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EVALUATION OF ANALGESIC, ANTI-PYRETIC ACTIVITY AND TOXICITY STUDY OF OSCILLATORIA *ANNAE* IN MICE AND RATS

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ABSTRACT

Medicinal herbs have been used as form of therapy for the relief of pain throughout histry. Analgesic, Anti-pyretic activity and toxicity study of *oscillatoria annae* was evaluated in the standard animal models. The methanolic extract of *oscillatoria annae* (MEOA) was evaluated by hot plate and acetic acid-induced writhing methods to assess analgesic activity. The anti-pyretic activity of the extract was also evaluated by normal body temperature and the extract showed significant analgesic and anti-pyretic activity. The MEOA was further evaluated for toxicity at the dose of 100 and 300 mg/kg administered by orally for 14 days in rats. At the end of experiments, the blood, liver function and kidney metabolism were observed. The hematological profile and different biochemical parameters such as SGOT, SGPT and SCP were estimated. The present study revealed that MEOA exhibited significant analgesic and antipyretic activity in the tested experimental animal models. The toxicity study indicates that the extract is not toxic at the tested dose.

Keywords: *Oscillatoria annae*, Analgesic, Anti-pyretic activity, Toxicity study

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. The *Oscillatoria annae* is a shrub found widely in India. *Oscillatoria annae* is a well-known medicinal used as an anti-inflammatory drug **[1]**. It has considerable reputation as a potent adjust in the treatment of various aliments such as inflammation and fever **[2]**. The *Oscillatoria annae* extract for the isolation and identification of steroids in insect and algae lipids [3]. We have reported on the potential of plants for its anti-inflammatory activity **[5]**. Hence, the present study was undertaken to evaluate the effect of the extract for its analgesic and anti-pyretic activity in mice and rats. Further, the toxicological studies were performed in serum sample following the administration of the regiments of the extract in rats for 14 days. Hepatic and renal toxicity was evaluated by measuring enzyme activity such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and hematological profile in blood. The effect of the extract was also compared with that of the standard drugs were used. They were maintained under standard environmental conditions and were fed with a standard pellet diet with water *adlibitum*.

Analgesic and Anti-Pyretic Activities

Acetic Acid-Induced Writhing Response in Mice

Mice were divided in to five groups of seven animals. Writhing test was used according to the method of turner with slight modification **[5]**. The animals were injected intraperitoneally (i.p.) of MEOA (100, 300 mg/kg) or standard drug aspirin (200 mg/kg). 1 hour prior to the injection of acetic acid while control group received vehicle (Normal saline). Writhing was induced by 10 mg/kg of acetic acid solution (0.6%) i.p. to mice, after acetic acid injection the mice were placed in a transparent box and the number of writhes was counted for a period of ten minutes. Writhing movement was accepted as contraction of the abdominal muscle 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. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically.

Hot Plate Reaction Time in Mice

The hot-plate test was assessed on groups of seven mice. The temperature of the metal surface was maintained at 55 ±1ºC. Latency to discomfort reaction (forepaw licking or

jumping) was determined by the method of turner **[5]**. The cut-off time was 20 seconds.

MATERIAL AND METHODS

Plant Material

The *Oscillatoria annae* was collected in the month of January 2007 from trichy NFMC, Tamil Nadu, India.

Chemicals and Reagents

The chemicals used in the present study were Morphine (S.D. Fine chemicals Limited, Bombay), Diclofenac sodium and Paracetamol (Prim research laboratories (Uthrangal).

Preparation of Extract

The *Oscillatoria annae* was dried. The dried algae was powdered with a mechanical grinder and stored in an air tight container. The dried powdered material of the algae was defatted with 800 ml methanol and macerated for seven days. During maceration the whole content was warmed twice a day with an interval of 12 hours. At the end of seventh day the extract was filtered through muslin cloth while hot and the extract was concentrated to a semi-solid mass.

The dried MEOA was dissolved in normal saline and used for the present study. On preliminary phytochemical qualitative analysis, the MEOA extract shows the presence of steroids, phenols, terpenoids and lipids.

Animals

Swiss albino mice of either sex weighing between 18 – 22 gms or albino wister Rats of either sex (180 – 200 gms) after the MEOA (100, 300 mg/kg) and standard drug morphine (5 mg/kg) administered by i.p. route. The prolongation of the latency times Compared with values of the control was used for statistical comparison.

Effect of MEOA in Animal Body Temperature

Rats were divided into four groups, comprising six in each group. The rectal temperature of each rat was measured initially and at 1 hour intervals for 5 hours after administration of either 0.25% Normal saline solution (control), or MEOA injected i.p. at 100 and 300 mg/kg to three groups of rats respectively.

Yeast – Induced Hyperpyrexia in Rats

The rats were divided into five groups containing six in each and trained to remain quiet in a restriaint cage. A thermoster probe was inserted 3 to 4 cm into the rectum and fastened to the till with adhesive tape. After measuring the basal rectal temperature, the animals were given subcutaneous injection of 10 ml/kg of 15% w/v yeast suspended in a 0.5 % w/v Normal saline solution. Then 19 hours after yeast injection, rectal temperatures was recorded, and the MEOA was injected i.p. at 100 and

300 mg/kg to 2 groups of rats, respectively similar volumes (5 ml/kg) of normal saline were injected to the controls. The fifth group of the rats received the antipyretic agent paracetamol, at a dose of 150 mg/kg i.p. Rectal temperatures were recorded immediately before MEOA, Paracetamol or saline administration and again at 20, 21, 22 & 23 hours after yeast injection.

Sub-Acute Toxicity Study

Wistar albino rats of either sex weighing 150-200 g of 6 animals were assigned to each group. Group I served as control received normal saline (5ml/kg). Group II &III received the extract at the dose of 100 and 300 mg/kg respectively. The vehicle and test drugs were administered orally, once daily for 14 days. On 15^{th} day, blood was collected by sinus puncture under ether anaesthesia for biochemical studies.

The hematological parameters such as, hemoglobin(Hb), red blood cell (RBC) count , white blood cell (WBC) count, clotting time and bleeding time were determined by standard method **[6]**.

The serum was separated, subjected to liver function tests [serum glutamate oxaloactate (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP)and Protein] and kidney function tests [blood urea nitrogen (BUN), and Creatinine]

Short Term Toxicity Study

The extract was administrated at the doses of 100 and 300 mg/kg orally once a day for 14 days to observe any short term toxicity. And at the end of the experiments, the hematological profile and different bio-chemical parameters of hepatoreneal functions were observed.

Hematological and Bio-Chemical Estimation

Hemoglobin content, leucocyte counts, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by standard methods **[7],** Bilirubin was measured by the method of malloy and evelyn **[8]**. Blood urea was determined by the diacetyl monoxime method of leclerc and Schwarz **[9]**. Protein content was measured by the method of lowry **[10]** using bovine serum albumin (BSA) as the standard.

Assessment of Liver Functions

The blood was centrifuged at 3000 rpm at 4ºC for 10 min to separate serum. The activities of SGOT level and SGPT were assayed by the method of **[11]**. The ALP activity in the serum was measured according to kings **[12]** method by spectrophotometer.

Statistical Analysis

Values were expressed, as mean ± SEM. Statistical values was determined using the student's t-test.

RESULTS

The effect of the MEOA on writhing response of mice was shown in **Table 1**. It was found that the extract caused as inhibition of writhing response induced by acetic acid in a dose-dependent manner. A dose of 300 mg/kg MEOA and aspirin could block the writhing response by 38.11 % and 67.87 % respectively. **Table 2** shows that MEOA had significant antinociceptive action in the hot plate reaction time method in mice. This effect was comparable to that of the standard drug morphine treated controls, suggesting the central activity of MEOA.

Table1: Effect of Methanolic Extract of *Oscillitoria annae* on an Acetic Acid Induced Writhing Test in Mice

Treatment	Dose (mg/kg)	No. of writhing	Percentage of inhibition
Control	**-**	**35.01 ± 1.23**	**-**
Aspirin	**200**	**11.0 ± 0.20**	**67.87**
MEOA	**100**	**28.6 ± 2.10**	**17.18**
MEOA	**300**	**21.1 ± 1.18**	**38.11**

Values shown are mean ± SEM (n= 7) P < 0.01 + P< 0.01 + P < 0.05 experimental groups were compared with control MEOA – OBRI

Table 2: Analgesic Activity of Methanol Extract of *Oscillitoria annae* on Hot Plate Method

Treatment	Dose (mg /kg)	Retention time (sec)			
		15 min	30 min	60 min	90 min
Control	**-**	**2.48 ± 0.12**	**3.06 ±0.20**	**2.93 ± 0.25**	**3.97 ±0.18**
Morphine	**2**	**3.45± 0.24**	**5.16 ± 0.52**	**6.21± 0.50**	**7.60 ± 0.66**
MEOA	**100**	**3.18 ± 0.32**	**5.26 ±0.43**	**6.28± 0.75**	**9.45 ±0.94**
MEOA	**300**	**3.58 0±.17**	**5.52 ± 0.62**	**8.60 ± 0.69**	**10.52 ± 1.2**

Values shown are mean ± SEM (n= 7)* P < 0.01 +P< 0.01 experimental groups were compared with control; MEOA - methanol extract of *oscillatoria anne*

Table 3: Effect of Methanol Extract of *Oscillatoria annae* on Normal Body Temperature in Rats Rectal Temperature in °C Before and After the Medication

Treatment	Dose (mg/kg)	Rectal Temperature in °C Before and after the medication					
		0 hr	1 hr	2 hr	3hr	4 hr	5 hr
Vehicle control	5 ml/kg	36.2±0.1	36.2±0.2	36.3±0.2	36.2±0.3	36.4±0.3	36.2±0.3
MEOA	100	36.2±0.2	36.4±0.3	35.4±0.3	35.2±0.2	35.3±0.6	35.2±0.2
MEOA	300	36.3±0.2	35.4±0.5	34.6±0.4	35.1±0.4	34.6±0.3	35.5±0.2

Each value represents mean ± SEM of six rats *P < 0.01, P< 0.01 as compared to the control values of corresponding hours; MEOA: methanol extract of *Oscillatoria annae*

Table 4: Effect of Methanol Extract of *Oscillatoria annae* on Yeast-Induced Pyrexia in Rats

Treatment	Dose mg/kg	Rectal Temperature in °C before and after the medication					
		0 hrs	19 hrs	20 hrs	21 hrs	22 hrs	23 hrs
Vehicle control	-	36.1±0.1	38.3±0.2	38.3±0.2	38.2±0.3	38.3±0.3	38.3±0.2
MEOA	100	36.2±0.2	38.7±0.1	38.1±0.2	37.5±0.4	37.2±0.5	36.9±0.1
MEOA	300	36.1±0.2	38.6±0.5	37.6±0.3	37.1±0.4	36.7±0.2	36.4±0.3
Paracetamol	150	36.3±0.3	38.6±0.4	37.4±0.3	36.8±0.3	36.5±0.3	36.2±0.3

Each value represents mean ±SEM of six rat. Statistical significance test for comparison of test with control was performed by student's test *p ‹ 0.001, +p‹0.01 compared to 0 hrs value; MEOA: Methanol extract of *Oscillatoria Annae*

Table 5: Hematological and Different Biochemical Parameters in Effect of MOAE in Rats

S.No	Parameters	Experimental		
		Control (normal saline 5 ml/kg)	MEOA (100 mg/kg)	MEOA (300 mg/kg)
1	Haemoglobin (g %)	14.6 14.3 ±0.27	12 14.10±0.21	13.6 14.18±0.25
2	WBC cells/cmm)	13550 13712±292.15	15800 14218±354.5	13800 14498±326.86
3	RBC(million cells/cmm)	7.8 7.33 ± 0.21	6.45 7.36 ± 0.28	8.1 7.48 ± 0.24
4	Clotting time (sec)	150 ± 10.95	145 ± 9.22	145 ± 10.02
5	Bleeding time (sec)	145 ± 9.22	140 ± 6.33	130 ± 6.33
Liver and kidney Function tests				
6	SGOT (u/l)	46.33 ± 2.58	43.67 ± 1.09	40.17 ± 1.62
7	SGPT (u/l)	131.33 ± 1.91	124.33 ± 2.75	112.0 ± 4.18**
8	ALP(u/l)	159.0 ± 3.29	148.5 ± 3.30	119.67 ± 2.85***
9	Creatinine(mg/dl)	17.83 ± 1.33	14.50 ± 1.29	14.33 ± 0.84
10	BUN (mg/dl)	2.51 ± 0.098	2.25 ± 0.094	2.15 ± 0.053
11	Urea (mg/dl)	33.01 ± 1.75	29.17 ± 1.28	26.17 ± 1.45*
12	Protein (g/dl)	6.2 ± 0.03	6.23 ± 0.62	5.82 ± 0.53

p < 0.05; **p < 0.01;p < 0.001; Values are mean SEM (n=6); Group was compared with normal control group; MEOA: Methanolic extract of *Oscillatoria Annae*; SGOT: Serum glutamate oxaloacetate transaminase; SGPT : Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase**

Effect of MEOA on normal body temperature and yeast induced hyperpyrexia in rat is shown in **Table 3 & 4**. It was found that MEOA at 100 mg/kg dose caused significant lowering of body temperature up to 4 hours following in administration. This effect was maximal at doses 200 mg/kg and it caused significant lowering of body temperature 5 hours after its administration. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 19 hours of administration. The antipyretic effect started as early as 1 hour and was maintained for 4 hours after MEOA administration. The standard drug (Paracetamol) and MEOA significantly reduced the yeast – provoked elevation of body temperature.

MEOA was also evaluated for its short term toxicity in rats. The hematological profile and bio-chemical parameter were shown in **Table 5**. No harmful effect was noted in either liver or kidney function of the extract-treated animals.

DISCUSSION

The potential of MEOA for its analgesic, antipyretic effect and short term toxicity was investigated. MEOA at doses of 100 and 300 mg/kg showed the significant analgesic and antipyretic activity. The analgesic test used in the present study was chosen in order to test different nociceptive stimuli, namely cutaneous thermic (hot plate) and chemical visceral (writhing) stimuli. Acetic acid induced abdominal writhing cause's analgesia by liberating endogeneous substances and many others excite pain to the nerve endings **[13]**. According to the percentage of inhibition on the number of writhes obtained with different doses of MEOA, we found that the intensity of the analgesic effect was similar to that of aspirin. Aspirin and related drugs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with mechanism transduction in primary afferent nociceptors. Results of the present study show that all the dosage of the MEOA producing a significant anti nociceptic effect may be due to blockade or release of endogenous substances that excite pain nerve endings similarly for aspirin or other non-steroidal anti inflammatory drugs (NSAIDS)

The hot plate method was originally described by woolfe and macdonald **[14]**. This test has been found to be suitable for evaluating centrally, but not peripherally acting analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance. The present study findings indicate that MEOA may be centrally acting.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation and graft rejection or other disease states. Antipyretics are drugs, which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulates the set point of body temperature. In fever, this set point is elevated. Drugs like paracetamol do not influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature **[15]**. Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds, which have an inhibitory effect on prostaglandin bio-synthesis **[16]**. The yeast induced hyperthermia in a rat model was employed for investigating the antipyretic activity of the extract. The present investigation indicated that MEOA showed significant antipyretic effect, again yeast induced pyregesic in rats. It was found that MEOA administration caused a significant decrease in rectal temperature similar to that of standard drugs. The result indicates that the extract has some influence on prostaglandin bio-synthesis because prostaglandin is believed to be a regulator of body temperature **[17]**.

MEOA was also evaluated for its chronic toxicity in rats. The results are summarized in **Table 5**. Serum enzyme elevation has been reported to be associated with a number of inflammatory disorders **[18]** with increased level of serum ALP and serum creatinine **[19]** are noted in chronic inflammatory disease such as rheumatoid arthritis, which is usually associated with anorexia and weight loss. Methyl prednisoly therapy in rheumatoid arthritis produced elevation of serum ALP and no changes in SGOT, ALP, creatinine or urea nitrogen also methyl prednisolone **[20]** was reported to lower the level of serum protein content in rheumatoid arthritis in humans the present study revealed that MEOA is non-toxic at the tested doses.

From the above discussion, the extract exhibited significant analgesic and antipyretic activity and did not affect the hematological and bio-chemical profiles of hepato-renal function. Thus this study substantiates the use of this plant as an analgesic and febrifuge in folklore medicine it can be used as a remedy not only for analgesic and antipyretic but also anti-inflammatory agent. Further detailed investigation is underway to determine the exact phyto-constituents responsible for the analgesic and antipyretic activity.

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