



Antinociceptive and Antiinflammatory Effects of the Methanolic extract of *Oscillatoria annae*

Rajavel R.¹, Mallika P.², Rajesh V.³, Pavan Kumar K.¹, Krishna Moorthy S.² and Sivakumar T.¹

¹Department of Pharmacy, Nandha College of Pharmacy and Research Institute, Erode-638 052, Tamil Nadu, INDIA

²Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, INDIA

³Department of Pharmacology, J.K.K. Nataraja College of Pharmacy, Komarapalayam-638183, Tamil Nadu, INDIA

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Abstract

The present study was designed to investigate antiinflammatory and antinociceptive activities of *Oscillatoria annae*. The antiinflammatory activity were studied in both acute and chronic inflammation models, where as by using four different animal models were employed to evaluate the antinociceptive activity of the extract in the acute model, carrageenan, dextran, histamine and serotonin were used to induce the inflammation in rat hind paw and cotton pellet enhanced granuloma method was used for chronic inflammation model. Acetic acid-induced writhing method, hot plate method, tail flick response and tail immersion method were used to evaluate the antinociceptive effect of the extract. The methanolic extract of *Oscillatoria annae* exhibited significant dose-dependent activity on the tested experimental animal models, and also on the extract significantly reduced in the acetic acid-induced abdominal contractions and the increased reaction time of mice in hot plate method, tail flick response and tail immersion method. This study has shown that the methanolic extract from the *Oscillatoria annae* does possess significant antiinflammatory and antinociceptive activity in animal models at the doses tested and the results were comparable to those observed for the standard drugs indomethacin, acetyl salicylic acid and morphine.

Key words: *Oscillatoria annae*, antinociceptive, antiinflammatory.

Introduction

Medicinal herbs have been used as form of therapy for the relief of pain throughout history¹. A case in point is *Oscillatoria annae* popularly known as blue green algae. The exploration of the chemical constituents from plants, pharmacological and phytochemical screening would provide the basis for developing the new lead molecules in strategic favor of natural product drug discovery². The discovery and development of isolating a new efficient, active and less toxic molecule for systemic activities was the aim and subject of many researchers^{3,4}. It is represented by a set of much more varying forms regarding with their morphology and physiology⁵. The algae, widely used in the human body and is constantly being exposed to foreign organism such as bacteria, virus, fungi and number of parasites, which co-exists to a certain degree in the skin, mouth, respiratory tract, intestinal tract and genital tract. Some of the microorganisms are essential for optimal health care for a healthy individual human but, and are well placed to keep on organisms from becoming a problem. The biologically active agents from natural sources have always been of great interest to working on various diseases⁶.

On the other hand, the natural barriers are being exposed to highly effective infectious microbes, severe disease may result, and the algae is useful in the management of enlargement and

obstruction of liver, spleen and kidney. *O. annae* are among the one of the worlds most ancient inhabitants. The algae which are singled celled that live in the fresh water, brackish water and marine water. In the presence of sunlight it can prepare their food for their survival. In warm nutrient rich environments, microscopic cyanobacteria can grow quickly, thus providing nitrogen fertilizer for rice and beans.

Evidence from the existing information shows the plant may possess hepatoprotective effect and is used in the treatment of chronic diarrhoea. There is no scientific information about the traditional, phytochemical, pharmacological and toxicological activities of this plant. As a part of continuing research for a novel plant derived agent, a methanolic extract of *O. annae* was preliminarily screened for antiinflammatory and antinociceptive activities and it showed promising effects on the various animal models. This present study was preformed in order to investigate its antiinflammatory and antinociceptive activities on both acute and chronic inflammation.

Material and Methods

Plant material: The *O. annae* was collected from Trichirappalli district of TamilNadu, India. The material was taxonomically identified by Botanical Survey of India, Trichy. The collected material was dried under shade; it was powered with a

mechanical grinder and stored in an air tight container. The dried powder material of the algae was macerated for seven days. During maceration the whole content was warmed twice a day at an interval of 12 hrs. At the end of seventh day the extract was filtered by using the muslin cloth. The extract was concentrated at the hot condition and it changes to a semisolid mass. The dried MEOA was suspended in normal saline and used for the present study.

Screening of the Phytochemical Compounds: The extract was screened for the presence of various constituents employing standard screening tests⁷. Conventional protocol was used for detecting the presence of steroids, alkaloids, tannins, flavonoids, and glycosides.

Animals: Studies were carried out by using male wistar albino rats weighing 180-200 gm and male swiss albino mice weighing 20-22 gm. They were obtained from the animal house of IRTT Medical College, Erode. The mice were grouped and housed in poly acrylic cages (38×23×10 cm) with not more than six animals per cage and maintained under standard laboratory condition (temperature 25 ± 2 °C) with dark/light cycle (14/10 hr.). The animals were observed under laboratory conditions for ten days before commencement of the experiment. All the procedures described, were reviewed and approved by the university Animal Ethical Committee.

Chemicals: Carragenin (SD fine chemicals limited, Bombay), 5-hydroxy tryptamine hydrochloride (serotonin), histamine (Sigma USA) were used in this study. The drugs of Indomethacin (Recon, Bangalore), Aspirin (USV, Bombay) and Morphine (MM Pharma, New Delhi), were used as standard drugs.

Acute toxicity test: The animals were divided into six groups each containing eight animals. MEOA was suspended in normal saline and administered orally as a single dose to each group of mice at different concentrations (250, 500, 750, 1000, 1250 and 1500 mg/kg b.w.) and the animals were observed for a 72 hr period. The number of deaths were expressed as a percentile and LD₅₀ was determined by probit a test, using the death percentage Vs the log dose⁸.

Antiinflammatory activity: Carrageenan induced rat hind paw oedema model: The rats were alienated into five groups (n=6). Group 1 served as a control and received the normal saline (0.9% NaCl, 5 ml kg⁻¹ b.w.) and the group 2, 3 and 4 were treated orally with MEOA 200, 400 and 600 mg/kg b.w. respectively. Group 5 received the standard drug indomethacin (10 mg kg⁻¹ b.w.). The administration of test drug was 30 minutes prior to injection of 0.1 ml of 1% w/v freshly prepared suspension of carrageenan in normal saline to the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1st, 2nd, 3rd and 4th hr, after the carrageenan injection by using plethysmometer⁹. The antiinflammatory effect of MEOA was calculated by the following equation.

Antiinflammatory activity (%) = $(1-D)/C \times 100$, where D represents the percentage difference in paw volume after administration of the drugs to the rats, C represents the percentage difference of volume in the control groups¹⁰.

Dextran-induced rat hind paw oedema model: The animals were treated as like to that of carrageenan induced paw oedema model, dextran (0.1, 1 % w/v in normal saline) was used in the place of carrageenan¹¹.

Histamine-induced rat hind paw oedema model: The right feet of the rat were induced by subplantar injection of 0.1 ml of 1% w/v of freshly prepared histamine in normal saline and the paw volume was measured as mentioned earlier⁵.

Serotonin-induced rat hind paw oedema model: In serotonin model, oedemas of the right hind paw of the rat were induced by subplantar injection of 0.1 mL of 1% w/v freshly prepared serotonin in normal saline. The groups and the treatment procedure of the animals were same as like the carrageenan-induced rat hind paw oedema model and the paw volume was observed¹¹.

Cotton pellet induced granuloma: The cotton pellets induced granulomas in rats were studied¹². Five groups of six animals in each group were selected. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were injected subcutaneously on either sides of the groin region of each rat. Group 1 which served as control and received the vehicle (normal saline, 5 mL kg⁻¹ b.w.). The extract of MEOA at the concentration range of 200, 400 and 600 mg kg⁻¹ b.w was administered orally to groups 2, 3 and 4 respectively for seven consecutive days from the day of cotton pellet implantation. Group 5 received standard Indomethacin (10 mg kg⁻¹ b.w.) at the same period.

On the 8th day, animals were anaesthetized and the granuloma tissues with their pellets are carefully removed and are separated from the extraneous tissues. The wet pellets were collected, weighed and then dried at 60°C for 24 h to a constant weight and then dried cotton pellets were weighed again. The increment in the dry weight of the pellets was taken as a measure the granuloma formation. The antiproliferative effect of MEOA compared with control.

Antinociceptive activity: Evaluation of antinociceptive properties of *O. annae* extract was carried out by chemical, mechanical and thermal noxious stimuli.

Acetic acid-induced writhing method: Mice were divided into 5 groups of 6 animals in each group were selected¹³. The 3 groups receive the different doses MEOA (200, 400, and 600 mg kg⁻¹ b.w.) and group-4 administrated with standard drug acetyl salicylic acid (100 mg kg⁻¹ b.w.) orally in prior to the injection of acetic acid; and the other group received the normal saline (0.9 % NaCl 5ml kg⁻¹ b.w.). Writhing was induced by

administering 10 ml kg^{-1} b.w. of acetic acid solution (0.6%) intraperitoneally. After the small interval time (in minutes) after acetic acid injection the mice were observed in a transparent box and then the number of writhes were counted for a period of 10 min. The writhing movements were measured as concentration of the abdominal muscles accompanied by stretching of hind limbs. There was a significant reduction in the number of writhes by drug treatments as compared to vehicle-treated animals. As per above considerations a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically.

Hot plate method: The 5 groups of 6 mice in each were selected for the present study¹⁴. The group 1 animals served as control which received the vehicle (normal saline, 5 ml kg^{-1} b.w.). The extract MEOA at concentrations of 200, 400 and 600 mg kg^{-1} b.w. were administered orally to group 2, 3, and 4 respectively and group 5 received the standard drug morphine (5 mg kg^{-1} b.w, s.c). The mice was placed on an aluminum hot plate and kept the temperature for 55 ± 0.5 °C for a maximum time of 30 sec¹⁵. The reaction time was noted at the time of animals showing the licked response of their fore and hind paws. The responses were recorded at 0, 15, 30, 45 and 60 min after administration of test drug. The mice reacted within 15 sec and did not show large variation when tested on four separated occasions.

Tail flick response: Mice were randomly assigned to five groups of six animals each. The control group received normal saline (0.9 % NaCl, 5 ml kg^{-1} b.w.). The methanol extract of *O. annae* were administered at the doses of 200, 400 and 600 mg kg^{-1} b.w. to the 2nd, 3rd and 4th group respectively, standard drug morphine (5mg kg^{-1} b.w, s.c) was given to the 5th group, which served as standard. Analgesic activity was measured after 30 minutes of administration of test and standard drugs¹⁵. The tail of each mouse was placed on the nichrome wire of an analgesiometer and it was fixed at 5.5 ± 0.5 amp. The time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The reading was taken after 30 min of administration of test drugs. The mice reacted within 15 sec and which did not show any large variation in the studies.

Tail immersion method: The 5 groups of 6 animals each in a group were selected. Group 1 received normal saline (0.9 % NaCl, 5 ml kg^{-1} b.w.) (control) and group 2, 3 and 4 received 200, 400 and 600 mg kg^{-1} b.w. of MEOA respectively. Group 5 received the standard drug morphine (5 mg kg^{-1} b.w, s.c). The tail (upto 5 cm) was dipped into a pot of water to maintain at 55 ± 0.5 °C. The time in seconds to withdraw the tail from the water was observed as the reaction time. The readings were taken after 30 hrs of administration to that of test drug. The mice reacted within 15 sec and did not show any large variation.

Statistical analysis: The values are mean \pm SEM statistical significance was determined by ANOVA, followed by student t-test value with $p < 0.001$.

Results and Discussion

Phytochemical screening: Preliminary phytochemical screening of the methanol extract *Oscillatoria annae* revealed the presence of tannins, steroids, alkaloids, glycosides and flavonoids. Further separation of the specific phytochemical studies is in progress.

Acute toxicity study: In the acute toxicity assay, there no deaths were observed in period of the 72 hrs, the experimental animals showed there no stereotypical symptoms associated with toxicity such as convulsion ataxia, diarrhoea or increased diuresis. The medium lethal doses (LD50) were determined to be higher than the highest dose tested that is 2.09 kg^{-1} b.w.

Anti-inflammatory activity: Carrageenan-induced rat hind paw oedema model: In these studies, the extract of *O. annae* by using methanol showed a significant inhibitory effect on the oedema formation from 1 to 4 hrs. The highest inhibitory effect were found during the period of 3rd hour, the inhibition was found to be 16.13, 27.42 and 33.87 % ($p < 0.001$) at the given doses of 200, 400 and 600 mg kg^{-1} b.w. of MEOA, respectively. These findings were comparable to the standard drug indomethacin (10 mg kg^{-1} b.w.) and the inhibition was found to be 38.71 % as shown in table 1.

Dextran-induced rat hind paw oedema model: Both of MEOA (200, 400 and 500 mg kg^{-1} b.w.) and indomethacin (10mg kg^{-1} b.w.) significantly decreased in the dextran-induced paw oedema ($p < 0.001$) MEOA at the dose of 17.65, 39.22 and 47.06 % respectively and results were compatible to that of the standard drug indomethacin (50.98%) as shown in table 2.

Histamine-induced rat hind paw oedema method: The results showed that the methanol extract at the doses of 200, 400 and 600 mg kg^{-1} b.w, significantly ($p < 0.001$) reduced the oedema formation of the rat paw at 1, 2 and 3 hr, after histamine injection. The effects were dose-dependent at the doses tested (200, 400 and 600 mg kg^{-1} b.w.) peak inhibitory effects 33.33 % and 43.86 % were observed for 200, 400 and 600 mg kg^{-1} b.w respectively as shown in table 3.

Serotonin induced rat hind paw oedema model: There was a dose dependant significant ($p < 0.01$) reduction in serotonin induced rat paw oedema at 200, 400 and 600 mg kg^{-1} b.w. of MEOA and at 10 mg/kg indomethacin over a period of 4 hr. as shown in table 4. The MEOA at the dose of 600 mg kg^{-1} b.w. exhibited maximum activity of 41.67 % while the standard drug Indomethacin (10 mg kg^{-1} b.w.) showed a 45.83 % inhibition.

Cotton pellet induced Granuloma: It was seen that MEOA was responsible for anti-inflammatory effect which would be calculated depending on the wet and dry weight of cotton pellets. According to these results the antiproliferative effect of MEOA (500 mg kg^{-1} b.w) and indomethacin (10 mg kg^{-1} b.w.) were calculated as 58.82 % and 61.03 % respectively. The dried

cotton pellets which induce the antiproliferative effect and it were calculated on the basis of dry weight pellets. The MEOA inhibited portion of inflammation was found to be 600 mg kg⁻¹ b.w and indomethacin (10 mg kg⁻¹ b.w.) were established as 53.14 % and 60.62 % (p<0.01), respectively as shown in table 5.

Antinociceptive activity: The extract at the dose of 200, 400, and 600 mg kg⁻¹ b.w. showed a significant antinociceptive effect in all the four different models for nociception used to investigate the antinociceptive effect of the MEOA results were dose dependant.

Acetic acid induced writhing method: The methanol extract from *O. annae* strongly reduced the abdominal contractions by inducing the intraperitoneal administration of acetic acid solution. The effects produced by MEOA were dose-dependant and the values were found to be significant (p<0.01) at the doses tested, when compared to control. MEOA at the doses of 200, 400 and 600 mg kg⁻¹ b.w. exhibited 21.15, 42.31 and 59.62 inhibition respectively, where the inhibition of the standard drug acetyl salicylic acid was found to be 69.23 % as shown in table 6.

Hot plate method: The MEOA produced significant (p<0.01) analgesic activity at all the doses tested. In this method MEOA considerably increased the animal's reaction time to the heat stimulus. The values were found to be significant and dose-

dependant. The highest reaction time of 7.81 ± 0.1* was observed at a dose of 600 mg kg⁻¹ b.w. compared with the control group value of 2.12 ± 0.1. The results were comparable to that of the standard drug morphine (8.65 ± 0.1) as shown in table 7.

Tail flick response: The results showed that the administration of MEOA at different doses (200, 400 and 600 mg kg⁻¹ b.w.) and standard drug morphine (5 mg kg⁻¹ b.w, s.c.). The mouse tail reaction time were noted when the animal's tail was subjected to heat generated by the tail flick apparatus meanwhile the response to heat stimuli of the control group was not altered during the period of an experiment. The administration of MEOA at the doses of 200, 400 and 600 mg kg⁻¹ b.w. exhibited 34.38, 68.75 and 84.38 % inhibition respectively, while as the standard drug morphine produced 90.63 % inhibition as shown in table 8.

Tail immersion method: In the tail immersion method the extracts were considerably increased, when the animal's subjected to heat stimulus and reaction time were noted. The values were found to be significant (p<0.01) and the effects were dose-dependant at the dose tested. Pre-treatment with MEOA at the doses of 200, 400 and 600 mg kg⁻¹ b.w. showed the 41.18, 64.71 and 82.35 % inhibition respectively and the % inhibition produced by standard drug morphine was found to be 91.18 % as shown in table 9.

Table-1
Effect of *Oscillatoria annae* extract on carrageenan-induced rat hind paw oedema

Treatments	Dose (mg kg ⁻¹)	Paw volume differences (ml)			
		1h	2h	3h	4h
Control (0.9%NaCl)	5ml	0.61±0.03	0.65±0.9	0.62±0.2	0.61±1.2
MEOA	200	0.59±0.10 (3.28)	0.54±0.08 (16.92)	0.52±1.6** (16.13)	0.49±1.6* (19.67)
MEOA	400	0.55±0.32 (9.84)	0.49±1.4** (24.62)	0.45±1.2* (27.42)	0.41±0.8 (32.79)
MEOA	600	0.51±0.9* (16.39)	0.46±1.2 (29.23)	0.41±0.8* (33.87)	0.38±0.5* (37.70)
Indomethacin	10	0.50±1.6* (18.03)	0.43±0.08* (33.85)	0.38±0.9* (38.71)	0.37±0.7 (39.34)

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, *p<0.001;**p<0.01 experimental groups compared with control group

Table-2
Effect of *Oscillatoria annae* extract on Dextran--induced rat hind paw oedema

Treatment	Dose (mg kg ⁻¹)	Paw volume differences (ml)			
		1h	2h	3h	4h
Control (0.9%NaCl)	5ml	0.54±0.01	0.55±0.02	0.52±0.01	0.51±0.02
MEOA	200	0.48±0.08 (11.11)	0.47±0.1 (14.55)	0.45±0.02** (13.46)	0.42±0.01 (17.65)
MEOA	400	0.45±0.1* (16.67)	0.38±0.01* (30.91)	0.32±0.03* (38.46)	0.31±0.01* (39.22)
MEOA	600	0.41±0.02* (24.07)	0.35±0.02* (36.36)	0.28±0.04* (46.15)	0.27±0.01* (47.06)
Indomethacin	10	0.39±0.03* (27.78)	0.31±0.04* (43.64)	0.26±0.01* (50.00)	0.25±0.01 (50.98)

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, *p<0.001;**p<0.01 experimental groups compared with control group

Table-3
Effect of *Oscillatoria Annae* extract on Histamine-induced rat hind paw oedema

Treatments	Dose (mg kg ⁻¹)	Paw volume differences (ml)			
		1h	2h	3h	4h
Control (0.9%NaCl)	5ml	0.58±1.0	0.59±1.2	0.57±1.1	0.57±0.72
MEOA	200	0.51±1.2 (12.07)	0.49±1.3** (16.95)	0.46±0.9** (19.29)	0.42±0.82 (26.32)
MEOA	400	0.48±1.1* (17.24)	0.41±1.2 (30.51)	0.38±1.1* (33.33)	0.34±0.9* (40.35)
MEOA	600	0.42±1.2* (27.59)	0.37±1.5 (37.29)	0.32±1.2* (43.86)	0.29±0.1* (49.12)
Indomethacin	10	0.40±0.1* (31.03)	0.35±1.0* (40.68)	0.30±1.3* (47.37)	0.27±0.1* (52.63)

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, *p<0.001;**p<0.01 experimental groups compared with control group

Table-4
Effect of *Oscillatoria Annae* extract on Serotonin-induced rat hind paw oedema

Treatments	Dose (mg kg ⁻¹)	Paw volume differences (ml)			
		1h	2h	3h	4h
Control (0.9%NaCl)	5ml	0.45±1.2	0.48±1.4	0.46±1.3	0.45±1.6
MEOA	200	0.41±0.9* (8.89)	0.39±1.2 (18.75)	0.37±1.4** (19.57)	0.35±1.6** (22.22)
MEOA	400	0.38±0.8* (15.56)	0.32±1.1* (33.33)	0.30±0.88* (34.78)	0.29±1.2* (35.56)
MEOA	600	0.36±0.2* (20.00)	0.28±1.2** (41.67)	0.26±0.7* (43.48)	0.25±0.2* (44.44)
Indomethacin	10	0.32±1.4* (28.89)	0.26±1.7* (45.83)	0.23±1.2* (50.00)	0.22±1.4* (51.11)

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, *p<0.001;**p<0.01 experimental groups compared with control group

Table-5
Effect of *Oscillatoria Annae* extract on cotton pellet-induced granuloma in rat

Treatment	Dose (mg kg ⁻¹)	Paw volume differences (ml)			
		1h	2h	3h	4h
Control (0.9 % NaCl)	5ml	175.14±0.31	--	46.12±0.78	--
MEOA	200	127.15±0.15*	27.40	31.36±0.13*	32.00
MEOA	400	85.17±0.03*	51.37	25.12±0.12*	45.53
MEOA	600	72.12±0.1	58.82	21.61±0.05*	53.14
Indomethacin	10	68.25±2.3*	61.03	18.16±0.14*	60.62

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, *p<0.001 experimental groups compared with control group

Table-6
Effect of *Oscillatoria Annae* extract on acetic acid-induced writhing test in mice

Treatments	Dose (mg/kg ⁻¹)	No. of Writhes	Percentage inhibition
Control (0.9 % NaCl)	5 ml	52 ±0.01	--
MEOA	200 ml	41±0.02**	21.15
MEOA	400 ml	30 ±0.04**	84.38
MEOA	600 ml	21 ±0.01**	59.62
ASA	100	16 ±0.02***	69.23

Values are mean ±SEM (n=6) for control and mean ±SEM (% of inhibition) (n=6) for each treatment, **:p<0.01; ***:p<0.05 experimental groups compared with control group.

Table-7
Effect of *Oscillatoria Annae* extract on hot plate test in mice

Treatments	Dose (mg kg ⁻¹)	Latency (S)				
		0min	15min	30min	45min	60min
Control (0.9%NaCl)	5ml	2.32±0.1	2.25±0.1	2.61±0.1	2.42±0.1	2.12±0.1
MEOA	200	2.62±0.3	3.45±0.1*	3.9±0.21*	4.16±0.1*	4.21±0.2*
MEOA	400	2.41±0.5	4.92±0.2*	5.45±0.16*	5.82±0.2*	6.12±0.3*
MEOA	600	2.43±0.2	5.71±0.3*	6.25±0.4*	7.12±0.1*	7.81±0.1*
Morphine	5	2.42±0.2*	6.21±0.21*	7.54±0.1	8.16±0.2	8.65±0.1

Values are mean ±SEM (n=6) *:p<0.01 experimental groups compared with control group

Table-8
Effect of *Oscillatoria Annae* extract on tail flick response in mice

Treatments	Dose (mg kg ⁻¹)	Reaction Time (s)	Percentage Inhibition
Control (0.9%NaCl)	5ml	3.2 ±0.22	--
MEOA	200	4.3 ±0.21**	34.38
MEOA	400	5.4 ±0.20**	68.75
MEOA	600	5.9 ±0.1**	84.38
Morphine	5	6.1 ±0.31**	90.63

Values are mean ±SEM (n=6), **:p<0.01 experimental groups compared with control group

Table-9
Effect of *Oscillatoria Annae* extract on tail immersion in mice

Treatments	Dose (mg kg ⁻¹)	No. of writhes	Percentage Inhibition
Control (0.9%NaCl)	5ml	3.4 ±0.31	--
MEOA	200	4.8 ±0.16*	41.18
MEOA	400	5.6 ±0.21**	64.71
MEOA	600	6.2 ±0.24**	82.35
Morphine	5	6.4 ±0.31**	91.18

Values are mean ±SEM (n=6), *p<0.001; **p<0.01 experimental groups compared with control group

In living animal tissues, inflammatory processes involve the release of several mediators, including prostaglandins, histamines, thermo-attractants, cytokines, proteinases and so on, as well as substance that regulate adhesion of molecules and the processes of cell migration activation and degranulation¹⁶. Among several traditional claims, the usefulness of the extract in inflammation and pain has been emphasizing more literature. Hence it was consider that investigation for these medicinal properties might give scientific authentication to the traditional claims moreover. The extract of MEOA has not been subjected to the above mentioned systemic pharmacological screening so far. The inflammatory responses observed from asthatic patients, bone and joints inflammation, microbial infection, anaphylaxis and allergic conditions and so forth. Thus the adoption of different anti-inflammatory and analgesic experimental model for the assessment of phytomedicines used in the traditional health care system for the management of pain, asthma, arthritis rheumatism and so on, are considered desirable and justifiable¹⁷.

The present studies were established for anti-inflammatory activity of the methanol extract of *O. annae*. Carrageenan-induced rat paw oedema model is one of the most widely used primary tests for the screening of new anti-inflammatory agents¹⁸. The method used frequently to assess the anti edematous effects of natural products. Development of edema in the paw of the rat after injection of carrageenan is a biphasic event¹⁴. The initial phase hyperemia observed during the first hour, is attributed to the release of histamines and serotonin¹⁹. The delayed oedema can also be observed due to the release of bradykinins and prostaglandins²⁰. The second phase may be explained by injecting dextran in induced paw oedema and is known to be mediated both by histamines and serotonin²¹. Dextran which induces the fluid and accumulated due to mast cell generation in protein rich exudate containing of large number of neutrophils²². The present study established indicates that MEOA (200, 400 and 600 mg kg⁻¹ b.w.) and indomethacin play a crucial role as protective factors against carrageenan induced acute inflammation. The extract produced marked inhibition a test which has significant observable value for anti-inflammatory agents acting by mediator inhibiting of acute inflammation²¹.

The carrageenan-induced paw inflammation test largely used to study both steroidal and non-steroidal anti-inflammatory drugs. Carrageenan induces anti-inflammatory reaction in 2 different phases. The initial phase occurs between 0 and 2 hr after injection of carrageenan has been suggested to the release of histamines, serotonin and bradykinins on vascular permeability¹⁹. The inflammation volume reaches its maximum approximately 3 hr post treatment after which it begins to decline²³. The response of late phase which acted as complement dependent reaction has been shown to be due to over production of prostaglandin in tissues²⁰. The result obtained from the present study suggests that the inhibitory activity of the extracts compared in the first phase of

carrageenan-induced inflammation may be due to inhibition of early mediators such as histamine and serotonin. The action on second phase study involved due to the inhibition of bradykinins and prostaglandins. The extract can be able to inhibit the inflammation from the first hr acting on both the early as well as the late phases. The extract also effectively inhibited the inflammation produced by dextran, histamine and serotonin which suggest that the anti-inflammatory activity of methanol extract of *O. annae* is possibly mediated by inhibiting the action of these mediators.

Therefore, it is explained about that the mechanism of action of, MEOA may be related to prostaglandin synthesis inhibition as described for the anti-inflammatory mechanism of indomethacin in the inhibition of the inflammatory process induced by carrageenan²⁴. Likewise the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases. Thus the result of the cotton pellet implantation model for anti-inflammatory activity supports the crude methanolic extract of *O. annae* anti-inflammatory activity. Inhibition of prostaglandin synthesis could give rise to analgesic activity¹⁹. The extract was further investigated for its possible antinociceptive activity. Four different animal models were employed to investigate the potential antinociceptive activity of methanol extract of *O. annae* in this present study. The methods for investigating antinociception were selected such that both centrally and peripherally mediated effects were investigated. Acetic acid-induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity²⁵. It is also called the abdominal constriction response, and it was very sensitive and can able to detect antinociceptive effect of compounds and dose levels that may appear inactive in other models like the tail flick test²⁶. Acetic acid is used to induce writhing which causes algesia by liberation of endogenous substances which in turn excite the pain nerve endings. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response. This method has been associated with prostanoids in general examples increase level of PGE₂ and PGF₂ in the peritoneal fluids²⁷. According to the percentage of inhibition on the number of writhes obtained with different doses of MEOA, we found that the response of the analgesic effect was equivalent to that of standard drug acetyl salicylic acid.

Aspirin and related drugs can inhibit cyclo-oxygenase in peritoneal tissues thus interfering with mechanism transduction in primary afferent nociceptors. The responsive results of acetic acid induced writhing strongly recommends the mechanism of *O. annae* extract may be linked partly to the blockade or release of endogenous substances like lipoxygenase and cyclo-oxygenase.

The analgesic activity were characterized by hot plate test of the extract by using morphine, as reference drug also produced a significant antinociceptive effect during all the observation

times when compared with control values. The hot plate method is considered to be selective for opioid like compounds in several animal species, but other centrally acting drugs including sedatives and muscles relaxants have also shown activity in this test²⁸. The validity of this test has been shown even in the presence of substantial impairment of motor performance²⁹. Thus the result obtained from the present study indicates that the methanol extract of *O. annae* relived the pain through both central and peripheral mechanisms.

The extract also had a significant and dose-dependent effect on the various acute pain models namely tail flick and tail immersion tests. The centrally acting analgesic agents promotes pain threshold in animals towards effect of heat and pressure. The effect of the extract on these pain models indicates that it might be centrally acting.

The plants which content natural product such as flavonoids, terpenoids, and steroids etc. can be isolated from medicinal plants have been discovered to possess significant anti-inflammatory and antinociceptive effect³⁰. It is therefore possible that both the anti-inflammatory and antinociceptive effects observed with this extract may be attributable to its flavonoids components, shown to be present during phytochemical analysis. The oral LD₅₀ obtained with this study also suggest it may have reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lacks of any reported side effect with the traditional use of this extract.

Conclusion

The study has shown that the methanol extract from the *O. annae* does possess significant anti-inflammatory and antinociceptive activity in the laboratory animals at the doses tested and the results were comparable to those observed for the standards drugs indomethacin, acetyl salicylic acid and morphine. It is also suggested the mechanism of anti-inflammatory action of MEOA might to be associated with the inhibition of prostaglandin synthesis and as observed for most non-steroidal drugs. It can also indicates that the extract possess analgesic properties which are mediated via peripheral and central inhibitory mechanisms. Thus the results obtained from the present study support the traditional use of this extract in some painful and inflammatory condition. It is important to point out that work is in progress to isolate and to characterize the active compounds present in the methanol extract of *O. annae*.

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