4.1 Introduction

The term depression indicates a dampened mood and pervasive unhappiness. However, medical science generally reserves this term for the catastrophic state of major dejection or discomport. Most popular form of depression is called dysthymia. Dysthymia (mild to moderate depression) is characterized by chronic depressed mood, low self-esteem, poor concentration, difficulty in making decisions, feeling hopelessness, poor appetite (or overeating) and insomnia (or excessive sleep) (Diagnosis and Statistical Manual of Mental Disorders, 1994). To overcome depression, many drugs are available. The Food and Drug Administration in United States started issuing warnings about conventional drugs to doctors in 2003, and in October 2004 it ordered “black box” labels, the strongest warning on all conventional antidepressants. The main side effect of conventional antidepressant drugs is suicidal behavior by children and teenagers. Using medicinal plants for treatment of depression is safe alternative to conventional drugs till now. There are a wide range of herbal products for use as antidepressants; whereas the animal based drugs (especially aquatic animals) are not available in market yet.

Postpartum Depression (PPD) is one of the global public health issues and affects 15% of child-bearing women (Almond, 2009). The etiology of PPD was linked with abrupt decrease in the estradiol level after the delivery (O’Hara et al., 1991; Bloch et al., 2000; Abou-Saleh et al., 1998), alterations in the hypothalamo pituitary-adrenal axis (Okano and Nomura, 1992), decrease in docosohexaenoic acid level in the brain and involvement of serotonergic system (Levant et al., 2008). Although,
Selective Serotonin Reuptake Inhibitors (SSRI's) and tricyclic antidepressants are indicated for the treatment of postpartum depression, their safety is not well established on neurological development of infants during the breastfeeding period (Tackett and Hale, 2010; Gentile et al., 2007). Hence, a safe antidepressant is warranted for the treatment of postpartum depression.

The traditional usage of *C. striatus* for post parturition rejuvenation effect suggests a central effect. In earlier reports, the Aqueous Extract of *Channa striatus* Fillets (AECSF) produced anti-nociception in mice synergistically through various receptor systems including serotonergic receptor system (Zakaria et al., 2006; Zakaria et al., 2008). The observation that *C. striatus* is already used by the local Malay population during the postpartum period without any reported side effects, involvement of serotonergic system in a previous report and lack of scientific studies on its neuropharmacological effects, stimulated an interest to study the antidepressant activity of *C. striatus*. Moreover, there is no report on antidepressant activity of *C. marulius*. Hence, a study was set up to examine antidepressant-like effect of both *C. striatus* and *C. marulius* in mice models of depression.

Diabetes is one of the most common health problem affecting approximately 200 million people worldwide and about 300 million people are at the risk. Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water and includes a group of metabolic diseases characterized by hyperglycemia. Plants contain many phytochemical constituents that help to maintain the blood glucose level. But the knowledge on animal based remedies is scarce.

Inflammation may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage (Robbins et al., 2008).
NSAIDs are reported to support denaturation of proteins, which act as antigens and lead to auto-immune diseases (Insel, 1996). Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis (Mizushima, 1966). Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Grant et al., 1970). In the previous chapters (Chapter 1 and 2), the fatty acids identified in both the species were found to possess anti-inflammatory properties and hence, the present study was carried out to assess the in vitro anti-diabetic and anti-inflammatory activities of *C. striatus* and *C. marulius*.

4.2 Materials and Methods

4.2.1 Preparation of *C. striatus* and *C. marulius* crude extract

Please refer section 1.2.1.

4.2.2 Experimental animals

Please refer section 1.2.3.

4.2.3 Experimental design

Wistar albino male rats (150-180 g) were divided into six groups of six animals each (n=6). The experiment was designed as follows: Group I served as control and received normal water daily for 21 days. Groups II animals received the standard drug imipramine (15 mg/kg p.o.) daily for 21 days. Group III and IV were orally administered with extract of *C. striatus* at concentrations of 200 mg/kg b.w. (CS LD) and 400 mg/kg b.w (CS HD) respectively for 21 days. Group V and VI were orally administered with extract of *C. marulius* at concentrations of 200 mg/kg b.w. (CM LD) and 400 mg/kg b.w. (CM HD) respectively for 21 days.
4.2.4 Induction of stress

The Chronic Unpredictable Mild Stress (CUMS) was induced based on the method originally used by Pal and Dandiya (1993) with minor modifications. The protocol was designed to maximize the unpredictable nature of the stressors. Each stress regimen was carried out for 2 periods with the following stressors in random order: food deprivation for 24 h, day-night reversal, soiled bedding (~150 ml water per cage) for 22 h, cage tilting (~45° inclined) for 22 h, crowded housing (10 animals per cage) for 12 h, exposure to a novel odor (household air freshener) for 12 h, restraint stress for 20 min, cold stress 4–8°C and heat stress 38–39°C for 20 min. One stressor was applied per day and the whole stress procedure lasted for 21 days with a completely random order. After induction of stress in rats with above stress regimen for 21 days and with simultaneous drug administration, animals were subjected to forced swimming test and sucrose consumption test to study the changes in behaviour.

4.2.5 Sucrose Consumption Test (SCT)

During this test, rats were given, for 24 h, a free choice between two bottles, one with 1% sucrose solution and another with normal water. To prevent possible effects of side preference in drinking behaviour, the position of the bottles was changed after 12 h. No previous food or water deprivation was applied before the test. The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups by weighing the bottles. The sucrose intake was calculated as an amount of consumed sucrose in mg per gram body weight. The preference for sucrose was calculated as percentage of consumed sucrose solution of the total amount of liquid drunk.
4.2.6 Forced Swimming Test (FST)

The test was performed according to the method described by Porsolt et al. (1977a). Antidepressant-like behavior is observed as decreased immobility of the rats. Thirty minutes after the drug administration, each rat was placed in an open cylindrical container (45 × 12 × 45 cm³) filled with 35 cm of water (room temperature). A rat was judged to be immobile when it remained floating in the water, making only the necessary movements to keep its head above water. Duration of immobility of the last 4 min of the total 6 min of swimming time was recorded by a blinded experimenter. The water was changed after each rat was tested. The animals were constantly watched to ensure no contact was made between their paws and the base of the cylinder during FST (Lucki et al., 2001).

4.2.7 In vitro Anti-diabetic activity (Miller, 1959)

4.2.7.1 Inhibition of α-amylase

The total assay mixture containing 200 μl of 0.02 M sodium phosphate buffer, 20 μl of enzyme (0.5 mg/ml), and the sample in the concentration range 100-500 μg/ml were incubated for 10 min at room temperature followed by addition of 200 μl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 μl of 3,5-dinitrosalycylic acid color reagent, placed in boiling water bath for 5 min, cooled to room temperature and diluted with 15 ml of distilled water and the absorbance was measured at 540 nm. The control samples were also prepared accordingly without any sample and were compared with the test samples containing various concentrations of the sample. The results were expressed as % inhibition using the formula:

\[
\text{% Inhibition} = \left(\frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}}\right) \times 100
\]
The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC$_{50}$) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

**4.2.7.2 Inhibition of α-glucosidase**

The enzyme α-glucosidase inhibitory activity was determined by premixing α-glucosidase (0.07 Units) with various concentrations of sample (100-500 μg/ml). Then, 3 mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37°C for 30 min and the reaction was terminated by addition of 2 ml of sodium carbonate. The α-glucosidase activity was determined by measuring the p-nitrophenyl released from p-nitrophenyl glucopyranoside at 400 nm after which the IC$_{50}$ value was calculated.

\[
\text{% Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100
\]

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC$_{50}$) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

**4.2.8 In vitro Anti-inflammatory study** (Kumar et al., 2011)

**4.2.8.1 Inhibition of protein denaturation**

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of the sample (100-500 μg/ml). pH was adjusted to 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests, 0.05 ml of distilled water was
used instead of the sample while product control tests lacked bovine serum albumin.

The percentage inhibition of protein denaturation was calculated as follows:

\[
\% \text{ inhibition} = 100 - \left( \frac{\text{OD of test} - \text{OD of product control}}{\text{OD of control}} \right) \times 100
\]

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC\textsubscript{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

**4.2.8.2 Proteinase inhibitory action**

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml of 25 mM Tris – HCl buffer (pH – 7.4) and 1.0 ml aqueous solution of the sample (100-500 μg/ml). The mixtures were incubated at 37°C for 5 min and then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 min. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated as follows:

\[
\% \text{ inhibition} = 100 - \left( \frac{\text{OD of test} - \text{OD of product control}}{\text{OD of control}} \right) \times 100
\]

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC\textsubscript{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

**4.2.9 Statistical Analysis**

Data analysis was performed using SPSS package. Values are expressed as mean±SD. Data from the control, standard, treated and untreated groups were compared by Analysis of Variance (ANOVA) with p<0.001.
4.3 Results

The *in vitro* anti-diabetic activity was assessed as $\alpha$-amylase activity and $\alpha$-glucosidase activity for five different concentrations of *C. striatus* and *C. marulius* extracts. There was a dose-dependent increase in percentage inhibitory activity against $\alpha$-amylase enzyme. At 100 $\mu$g concentration of *C. striatus* extract, the activity was inhibited by $1.22 \pm 0.26\%$, whereas at the highest concentration (500 $\mu$g), the activity was recorded as $31.96 \pm 0.46\%$. For *C. marulius*, the activity was inhibited by $5.78 \pm 0.26\%$ at 100 $\mu$g and the highest inhibition was observed at 500 $\mu$g concentration ($50.23 \pm 0.79\%$). At each concentration, the activity was higher in *C. marulius* extract when compared to *C. striatus* as shown in Figure 4.1. When comparing both the species, better results were obtained in *C. marulius* extract. The IC$_{50}$ value of *C. striatus* was $66.85 \pm 0.62$ $\mu$g/ml and *C. marulius* extract was $42.67 \pm 0.90$ $\mu$g/ml.

In $\alpha$-glucosidase activity, the inhibitory action increased in a dose dependent manner similar to $\alpha$-amylase activity. At 100 $\mu$g concentration of *C. striatus* extract, the activity was inhibited by $8.66 \pm 0.52\%$, whereas at the highest concentration (500 $\mu$g), the activity was recorded as $20.88 \pm 0.31\%$. For *C. marulius* extract, the activity was inhibited by $7.67 \pm 0.16\%$ at 100 $\mu$g and the highest inhibition was observed at 500 $\mu$g concentration ($15.13 \pm 0.39\%$). At each concentration, the activity was higher in *C. striatus* when compared to *C. marulius* as shown in Figure 4.2. When comparing both the species, better results were obtained for *C. striatus* extract. The IC$_{50}$ value of *C. striatus* was $493.99 \pm 10.98$ $\mu$g/ml whereas *C. marulius* extract was $724.81 \pm 13.80$ $\mu$g/ml.

The *in vitro* anti-inflammatory activity was assessed by protein denaturation and proteinase inhibitory activity for five different concentrations of *C. striatus* and
C. marulius extracts. The protein denaturing activity increased in both the extracts of C. striatus and C. marulius in a dose dependent manner. The least denaturing activity was recorded at 100 µg concentration (C. striatus- 8.33 ± 3.61% and C. marulius - 8.89 ± 3.84%). At the highest concentration of the extract (500 µg), C. striatus produced 45.83 ± 3.61% activity which was higher than C. marulius (37.78 ± 7.70%). At each concentration, the activity of C. striatus was higher than C. marulius as shown in Figure 4.3. The IC₅₀ value of C. striatus was 191.62 ± 3.77 µg/ml whereas C. marulius extract was 232.71 ± 41.35 µg/ml.

The anti-proteinase activity increased with increase in concentration of both the extracts of C. striatus and C. marulius as shown in Figure 4.4. Maximum anti-proteinase activity was observed at 500 µg of C. striatus extract (95.73 ± 0.46%) whereas the activity was lesser in C. marulius (82.58 ± 3.47%) in the same concentration. At 100 µg concentration, the activity of C. striatus was least (5.20 ± 2.43%) and C. marulius had higher activity (39.39 ± 2.62%), but gradually decreased by 500 µg concentration. The IC₅₀ value of C. striatus was 50.51 ± 0.44 µg/ml whereas C. marulius extract was 52.27 ± 0.32 µg/ml.

In the forced swimming test, a highly significant (p<0.001) reduction in the immobility time was recorded in both the standard drug treated group (56.17 ± 14.22 sec) as well as the CM HD group (63.67 ± 12.89 sec). The immobility time was higher in both the low doses of C. striatus (108.17 ± 10.80 sec) and C. marulius (100.83 ± 7.28 sec) whereas the immobility time was less in the CS HD group (83.33 ± 14.92 sec) as given in Table 4.1. The percentage protection is in the order of Imipramine (73.69%) > CM HD (70.18%) > CS HD (60.97%) > CM LD (52.77%) > CS LD (49.34%) as shown in Figure 4.5.
The Sucrose Consumption Test (SCT) results indicate that the sucrose preference was more or less the same in all the treated groups as shown in Figure 4.6. The preference was less in the induced group (79.76 ± 5.11% with 26.11 ± 5.71 ml/24 h sucrose consumption). In all other groups, the preference was high indicating high sucrose consumption as follows: Imipramine treated group (93.87 ± 1.72% with 69.60 ± 6.16 ml/24 h sucrose consumption), CM HD (92.31 ± 1.46% with 55.77 ± 7.12 ml/24 h), CS HD (92.76 ± 0.96% with 57.53 ± 5.40 ml/24 h), CM LD (90.81 ± 1.88 % with 48.12 ± 5.34 ml/24 h) and CS LD (89.90 ± 2.35% with 48.59 ± 6.68 ml/24 h).

The preference for water is higher in the induced group (6.55 ± 1.68 ml/ 24 h) than all the other groups. The lowest preference for water was observed in the *C. striatus* treated group (CS HD- 4.46 ± 0.41 ml/24 h) followed by imipramine treated group (4.47 ± 1.04 ml/24 h), CM HD (4.59 ± 0.76 ml/24 h), CM LD (4.82 ± 0.84 ml/24 h) and CS LD (5.41 ± 1.30 ml/24 h). The overall fluid consumption was higher in the standard group (74.08 ± 5.25 ml/ 24 h) followed by CS HD (61.99 ± 5.33 ml/24 h), CM HD (60.36 ± 7.04 ml/24 h), CS LD (54.00 ± 6.85 ml/24 h) and CM LD (52.94 ± 5.26 ml/24 h). The least fluid consumption rate was recorded in the induced untreated group (32.66 ± 6.28 ml/24 h) as given in Table 4.2.
Table 4.1 Effect of *C. striatus* and *C. marulius* extracts on forced swimming test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
<th>Immobility Time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced</td>
<td>-</td>
<td>213.50 ± 15.35</td>
</tr>
<tr>
<td>Imipramine</td>
<td>15 mg/kg b.w</td>
<td>56.17 ± 14.22*</td>
</tr>
<tr>
<td>CS LD</td>
<td>200mg/kg b.w</td>
<td>108.17 ± 10.80*</td>
</tr>
<tr>
<td>CS HD</td>
<td>400mg/kg b.w</td>
<td>83.33 ± 14.92*</td>
</tr>
<tr>
<td>CM LD</td>
<td>200mg/kg b.w</td>
<td>100.83 ± 7.28*</td>
</tr>
<tr>
<td>CM HD</td>
<td>400mg/kg b.w</td>
<td>63.67 ± 12.89*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 6). *Significant at p<0.001

Table 4.2 Effect of *C. striatus* and *C. marulius* extracts on sucrose consumption test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
<th>Consumption (ml/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fluid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
</tr>
<tr>
<td>Induced</td>
<td>-</td>
<td>32.66 ± 6.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.55 ± 1.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.11 ± 5.71</td>
</tr>
<tr>
<td>Imipramine</td>
<td>15 mg/kg b.w</td>
<td>74.08 ± 5.25*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.47 ± 1.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69.60 ± 6.16*</td>
</tr>
<tr>
<td>CS LD</td>
<td>200mg/kg b.w</td>
<td>54.00 ± 6.85*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.41 ± 1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.59 ± 6.68</td>
</tr>
<tr>
<td>CS HD</td>
<td>400mg/kg b.w</td>
<td>61.99 ± 5.33*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.46 ± 0.41*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.53 ± 5.40*</td>
</tr>
<tr>
<td>CM LD</td>
<td>200mg/kg b.w</td>
<td>52.94 ± 5.26*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.82 ± 0.84*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.12 ± 5.34</td>
</tr>
<tr>
<td>CM HD</td>
<td>400mg/kg b.w</td>
<td>60.36 ± 7.04*</td>
</tr>
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<td></td>
<td></td>
<td>4.59 ± 0.76*</td>
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<tr>
<td></td>
<td></td>
<td>55.77 ± 7.12*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 6). *Significant at p<0.001
Figure 4.1 α - Amylase activity of *C. striatus* and *C. marulius* extracts

![Bar graph showing amylase activity of C. striatus and C. marulius extracts.](image)

Figure 4.2 α - Glucosidase activity of *C. striatus* and *C. marulius* extracts

![Bar graph showing glucosidase activity of C. striatus and C. marulius extracts.](image)
Figure 4.3 Protein denaturation activity of *C. striatus* and *C. marulius* extracts

Figure 4.4 Proteinase inhibition activity of *C. striatus* and *C. marulius* extracts
Figure 4.5 Percentage protection of *C. striatus* and *C. marulius* extracts in Forced Swimming Test

![Bar chart showing percentage protection of *C. striatus* and *C. marulius* extracts in Forced Swimming Test.](image)

Figure 4.6 Percentage preference of sucrose by *C. striatus* and *C. marulius* extracts in sucrose consumption test

![Bar chart showing percentage preference of sucrose by *C. striatus* and *C. marulius* extracts in sucrose consumption test.](image)
4.4 Discussion

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Gandhi and Sasikumar, 2012). It was proposed that inhibition of the activity of α-amylase and α-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose; as a result, the reduction of postprandial blood glucose level occurs (Lhoret and Chiasson, 2004). In the present study, both the extracts of C. striatus and C. marulius inhibited α-amylase and α-glucosidase in a dose dependent manner and thus, they are found to possess anti-diabetic activity.

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of C. striatus and C. marulius extracts on protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries many serine proteinases in their lysosomal granules. It was previously reported that leukocyte proteinase plays important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995).

Forced swimming test is one of the well-established animal models of depression (Porsolt et al., 1977b; Porsolt et al., 1978) which is used to screen the potential drugs for antidepressant activity. In the present study, extracts of both the
species significantly reduced the immobility time in FST in all the two concentrations when compared with the control group. The positive control drug Imipramine significantly (p<0.001) reduced the immobility time in FST. The pattern of dose-response produced by the extracts of \textit{C. striatus} and \textit{C. marulius} used in this study in FST and SCT showed a nonlinear relationship with a maximum response at high doses.

In a previous study, Aqueous Extract of \textit{C. striatus} Fillet (AECSF) produced maximum anti-nociceptive effect at 40% w/v concentration when compared to 30% and 50% w/v concentrations (Jais \textit{et al.}, 1997). Interestingly, similar findings were observed in another study by Saleem \textit{et al.} (2011), with a maximum antidepressant-like effect at 40% w/v concentration when compared to 30% and 50% w/v concentrations. The pharmacological mechanism of the observed antidepressant-like effect of \textit{C. striatus} and \textit{C. marulius} is not clear from this study. The level of palmitic and oleic acid concentrations seem to be relevant for sleep disturbances in depressive subjects, which may be due to their function as precursors of the sleep inducing oleamide (Irmisch \textit{et al.}, 2007), a fatty acid amide that has been shown to produce antidepressant-like effects (Akanmu \textit{et al.}, 2007).

Omega-3 fatty acid is also shown to be effective for depression (Parker \textit{et al.}, 2006). Oral treatment with lysine and L-arginine were reported to reduce anxiety and stress (Smriga \textit{et al.}, 2007). Treatment with yeast hydrolysate, containing high concentrations of glutamic acid and aspartic acid, was reported to exhibit anti-stress activity in humans (Lee \textit{et al.}, 2009). \textit{C. striatus} was reported to contain all these fatty acids and amino acids as major components (Jais \textit{et al.}, 1994; Jais \textit{et al.}, 1998; Zuraini \textit{et al.}, 2006) and as per the data obtained for \textit{C. striatus} and \textit{C. marulius} in the previous chapter (Chapter 1 and 2) dealing with the fatty acids of both the species. In
the present study, the immobility time was greatly reduced as a function of *C. marulius* high dose than *C. striatus* high dose which might be due to the presence of large amount of fatty acids and amino acids in *C. marulius*. Although the possible involvement of these fatty acids and amino acids might be anticipated in the observed antidepressant-like activity of both the species, it cannot be concluded from this study.

In a previous study by Zakaria *et al.* (2005a), AECSF exhibited antinociception in mice synergistically through muscarinic, GABAA-ergic, alpha-adrenergic and serotonergic receptor systems and not through opioid receptor system. The anti-nociception effect was observed to act through nitric oxide/ cyclic Guanosine Monophosphate (cGMP) pathway also (Zakaria *et al.* 2005b). Hence, in an attempt to explain the mechanism of action, it may be speculated that the AECSF might have acted through one or more of these receptor systems to produce the observed antidepressant-like effect. However, it cannot be concluded from this study.

Agents that enhance locomotor activity in open-field test including psychomotor stimulants, convulsants and anti-cholinergics, tend to produce a false positive result in FST and TST (Tail Suspension Test) (Bourin *et al.*, 2001). Saleem *et al.* (2011) reported that AECSF produced a dose-dependent decrease in number of crossings and number of rearings in the open-field test. This hypolocomotion effect of the AECSF treated animals when compared with the control group animals in open-field test indicated the absence of any psychomotor stimulant activity, thereby supporting the antidepressant-like effect of the AECSF observed in the FST and TST. Although the pharmacological mechanism by which the AECSF caused a significant decrease of locomotion in the open-field test is not clearly understood from their study, decreased spontaneous locomotor activity suggests a possible sedation effect (Zapata-Sudo *et al.*, 2010). David *et al.* (2003) suggested that even if a sedative effect
is observed in the open-field test, antidepressant-like activity may be perceived in the FST. In the previous studies, clonidine (Malinge et al., 1988), imipramine (Wieland and Lucki, 1990), desipramine (David et al., 2003), buspirone, ipsapirone and gepirone (Wieland and Lucki, 1990) produced significant decrease in immobility time in FST and significant decrease in locomotor activity (number of crossings and number of rearings) at doses similar to those which decreased immobility.

In a study by Diers et al. (2008), two marine natural compounds, aaptamine and 5,6-dibromo-N,N-dimethyltryptamine, also produced significant antidepressant-like activity in the forced swim test. The antidepressant positive controls, citalopram (selective serotonin reuptake inhibitor) and desipramine (tricyclic antidepressant) both dose-dependently reduced immobility time in the forced swim test. They also highlighted the potential to rationally select marine derived compounds for treating depression and other neuropsychiatric disorders though there is very little published data on the potential application of marine natural products to treat neuropsychiatric disorders. They also reported that many natural products derived from chemically defended organisms in the marine environment have pharmacophores related to serotonin or clinically utilized antidepressant drugs.

Collectively, these data indicate that antidepressants can produce decreased locomotor activity in open-field test in rodents. Hence, the decreased immobility time produced by *C. striatus* and *C. marulius* extracts in this study are similar to the previous findings (David et al., 2003; Malinge et al., 1988; Wieland and Lucki, 1990). Alternatively, the presence of high concentration of arachidonic acid (19.02% of total fatty acids) in *C. striatus* (Jais et al., 1994; Jais et al., 1998; Zuraini et al., 2006; Zakaria et al. 2006) may be considered as a reason for the decreased locomotor activity, since arachidonic acid was found to reduce locomotor activity in mice in
open-field test in a previous study (Laborit et al., 1975). However, no conclusion can be derived from the present study regarding the mechanism of the observed hypolocomotion, since it was a basic behavioural investigation to evaluate the potential antidepressant activity of *C. striatus* and *C. marulius*.

In conclusion, this study demonstrated that the aqueous extracts of *C. striatus* and *C. marulius* produced antidepressant-like effect in mice models of depression which was not due to any psychomotor stimulant activity thereby supporting the traditional usage of them. Despite the positive results of this research, only male albino rats were used, a suitable animal model of induced- postpartum depression may be used in future to assess the postpartum antidepressant-like effect. Hence, further investigations are necessary to identify the bioactive principles, their respective mechanism of action and also toxicological assessment of *C. striatus* and *C. marulius* extracts. The present research findings have clinical importance since *C. striatus* is currently used by the local Malay population during postpartum period.