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ORIGINAL ARTICLE

A Comparison of Analgesic and Anti-Inflammatory Activities of *Portulaca oleraceae* Leaf and Seeds

Nudrat Fatima[1,]*, Shazia Syed[2], Mansoor Ahmad[3], Mehjabeen[4], Noor Jahan[5]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan*

[2] *Department of Chemistry, University of Karachi, Karachi, Pakistan*

[3] *Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan*

[4] *Department of Pharmacology, Federal Urdu University of Science and Technology, Karachi, Pakistan*

[5] *Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan*

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Address of Correspondence Author:
drnudrat_ekq@yahoo.com

ABSTRACT

Background: *Portulaca oleraceae* is a widely utilized herb as nutrition and medicine. It is an important fragment of Chinese and Ayurveda medicines since centuries. Whole plant and seeds of *P. oleraceae* has been reported for a number of medicinally important phytoconstituents.

Objective: A lot of research work is carried out on the whole plant regarding pharmacological activities including analgesic and anti-inflammatory activities, but as far as seed part is concerned, a little work is carried out. So a comparative study was designed to investigate the pain relieving and edema reducing properties of leaf versus seeds of *P. oleracea*.

Methods: Extract was prepared by classical reported method. Analgesic activity was conducted by two different methods i.e. Hot plate and Writhing tests, while anti-inflammatory activity was conducted using Formalin test. Dunnett's *t*-test was used to analyze the data.

Results: The result indicated that seed extract inhibits inflammation to a greater extent than leaf extract. Regarding analgesic activity by both methods, leaf extract is proven to be more potent analgesic than seed extract in early phase while again seed extract is better analgesic in late phase.

Conclusion: Comparative results show that *Portulaca oleraceae* seed extract possess persistent higher analgesic effect in high doses and significant anti-inflammatory activity than leaf extract. So further work can be carried out in combination of leaf and seed extract to obtain continuous activity in both early and late phases of analgesia.

Keywords: Anti-inflammatory, analgesic, *Portulaca oleraceae*, seed extract, leaf extract.

INTRODUCTION

Inflammation and pain are interrelated to each other. In most cases after an injury some inflammatory mediators like prostaglandins, tumor necrosis factor-α, interleukin 1β and interleukin-6 are released at the site, which interact with special receptors for pain called nociceptors. As a result nervous system transfer signal of pain, when there is chronic inflammation, there is increased sensation of pain. The strategy for decreasing inflammation and pain is usually the control of these biochemical mediators release. A number of natural compounds possess anti-inflammatory activity following this route [1].

Portulaca oleraceae is a plant used for thousands of years as food and medicine. Many researchers worked on anti-inflammatory activity on aerial parts of the plant. Chan K *et al*. in 2000 reported anti-

inflammatory activity of *P. oleraceae* leaves and stem [2]. Lee *et al*., 2012 worked on aqueous extract of dried aerial parts of *Portulaca oleracea* and found that it prevents the TNF-α-induced vascular inflammatory process [3], Seo in 2015 found that *Portulaca oleracea* treatment suppresses the LPS-induced inflammation [4]. No researcher has worked on seeds for anti-inflammatory activity but the analgesic activity of seeds was reported in literature [5]. So it was assumed that seeds also contain anti-inflammatory activity and hence comparative study was conducted.

P. olaraceae contains almost all major phyto-constituents including alkaloids oleracimine, oleracimine A, and oleracone A, oleracone B and βcarboline among which the first two mentioned are responsible for anti-inflammatory action [6], glycoside, carbohydrate [7, 8], sterols [9], mono, di and triterpenes [10], flavonoids [11, 12], vitamins [13], minerals [14] and amino acids [15]. It is an herb used for dietary and therapeutic purposes in liver, kidney and spleen disorders. The plant is reported to possess refrigerant, exhilarant and diuretic action. Some beneficial pharmacological effects are also described in literature like antihypoxia neuroprotective [16], lipid lowering [17], hypocholesterolemic [18], antiulcerogenic [19], antiasthma [20], wound healing [21], antifungal [22], antibacterial [23], anti-diabetic [24] and muscle relaxant property [4].

Taking into account high pharmacological profile of *P. oleraceae*, it was decided to explore seed part regarding pharmacological screening and comparison with whole plant. So at the first step, analgesic and anti-inflammatory activities were chosen.

MATERIALS AND METHODS

Plant Extract

Portulaca oleraceae, Linn whole plant with seeds was purchased from Jodia Bazar, Karachi and identified by Prof. Dr. Mansoor Ahmad (Professor Pharmacognosy in Research Institute of Pharmaceutical Sciences). A voucher sample was kept in departmental Herbarium for future issues. Methanol extract was prepared according to classical method [25].

Research Animals

The research animals were procured from animal house of HEJ Research Institute of Chemistry. The strain used was Swiss albino mice and weight range was 25-30g. All animals were male, adult, healthy, active and alert which were observed in quarantine for one week before the experiment. Grouping was carried out for the three activities according to following plan containing 5 animals in each group (Table **1**).

Table 1. Grouping of research animals.

S. No.	Activity	Group	Test Substance	Dose
1	Writhing Test	1A	Normal Saline	0.5ml
2		2A	*P. oleraceae* leaf extract	300mg/kg
3		3A	*P. oleraceae* leaf extract	500mg/kg
4		4A	*P. oleraceae* seed extract	300mg/kg
5		5A	*P. oleraceae* seed extract	500mg/kg
6		6A	Aspirin	300mg/kg
7	Hot Plate Method	1B	Normal Saline	0.5ml
8		2B	*P. oleraceae* leaf extract	300mg/kg
9		3B	*P. oleraceae* leaf extract	500mg/kg
10		4B	*P. oleraceae* seed extract	300mg/kg
11		5B	*P. oleraceae* seed extract	500mg/kg
12		6B	Aspirin	300mg/kg
13	Formalin Test	1C	Normal Saline	0.5ml
14		2C	*P. oleraceae* leaf extract	300mg/kg
15		3C	*P. oleraceae* leaf extract	500mg/kg
16		4C	*P. oleraceae* seed extract	300mg/kg
17		5C	*P. oleraceae* seed extract	500mg/kg
18		6C	Aspirin	300mg/kg

Analgesic Activity

Writhing Test

The Writhing Test was carried out on animals of group 1A-6A and the test compound was orally administered in selective dose mentioned in Table **1**. After thirty minutes 0.25ml of acetic acid was injected intraperitonially to induce writhes. Soon after acetic acid injection number of writhes were counted for thirty minutes. If the number of writhes in test drug treated animal is less than that of control group, it was a sign of analgesia [26].

Hot Plate Method

Hot plate method is another method for detection of analgesia. In this method, hot plate is maintained at 55±1ºC. An empty beaker of two liter volume is used to place the animal on hot plate. Animals of group 1B-6B (Table **1**) were given test compound orally immediately before hot plate test. They were then placed into beaker on hot plate and reaction time was noted for each animal. Licking and raising of front paw was termed as reaction after which animal was removed from hot plate. Similar procedure was repeated after every 30 minutes up to 4 and half hours post dosing [27].

Anti-Inflammatory Activity

Formalin Test

For Formalin Test the animals of group 1C-6C (Table **1**) were administered test compound orally and the after thirty minutes injected with 20µl 1% formalin in right hind paw and normal saline in left hind paw. The reaction was noted in two phases *i.e*. first phase of 0-10 minutes and second phase of 11-30 minutes after injection. Intensive licking and biting of right hind paw was termed as reaction and how many times the animal licks /bite was noted to compare with control using following formula:

% Inhibition = Mean no. of licking (control) - Mean no. of licking (drugs) / Mean. no of licking (control) [28].

Statistical Analysis

All data is presented as Mean ± S.E.M. Dunnett's *t*-test was used to analyze the data and $p \leq 0.05$ was considered as significant while $p \leq 0.01$ was considered as highly significant* [29]. All results were found significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Analgesic Activity

Writhing Test

In Writhing Test leaf extract at lower dose exhibited 75.86% and 46.3% while seed extract showed 50.8% and 60.74% pain inhibition at early and late phases respectively (Table **2**). At higher dose the inhibition percentage was 89.19% and 81.48% for leaves and 67.35 and 61.48% for seeds. The following formula is used for calculation of % inhibition;

% Inhibition = A-B / A X 100; where A = Mean no. of writhing (control) and B = Mean no. of writhing (test)

Table 2. Writhing test observations.

Group	No. of Writhes		% Inhibition	
	Early Phase	Late Phase	Early Phase	Late Phase
1A	87±5.67	54±4.67	0	0
2A	21±1.67*	29±3.30	75.86	46.29
3A	9.4±3.09	10±3.14	89.19	81.48
4A	42.8±1.67*	21.2±4.45	50.80	60.74
5A	28.4±4.7	20.8±2.06	67.35	61.48
6A	50.6±1.35*	14±0.45*	41.83	74.07

All data is presented as Mean ± S.E.M.

Hot Plate Test

By hot plate method the results were slightly different showing higher activity in seed extract as compared to that of leaf extract (Table **3**). If we assume 0.5 hour as early and 4.5 hours as late phase then % inhibition was at early phase the inhibition percent was 0, 6.66, 46.66, 21.66 and 225% for leaf extract 300 and 500mg dose, seed extract 300 and 500mg dose and standard drug aspirin respectively. While at late phase inhibition percent was 1.72, 15.52, 50, 18.79 and 89.65% in similar sequence.

Table 3. Hot plate test.

Gp	Reaction Time in Seconds ± S.E.M										Early Phase % inh.	Late Phase % inh.
	0 Hr	0.5 Hr	1 Hr	1.5 Hr	2 Hr	2.5 Hr	3 Hr	3.5 Hr	4 Hr	4.5 Hr		
1B	11.6 ± 1.029	12 ± 1.04	11.8± 1.583	10.8± 1.969	12.6± 1.777	11.8± 2.26	11.6± 1.024	11.6± 1.013	11.6± 1.013	11.6± 1.013	-	-
2B	11.4 ± 2.35	11.4 ± 2.01	12.2± 1.93	11.4± 2.23	11.2± 1.93	10.4± 2.06	11.6± 2.25	11.2± 2.03	11.2± 2.03	11.8± 2.28	0%	1.72%
3B	15.8 ± 1.20	12.8 ± 1.68	12.2± 1.56	13.2± 1.88	12.2± 1.83	11.6± 1.17	18.4± 3.34	17.2± 2.17	14.4± 1.47	13.4± 1.54	0, 6.66%	15.52%
4B	14.6 ± 2.27	17.6 ± 1.20	16.8± 1.56	17.4± 1.53	18.0± 0.63	17.4± 1.16	17.6± 0.74*	17.6± 0.74*	18.6± 0.63*	17.4± 1.16	46.66%	50%
5B	14.6 ± 1.40	14.6 ± 1.53	14.0± 1.64	13.4± 1.12	13.4± 1.50	14.2± 1.24	13.2± 1.93	13.8± 1.49	12.4± 1.49	13.78±1.22	21.66%	18.79%
6B	15.0 ± 1.43	39.0 ± 0.36*	38.0± 0.82	39.0± 0.12*	44.0± 0.51*	45.0± 0.86	33.0± 0.81	22.0± 0.91	22.0±0.91	22.0± 0.91	225%	89.65%

All data is presented as Mean ± S.E.M.

Table 4. Formalin test observations.

Group	No. Licking and Biting		% Inhibition	
	Early Phase	Late Phase	Early Phase	Late Phase
1A	73±1.16	22±2.01	0	0
2A	42.6±2.16	17.6±1.29	41.64	20.0
3A	51.4±4.68	29.8±2.71	29.58	35.45
4A	48.0±5.01	12.2±3.02	34.24	44.54
5A	42.2±1.96	12.0±0.37	42.19	94.54
6A	57.0±1.19	19±0.29	21.91	13.63

All data is presented as Mean± S.E.M.

Anti-Inflammatory Activity

Paw Licking Test

In case of anti-inflammatory activity by paw licking method, if there is reduction in licking and biting of animal as compared to control, then a positive result is concluded. Both leaf and seed extract exhibited positive result. The percentage inhibition of leaf extract (300 mg dose) was 41.64% at early phase and 20.0% at late phase while leaf extract (500 mg dose) was 29.58% at early phase and 35.45% at late phase. For seed extract the observations were 34.24% and 44.54% for 300mg and 42.19% and 94.54% for 500mg dose at early and late phases (Table **4**).

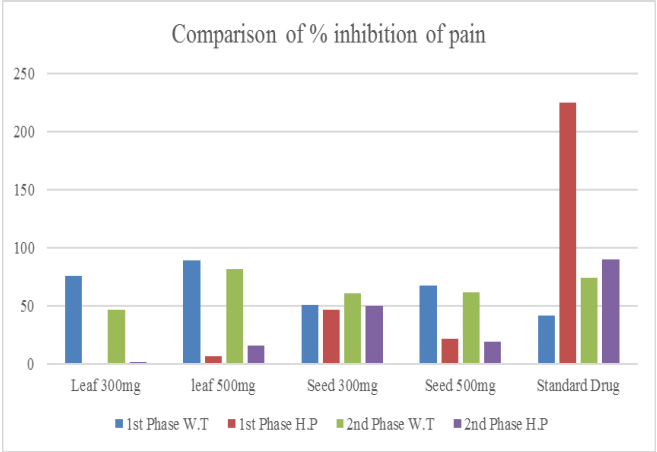

Figure 1. The comparison of analgesic activity.

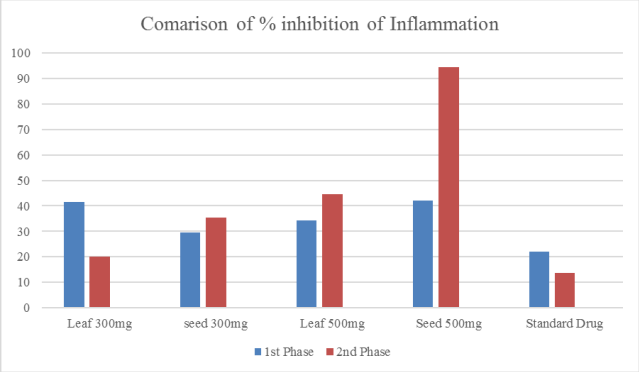

Figure 2. The comparison of anti-inflammatory activity.

Comparing the data of analgesic activity by both methods, it is very clear that in early phase leaf extract at 300 and 500mg doses exhibited higher activity than seed extract, while in late phase the situation is vice versa (Figure **1**). So further work can be carried out in combination of leaf and seed extract to obtain continuous activity in both early and late phases. The analgesic activity may be due to the presence of Allantoin as reported by Heng-Zhi Wang and Chuan-Jin Wang, 2018 [5].

Comparing the data for anti-inflammatory activity, leaf extract at 300mg dose exhibited higher activity than seed extract but at 500mg dose the seed extract exhibited better results. At 500mg dose seed extract showed increased potency than leaf extract in both early and late phases (Figure **2**). The anti-inflammatory activity could be a result of steroids which inhibits release of arachidonic acid. The mechanism of action may be prevention of phopholipase A2 which results in reduced production of prostaglandins and thromboxanes. Our standard compound Aspirin also works in a similar mechanism of inhibition of prostaglandins and thromboxanes through cycloxygenase enzymes inhibition [30]. Our assumption was supported by the results i.e. seeds also possess anti-inflammatory activity along with aerial parts of *P. olaraceae*.

CONCLUSION

Comparative results show that *Portulaca oleraceae* seed extract possess persistent higher analgesic effect in high doses and significant anti-inflammatory activity than leaf extract.

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