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Pharmacology Section

Evaluation of the Anti-nociceptive and Anti-inflammatory Activities of the Pet: Ether Extract of *Portulaca Oleracea* (Linn.)

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ABSTRACT

To investigate the anti-nociceptive and the anti-inflammatory activities of the petroleum-ether extract of Portulaca oleracea. The petroleum-ether extract of Portulaca oleracea was subjected to a preliminary phytochemical screening. Acute toxicity studies were carried out in Swiss albino mice. The analgesic activity of the petroleum-ether extract of Portulaca oleracea was evaluated by using well established models like acetic acid induced writhing, the formalin test and the tail immersion method in mice. Its acute anti-inflammatory effect was studied by the Carrageenan induced hind paw oedema method in rats. Its phytochemical evaluation revealed the presence of alkaloids, tannins, flavonoids, saponins and triterpenoids. The acute toxicity studies showed that the extract was non-toxic upto a maximum dose of 2000 mg/kg body weight. The petroleum-ether extract exhibited significant inhibition of the acetic acid induced writhing, it reduced the paw-licking response time significantly in the formalin test and it increased the withdrawal latency time in the tail immersion test. The Carrageenan induced hind paw oedema was significantly reduced in rats. The present study indicated that the petroleum-ether extract of Portulaca oleracea had potential anti-nociceptive and anti-inflammatory activities.

Key Words: Portulaca oleracea (Linn.), petroleum-ether extract, anti-nociceptive, antiinflammatory

INTRODUCTION

The classical NSAIDs (aspirin like drugs) are among the most widely prescribed drugs worldwide as analgesic and anti-inflammatory agents and they have become an important weapon in the control of inflammation and the pain which is associated with musculoskeletal pathologies like; rheumatoid arthritis, osteoarthritis, gout, tendonitis, muscle strain, post-operative and post-traumatic inflammation, thrombophlebitis and vasculitis. Being the drug of choice for the treatment of rheumatic disorders and other degenerative inflammatory diseases or being an abused drug, the consumption of NSAIDs has been rated as high as compared to that of other drugs. However, their prolonged clinical use is hampered by their side effects, most notably, gastric erosion, ulceration, haemorrhage, bronchospasm and kidney and liver dysfunction [1]. This has been the rational behind the development of new analgesic and anti-inflammatory drugs. The search for novel molecules has been extended to herbal drugs that offer better protection against pain and inflammation with minimal unwanted effects.

Portulaca oleracea (P. oleracea) which belongs to the family "Portulacaceae", is a herbaceous plant which is widely distributed throughout the world. It contains many biologically active compounds and it is a source of many nutrients like free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinones, proteins [2], a-linolenic acid, b-carotene [3,4] mono terpene glycoside [5] and N-trans-feruloyltyramine [6]. It has also been found to contain vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, 0a-tocopherol and glutathione [7,8,9]. The high contents of a variety of phytoconstituents which are present in this plant have been considered to be responsible for the biological activities which have been reported as characteristic of this plant like antibacterial, antifungal [10], anti-fertility [11], muscle relaxant [12] and wound healing properties [13] (This plant which is normally used as a vegetable to prepare curry by the native people of Andhra Pradesh has been used in combination with tomato). Previous studies have revealed the above mentioned pharmacological properties of Portulaca oleracea. However, no study was done to assess the combination of the analgesic and the anti-inflammatory activities of the petroleum-ether extract of Portulaca oleracea. Therefore, the present study was designed to investigate the petroleum-ether extract of Portulaca oleracea for its anti-nociceptive and anti-inflammatory activities.

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 the authentication of this plant were done at the Department of Botany, Government Degree College, Kanchipuram.

Animals

Swiss albino mice (25-30g) and male Wistar rats (150-175 g) were procured from the institutional animal house. The animals had free access to the standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions, and they were maintained at a room temperature of 25 ±1°C; a relative humidity of 45-55% and in 12:12 light/dark cycles. All the experiments were conducted in strict compliance according to the ethical principles and the guidelines which were provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the institutional animal ethical committee (T.C/COL/2331/2011/HOSF-378).

Preparation of the Extract

The Portulaca oleracea leaves were shade dried and one kg of coarse powder was soaked in 4 liters of petroleum-ether for 3 days at room temperature. The cold macerate was extracted by using

the Soxhlet apparatus. The extract was evaporated to dryness by using a rotary vacuum flask evaporator and the yield was10% w/w.

Phytochemical Screening

The petroleum-ether extract from the Portulaca oleracea leaves was subjected to a qualitative chemical investigation for the identification of its phyto constituents [14] like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides by using the appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate was used for the following tests.

Test for Alkaloids (Mayer's Test)

The extract was treated with Mayer's reagent and the appearance of a cream colour indicated the presence of alkaloids.

Test for Tannins

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

Test for Flavonoids (Shinoda Test)

In the extract, 5ml of 95% ethanol, few drops of conc. HCl and 0.5g of magnesium turnings were added. The formation of a pink colour indicated the presence of flavonoids.

Test for Saponins (Froth Test)

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

Test for Terpenoids (Salkowski Test)

5ml of the extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face which was formed indicated 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. The formation of a violet coloured ring at the junction of the two liquids indicated the presence of carbohydrates.

Test for Glycosides (Modified Borntrager's Test)

To 5 ml of the extract, 5ml of 5% $FeCl_3$ and 5ml of dil. HCl were added. This was heated for 5 min. in a boiling water bath. It was then cooled and benzene or any organic solvent was added to it. The mixture was shaken well. The organic layer was separated and an equal volume of dil. Ammonia was added to it. The ammonical layer showed a pinkish red colour.

Acute Toxicity Studies

Acute oral toxicity studies were performed according to the Organization for Economic Cooperation and Development (OECD 423) guidelines [15] . Albino mice of either sex which weighed 25-30 g and which were of 90 days of age were used to determine the LD_{50} of the 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 mice. The animals were continuously observed for changes in their autonomic or behavioural responses for 6 hours. The animals were kept under observation for 14 days to detect any mortality. The petroleum-ether extract was found to be non-toxic with a dose of up to 2g/kg of body weight.

Anti-nociceptive Activity

The acetic acid induced writhing, formalin induced nociception and the hot water tail immersion methods were used to evaluate the potential anti-nociceptive activity of the petroleum-ether extract of the leaves of Portulaca oleracea. The petroleum-ether extract was prepared as a suspension in 1% Tween 80 and it was administered orally to the experimental animals. A group of animals was treated with the vehicle (1% Tween 80) and this served as the control group. Morphine (5mg and 10mg/kg sc) was used as a standard drug for comparison.

Acetic Acid Induced Abdominal Constriction

The mice were treated with the petroleum ether extract of Portulaca oleracea in different doses (50,100 and 200 mg/kg) per oral. Sixty minutes later, acetic acid (0.6% v/v in saline) was injected intraperitoneally in a dose 10ml/kg [16]. The number of abdominal constrictions (writhing) in the mice was counted for 15 min following the acetic acid injection. Any significant reduction in the number of abdominal constrictions by the treatment as compared to that in the vehicle treated animals was considered as an anti-nociceptive response. The percentage inhibition of the writhings as compared to the vehicle treatment was calculated by using the formula,

(C-T/C) x100 where C is the number of abdominal constrictions which were recorded in the vehicle treated animals and T is the number of abdominal constrictions in the treatment group. The highest dose which was employed (200mg/kg) was chosen for further anti-nociceptive assessments.

Formalin-induced Paw-licking

20 microlitres of 1% formalin in saline was administered s.c. in the plantar surface of the left hind paw of mice [17] 60 min after the treatment with the petroleum-ether extract of Portulaca oleracea (200mg/kg).The time which was spent in licking or biting the injected paw was recorded every 5 min for a period of 30 minutes and it was considered as the response to the nociception. The early phase of the nociceptive response normally peaks from 0-10 min and the late phase normally peaks from 10-30 min after the formalin injection. The paw licking time in the mice after the petroleum-ether extract/morphine (5mg/kg) treatment was compared with that in the vehicle treated mice. The percent inhibition of the paw licking time as compared to the vehicle treatment was calculated by using the formula, Percent inhibition = C-T/Cx100, where C was the biting/paw licking response time (seconds) in the vehicle treated group and T was the biting/paw licking response time (seconds) in the petroleum-ether treated group.

The Tail Immersion Test

The tail immersion test [18] was used to evaluate the anti-nociceptive activity of Portulaca oleracea in a dose of 200mg/kg p.o. The tail of a mouse was immersed in a water bath which was maintained at 55 ±0.5°C. The time for the withdrawal of the tail was taken as the reaction time. A cut off period of 10 seconds was maintained. The reaction time was measured just before the administration of the test substances (0 min) and then at intervals of 30 min up to a period of 90 min. The increase in the latency period was compared to that in the vehicle treated group. Morphine, in a dose of 10mg/kg s.c, was used as a standard drug.

Antiinflammatory Activity

Carrageenan-induced Hind Paw Oedema: Male Wistar rats (150-175 g) were fasted overnight and the paw oedema was developed by injecting carrageenan 0.1ml of 1% w/v suspension in sterile normal saline into the subplantar tissue of the right hind paw [19]. One hour before the carrageenan injection, the petroleum-ether extract of Portulaca oleracea which was suspended in Tween 80 was administered orally in a dose of 200mg/kg .The control group of rats received 1% Tween 80 v/v 1ml/100gm per oral. Another group of rats was administered the standard drug diclofenac 10mg/kg i.p 30 minutes prior to the carrageenan injection.

The diameter of the paw was measured by using digital Vernier calipers before the administration of carrageenan and at 1, 2, 3, 4, 5 and 6 hour intervals after the administration of carrageenan. The thickness of the oedema in (mm) at various time intervals was calculated by subtracting the zero hour reading.

The oedema (ΔT) was calculated as follows,

$\Delta T = Tt - T0$

Tt is the right hind paw thickness in mm at time t

T0 is the right hind paw thickness before the sub-plantar carrageenan injection

The % reduction of the oedema was calculated as follows,

Mean oedema in the untreated control group (C) – mean oedema in the drug treated group (T) / Mean oedema in the untreated control group×100 = (C-T/C×100)

STATISTICS

The data were expressed as mean ± standard error of the mean (S.E.M).The results were analyzed by One Way Analysis of Variance, followed by the Post hoc Bonferroni Test. A value of $p<0.05$ was considered as significant.

RESULTS

Phytochemical Screening

The percentage yield of the petroleum-ether extract of the leaves of Portulaca oleracea was found to be 10.6% w/w. The chemical tests indicated the presence of phytoconstituents like flavonoids, tannins, saponins, terpenoids and alkaloids in the petroleum-ether extract.

Acute Toxicity Studies

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

Anti-nociceptive Activity

Acetic acid induced abdominal constrictions: The mean number of abdominal constrictions in the vehicle treated control animals was 36.50 ± 0.42 [Table/Fig-1]. A significant reduction in the number of abdominal constrictions was recorded for the morphine treated mice, the mean value being 4.83 ± 0.54 and the percentage inhibition of nociception being 86.76%. A dose dependent reduction in the number of abdominal constrictions was noticed after the administration of the petroleum-ether extract of Portulaca oleracea.The reduction was significant with 100mg/kg (30.45 ± 0.56) and 200mg/kg (14.83 ± 1.49) of the extract. In the above doses, the percentage inhibitions of nociception were 16.57% and 59.36% respectively.

Treatment	Number of abdominal constriction
Vehicle (Tween 80 1% v/v)	36.50 ± 0.42
Morphine 1 mg/kg s.c	4.83 ± 0.54* (86.76%)
Petroleum ether extract 50mg/kg p.o	35.62 ± 0.21 (2.41%)
Petroleum ether extract 100mg/kg p.o	30.45 ± 0.56* (16.57%)
Petroleum ether extract 200mg/kg p.o	14.83 ± 1.49* (59.36%)

[Table/Fig-1]: Effect of Portulaca oleracea extract on acetic acid induced abdominal constrictions in mice
Each value represents the mean ± SEM of six observations
($p^*<0.05$) compared to vehicle treatment One way Anova followed by Bonferonni test
The value in parenthesis indicates the percentage of nociception.

Formalin-induced Nociception

In the vehicle treated control animals, the paw licking response time was 46.33 ± 2.6 in the early phase (0-10 min) and it was 85.8 ± 3.58 in the late phase (10-30 min). In the morphine treated animals, the paw licking response time was significantly reduced, both in the early (10 ± 0.73) and the late phases (3.33 ± 0.49). A significant reduction in the paw licking response time was evident in the early phase (37.16 ± 1.16) and in the late phase (27.33 ± 5.31) after the treatment with the petroleum-ether extract of Portulaca oleracea. The extract nearly produced 19% and 33% inhibitions of the nociceptive response in the early and late phases respectively [Table/Fig-2].

Sl. No.	Treatment	Dose (mg/kg)	Early phase 0-10min	Late phase 10-30min
1	Vehicle (Tween-80 1% v/v)	0.1ml/10g	46.33 ± 2.61	85.8 ± 3.58
2	Morphine	10mg,sc	10 ± 0.73* (82%)	3.33 ± 0.49* (96.5%)
3	Pet-Ether Extract	200mg,p.o	37.16 ± 1.16* (19.79%)	27.33 ± 5.31* (33.18%)

[Table/Fig-2]: Effect of extract of Portulaca oleracea on formalin induced nociception in mice
Each value is presented as mean ± SEM of six observations.
($p^*<0.05$) compared to vehicle treatment One way Anova followed by Bonferonni test
The value in parenthesis indicates the percentage of nociception.

The Tail Immersion Test

The mean reaction time in the vehicle treated mice during the observation periods (30, 60 and 90 min.) were 1.78 ± 0.03, 1.73 ± 0.07 and 1.76 ± 0.02 respectively [Table/Fig-3].

The Morphine treatment significantly increased the reaction time in all the observation periods. The reaction time with the 200 mg/kg petroleum-ether extract of Portulaca oleracea was also significantly increased as compared to that in the vehicle treated mice. It showed the maximum latency period at the 60th minute.

Carrageenan Induced Hind Paw Oedema

Intra-plantar carrageenan administration increased the diameter of the paw significantly over the period of observation in the vehicle treated control animals [Table/Fig-4].This increase was significantly lesser at all the observation periods with indomethacin treatment. A maximum of 48% inhibition was observed at five hours. In a similar fashion, the treatment with the petroleum-ether extract of Portulaca oleracea also reduced the increase in the paw diameter due to the carrageenan administration. A maximum of 26% inhibition was observed at the 5th hour.

Sl. No.	Treatment mg/kg	O hours	30 min	60 min	90 min
1	Vehicle (Tween 80, 1%v/v)	1.71±0.03	1.78±0.07	1.73±0.04	1.76±0.02
2	Morphine 10mg,sc	1.80±0.05	2.30±0.03*	4.13±0.08*	8.16±0.08*
3	Pet-Ether Extract 200mg, po	1.92±0.07	2.03±0.07*	2.76±0.10*	2.42±0.05*

[Table/Fig-3]: Effect of Portulaca oleracea extract on Thermal nociception in mice

Each value is presented as mean ± SEM of six observations.
(p*<0.05) compared to vehicle treatment One way Anova followed by Bonferonni test
The value in parenthesis indicates the percentage of nociception.

DISCUSSION

The present study was undertaken to scientifically validate the traditional claims of Portulaca oleracea with particular references to its anti-nociceptive and anti-inflammatory effects by different methods, viz. visceral nociception (acetic acid induced abdominal constriction), thermal nociception (tail immersion test) and neurogenic and inflammatory nociception (formalin induced paw licking) and to its anti-inflammatory effect (the carrageenan induced hind paw oedema test).

In the acute toxicity testing, no mortality was observed in the mice, even in a dose of 2g/kg of petroleum-ether extract of Portulaca oleracea, which indicated the safe nature of the extract.

The acetic acid induced abdominal constriction [16] method is a very sensitive one and it can detect the anti-nociceptive effect of substances at a dose that cannot be detected by other methods such as the tail-flick test [20]. The abdominal constriction responses were found to partly involve the local peritoneal receptors [21]. This method has been associated with prostanoids in general; e.g increased levels of PGE2 and PGEF2α in the peritoneal fluids [22] as well as lipoxygenase products which were reported by some researchers [23, 24]. The mean number of abdominal constrictions after the acetic acid injection in the mice was significantly (P<0.05) reduced by the petroleum-ether extract of Portulaca oleracea, either due to the inhibition of prostaglandins or due to leukotriene synthesis.

The formalin induced paw licking was a persistent-pain model which was used to evaluate the neurogenic and inflammatory nociception in 2 phases (i.e.) in the early (0-10 min.) and the late phases (10-30 mts) respectively. The early phase of the formalin response was attributed to the direct stimulation of the nociceptors [17,25,26]. The late phase was the result of an inflammatory reaction which was caused by tissue injury, leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids. The centrally acting analgesic drugs like the narcotic analgesics inhibit both the phases equally, while the peripherally acting drugs such as steroids and NSAID's suppress mainly the late phase. The petroleum-ether extract of Portulaca oleracea markedly attenuated the formalin induced paw licking response time in the late phase as compared to that in the early phase. This revealed that Portulaca oleracea may be more effective in alleviating the pain which was caused by inflammation.

The carrageenan induced paw-oedema model has gained greater importance and support over the years, because the oedema which is induced by carrageenan is reported to have been inhibited by a majority of the steroidal and the non-steroidal anti-inflammatory drugs. Moreover, the lesions which were induced by carrageenan have been said to resemble those of rheumatoid arthritis histologically in human beings, at least to a certain extent. These observations have justified the use of carrageenan as the prime oedemogen. It has a biphasic effect. The first phase (0-3hr) is due to the release of histamine and serotonin, the plateau phase is maintained by a kinin like substance (3hours) and the late phase (4.5-5hr) of the inflammation is attributed to prostaglandin release [27]. In the intial phase, the mast cells are activated and degranulated, thus releasing histamine and serotonin. These mediators increase the vascular permeability of the blood vessels, which facilitates the infiltration of neutrophils and the accumulation of plasma fluids and proteins into the interstitial spaces. This is followed by the release of kinins after a certain time. These events lead to the development of oedema which is reduced by anti-inflammatory agents. The drugs which inhibit the initial phase of the carrageenan induced hind paw oedema indicate that they exert an anti-inflammatory effect. The oedema in the late phase is due to a massive infiltration of neutrophils and the overproduction of prostaglandin. NSAIDs predominantly inhibit the late phase of the inflammation in the carrageenan induced hind paw oedema.

The administration of the Portulaca oleracea extract consistently reduced the paw oedema in rats after the carrageenan administration [Table/Fig-4].This observation indicated the potent anti-inflammatory effect of the petroleum-ether extract of Portulaca oleracea.Thus, the present study revealed the potential analgesic and anti-inflammatory effects of the petroleum-ether extract of Portulaca oleracea.

The phytochemical screening of the petroleum-ether extract of Portulaca oleracea revealed the presence of flavonoids, tannins, saponins, terpenoids and alkaloids. The phytochemical constituents are physiologically active compounds which possess a great potential for therapeutic and prophylactic uses. The analgesic and the anti-inflammatory effects of flavonoids, tannins and saponins have been reported [28, 29]. The anti-inflammatory activity of flavonoids may be mainly due to the inhibition of the prostaglandin synthesis [30,29,31]. Terpenoids have also been reported to possess anti-inflammatory, anti-oxidant and neuroprotective activities [32]. Hence, the analgesic and the anti-inflammatory

Sl. No.	Treatment	1 hour	2 hour	3 hour	4 hour	5 hour
1	Vehicle (Tween 80, 1% v/v)	1.43 ±0.84	2.20 ±0.27	2.27 ±0.28	2.31 ±0.35	2.31 ±0.39
2	Indomethacin 10mg/kg, s.c	1.29 ±0.3 39.2%	1.49 ±0.48 32.27%	1.38 ±0.49 39.20%	1.31 ±0.49 43.29%	1.20 ±0.35 48.05%
3	Pet-Ether Extract 200 mg/kg,po	1.30 ±0.12 15.84%	2.0 ±0.15 9.09%	1.91 ±0.19 15.85%	1.81 ±0.88 21.64%	1.71 ±0.79 25.97%

[Table/Fig-4]: Carrageenan induced paw edema

Each value is presented as mean ± SEM of six observations.
(P*<0.05) compared to vehicle treatment One way Anova followed by Bonferonni test
The value in parenthesis indicates the percentage reduction of paw edema.

effects which were produced by the extract may be attributed individually or collectively to the flavonoids, tannins, terpenoids and the saponins.

CONCLUSION

From the present study, it is quite apparent that the petroleum-ether leaf extract of Portulaca oleracea possesses significant analgesic and anti-inflammatory effects against different stimuli. This was evidenced by a significant increase in the reaction time by the stimuli in different experimental models and by the significant analgesic and anti-inflammatory activities. Apart from the huge number of research studies in the field of analgesic and anti-inflammatory discoveries, this field still needs more attention from scientists around the world.

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