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IN VITRO AND *IN VIVO* ANTI-INFLAMMATORY POTENTIAL OF *OCTHOCHLOA COMPRESSA* EXTRACTS IN CARRAGEENAN INDUCED RATS

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Abstract

Octhochloa compressa (Poaceae) is a desert plant of Cholistan traditionally used as a food source by local nomads. Different extracts of Octhochloa compressa: aqueous (Aqu), methanol (MetOH), n-butanol (n-But), ethyl acetate (EtAc), n-hexane (n-Hex) and dichloromethane (DCM)) were examined for anti-inflammatory activity by in vitro human red blood cell (HRBC) membrane stabilization assay and invivo carrageenaninduced oedema in rat paw. The potency of O. compressa extracts was compared with diclofenac at 15mg/ml and 10mg/kg for invivoand invitrotrials, respectively. According to anti-inflammatory activity in the in vitro experiment, MetOH extract was exhibited the remarkable potential among all extracts with 85% protection of HRBC lysis (IC_{50} 231.7±0.678µg/ml; $p<0.001$), even better than standard drug (diclofenac). Therefore, the most promising and effective MetOH and EtAc extracts in the in vitro experiments were selected for further testing of in vivo anti-inflammatory activity. In the in vivo assay, among three doses of MetOH extracts, 300mg/kg BW dose of MetOH extract shown highly significant ($p<0.001$) reduction in the carrageenan induced paw oedema (from 8.48±0.20mm at 1h to 7.67±0.44mm at 4h) compared to diclofenac (from 8.13±0.55mm at 1h to 7.48±0.21mm at 4h). Hence, MetOH extract showed the most promising anti-inflammatory activity in the membrane stabilizing action on HRBC membrane and reduction of oedema in carrageenan induced rat paw model. Hence, the plant could be recommended as a therapeutic agent against the inflammatory diseases.

Keywords: *Anti-inflammatory, Carrageenan, Cholistan desert, Octhochloa compressa Plant extracts, Rats*

INTRODUCTION

Inflammation is a complex pathway of tissue damage occurred in response to harmful stimuli like

pathogens, effects of damaged cells or irritants (1). Inflammation hastwo main classes: acute and chronic; wherein acute inflammation occurs due to any cut or scrape having short duration of action (2). Repeated incidents of acute inflammation give rise to chronic inflammation which is an independent long term response (3). Oxidative stress and inflammation both are inter-linked pathways that lead to pathological conditions like asthma, atherosclerosis, arthritis, liver and kidney diseases, aging, cancer and cardiovascular disease (4). The production of reactive oxygen species is increased during inflammation which led to productionof inflammatory mediators such as chemokines, pro-inflammatory and cytokines that result in the migration of inflammatory cells towards the site (5). So, inflammatory complications are a foremost burden on health and social system (6).

Due to elevated cost and adverse side effects of synthetic steroidal and non-steroidal drugs used to treat these complications, the search for new alternative treatments from plant sources is getting popular with the objectives of minimum side effects and healthier tolerability by the patients (5, 7, 8). The medicinal plants encompass an assembly of diverse compounds that act cooperatively or synergistically on target components of the intricate cellular pathways (9). The*in vitro* and *in vivo* anti-inflammatory potential of different extracts of *S. mukorossi* were assessed and among all extracts, the maximum potential was shown by aqueous extract of the plant (10). While in another study, MetOH extract of *Alpinia zerumbet* leaves showed remarkable *in vitro* antioxidant and anti-inflammatory effects (11).

Pakistan is a country enriched with a heritage of enormous herbal medicines, just like the trend prevailing in most of the developing countries (12). In the recent times, desert plants have gained more attentions due to their survival in the harsh environment and considered as rich source of various active metabolites especially affective in anti-oxidant, anti-microbial, anti-tumour, anti-inflammatory, anti-pyretic, anti-diabetic, antiviral and antifungal potentials (13-15). A wide variety of plant species have been utilized as therapeutic purpose by local nomads (16). *Octhochloa compressa* is a perennial, flowering desert plant (family: *Poaceae*) commonly known as Ghoradhob or Hilu anddistributed in the sandy areas of North-West India, Pakistan and North-East Africa (17). In Pakistan, this plant is distributed in the sandy area of Thailand Cholistan; where it is used as a food source (18, 19). To the best of our knowledge, its comprehensive anti-inflammatory potential has not yet been explored. As part of continuous efforts to explore the new sources of natural compounds with potential biological applications, the current study was aimed to evaluate the*in vitro* and *in vivo* anti-inflammatory potential of *O. compressa* extracts.

MATERIALS AND METHODS

CHEMICAL AND EQUIPMENTS

Methanol, ethyl acetate, *n*-butanol, *n*-hexane and dichloromethane were purchased from Sigma-Aldrich (USA). The equipment used in the study like digital weighing balance (Shimadzu, AY62 Japan), incubator (Memmert, Beschickung-Loading Model 100-800), vernier callipers, rotary evaporator (Heidolph, Laborota 400-efficient) and UV-visible spectrophotometer (Irmeco, U2020) were of international research standards. All the chemicals used in the study were pure and of research grade quality.

PLANT COLLECTION AND PREPARATION OF EXTRACTS

O. compressa whole plant was collected from Cholistan desert in the Bahawalpur region. The plant was cleaned with distilled water and dried under the shade for 10-15 days. Dried plant material was grounded to coarse powder and soaked in a series of non-polar to polar solvents separately; *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, methanol and distilled water. After 3days of occasional shaking at room temperature, the plant material was filtered through muslin cloth and filter paper. Each collected filtrate extracted through a solvent was properly labelled and concentrated to form of a semi-solid paste by subjecting to rotary evaporation (20) and then stored in -20ºC freezer until further studies. The plant yield was calculated by the following formula:

Plant yield = weight of extracted plant residue / weight of plant extract

IN VITRO ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory potential of *O. Compressa* extracts (Aqu, MetOH, *n*-But, EtAc, *n*-Hex and DCM) was evaluated by human red blood cells (HRBC) membrane stabilization method (21, 22). The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment. Collected blood was mixed in equal volume of Alsever solution, centrifuged for 10min at 3000 rpm, packed cells were washed with iso-saline solution and 10% suspension was made. Various concentrations of extracts were prepared (4000, 2000, 1000, 500 and 250µg/ml) and to each concentration 1ml of phosphate buffered saline (PBS), 2ml hyposaline and 0.5ml of HRBC suspension were added. It was incubated at 37ºC for 30min, centrifuged at 3000rpm for 20min and haemoglobin contents of the supernatant solution was estimated at 560nm. Diclofenac was used as a standard reference or control (22). Percentage protection was calculated as:

Percentage protection = 100 - (OD sample / OD control) ×100, where OD = optical density

IN VIVO ANTI-INFLAMMATORY ACTIVITY

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

The albino rats of either sex weighing 170-250g were housed in the animal house zone-2 research laboratory, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. All the rats were placed in polycarbonate cages (size 47×34×18cm^3) by following Organisation for Economic Co-operation and Development (OECD) guidelines under standard laboratory conditions of 12h light/dark cycle at 22±2ºC and 35-60% humidity with free access to standard diet with water ad libitum. Animals were deprived of food but not from water for 8h before the experiment. All the animal experimentation and treatment protocols were ethically approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

CARRAGEENAN-INDUCED OEDEMA MODEL

The extract of *O. compressa* that gave maximum anti-inflammatory potential in the *in vitro* experiment was subjected to *in vivo* anti-inflammatory testing in the Albino rats weighing between 200-250g of either sex as previously described (23). The animals were divided into nine groups with five animals in each group (Table I). According to the protocol, the size of right hind paw of each rat was measured with vernier calliper. The vehicle (Distilled water 5ml/kg BW), diclofenac (15mg/kg BW), MetOH and EtAc extracts of *O. compressa* doses (100, 200 and 300mg/kg BW) were administered intra-peritoneally to each respective group. After 30min of dose administration, the oedema was induced into the supplanter tissue of right hind paw of each rat by injecting 0.1ml carrageenan (1% in saline). The paw size of each rat was measured after 1, 2, 3 and 4h of carrageenan injection and % inhibition was calculated by following formula:

$$\% \text{ inhibition} = N - N_t / N \times 100$$

Where, N = paw size of control group, Nt = paw size of treatment group

STATISTICAL ANALYSIS

The collected data of various experimental studies were interpreted by using two-way Analysis of Variance (ANOVA) through SPSS 20. IC_{50} was calculated from Graph Pad Prism software version 8 and the graphical figures were operated (24).

RESULTS AND DISCUSSION

PLANT EXTRACTION AND YIELD OF EXTRACTS

Medicinal plants comprise great number of chemical substances which differ from each other regarding polarity and chemical properties. Due to different polarity and nature of solvents, each extractyield differently (25). In case of *O. compressa*, the maximum % of extract yield was obtained in the Aqu extract, followed by DCM and MetOH extracts. The minimum extract yield was obtained in *n*-Hex

extract. The overall trend of % yield extract was Aqu>DCM>MetOH>*n*-But>EtAc>*n*-Hex (Table II). The extract yield from same plants highly depends on nature of solvents. The different extracts of same plants having different level of activities are due to presence and abundance of various active metabolites (25).

Table I. Experimental design and treatments of rats for *in vivo* anti-inflammatory experimentation

Group number	Treatment post-Carrageenan induction
Group Ⅰ	Non Carrageenan (Normal saline 5mL/kg)
Group ⅠⅠ	-ve control (Normal saline 5 mL/kg BW)
Group ⅠⅠⅠ	+ve control (Diclofenac 15 mg/kg BW)
Group Ⅳ	100 mg/kg BW of MetOH extract
Group Ⅴ	200 mg/kg BW of MetOH extract
Group Ⅵ	300 mg/kg BW of MetOH extract
Group Ⅶ	100 mg/kg BW of EtAc extract
Group Ⅷ	200 mg/kg BW of EtAc extract
Group ⅠⅩ	300 mg/kg BW of EtAc extract

Table II. Extraction yield of *O. compressa* extracts by various extracting solvents

Solvent	Extraction yield (%)
Aqu	7.16
MetOH	5.80
n-But	4.73
EtAc	3.31
n-Hex	2.52
DCM	6.10

IN VITRO ANTI-INFLAMMATORY ACTIVITY

All six extracts were exhibited moderate to good stabilization effectsin a dose dependent manner by inhibiting hypo-tonicity induced membrane lysis of HRBC. The most significant anti-inflammatory potential was observed by MetOH extract with IC_{50} 231.7±0.678 µg/ml followed by EtAc and DCM extracts with IC_{50} 398.5±1.12 and 442.8±0.97µg/ml, respectively. The overall trend of *in vitro* anti-inflammatory potential by *O. compressa* extracts was: MetOH>EtAc>DCM>Aqu>*n*-Hex>*n*-But (Table III & Fig. 1).

Table III. Anti-inflammatory activity of *O. compressa* extracts and standard drug (diclofenac) by HRBC membrane stabilization method

	% Protection±SEM					
Samples	**4000 µg/ml**	**2000 µg/ml**	**1000 µg/ml**	**500 µg/ml**	**250 µg/ml**	**IC_{50} µg/ml**
Diclofenac	86.15±0.45	75.62±1.28	64.95±0.55	59.35±0.65	44.25±0.95	369.30±0.88
Aqu	79.75±0.45**	72.82±0.52	61.95±0.55	51.45±1.25	29.00±0.50	492.30±1.59
MetOH	85.15±0.35***	77.65±0.66**	71.23±0.44**	60.95±0.22	54.25±0.76	231.70±0.68***
n-But	63.30±0.26	49.50±0.80	37.75±0.55	–	–	2452.40±0.79
EtAc	83.15±0.43***	73.62±0.98**	62.25±0.33	56.35±0.65	44.25±0.95	398.50±1.12*
n-Hex	69.00±0.50	57.88±0.68	45.85±0.25	–	–	1788.10±0.82
DCM	83.15±0.35***	74.82±0.32**	60.95±0.53	55.45±1.25	42.50±0.50	442.80±0.97*

The values are represented as Mean±SEM of triplicate in each group. The results were analysed using two-way ANOVA. IC50 was calculated by Graph pad prism software. Different comparisons between various treatment groups were made with control group. *=P<0.05, **=P<0.01, ***=P<0.001.

Lysozyme (a bactericidal enzyme) and *b*-glucuronidase (an acid protease) are found in the neutrophils, released extra-cellularly and cause tissue inflammation (26). This inflammation can be prevented by stabilization the lysosomal membrane by preventing the release of lysosomal constituents of activated neutrophils (27). Since the erythrocyte membrane is analogous to the lysosomal membrane, therefore the extracts might be able to stabilize the erythrocyte membrane and may also be able to stabilise the lysosomal membranes (22). Several studies have reported that different extracts of medicinal plants mediate their anti-inflammatory effects through RBC membrane stabilization (28-30). MetOH extract of *O. compressa* exhibited maximum *in vitro* anti-inflammatory potential which may be caused due to presence of

active metabolites like terpenoids, flavonoids and other related polyphenols (21). The promising potential of MetOH and EtAc extracts prompted to investigate their *in vivo* anti-inflammatory potential.

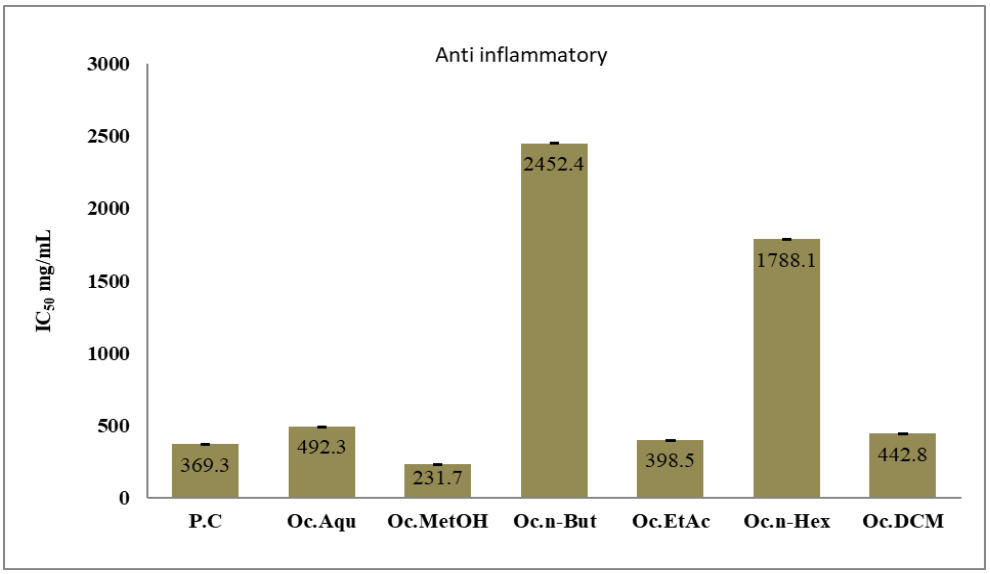

Fig. 1. IC_{50} of anti-inflammatory potential by crude extracts of *O. compressa* compared to positive control (P.C. = Diclofenac)

CARRAGEENAN INDUCED PAW OEDEMA MODEL

The size of rat paw was extensively measured to test the anti-inflammatory potential of compounds compared to the drug. After 1h of carrageenan induction, the paw oedema was progressively increased, giving the maximum appearance at 4thh and later reduced toward basal point after 5thh (31-33). The standard drug Diclofenac (15mg/kg BW) in the +ve control group showed a significant reduction ($p<0.001$) of rat paw inflammation at 2ndh, measured after each hour up-to 4thh when compared with -ve control group. It was observed that the MetOH extract of *O. compressa* at the doses of 200mg/kg and 300mg/kg BW have shown significant and highly significant decrease in paw oedema with maximum effect at 3rdh ($p<0.01$) and 4thh ($p<0.001$) of carrageenan administration, respectively. Among all doses of MetOH extract, 300mg/kg BW showed maximum reduction of right hind paw oedema ($p<0.001$) (Table IV & Fig. 2). The EtAc extract of *O. compressa* at the dose of 100mg/kg didn't show any reduction in paw oedema, rather, it caused an increase in the size of oedema, while the 200mg/kg BW and 300mg/kg BW doses have shown somewhat in-significant reduction in the paw oedema with max effect at 4thh ($p<0.05$) (Table V & Fig. 3). The extract showing the reduction in the paw size by carrageenan induced inflammation showed inhibitory effects on the release of serotonin and histamine in early phase and inhibition of arachidonic acid in the later phases. Therefore, from these results it was concluded that MetOH extract of *O. compressa* has the ability to reduce the inflammation in all phases by inhibiting the inflammatory mediators.

Table IV. Anti-inflammatory activity of crude methanol extracts of *O. compressa* in the carrageenan induced paw oedemamodel in rats

Treatment	Paw size (mm)				
	0 h	1 h	2 h	3 h	4 h
Normal control	7.33±0.37	7.33± 0.37	7.34±0.56	7.33±0.29	7.33±0.421
Negative control	7.45±0.33	9.08±0.03	9.21±0.48	10.11±0.38	10.55±0.44
Positive control	7.22±0.32	8.13±0.55	7.67±0.39	7.59±0.44	7.48±0.21
MetOH (100 mg/kg)	7.37±0.41	8.73±0.09	8.66±0.24	8.53±0.21	8.33±0.18
MetOH (200 mg/kg)	7.33±0.27	8.43±0.27	7.83±0.37*	7.82±0.33*	7.78±0.30*
MetOH (300 mg/kg)	7.39±0.23	8.481±0.20	7.71±0.38***	7.69±0.08***	7.67±0.44***

The values are represented as Mean±SEM of triplicate in each group. The results are analysed using two-way ANOVA. Different comparisons between various treatment groups were made with control group. *=P<0.05, ***=P<0.001. Positive control: Diclofenac, Negative control: normal saline.

Development of inflammation in the paw of a rat is a tri-phasic event with contribution of various inflammatory mediators. The initial phase-I during the first 2h after injection of carrageenan is due to

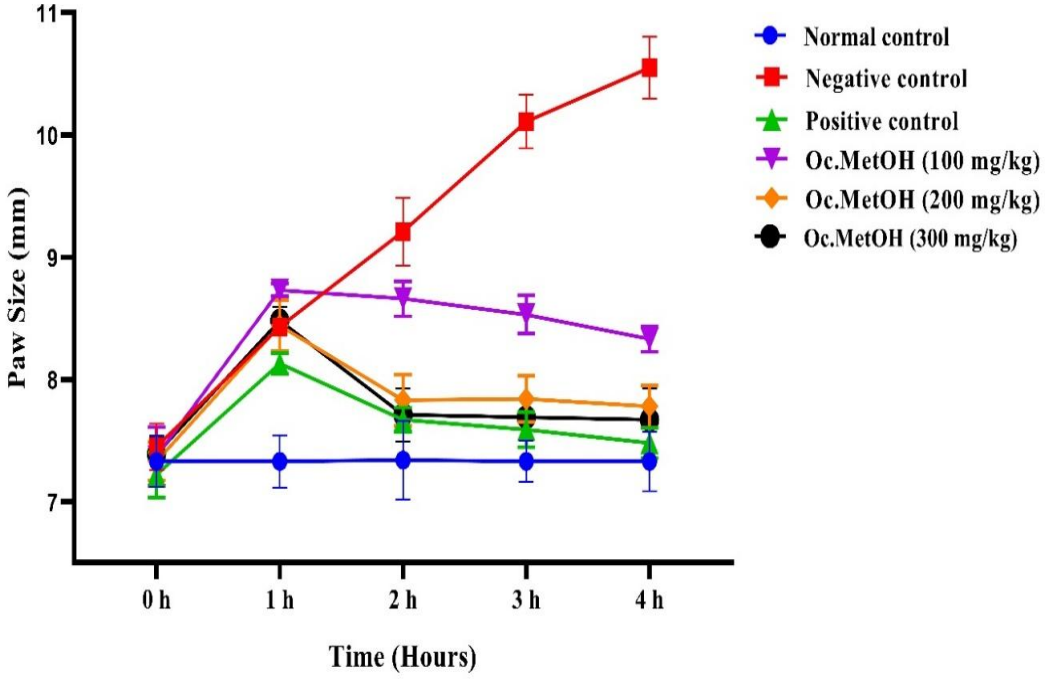

Figure 2: Anti-inflammatory effects of *O. compressa* MetOH (methanol) extracts on the inflammation of carrageenan induced rat paws

production of specific inflammatory mediators *i.e.* histamine and serotonin. The intermediary 2nd phase of oedema (2-2.5h) occurred due to production of lysosome, kinin and histamine and the 3rd phase of oedema (2.5-6h) is due to production of prostaglandins; PGE2, this last phase is quite sensitive to most of the clinical anti-inflammatory drugs (34). Prostaglandins are the major causative agents of acute inflammation. Although, inflammation proceeds by cyclo-oxygenase and lipoxygenase pathways (35, 36), Nitic oxide is formed at the site of inflammation due to various factors including endothelial cells, sensory nerve cells and leukocytes (31). Following the boost up of vascular permeability, cell infiltration proceeds chiefly due to neutrophils in the acute phase, that results in the inflammation by release of mediators such as oxygen-derived free radicals (2). Previously, it was observed that paw oedema induced in rat by Carrageenan showed all the cellular and biochemical features that are usually present in the inflammatory phases (37). Therefore, it could be concluded that the anti-inflammatory potential of selected plant extracts is due to inhibition of these mediators like kinin, histamine and prostaglandins (38). All doses of extract showed a dose dependant anti-inflammatory potential at 3rdh to 4thh post-injection might be due to existence of polyphenols and flavonoids in the extracts. Flavonoids also have an important role in the inflammatory process via various routes due to the blockage of COX (38, 39).

Table v. Anti-inflammatory activity of crude ethyl acetate extract of*O. compressa* in the carrageenan induced paw oedema model in rats

Treatment	Paw size (mm)				
	0 h	1 h	2 h	3 h	4 h
Normal control	7.33±0.365	7.33±0.37	7.34±0.56	7.33±0.29	7.33±0.421
Negative control	7.45±0.33	9.08±0.03	9.21±0.48	10.11±0.38	10.55±0.44
Positive control	7.22±0.32	8.13±0.55	7.67±0.39	7.59±0.444	7.48±0.21
EtAc (100 mg/kg)	7.39±0.43	8.33±0.28	8.55±0.32	8.65±0.19	8.77±0.28
EtAc (200 mg/kg)	7.22±0.22	8.66±0.16	8.43±0.41	8.18±0.21*	8.10±0.15*
EtAc (300 mg/kg)	7.28±0.33	8.54±0.25	8.55±0.43	8.48±0.35	8.39±0.13

The values are represented as Mean±SEM of triplicate in each group. The results are analysed using two-way ANOVA. Different comparisons between various treatment groups were made with control group. *=P<0.05. Positive control: Diclofenac, Negative control: normal saline.

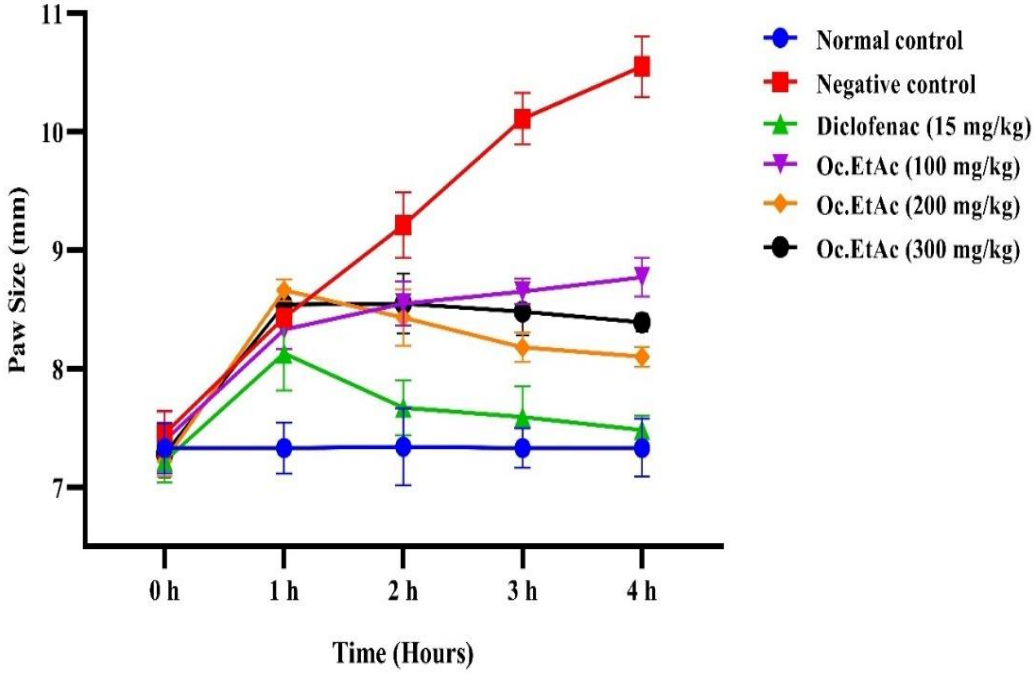

Figure 3: Anti-inflammatory effects of EtAc (ethanol) extracts of *O. compressa* on the inflammation of carrageenan induced rat paws

CONCLUSION

The current study has established the medicinal importance of *O. compressa* through demonstration of its *in vitro* and *in vivo* anti-inflammatory activities. According to the anti-inflammatory activity, among all extracts, the MetOH extract has exhibited remarkable potential with 85% protection of HRBC lysis (IC_{50} 231.7±0.678µg/ml; $p<0.001$), even better than standard drug (diclofenac) by *in vitro* method. In case of *in vivo* anti-inflammatory assay, among three doses of MetOH extracts, 300 mg/kg BW dose has shown highly significant ($p<0.001$) reduction in the carrageenan induced paw oedema (from 8.48±0.20mm at 1h to 7.67±0.44mm at 4h) as compared to diclofenac (from 8.13±0.55mm at 1h to 7.48±0.21mm at 4h). Hence, the study validates that the use of *O. compressa* could be an interesting source of anti-inflammatory agent with a potential use in different pharmaceutical preparations.

Conflict of interest:

There is no conflict of interest among the authors.

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