Antifatigue Activity of Ethanolic Extract of *Ocimum sanctum* in Rats

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**ABSTRACT**

*Ocimum sanctum* L. is known for its medicinal properties and also has been considered sacred in India since Vedic times. The aqueous suspension of 70% alcoholic extract of *Ocimum sanctum* L. was investigated for antifatigue activity. Rats were subjected to Weight-loaded Forced Swim Test (WFST) every alternate day for 2 weeks. *O. sanctum* at a dosage of 150, 300 and 450 mg kg⁻¹ b.wt. was administrated to rats every day. Swimming time, change in body weight, lipid peroxidation, Lactate Acid (LA), glycogen and blood biochemical parameters namely heamoglobin (Hb%), Blood Urea Nitrogen (BUN) and Creatine Kinase (CK) were evaluated as biomarkers of physical fatigue. The *O. sanctum* treatment was found to lower malondialdehyde (MDA) and lactic acid levels in liver and muscle tissues compared with control exercise group (p<0.05). Serum biochemical parameters viz., BUN and CK activity were also reduced as compared with control exercise group (p<0.05). The present study thus indicates that out of three studied treatments of *O. sanctum* (*O. sanctum* 150, 300 and 450); the *O. sanctum* at the dose of 300 mg kg⁻¹ b.wt. showed better performance against fatigue when compared with other two treatments. As treatment at 300 mg kg⁻¹ b.wt. facilitated the aerobic glucose metabolism and promote swimming time, suggesting that *O. sanctum* ameliorates the various impairments associated with physical fatigue.

**Key words:** Medicinal plants, *Ocimum sanctum*, anti-fatigue, forced swimming test, endurance capacity, performance enhancement

**INTRODUCTION**

Widely held world’s population rely on traditional medicine for treatment of various diseases (Abdel-Azim *et al.*, 2011; Kumar *et al.*, 2011). Medicinal plants have attained amplifying significance in development co-operation for few years (Karim *et al.*, 2011; Malik *et al.*, 2011). *Ocimum* is well-known genus and widely distributed throughout the world (Oboh *et al.*, 2009). In India *Ocimum sanctum* L. (Lamiaceae) has been considered sacred and worshipped since Vedic times. The plant is a part of Indian and Unani system of medicine and is well recognized in Ayurveda for its medicinal properties (Satyavati *et al.*, 1987). The leaves of the plant have long been used to treat a variety of ailments, including skin diseases, gastric and hepatic disorders and are used as a diaphoretic, an anti-periodic and as an expectorant. *O. sanctum* is an important botanical supplement used in combination with other plants for the treatment of various stress-induced disorders in India and other Asian countries (Satyavati *et al.*, 1987; Mediratta *et al.*, 2002). *O. sanctum* has been assessed for its pharmacological activities like anti-stress (Anbarasu and Vijayalakshmi, 2007; Gupta *et al.*, 2007), anti-ulcerogenic (Liu, 1995; Thaker and Anjaria, 1986),
radio protective (Devi and Ganasoundari, 1995), anti-inflammatory effects (Singh and Majumdar, 1999), immune modulatory activity (Mediratta et al., 2002; Hemalatha et al., 2011) and nootropic potential (Joshi and Parle, 2006). Anti-bacterial activity of O. sanctum against gram positive and gram negative bacteria was reported by Mishra and Mishra (2011).

The phytochemical constituents of O. sanctum are ursolic acid, α-triterpenoid and rosmarinic acid (phenylpropanoid) (Gupta et al., 2002). The stem and leaf of O. sanctum contains a variety of chemical compounds which include saponins, flavonoids, terpinoids and tannins (Thaker and Anjaria, 1986). It contains volatile oil comprising mainly of eugenol and β-caryophyllene with minor terpenes like bornyl acetate, β-elemene, methyl eugenol, neral, β-pinene etc. (Gupta et al., 2002). The ethanol extract of O. sanctum leaves was found to prevent the reduction in adrenergic neurotransmitters in brain of rat exposed to swimming stress and gravitational stress (Singh et al., 1991).

Physical fatigue has been shown to be accompanied by deterioration in performance (Tanaka et al., 2008). Fatigue can cause release of Reactive Oxygen Species (ROS) which leads lipid peroxidation of membrane structure. Malondialdehyde (MDA), an oxidative degradation product of the cell membrane lipids, is generally considered as an indicator of lipid peroxidation (Alessio and Goldfrab, 1988). The fatigue conditions are also marked by the release of Blood Urea Nitrogen (BUN) and Creatine Kinase (CK) into the serum which serves as indirect index of membrane damage (Passarella et al., 2008). Free radical formation will be more in the stress condition during over exercises, if these free radical formations were not controlled with in the muscle, they may cause muscle damage. Damaged muscle could in turn inhibit performance by induction of fatigue and also these disorders can lead to a reduction in exercise intensity or even to the interruption of activity (Davis and Bailey, 1997). However, there is paucity of information available concerned with O. sanctum anti fatigue properties. The purpose of this study was to evaluate the effect of O. sanctum on physical strength and endurance in rats during forced swimming experiment.

MATERIALS AND METHODS

Plant material: Ocimum sanctum L. leaf material was procured from Mysore and identified with the help of herbarium collection, Department of Botany, Mysore University, Mysore, India. The plant material was allowed to dry in shade for three days. The air-dried plant material was powdered and extracted on an orbital shaker with 70% ethanol over night, filtered. The extract was flash evaporated to evaporate excess alcohol and then lyophilized to make the extract into powdered form. The final yield of the lyophilized powder was 5.84%.

Animals: Animal studies were conducted according to the guide lines from Institute Animal Ethical Committee and Committee for the Purpose of the Control and Supervision of Experiments on Animals (CPCSEA). Male albino rats of Wistar strain weighing 100-120 g were selected from the stock colony, Defence Food Research Laboratory, Mysore, India, housed in an acrylic fibre cage in a temperature controlled room (temperature 26±2°C) and was maintained in 12 h light/dark cycle with free access to diet and drinking water.

Experimental design: Thirty male Wister rats were randomly divided into the following 5 experimental groups. 1) Sedentary, ii) Control, iii) O. sanctum - 150, iv) O. sanctum -300 and v) O. sanctum - 450. The treatment group rats were administered orally with aqueous suspension of
lyophilized 70% alcoholic extract (150, 300 and 450 mg kg⁻¹ b.wt. per day for O. sanctum 150, 300 and 450, respectively) for a period of two weeks. Control and sedentary rats were orally administered with equal amount of distilled water. Body weights were observed daily during the experimental period.

**Weight-loaded forced swim test (WFST):** The weight-loaded forced swim test (WFST) was performed as described previously with some modifications (Jung et al., 2007). The rats of O. sanctum administered groups and control group were allowed for swimming exercise with support constant loads (tagged to the tails) corresponding to 10% of their body weight. The swimming exercise was carried out in small tank with 30 cm deep with water maintained at 25±2°C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec (Wang et al., 2006). This experiment was repeated every alternate day for a period of two weeks. Animals were sacrificed under mild anaesthesia. Blood samples were collected immediately after the last exercise. Blood was collected from heart using a heparinized syringe into centrifuge tubes. Plasma was obtained after centrifugation at 3000 rpm and used for measurement of Blood Urea Nitrogen (BUN) and Creatine Kinase (CK). Liver and muscle tissues were removed and frozen immediately at -80°C for estimation of lipid peroxidation, lactic acid and glycogen.

**Determination of biochemical parameters:** Haemoglobin content in all rat groups was measured with the help of blood analyzer. Blood Urea Nitrogen (BUN) and Creatine Kinase (CK) were determined according to the procedures provided by the kits (Biosystems and Agappe, respectively). BUN contents was expressed in U dL⁻¹. The haemoglobin and serum CK activity was expressed in g dL⁻¹ and U L⁻¹, respectively. TBARS in terms of malondialdehyde (MDA µmol cm⁻¹ g⁻¹) was analyzed by Buege and Aust (1978) method. Lactic acid and glycogen levels were estimated spectrophotometrically by Sawhney and Singh (2005) and Miller (1959), respectively.

**Statistical analysis:** The data are expressed as Mean±Standard Deviation of the mean. Data were analyzed using Student's t-test with the help of statistical programme for social sciences (SPSS-10 software). Differences at p<0.05 were considered to be significant.

**RESULTS AND DISCUSSION**

**Effect of Ocimum sanctum on body weight and swimming time:** The present study focuses the potential of O. sanctum in preventing various types of damages from the exercise stress and to enhance physical endurance. Swimming until exhaustion is an experimental protocol used for inducing stress in laboratory animals. In these studies, biochemical, hormonal and behavioural changes were recorded to evaluate the physiological responses to a stressful situation (Marcondes et al., 1996). There was no significant change in body weight between the sedentary group, control group and the O. sanctum treatment groups in experimental period (p>0.05) (Table 1). Bhattacharya and Ghosal (2000) reported that the amines were more utilized in stress conditions which resulted depletion of amines in the body. Moreover, the depletion of amines are responsible for fatigue, reduced stamina, lowered mood (hopelessness) or despair seen in individuals under intense stress. The same has been confirmed by Ishola and Ashorobi (2007) and Ishola et al. (2008) based on forced swim test on root extracts of Alchornea cordifolia. The previous experiments
Table 1: Effect of *Ocimum sanctum* on body weight in weight loaded forced swim test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>140±9.30</td>
<td>187±9.94</td>
</tr>
<tr>
<td>Control</td>
<td>146±8</td>
<td>165±11.3</td>
</tr>
<tr>
<td><em>O. sanctum</em> -150</td>
<td>150±15.05</td>
<td>170±5±18.01</td>
</tr>
<tr>
<td><em>O. sanctum</em> -300</td>
<td>162±13.37</td>
<td>180±5±13.03</td>
</tr>
<tr>
<td><em>O. sanctum</em> -450</td>
<td>154.3±11.25</td>
<td>191±15.59</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D, n = 6 in each groups. Sedentary: Rat unexposed to the WFST and treated with distilled water; Control: Rat exposed to the WFST and treated with distilled water; *O. sanctum* -150, *O. sanctum* -300, *O. sanctum* -450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ b.wt. respectively. Non significantly different vs. control (p>0.05)

Fig. 1: Effect of OS on endurance capacity. Values are expressed as Mean±S.D, n = 6 in each groups; significant difference is p<0.05 vs. control. Sedentary: Rat unexposed to the WFST and treated with distilled water, Control: Rat exposed to the WFST and treated with distilled water, *O. sanctum* -150, *O. sanctum* -300, *O. sanctum* -450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ b.wt., respectively

in rats have demonstrated a protective effect of *Ocimum sanctum* extracts against different types of stresses (Ravindran et al., 2005; Samson et al., 2007; Gupta et al., 2007). Stress-induced increases in circulating corticosterone, dopamine and serotonin levels were prevented with *O. sanctum* extract pretreatment and other adaptogenic effects on biochemical changes in acetylcholine, acetylcholinesterase and creatine kinase were observed (Sembulingam et al., 2005). The anti fatigue activity of the *O. sanctum* was measured as the swimming endurance capacity of rat with 10% tail load. It was observed that the swimming time was significantly increased from first day to the last day (day 14) in all treatments (p<0.05). The increase essentially seemed to be a function of concentration of the *O. sanctum* extract up to 10th day. However, with *O. sanctum* -450 was showed insignificant reduction in time after the day 10 and the maximum performance was observed in *O. sanctum* -300 group (Fig. 1).

**Effects of *O. sanctum* on blood biochemical parameters:** Haemoglobin (Hb) is one of the indicators that reflect the degree of recovery from fatigue after exercise (Gao and Wu, 2008). Its main function is to serve as the carrier for the erythrocyte to transport oxygen and carbon dioxide (Nikinmaa, 1997). Apart from maintaining the acid-alkali balance the body fluid acid-base balance is very important because a small change can produce major disturbance since it can affect the electrolytes and functionality of enzymes. In the present study, swimming exercise lowered the Hb
### Table 2: Effect of *O. sanctum* on blood biochemical parameters after the WFST

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb levels (g dL⁻¹)</th>
<th>BUN (U dL⁻¹)</th>
<th>CK (U L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>13.35±1.21</td>
<td>2.31±0.53</td>
<td>106.68±8</td>
</tr>
<tr>
<td>Control</td>
<td>12.55±1.00</td>
<td>4.11±0.81</td>
<td>219.00±27</td>
</tr>
<tr>
<td><em>O. sanctum</em>-150</td>
<td>13.20±1.22</td>
<td>3.82±0.69</td>
<td>164.10±10</td>
</tr>
<tr>
<td><em>O. sanctum</em>-300</td>
<td>14.70±1.35</td>
<td>3.35±0.55</td>
<td>147.60±18</td>
</tr>
<tr>
<td><em>O. sanctum</em>-450</td>
<td>14.70±1.32</td>
<td>3.41±0.58</td>
<td>154.68±7</td>
</tr>
</tbody>
</table>

Values are expressed as Means±S.D. n = 6 in each group; significant difference is p<0.05 vs. control. Sedentary: Rat exposed to the WFST and treated with distilled water; Control: Rat exposed to the WFST and treated with distilled water; *O. sanctum*-150, *O. sanctum*-300, *O. sanctum*-450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ bw, respectively.

Levels in control group (12.55±1.0 g dL⁻¹) compared with sedentary group (13.20±1.21 g dL⁻¹).

Higher level of Hb is helpful to improve the exercise ability (Gao and Wu, 2008) and the same was observed in the present study with the supplementation of *O. sanctum*-150, 300 and 450 compared to the control group (p<0.05). However, *O. sanctum*-300 and -450 treatment groups were observed with similar Hb concentrations (Table 2). The previous immunomodulatory studies of *Ocimum sanctum* were also supported the present study that the *O. sanctum* could enhance the production of RBC, WBC and haemoglobin (Jebs et al., 2011).

Mostly BUN represents the renal function; however, the levels of BUN changes in stress conditions (Tanno et al., 2002). The normal function of CK in cells is to add a phosphate group to creatine, converting it into the high-energy molecule phosphocreatine. Phosphocreatine may be utilized as a quick source of energy by muscle cells. Most of the CK in the body normally present in muscle, an increase in the amount of CK in the blood indicates the muscle damage. The most intriguing findings were the significant increase in BUN and serum CK with swimming exercise (Table 2). These BUN and CK contents were decreased with the treatment of *O. sanctum* (all doses), however, *O. sanctum*-300 showed significantly lowered BUN and CK when compared to the other two doses i.e., *O. sanctum*-150 and -450 (Table 2).

**Effect of *O. sanctum* on lipid peroxidation:** Lipid peroxidation products in liver and muscle increase after severe exercise in liver and muscle (Keenoy et al., 2001). There is an evidence that mitochondrial dysfunction is directly related to fatigue in humans. Thio-Barbituric Acid Reactive Substances (TBARS) studied represents lipid peroxidation in liver, brain and muscle tissues as malondialdehyde (MDA μmol cm⁻¹ g⁻¹) and results are shown in Fig. 2. Swimming exercises significantly increased MDA concentration in muscle (40 μmol cm⁻¹ g⁻¹), liver (55 μmol cm⁻¹ g⁻¹) and brain (51 μmol cm⁻¹ g⁻¹) when compared with sedentary group (p<0.05). The *O. sanctum*-450 treatment reduced more lipid peroxidation when compared with other two treatments of *Ocimum sanctum* in liver, brain and muscle tissues (40, 39 and 36 μmol cm⁻¹ g⁻¹, respectively). The results of TBARS levels are in line with several authors (Kumar et al., 2009). Presence of flavonoids in *O. sanctum* may be held responsible for its attenuating activity because flavonoids have been reported as potentially useful exogenous agents in protecting the organs against free radical induced damage (Blaylock, 1999). So, it is evident that the *O. sanctum* could effective in increased exhaustive exercised endurance capacity.

**Effect of *O. sanctum* on lactic acid:** Lactic Acid (LA) is one of the glycolytic products of carbohydrate metabolism under anaerobic condition and may be considered as one of the biomarkers for judging the degree of fatigue (Wang et al., 2006; Yu et al., 2008). Since, ATP
Fig. 2: Effect of *O. sanctum* on lipid peroxidation. Values are expressed as Mean±S.D, n = 6 in each groups; significant difference is p<0.05 vs. control. Sedentary: Rat unexposed to the WFST and treated with distilled water, Control: Rat exposed to the WFST and treated with distilled water, *O. sanctum* -150, *O. sanctum* -300, *O. sanctum* -450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ b.wt., respectively.

Fig. 3: Effect of *O. sanctum* on Lactic acid content. Values are expressed as Mean±S.D, n = 6 in each groups; significant difference is p<0.05 vs. control. Sedentary: Rat unexposed to the WFST and treated with distilled water, Control: Rat exposed to the WFST and treated with distilled water, *O. sanctum* -150, *O. sanctum* -300, *O. sanctum* -450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ b.wt., respectively.

Production is fast under anaerobic glycolysis this becomes the major source of energy supply during short time exercise. As shown in Fig. 3, there was a significant decrease in the concentration of LA in all *O. sanctum* treated groups (*O. sanctum* -150, -300 and -450) compared to the control group in liver and in muscle. However, liver and muscle tissues of *O. sanctum* -300 treated rats were showed significantly reduced levels of lactic acid when compared with other two treatments of *O. sanctum* (p<0.05). These reducing levels of lactic acid in *O. sanctum* -300 group confirms the anti fatigue property of *O. sanctum*.

**Effect of *O. sanctum* on Hepatic and muscle glycogen:** The glycogen contents are sensitive parameters related to fatigue, hence the liver and muscle tissues were used for its glycogen content.
Fig. 4: Effect of *O. sanctum* on Liver and muscle glycogen. Values are expressed as Mean±S.D., n = 6 in each groups; significant difference is p<0.05 vs. control. Sedentary: Rat unexposed to the WFST and treated with distilled water, Control: Rat exposed to the WFST and treated with distilled water, *O. sanctum* -150, *O. sanctum* -300, *O. sanctum* -450: Rat exposed to the WFST and treated with different doses of *Ocimum sanctum* extract i.e., 150, 300, 450 mg kg⁻¹ b.wt., respectively

(Fig. 4). It is known that endurance capacity decreases if the available energy is exhausted. Glycogen is the important resource of energy during exercise, the increase in glycogen stored in liver is an advantage to enhance the physical endurance (Dohm et al., 1983). Depletion of liver glycogen is an important factor in the exercised fatigue, may lead to hypoglycemia impairing nervous function (Dohm et al., 1983). Swimming exercise reduced the levels of liver and muscle glycogen when compared with sedentary group (p<0.05). All the three doses of *O. sanctum* were helped to improve the glycogen levels of both liver and muscle tissues (Fig. 4). However, *O. sanctum* -300 and -450 treatments were maintained more or less similar with respect to glycogen levels in liver (45.25±2.6 and 43±3.2 mg g⁻¹, respectively) and in muscle tissues (4.0±0.5 and 4.0±0.2 mg g⁻¹, respectively). These results also support that *O. sanctum* -300 is the concentration capable of improving the glycogen levels in exercised stress, showing the glycogen sparing action of *O. sanctum*.

**CONCLUSIONS**

In conclusion, the data suggested that leaf extracts of *Ocimum sanctum* could extend the swimming time to exhaustion of the rat with 10% tail load, as well as increase the tissue glycogen contents and decrease the Malondialdehyde (MDA), lactate, BUN and serum CK levels. These results also support that *O. sanctum* at 300 mg kg⁻¹ b.wt. is the optimum concentration to act
against fatigue. However, further studies are necessary to evaluate the detailed mechanism(s) involved in the anti-fatigue properties *O. sanctum*.

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