee A, et al., 1956, Simopoulous AP, et al., 1992, Prashanth KL, et al., 2005). The high contents of a variety of phytoconstituents present in this plant were considered to be responsible for the biological activities like antibacterial, antifungal (Oh KB, et al., 2002), anti-fertility (Verma OP, et al., 1982), muscle relaxant (Parry O, et al., 1993) and wound healing properties, (Rasheed AN, et al., 2003) analgesic and antiinflammatory activity (Jagan Rao N, et al., 2012). This plant which is normally used as a vegetable to prepare curry by the native people of Andhra Pradesh is used in combination with tomato. Earlier studies revealed the above pharmacological properties of Portulaca oleracea. However, no study was done on its antiarrthritic activity of petroleum-ether extract of Portulaca oleracea. Therefore, the present study has been designed to investigate the petroleum-ether extract of Portulaca oleracea for its antiarrthritic activity.

MATERIALS AND METHODS

The leaves of Portulaca oleracea were collected from a local vegetable market in Kanchipuram in the month of January 2011. The identification and authentication of the plant done at the department of Botany, Government Degree College, Kanchipuram.

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Animals

Male Wistar rats (150-175 g) were procured from the institutional animal house. The animals had free access to standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions, and maintained in room temperature of 25±1°C; relative humidity 45-55% and a 12:12 light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocol was approved by the institutional animal ethical committee (T.C/COL/2331/2011/HOSF-378).

Preparation of extract

Portulaca leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w.

Phytochemical screening:

The petroleum ether extract of Portulaca oleracea leaves were subjected to qualitative chemical investigation for the identification of phyto constituents (Khandelwal KR, et al., 2000) like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides using appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

Test for alkaloids (Mayer's test):

The extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

Test for tannins:

The extract was treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

Test for flavonoids (Shinoda test):

To the extract, add 5 ml 95% ethanol, few drops of conc. HCl and 0.5g magnesium turnings. Pink coloration indicates the presence of flavonoids.

Test for saponins (froth test):

1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test for terpenoids (Salkowski test):

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for carbohydrates (Molisch's test):

The extract was treated with 3ml of alpha–napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates

Test for glycosides (modified Borntrager's test):

To 5 ml of extract add 5ml of 5% FeCl3 and 5ml dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil.Ammonia. Ammonical layer shows pinkish red color.

Acute toxicity studies:

Acute oral toxicity studies were performed according to Organization for Economic Cooperation and Development (OECD 423) guidelines (Ecobichon DJ, et al., 1997). Male Wistar rats weighing 150-175 g were used to determine the LD_{50} of petroleum-ether extract of Portulaca oleracea. Tween-80 1% v/v was used as vehicle to suspend the petroleum-ether extract.The petroleum-ether extract was administered in a dose of 2g/kg orally to a group of three rats. The animals were continuously observed for changes in autonomic or behavioral responses for 6hrs.The animals were kept under observation for 14 days to detect any mortality. The petroleum-ether extract were found to be non-toxic up to dose of 2g/kg body weight.

Anti - arthritic activity

The method, by Fruends adjuant arthritis model, was followed. The albino rats of either sex were selected and divided into five groups, each comprising of six animals. Group I, served as control received vehicle 2ml/kg (0.3% carboxy methyl cellulose in distilled water), Group II, served as standard received indomethacin (100 mg/kg), Groups III, IV, and V, served as tests received 100mg/kg, 200mg/kg, 300mg/kg of petroleum ether extract of Portulaca oleracea respectively for 21 days.

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The experimental protocol was for 21days and on the day one, Fruends adjuant 0.1ml (1ml contains 1mg mycobacterium Tuberculosis (H37Ra, ATCC25177) heat killed and dried, 0.25 ml mineral oil and 0.15ml mannide mono oleate was administered into the sub plantar region of right hind paw. The paw volume and paw thickness was measured at day 4, day 8, day 14 and day 21.

Percentage inhibition of paw volume was calculated by the formula,

$$i = (1-\Delta V_{Treated} / -\Delta V_{Control})*100$$

Where, ΔV represents the mean change in paw volume

Statistics

The data were expressed as avg ± standard error of mean (S.E.M).The results were analyzed by one way Analysis of Variance followed by dunnet t test. A value of $p<0.05$ was considered significant.

RESULTS

Phytochemical Screening:

The percentage yield of petroleum-ether extract of leaves Portulaca oleracea was found to be 10.6%w/w.The chemical tests indicate the presence of Phytoconstituents like the flavonoids, tannins, saponins, Terpenoids and Alkaloids in the petroleum-ether extract.

Acute toxicity studies:

There was no significant alteration in autonomic or behavioral responses in the mice treated with pet-ether extract of the leaves of Portulaca oleracea. No mortality was recorded in these animals up to 14 days.

Anti - arthritic activity:

Sub plantar Fruends adjuant administration increased the diameter of the paw significantly over the period of observation in vehicle treated control animals.(Table 1).The increase was significantly less at all observation periods with indomethacin treatment. A maximum of 77.82% inhibition was observed on 21st day. In a similar fashion treatment with petroleum-ether extract also attenuated the increase in paw diameter due to Fruends adjuant administration, this was more pronounced at 300mg/kg of petroleum ether extract of portulaca oleracea. A maximum of 75.69% inhibition was observed on 21st day.

Table-1: Effect of petroleum ether extract of Portulaca oleracea in Adjuant induced arthritis model

Groups	Mean changes in paw oedema ± SEM				% inhibition of paw swelling on 21st day
	Day 4	Day 8	Day 14	Day 21	
Control (0.3% CMC)	4.23±0.06	4.44±0.15	4.50±0.09	4.69±0.11	0
Standard (indomethacin 100 mg/kg)	3.89±0.05**	3.05±0.09**	1.94±0.18**	1.04±0.14	77.82
Petroleum ether extract 100mg/kg p.o	3.82±0.03**	3.78±0.05**	3.53±0.16**	2.45±0.06**	47.76
Petroleum ether extract 200mg/kg p.o	4.07±0.08**	3.46±0.04**	2.42±0.12**	1.54±0.05**	67.16
Petroleum ether extract 300mg/kg p.o	4.12±0.02	3.86±0.02	2.08±0.06	1.14±0.12	75.69

n=6, Values expressed in avg ± S.E.M, p<0.01.**

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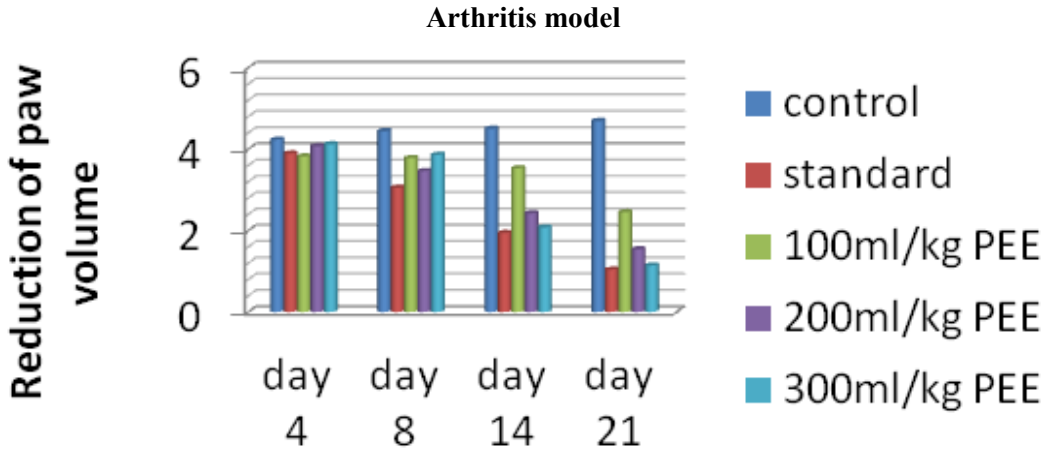

Figure-1: Histogram showing effect of petroleum ether extract of Portulaca oleracea in Adjuant induced

DISCUSSION

The present study was carried out to see the efficiency of petroleum ether extract of Portulaca oleracea against a chronic inflammatory disease, that is, arthritis. In the acute toxicity testing, no mortality was observed in the rat even in a dose of 2g/kg of petroleum ether extract of Portulaca oleracea, which indicates the safe nature of the extract.The phytochemical study revealed the presence of sterols, glycosides and flavonoids. The presence of sterols and flavonoids favoured further to pursue the study in search of a definite biologic activity. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of cartilage and bone destruction. It has closed similar to human rheumatoid diseases (Sing S, et al., 1996). The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and therapeutic effects of drugs.

The Freund's adjuvant induced arthritis model, originally developed by Pearson et al (Pearson CM, et al., 1963). The successful initiation of arthritis in animal model is capable of activating and synthesising mediators such as PGE2 and cytokines like TNF-alpha and IL-1. These prostaglandins with cytokines and other group of enzymes institute edema formation, cartilage and bone destruction (Hopkins SJ, et al., 1990).

The standard drug indomethacin and ethanolic extract significantly suppressed the swelling of the paw, which may be due to the suppression of inflammatory mediators released due to induction of freunds adjuvant. Though the actual mechanism of suppressing inflammation is not known but it can be correlated with presence of alkaloids and flavonoids in suppressing the inflammation and its antioxidant properties (Mu L, et al., 2007). To conclude that the petroleum ether extract of Portulaca oleracea possesses potentially useful antiarrthritic activity since it give a positive results in controlling inflammation in adjuvant induced arthritis model in rats.

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