6. Pharmacological activities of metabolites of *Aspergillus oryzae*
INTRODUCTION

Most of the pharmaceutical compounds have been derived from terrestrial sources. Unfortunately, they are not effective in treatment of human diseases due to the growing incidence of drug-resistance. Their inability to cure cancer, AIDS, Alzheimer’s disease and arthritis insists the importance of new drug discovery. In this scenario, marine animals have continuously provided broad and structurally diverse group of pharmacologically active compounds to the mankind.

Marine natural products chemistry began in 1951; Bergmann and Feeney (1951) isolated spongouridin and spongothymidin nucleosides from sponge Cryptotethya crypta and the discovery of prostaglandins from Caribbean gorgonian Plexaura homomalla (Weinheimer and Spraggins, 1969) served as a further stimulus in the search for new drugs from the sea. In this connection, many biomolecules have been isolated from marine organisms and few of them are currently under study at an advanced stage of clinical trials and some of them have already been approved.

FDA (Food and Drug Administration) from United States approved three marine derived drugs such as Cytarabine and Vidarabine from the Caribbean sponge Tethya crypta and Ziconotide obtained from marine snail Conus magus. Likewise, European Agency for the Evaluation of Medicinal Products (EMEA)
approved trabectedin from tunicate *Ecteinascidia turbinate*. Moreover, 13 marine derived compounds are under the clinical pipeline, that are either in Phase I, Phase II or Phase III clinical trials (Mayer *et al.*, 2010). Plinabulin isolated from *Aspergillus ustus* has been used as an anticancer agent which is under the clinical trails (Kanoh *et al.*, 1997).

Usually pharmacological properties of any drug is assessed through various common pharmacological tests such as anticoagulant, analgesic activity, anthelmintic activity, antipyretic activity, antiallergic activity, antiarhythmic activity, immunomodulatory activity, central nervous system activity, antiulcer activity, toxicity assay and antidiabetic activity (Kosta *et al.*, 2008; Mayer and Hamann, 2005; Bhakuni and Rawat, 2005). Mostly the isolated marine natural products exhibited pharmacological activities are under the four chemical classes, polyketides (fatty acids, macrolides), terpenes (diterpenes, sesterterpenes, sesquiterpenes and sterols), nitrogen-containing compounds (indoles, proteins, depsipeptides, peptides, amides, pyrrols) and polysaccharides (Mayer and Lehmann, 2000). Some of these compounds have structural similarities or even identical to natural products of microbial origin (Proksch *et al.*, 2003a).

Inflammation is typically a protective mechanism that is triggered by inflammatory mediators and controlled by proinflammatory cytokines. Overproduction of this leads to pathogenesis of various diseases. The untreated
inflammation leads to chronic inflammatory disorders (Watanabe et al., 2001; Hirose et al., 2001; Vane and Booting, 1987; Costa et al., 2004).

In general, non steroid drugs were being used for the treatment of inflammation (Vane and Booting, 1998) and found not to be very effective against inflammation. Nowadays, steroids and cyclooxygenase inhibitors are commonly being used as anti-inflammatory drugs. But they are causing side effects such as gastrointestinal irritation, ulcers, hypertension and cardiac abnormalities (Ramasekhara Reddy et al., 2011). Hence, there is greater interest to search more compounds of anti-inflammation properties without side effects.

Many anti-inflammatory compounds have been reported from marine microorganisms, for example Diazepinomicin from *Micromonospora* sp. (Lam, 2006), Cyclomarin A from *Streptomyces* sp. (Jensen et al., 2007), Salinamides A and B (Pietra, 1997) from marine actinomycetes and Malyngamides S from cyanobacteria (McPhail and Gerwick, 2003). Recently, many new compounds have been derived from marine-derived fungi. Approximately about 450 new compounds were reported between 2009 and 2010 (Rateb and Ebel, 2011). Fungal genera *Aspergillus* has been known to be a major contributor to the secondary metabolites of marine fungal origin (Belofsky et al., 1998; Toske et al., 1998; Suda et al., 2003; Kato et al., 2007; Fremlin et al., 2009). Among them
Oxygenated Hexylitaconates from a marine sponge-derived fungus *Penicillium* sp. exhibited potent anti-inflammatory activity (Lin Li *et al.*, 2011).

In the present study the antibacterial metabolites producing *Aspergillus oryzae* (AMS4) was extracted with ethyl acetate and screened for their pharmacological activities through basic pharmacological assays such as anti-inflammatory, analgesic, antipyretic, Central Nervous System (CNS) stimulant and anti ulcer.

**MATERIALS AND METHODS**

**Extraction**

*Aspergillus oryzae* (AMS4) was broth cultured in 100 ml Glucose Peptone Yeast extract broth for seven days at 120 rpm at room temperature. The culture broth was centrifuged at 5000 rpm for 15 min and then supernatant was extracted employing liquid-liquid extraction (Belofsky *et al.*, 1998). Equal volume of ethyl acetate solvent was added to culture broth and solvent phase was separated in a separating funnel and then concentrated by evaporation and used for the assessment of pharmacological activities through pharmacological assays.
Experimental animals

All the experiments were performed with the approval of the protocol by the Institutional Animal Ethics Committee (IAEC). Approximately, 100-200 g of adult albino rats (Thirunavukkarasu et al., 2010) were maintained in a room at a controlled temperature of 22±2°C for 12-h light/dark cycle and relative humidity of 60±5% in Department of Pharmacology, S.B. College of Pharmacy, Sivakasi and were given uniform pelleted diet and water ad libitum. Eight hours before each experiment, animals were given only water in order to avoid food interference with substance absorption.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

Carrageenan induced paw edema was done by following the method of Winter et al. (1962). Rats were divided into four groups of 4 animals each. Edema was induced in sub plantar region of right hind paw of rats by an injection of lamda carrageenin (sigma, 0.05 ml of 1% w/v in saline solution). Saline (1 ml/kg) given to Group I was used as control and standard drug Diclofenac sodium (10 mg/kg) was administered to Group II rats and considered as positive control. One hour prior to carrageenan injection, Group III and IV were treated with test extract of A. oryzae (AMS4) (in 0.5% gum acacia) at a dose level of 100 and 200 mg/kg p.o. All the doses were administered orally. The paw volume of each animal was estimated by mercury displacement method using
plethysmometer before and after injection at a time intervals of 0, 1, 2, 3, 4 and 5 hrs. Result values were expressed as Mean±S.E.M and analyzed statistically using one way ANOVA (Analysis of Variance) followed by Dunnett’s multiple comparison tests.

**Analgesic activity**

Tail immersion method was done to determine the analgesic activity by following the method of Hukkeri et al. (2004) and Jain et al. (2007). Adult albino rats were tested for their sensitivity by placing the tip of tail (last 1-2 cm) gently in warm water maintained at 55±2°C. Any rats flicking the tail within 5 sec were selected for this experiment. The selected rats were divided into four groups of four animals each and fasted overnight but during the experiment given water only. Group I (control group) received normal saline, Group II (standard reference group) was treated with Dichlofenac sodium (100 mg/Kg) p.o. Group III and Group IV received ethyl acetate extracts of *A. oryzae* (AMS4) at 100 and 200 mg/kg p.o. respectively. After drug treatment, the basal reaction time of all groups of rats was noted at different time intervals of 0, 1, 2 and 3 hrs. Results were expressed as Mean±S.E.M and analyzed statistically using one way ANOVA (Analysis of Variance) followed by Dunnett’s multiple comparison tests.
Antipyretic activity (Yeast induced pyrexia method)

Antipyretic activity was performed by following the method of Hukkeri et al. (2004) and Jain et al. (2007). The animals with rectal temperatures of 37.5±0.5°C were selected and divided into four groups each containing six rats. A suspension of Brewer’s yeast (15%) in saline (0.9%) was prepared. Fever was induced by the injection of brewer’s yeast suspension (10 mg/kg) subcutaneously in back below the nape of neck. The injected area was massaged in order to spread the suspension beneath the skin. After 18 hrs the animals develop 0.5°C rise in the rectal temperature were selected for further studies. Group I (control group) was given normal saline and Group II (standard reference group) was treated with Paracetamol (45 mg/Kg) p.o. Group III and Group IV were treated with A. oryzae (AMS4) extract at 100 and 200 mg/kg p.o. respectively. The rectal temperature was recorded using Digital Telethermometer (TNCO) after 1, 2, 3 and 4 hrs in all groups. The values were expressed as Mean±S.E.M and analyzed statistically using one way ANOVA (Analysis of Variance) followed by Dunnett’s multiple comparison tests.

Central nervous system stimulant activity

Locomotor activity was done by following the method of Santhanaramasamy and Senthilkumar, (2009). The computerized locomotion detection system (actophotometer) equipped with photosenser was used to measure spontaneous locomotor activity and rearing. In this experiment, the rat
was individually placed in a transparent cage (25 x 48 x 18 cm$^3$) before the administration of vehicle (1% saline) or test extracts and locomotor activity was recorded for 10 minutes. The animals were divided into four groups to assess the effectiveness of test compounds. Group I was served as an untreated control, Group II was treated with standard caffeine (30 mg/kg, i.p), Group III and IV were treated with test extract at 100 and 200 mg/kg of $A.\ oryzae$ (AMS4). The locomotor activity was observed after 30 minutes of extract administration for 10 minutes and the percentage change in activity was calculated by following formula

$$\text{Percentage} \ (%) = \frac{(A-B)}{A} \times 100$$

Where,

- A – Before drug treatment
- B- After drug treatment

**Anti ulcer activity**

**Pyloric ligation induced gastric ulceration**

Albino rats were divided into four groups of 4 animals each. Animals were fasted for 24 hrs before the study, but had free access to water. The Group I (control group) was given normal saline and Group II (standard reference group) received Ranitidine at 13.5 mg/Kg p.o. Group III and Group IV were treated with $A.\ oryzae$ (AMS4) extract at 100 and 200 mg/kg p.o. respectively. The animals were anaesthetized with the help of anesthetic ether; the abdomen was opened
by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to the method of Shay et al. (1945) and avoids traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. After the animals recovered from anesthesia, test extracts were given orally using gavages. The animals were deprived of food and water post operatively and then sacrificed after 19 hrs of pyloric ligation.

Blood samples were collected from marginal tail vein of rats and subjected for estimation of serum alkaline phosphatase by using the method of Kind and King’s (1954). Serum calcium was estimated by ortho cresolphthelin complexone method (OCPC) (Schwarzenbach, 1955; Biggs and Moorehead, 1974). The abdomen was opened and examined for lesion to determine ulceration score. The cardiac end of stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 3000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity.

**Determination of ulcer scores (Macroscopic evaluation of stomach)**

Estimation of ulcer score was carried out by following the method of Kunchandy et al. (1985). The pyroligated animal stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood.
clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted. Scoring of ulcer was made as follows: Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Deep Ulcers (2) and Perforation (3).

**Determination of pH**

1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using digital pH meter.

**Determination of total acidity**

Estimation of total acidity was performed by following the method of Raju *et al.* (2009). An aliquot of 1ml gastric juice was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink colour was established. The total acidity was expressed as mEq/L by following formula:

\[
\text{Acidity} = \text{Vol. of NaOH} \times N \times \frac{100}{0.1} \text{ (mEq/L)}
\]

**Determination of free acidity**

Determination of free acidity was done by following the method of Rajkapoor *et al.* (2002). One ml of gastric juice was taken into a 50 ml conical flask and two drops of Topfer’s agent was added and titrated with 0.01N NaOH until canary yellow colour was observed. Free acidity was calculated by the same
formula for the determination of total acidity. The values were expressed as Mean±S.E.M and analyzed statistically using one way ANOVA (Analysis of Variance) followed by Dunnett’s multiple comparison tests.

**RESULTS**

**Anti-inflammatory activity**

Anti-inflammatory effect of ethyl acetate extract of *A. oryzae* (AMS4) is given in the Table 5.1. The highest volume of increased paw edema was noticed at 2\textsuperscript{nd} hrs from injection of carragenan. Test extract showed reduction in paw volume, the activity exhibited at 2\textsuperscript{nd} hrs was higher than standard drug but not statistically significant. The ethyl acetate extract at 100 and 200 mg/kg and standard drug (Diclofenac sodium) showed highly significant (P<0.001) variation when compared to the control. In all cases, paw volume increased over the time, but their volume reduced while comparing with control. In general, the extract showed increasing activity with increase in concentration.

**Analgesic activity**

Analgesic activity of ethyl acetate extract of *A. oryzae* (AMS4) is represented in Table 5.2. The administration of standard drug Dichlofenac sodium at 100 mg/kg p.o., showed higher reaction time during the experiment. After one hour the percentage of analgesic activity of test extract at 200 mg/kg was equal to that of standard drug at 100 mg/kg p.o. but observed overall
analgesic activity of test extract was found to be lower than standard drug. The ethyl acetate extract at 100 and 200 mg/kg and standard drug Dichlofenac sodium at 100 mg/kg showed highly significant (P<0.001) reaction time when compared to control. The reaction time was increased when increasing extract concentration and time.

**Antipyretic activity**

Effect of ethyl acetate extract of *A. oryzae* (AMS4) on yeast induced pyrexia in rats is depicted in Table 5.3. Test extract showed dose dependent antipyretic activity in the present study. Almost equal effect was noticed at a dose of 200 mg/kg and standard drug Paracetamol at 45 mg/kg. The effect was significant (P<0.5) during 4 hrs after the brewer’s yeast administration when compared with control. Untreated normal rats did not show any decrease in the body temperature on oral administration of extract.

**CNS stimulant activity**

The locomotor activity was greatly stimulated by test extract and the higher dose showed decrease in stimulant activity (Table 5.4). The standard drug caffeine at 30 mg/kg concentration showed maximum stimulant activity (24%). The test extract showed comparatively lower activity than standard. However, ethyl acetate extract at 100 mg/kg concentration showed substantial stimulant activity (19.5%).
Anti ulcer activity

Anti ulcer activity of ethyl acetate extract of *A. oryzae* (AMS4) is summarized in Table 5.5. For control group, mean gastric juice volume was 9.50 ml and Ranitidine, a standard drug decreased the mean gastric volume to 2.75 ml. Ethyl acetate extract at 100 and 200 mg/kg decreased the mean gastric juice volume to 2.50 and 1.60 ml respectively. The reduction in gastric juice volume was statistically significant (*P*<0.001), when compared with control. Test extract reduced gastric juice volume almost equally at 100 mg/kg (2.50 ml) as that of Ranitidine (2.75 ml) and 200 mg/kg showed higher reduction when compared to standard but not statistically significant.

Test extract showed elevation in pH indicating their capacity to reduce the acidity of gastric juice. The mean pH value of ethyl acetate extract at 200 mg/kg (3.95) was almost equal as that of Ranitidine (4.89). Moreover, test extract at 200 mg/kg (45.35 meq/l) decreased the gastric free acidity than Ranitidine (33 meq/l) and total acidity (63.50 meq/l) also decreased at 200 mg/kg and observed to be almost equal to Ranitidine (58.50 meq/l).

The severity of gastric ulceration was assessed based on the mean ulcer index. Ethyl acetate extract at 200 mg/kg exhibited mean ulcer index of 0.75 Ul/L, which was almost comparable to that of Ranitidine (0.25 Ul/L) and the ulcer score was lower than control (2.50 Ul/L) indicating anti ulcer activity of the test extract.
Serum calcium level was induced by ethyl acetate extract at 200 mg/kg (12.05 mg/dl), which was almost equivalent to standard Ranitidine (11.57 mg/dl). Similar level decreases in alkaline phosphatase level was also observed for test extract at 200 mg/kg (18.44 ka) but it was lower than Ranitidine (12.78 ka). This obtained results indicated that ethyl acetate at 200 mg/kg significantly reduced (P<0.001) the volume of gastric juice, free and total acidity, alkaline phosphatase and increase calcium level thereby found to be potential against gastric ulcers in rats.

**DISCUSSION**

Marine environment continuously provides broad and structurally diverse array of pharmacologically active compounds to mankind. These compounds have been shown to exhibit anti cancer, antimicrobial, antifungal, anti-inflammatory and other pharmacological activities (Gul and Hamann, 2005; Mayer and Hamann, 2005). In the last three years there have been over 15 marine-derived secondary metabolites in human clinical trials (Saleem *et al.*, 2007). This number increases rapidly; approximately 700 novel marine natural products have been published and of which 16–18% were of microbiological origin (Blunt *et al.*, 2010). Natural products with pharmacological activity have been reported such as manoalide from Palauan sponge *Luffariella variabilis* (De Silva and Scheuer, 1980), pseudopterosin E from gorgonian *Pseudopterogorgia elisabethae* (Roussis *et al.*, 1990), polyketide salinipyrone A from actinomycete
Salinispora pacifica (Oh et al., 2008) and scytonemin from cyanobacteria (Proteau et al., 1993).

Anti-inflammation test is one of the most common and primary test for screening pharmacological properties of natural products. Prolonged inflammation leads to pathogenesis of various diseases such as rheumatoid arthritis, periodontitis, chronic inflammation, otitis, autoimmune diseases, hearing loss and bacterial sepsis (Watanabe et al., 2001; Hirose et al., 2001). In the present observation, ethyl acetate extract of A. oryzae significantly reduced the formation of paw edema induced by carrageenan. This study was done for 4 hrs because carrageenan induced hind paw edema in rat includes three distinct phases, such as release of histamine and serotonin in the first phase (0-120 min), kinins in the second phase (180 min) and prostaglandin in the third phase (240 min) (Olumayokun et al., 1999). The extract showed marked inhibition in edema formation. This observed inhibition of edema could be attributed to the activation of the kinin system, accumulation of neutrophils and the release of several mediators such as prostanoids and cytokines (Akkol et al., 2008).

The exhibited anti-inflammatory activity of fungus A. oryzae was dose dependent. In a similar study Lin Li et al. (2011) reported anti-inflammatory activity of oxygenated hexylitaconates from sponge derived fungi Penicillium sp. and few anti-inflammatory compounds derived from fungi such as Endolides A-J
from fungus *Stachylium* sp. (Almeida, 2011), Asperlin from marine-derived fungus *Aspergillus* sp. SF-5044 (Lee *et al.*, 2011) and also Mangicols A-G from *Fusarium heterosporum* (Renner *et al.*, 2000). Active extract at 200 mg/kg showed reduction in paw volume and the activity was relatively higher than standard drug Diclofenac sodium. Similar level activities by fungal and bacterial metabolites have been reported earlier. For example, Belofsky *et al.* (2000) reported that Oxepinamides isolated from tunicate fungi *Acremonium* sp. exhibited comparable higher activity than standard drug. Similarly, Santhana ramasamy and Senthilkumar (2009) remarked that 200 mg/kg of bacterial metabolites exhibited good anti-inflammatory activity. But, the observed activity was lower when compared to the anti-inflammatory effect of lobophorin A-B isolated from bacterial strains of brown alga *Lobophora variegata* which reduced inflammation at a dose of 50 µg (Jiang *et al.*, 1999). Emmanuel Joshua Jebasingh (2008) stated that bacterial metabolites showed reduction in paw volume at higher concentration than standard drugs. Thus this study suggested that the fungal metabolites could be used as effective drugs in the treatment of inflammation.

Both anti inflammatory and analgesic activities are mediated through a common mechanism. Hence, tail-flick behavior in rat was used to evaluate the analgesic activity of fungal extract which also showed increasing activity with increasing extract concentration but activity was observed to be lower than
standard drug. It may be attributed to the pure form of standard drug and crude form of extract. Extract at 200 mg/kg during 1 hr showed almost equal activity to standard drug. This observation of pain killing effect could be attributed to selective modulation of neuronal nicotinic receptors in the spinal cord and brain in rat (Marwick, 1998). Emmanuel Joshua Jebasingh (2008) observed higher analgesic activity than standard drug during 1 hr at 200 mg/kg of chloroform extracts from *Bacillus megaterium* and *Pseudomonas aeruginosa*. Analogously, Santhanaramasamy and Senthil Kumar (2009) reported similar pattern of analgesic activity in marine bacterial extracts from Tuticorin coast. The present study further coincides with the analgesic activities of pseudopterosins isolated from gorgonian *Pseudopterogorgia elisabethae* (Look et al., 1986). The results in this study evidenced that the fungal metabolites in crude extract hold pain killing effect at lower concentration.

Yeast induced pyrexia is a classical method for testing antipyretic activity of any compound. Ethyl acetate extract of *A. oryzae* showed comparable dose dependent antipyretic activity with higher activity at 200 mg/kg which was equal potent activity to standard drug. This activity of extract could be attributed to the ability of compound in inhibition of prostaglandin biosynthesis (Vane, 1987). Antipyretic observed was found to be higher than reports of Emmanuel Joshua Jebasingh (2008) who reported that marine bacterial chloroform extracts of *Bacillus megaterium* and *Pseudomonas aeruginosa* exhibited lower activity than
standard drug. This observation is corroborated with Sadish Kumar et al. (2009) who reported that cyclohexane extract of brown alga, *Turbinaria conoides* showed more significant antipyretic activity than standard drug. The results coincides with the study of Rajesh (2008) who remarked that methanol extracts of *Eudistoma viride* showed higher antipyretic activity compared with standard drug. This prominent activity observed in fungal extract indicates the presence of antipyretic compounds which may have an inhibitory effect on prostaglandin biosynthesis thereby could inhibit pyrexia in rats.

Locomotor activity of rats was stimulated by extract of *A. oryzae*. This observed CNS stimulant activity at 100 mg/kg was lower when compared with standard drug Caffeine (30 mg/kg). Some seaweed extracts have been shown to have similar activity. Arulsenthil (2008) reported that the seaweed *Laurencia papillosa* extract exhibited central nervous system stimulant activity lower than the standard drug. Kamat et al. (1991) reported that the seaweed extract of *Sargassum tenerrimum* and *Caulerpa sertularioides* and ethanolic extract of *Pocockiella variegata, Sargassum cinereum* from India showed a good CNS stimulant activity. It is suggested that the seaweed CNS stimulant active metabolites may be due to associated microbial source, because some of the molecules similar to marine microbial origin (Aneiros and Garateix, 2004). The possible mechanism of CNS stimulant activity by fungal extract may be attributed to the inhibition of phosphodiesterases or blockade of adenosine receptors or
increases in the rate of turnover of norepinephrine and dopamine or increases in the sensitivity of post-synaptic central catecholamine receptors or increases in the brain content in rats (Misra et al., 1986).

Extract of A. oryzae significantly reduced gastric ulcer formation, free acidity, total acidity and ulcer index in pylorus ligation model. Volume of gastric secretion is an important factor in the production of gastric ulcers which believed to be due to stress induced increase in gastric hydrochloric acid secretion and their accumulation in unprotected lumen of stomach. They lead to auto digestion of gastric mucosa and breakdown of gastric mucosal barrier. They also cause upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage (Sakat and Juvekar, 2009). In this observation, test extract showed almost equal effect compared to standard drug Ranitidine (13.5 mg/kg). At 200 mg/kg test extract significantly reduced the volume of gastric juice, free and total acidity of gastric secretion, alkaline phosphatase and showed increase in calcium level in rats. This present observation coincides with anti-ulcer activity exhibited by mangrove Avicennia officinalis extracts which significantly decreased the acidity at 125 mg/kg in pyloric ligation model (Thirunavukkarasu et al., 2010). Perera et al. (2001) remarked that 125 mg/kg of water extract from red mangrove showed equal anti ulcer activity when compared with standard drug Cimetidine (250 mg/kg). This obtained anti-ulcer activity of fungal extract suggests that fungal metabolites may have the ability to stimulate
mucus, bicarbonate and the prostaglandin secretion and also counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen thereby may inhibit ulcer formation (Borelli and Izzo, 2000).

The present study indicated the efficient nature of fungal metabolites of *A. oryzae* with anti inflammatory, analgesic, antipyretic, CNS stimulant and anti ulcer properties. Thus this work suggests that marine fungi could be used as a source for isolation of diverse compounds with pharmacological activities. Further exploration of this active crude extract would certainly lead to isolation of potentially useful compounds.
**Table 5.1: Anti-inflammatory activity of *Aspergillus oryzae* extract against carrageenan induced paw edema in albino rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Increase in paw volume (ml)</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
</tr>
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<td></td>
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<tr>
<td>Control</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.584±0.008</td>
<td>0.927±0.009</td>
<td>0.982±0.007</td>
<td>0.937±0.005</td>
<td>0.849±0.007</td>
<td>0.712±0.007</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.599±0.007 ns</td>
<td>0.765±0.004***</td>
<td>0.742±0.007***</td>
<td>0.643±0.008***</td>
<td>0.615±0.008***</td>
<td>0.595±0.008***</td>
<td></td>
</tr>
<tr>
<td>sodium</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASM4</td>
<td>100</td>
<td>0.594±0.014 ns</td>
<td>0.840±0.008***</td>
<td>0.705±0.005***</td>
<td>0.648±0.006***</td>
<td>0.608±0.007***</td>
<td>0.581±0.007***</td>
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<tr>
<td></td>
<td>200</td>
<td>0.588±0.009 ns</td>
<td>0.814±0.008***</td>
<td>0.703±0.009***</td>
<td>0.640±0.006***</td>
<td>0.601±0.005***</td>
<td>0.587±0.006***</td>
<td></td>
</tr>
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</table>

*p value calculated by one way ANOVA followed by Dunnett’s test
ns – non significant; ***p<0.001 (significant), n=4, values are mean±SEM

**Table 5.2: Analgesic activity of *Aspergillus oryzae* extract by tail flick method**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction time (sec) after drug administration</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.75±0.48</td>
<td>2.75±0.25</td>
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<tr>
<td>Diclofenac</td>
<td>100</td>
<td>6.5±0.65***</td>
<td>9.25±0.48***</td>
</tr>
<tr>
<td>sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASM4</td>
<td>100</td>
<td>5.75±0.48***</td>
<td>6.75±0.48***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.5±0.29***</td>
<td>8.75±0.63***</td>
</tr>
</tbody>
</table>

N=4, values are Mean±SEM ***p<0.001 (significant)
Table 5.3: Antipyretic activity of *Aspergillus oryzae* extract against Brewer’s yeast induced pyrexia in albino rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial temp. (°C)</th>
<th>Rectal temperature °C in hour ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hr 1 hr 2 hr 3 hr 4 hr</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>37.53±0.09 38.15±0.16 38.16±0.09 38.09±0.09 37.98±0.09</td>
<td>37.89±0.1</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>45</td>
<td>37.59±0.23 38.20±0.17 37.96±0.14 37.78±0.14 37.72±0.14</td>
<td>37.59±0.11</td>
</tr>
<tr>
<td>ASM4</td>
<td>100</td>
<td>37.34±0.12 38.00±0.06 37.78±0.08 37.67±0.09 37.59±0.1</td>
<td>37.41±0.11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>37.50±0.05 38.20±0.07 37.93±0.04 37.75±0.04 37.68±0.03</td>
<td>37.52±0.04</td>
</tr>
</tbody>
</table>

Mean ± SEM, (n=4), ns – non significant, *p<0.05, **P<0.01 (significant)

Table 6.1: Effect of *Aspergillus oryzae* extract on locomotor activity (CNS Stimulant activity)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean locomotor activity scores in ten minutes</th>
<th>Percentage of locomotor activity</th>
<th>Nature of action</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment After treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>163.5±1.708 162.5±1.708</td>
<td>0.6%</td>
<td>-</td>
</tr>
<tr>
<td>Caffeine</td>
<td>30</td>
<td>170.5±2.218 224±3.163</td>
<td>24%</td>
<td>Stimulant</td>
</tr>
<tr>
<td>AMS4</td>
<td>100</td>
<td>175.5±1.708 218±20.897</td>
<td>19.5%</td>
<td>Stimulant</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>188.3±1.315 190.5±2.5</td>
<td>1.15%</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean ± SEM, (n=4)
Table 5.5: Anti ulcer activity of *Aspergillus oryzae* extract by pyloric ligation induced gastric ulceration method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of gastric juice</th>
<th>pH</th>
<th>Free acidity (mEq/litre)</th>
<th>Total acidity (mEq/litre)</th>
<th>Ulcer scores (Ul/L)</th>
<th>Serum alkaline phosphate (Ka)</th>
<th>Serum calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>9.50±0.129</td>
<td>2.40±0.06</td>
<td>96.65±0.221</td>
<td>119±0.183</td>
<td>2.50±0.287</td>
<td>48.81±0.08</td>
<td>9.74±0.041</td>
</tr>
<tr>
<td>Ranidine</td>
<td>13.5</td>
<td>2.75±0.171***</td>
<td>4.89±0.013***</td>
<td>33±0.183***</td>
<td>58.50±0.129***</td>
<td>0.25±0.143***</td>
<td>12.78±0.081***</td>
<td>11.57±0.53***</td>
</tr>
<tr>
<td>AMS4</td>
<td>100</td>
<td>2.50±0.129***</td>
<td>3.55±0.013***</td>
<td>56.50±0.129***</td>
<td>69.85±0.171***</td>
<td>1±0.10***</td>
<td>25.41±0.461***</td>
<td>10.66±0.03***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.60±0.082***</td>
<td>3.95±0.013***</td>
<td>45.35±0.222***</td>
<td>63.50±0.238***</td>
<td>0.75±0.145***</td>
<td>18.44±0.067***</td>
<td>12.05±0.068***</td>
</tr>
</tbody>
</table>

N=4, values are Mean±SEM ***P<0.001 (significant)