Journal of Medicinal Plants Research Vol. 3(7), pp. 533-537, July, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 © 2009 Academic Journals

Full Length Research Paper

Evaluation of analgesic and anti-inflammatory activities of *Oscillatoria willei* in experimental animal models

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Accepted 3 October, 2007

The methanol extract of *oscillatoria willei* (MECO) was investigated by hot plate and acetic acid induced writhing method to assess its analgesic activity. The extract was evaluated using carrageenan induced paw oedema method and cotton pellet induced granuloma method for its effect on acute and chronic phase inflammation models in rat as well as analgesic activity in mice. It was found that extract caused an inhibition on writhing response induced by acetic acid in a dose dependant manner. Maximum inhibition (67%) was obtained at a dose of 200 mg/kg after 3 h of drug treatment in carrgeenan induced paw oedema whereas indomethacin (standard drug) produced 61.65% inhibition in chronic model (cotton pellet induced granuloma) the MECO and standard drug showed decreased formulation of granuloma tissue. The result indicates significant analgesic and anti–inflammatory effects and therapeutic efficacy of MECO on animal models which are comparable with those of standard drugs.

Key words: *Oscillatoria willei,* analgesic, carrageenan induced paw oedema.

INTRODCTION

The seas and oceans of the world represent a vast reserve of natural biologically active, useful pharmaceutical products. Among the various group of manner organism, the most conspicuous and very potent are *oscillatoria willei*. It is also known as blue green algae. It occurs across the world in a range of terrestrial, fresh water and marine environment (Alonse et al., 2001). Maizels and Budde was developed LC/MS method for the determination of cyanobacteria toxins in water (Maizels et al., 2004). Cyanobacteria are prokaryote in nature. They are photoautotrophs and show the gliding motility. They are quite small and usually unicellular. They often grow in colonies, some its fossils dating back to almost 4 million years, making them the oldest among the things in the fossil record. Most classification schemes for cyanobacteria are recognized by cell or colony shape. Cyanobacteria have been found among the oldest fossils on the earth and are one of the largest groups of bacteria. They also contain chlorophyll, the same photoharvesting pigment (Lemasson et al., 1973) that plants use.

Hypolith are the example of cyanobacteria living in extreme conditions and some cyanobacteria are sold as food, notably Aphanizomenon flos – aquae (spirulina). It has been suggested that they could be a much more substantial part of human food supplies, as a kind of "super food". They are providers of nitrogen, being used as fertilizers in cultivation of cereals. The generation of oxygen was ensured by cyanobocteria during Archean and proterazoic eras. In cyanobacteria, after transport by specific permeases, ammonium is incorporated into carbon skeletons by the sequential action of glutamate synthetase and glutamate synthase (Pastor et al., 2005). Cyanobacteria have a great deal of potential as a source of fine chemicals, a biofertilizer and as well as a source of renewable fuel (Monserrat et al., 2001).

Cyanobacteria find wide use in agriculture, bioremediation, clinical diagnosis; pharmacological aspect etc. potentially useful compounds including pharmaceuticals, industrial chemicals, restriction enzymes etc. have been identified largely through pioneering works of Moore and Patterion (Borowitzka, 1995). Among the many compounds found and characterized till date, many are toxic and have been suspected as the cause of deaths in animals including humans.

Cyanobacteria have been linked to human and animal illnesses around the world, including North and South

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America, Africa, Australia, Europe, Scandinavia, and China. Cyanobacteria are the most common, but not the only group of algae to form HABs. The cyanobacteria synchocysts SP PCC 6803 was the first photosynthetic organism whose genome was completely sequenced in 1996 by Kazusa Research Institute, Japan. It continues to be an important model organism. At least one secondary metabolite, cyanovirin has shown to posses Anti-HIV activity (David et al., 2000). Search for bioactive compounds from cyanobacteria has been reviewed extensively. The Red Sea gets its name from occasional blooms of a reddish species of *Oscillatoria*.

The research on photosynthesis and nitrogen fixation is now being utilized and further explored for biotechnological development. In the present work attempt has been made to find out from the *O. willei* (Family: Oscillatoriaceae) extract for its analgesic and anti-inflammatory using standard models.

EXPERIMENTAL

Marine *oscillatoria Willei* chosen for study

The marine *O. willei* was chosen for this study. It is an autotrophic, filamentous organism. The strain was obtained from National Facility for Marine Cyanobacteria (NFMC), Trichy.

Chemical and reagents

The Chemicals used in the present study were carrageenan (S.D. Fine Chemicals Limited, Bombay), and indomethacin (IPCA, Bombay).

Preparation of extract

About 50 g of powdered cyanobacteria was taken in a round bottom flask and add ethanol and macerated for 7 days. During maceration the whole content was warmed 2 times a day at an interval of six hours. At the end of 7^{th} day the extract was filtered through muslin cloth while hot and the extract was concentrated to a semisolid mass and dried in a desiccator. This extract has been used for various experimental purposes.

Phyto chemical profile

The phytochemical investigation showed the presence of the following constituents, the presence of carbohydrate (Molisch`s test, Benedicts test) protein (Biurete test), Phytosterol and steroid (Liberman burchard test) Phenolics and tannins (action with Ferric chloride), Fats (with alc. KOH phenolphthalein). The extract was subjected to thin layer chromatography.

Animals

Swiss albino mice of either sex weighing 20 - 25 g or albino wistar rats of either sex weighing 180 – 300 g were used for study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water *ad libitum*.

Toxicity study

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines in wistar mice at a dose level of fractions up to 3000 mg/kg. Mice were kept fasting for overnight and maintained with water *ad libitum*. The animals are kept fasting for 4 h before commencing experiments with free access to water. The animals are divided into 5 groups with 5 animals in each group. The test compounds at different doses were administered orally to each group of mice. The animals were observed for the number of death after 24 h. (Litchfield et al., 1949).

Evaluation of analgesic activity

Acetic acid induced writhing response in mice

Swiss albino mice of either sex were used and divided into groups of five animals. The animals were treated with methanol extract and paracetmol was administered orally 1 h prior to the injection of acetic acid. Writhing was induced by injecting acetic acid solution intra-peritonially. Ten minutes after acetic acid injection, mice were placed in transparent box and number of writhes was counted for a period of twenty minutes. Writhing movement was accepted as contraction of abdominal muscle accompanied by stretching of hind limbs. A significant reduction in number of writhes by drug treated as compared to vehicle treated animal which was considered as a positive analgesic response and percentage inhibition of writhing was calculated and evaluated statistically.

Hot plate reaction time in mice

The hot plate test was assessed with a group of mice. The temperature of metal surface was maintained at $55 \pm 0.2^{o}C$. Latency of discomfort reaction (fore paw licking or jumping) was determined (Turner et al., 1960). The latency was recorded before and after the MECO (50,100 and 200 mg/kg) and standard drug was administered by oral route. Morphine was used as reference standard.

Investigation of anti-inflammatory effect

Carrageenan induced paw odema method

The rats were divided into 5 groups. The extract and standard used for this study were prepared. Animals were deprived of food and water for 18 h before the experiment. On the day of the experiment each group is assigned with 6 rats. They were marked and numbered for identification. The test compound and standard drug were administered orally (Winter et al., 1957). After 60 mts, 0.9 % carregeenan was injected to the lateral mallelouls on the sub plantar region of the right hind paw of the rat and paw volume was measured at 1, 2 and 3 h after injection. The percentage inhibition of inflammation was calculated for comparison.

The ratio of the anti-inflammatory effect of MECO was calculated by the following equation:

Anti- Inflammatory activity (%) = (1-D/C) x 100

Where D represents the percentage difference in paw volume after MECO was administered to the rats and C represents the percentage difference of volume in the control groups.

Cotton pellets-induced granuloma

The rats were divided into 5 groups. The cotton pellet granuloma model investigated the proliferation phase of inflammation (Winter et al., 1957). The extract at different does and indomethacin at 10 mg/kg weight were given orally to animals. After 30 mts animals were anesthetized, shaving fur, 10 mg of sterile cotton pellets were inserted, one in each axilla. The extracts were administered orally

Table 1. Effect of methanol extract of *Oscillatoria willei* on acetic acid inducedwrithing test.

Treatment	Dose(mg/k)	Reaction Time (sec)			
		15 min	30 min	60 min	90 min
Control	----	2.43 ± 0.23	3.05 ± 0.27	2.86 ± 0.22	3.69 ± 0.22
Morphine	2	3.35 ± 0.28	5.29 ± 0.46	7.22 ± 0.68[a]	7.58 ± 0.58[a]
MECO	50	3.49 ± 0.22	5.42 ± 0.52	8.66 ± 0.58[b]	9.14 ± 1.85[b]
MECO	100	3.67 ± 0.29	6.18 ± 0.45[a]	9.05 ± 0.47[b]	10.31 ± 1.36[b]
MECO	200	3.69 ± 0.27	6.35 ± 0.56[a]	9.22 ± 0.44[b]	12.12 ± 1.32[b]

p< 0.05 when compared to control.

Table 2. Analgesic Activity of Extract of *oscillatoria willei* Extract on Hot Plate Method.

Treatment	Dose (mg/kg)	Reaction Time (sec)			
		15 min	30 min	60 min	90 min
Control	-	2.49 ± 0.12	3.06 ± 0.20	2.82 ± 0.23	3.76 ± 0. 17
Morphine	2	3.37 ± 0.24	5.26 ± 0.42	7.22 ± 0.50[a]	7.66 ± 0.65[a]
MECO	50	3.18 ± 0.30	5.35 ± 0.43	7.28 ± 0.74[a]	9.41 ± 0.84[a]
MECO	100	3.52 ± 0.17	5.44 ± 0.52	8.32 ± 0.67[b]	10.22 ± 1.2[b]
MECO	200	3.63 ± 0.25	6.25 ± 0.35[a]	9.11 ± 0.48[b]	11.42 ± 1.2[b]

Values shown are mean ± SEM (n= 7). [a] $p < 0.001$, [b] $p < 0.01$, Experimental groups were compared with control.

daily for a period of seven days. The next day rats were sacrificed and pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C for constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

RESULT AND DISCUSSION

Acute toxicity study

When the mice were observed for behavioural changes after oral administration of extract, none of the mice exhibited any abnormal behavioural response at lower dose. These include motor activity, tremor, convulsion, piloerection, sedation, muscle relaxation, analgesia etc. But those mice which received higher dose showed slight toxic symptoms, which reflect LD_{50}.

Phytochemical profile study

The active part of alcoholic extract was subjected to che mical analysis. It helped in determining various classes of compounds present in it. It was determined by identification reaction based on chemical group to be determined. Thin layer chromatography of extract was done. The phytochemical investigation indicated that it contains carbohydrate, protein, tannin, phenols, fats and steroid etc.

Analgesic studies

The extract caused an inhibition on writhing response induced by acetic acid on dose depend manner. Doses of 50,100, 200 mg/kg MECO and paracetamol could block the writhing response as given in Table. The effect of the MECO on writhing response of mice was summarized in Table 1. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. Dose of 200 mg/kg MECO and paracetamol could block the writhing response. As shown in Table 2, MECO showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug morphine treated controls, suggesting that central activity of MECO.

Anti-inflammatory studies

As shown in Table 3, the extract showed maximum inhibition of 55.85% at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas the standard drug showed 61.65% of inhibition.

As shown in Table 4, in the chronic model (cotton pellet induced granuloma), the MECO (200 mg/kg) and standard drug showed decreased formation of granuloma tissue at 52.92% and 56.48% ($p < 0.001$), respectively.

DISCUSSION

The potential of the MECO for its analgesic and anti-inflammatory effect was investigated. The effect of MECO at the dose of 50,100 and 200 mg/kg showed significant analgesic and anti-inflammatory activity. The analgesic test used in the present study was chosen in order to test the different nociceptive stimuli, namely cutaneous

Table 3. Anti - Inflammatory Activity of Extract of *Oscillatoria willei*.

Treatment	Dose (mg/k)	Paw volume (ml)	% of inhibition
Carrageenan Control	0	0.732 ± 0.071	-
Indomethacin	10	0.281 ± 0.016	61.65
MECO	50	0.398 ± 0.027	45.92
MECO	100	0.354 ± 0.026	51.77
MECO	200	0.324 ± 0.026	55.85

Values shown are mean ±SEM (n=6) experimental groups were compared with control $p<0.001$.

Table 4. Anti - inflammatory activity of extract of *oscillatoria willei* on cotton -pellets induced granuloma in rats.

Treatment	Dose (mg/k)	Weight of cotton pellets (mg)	% of inhibition
Control	-	47.8 ± 3.2	-
Indomethacin	10	20.8 ± 1.3	56.48
MECO	50	34.5 ± 2.6	27.82
MECO	100	26.75 ± 1.4	44.03
MECO	200	22.5 ± 1.9	52.92

Values shown are mean ±SEM (n=6) experimental groups were compared with control $p<0.001$.

thermic and chemical visceral stimuli (Turner et al., 1960). In acetic acid induced abdominal writhing causes algesia by liberating endogenous substances and many others excite pain to the never ending. Based on the percentage inhibition on the number of writhes obtained with different doses of MECO, it was found that the intensity of the analgesic effect was similar to that of the paracetamol. Paracetamol and related drugs can inhibit cyclooxygenase in peripheral tissues, thus interfering with mechanism transduction in primary afferent nociceptors (Raj, 1996). The ostagladina amplify the pain mechanism and enhance vascular permeability while the leukotriens contract smooth muscle of blood vessels. Prostaglandins also enhance the vascular permeability and mediate pro inflammatory and allergic response (Bisgaard, 2000; Bley et al., 1998). Results of the present study show that all the doses of the MECO produce significant antinociceptive effect which may be due to blockade or release of endogenoeus substances that stimulate pain in nerve endings similar to paracetamol and other NSAIDs. The hot plate method has been found reliable for evaluating centrally but not periphrally acting analgesics. The validity of test has been shown even in the presence of substantial impairment of motor performance. The present study indicates that MECO may be centrally acting.

The present study establishes the anti-inflammatory activity of methanol extract of *O. willei*. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation and is believed to be bi-phasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome. Based on this, it would be argued that suppression of Ist phase may be due to inhibition of release of early mediators, such a histamine, serotonin and action in IInd phase may be explained by an inhibition of cyclo-oxygenase. These mediators take part in inflammatory response and are able to stimulate nociceptive and thus reduce pain. It has been reported that second phase of oedema is sensitive to most clinically effective anti inflammatory drugs, which has been frequently used to access the anti-edematous effect of natural products (Logia et al., 1968). Based on these reports, it can be inferred that the inhibition effect of the extract of MECO on carrageenan induced inflammation in rats may by due to inhibition of the mediators responsible for inflammation.

The extract exhibited significant anti inflammatory activity on the cotton pellet test. The cotton pellet granuloma widely used to evaluate the transudative and proliferate components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues (Alcaraz et al., 1988, Linardi et al., 2000). Chronic inflammation occurs by means of the development of proliferate cells. These cells can exist either in spreaded form or in granuloma form. Non-steroidal anti inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides. The MECO showed significant anti-inflammatory activity in cotton

pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

So, we can conclude that the present study shows that MECO extract exhibit significant analgesic and anti-inflammatory activity against early phase (acute paw oedema), late phase (cotton pellet granuloma) of inflamemation models.

ACKNOWLEDGEMENTS

The authors are thankful to the Chairman Thiru. V. Shanmugan B.Com, Nandha Educational Trust, Erode and Mr. S. Nandhakumar Pradeep, M. B. A., Secretary, Nandha Educational Trust, for providing in all research aspects, regular encouragement and assistance to carry out this research successfully.

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