



## Original article

## Evaluation of antioxidant and anti-fatigue properties of *Trigonella foenum-graecum* L. in rats subjected to weight loaded forced swim test

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## ABSTRACT

**Background:** *Trigonella foenum-graecum* L. (Fabaceae) is commonly known as fenugreek. Aim of the present study was to assess anti fatigue potential of fenugreek hydro-alcoholic extract (FHE).

**Methods:** The anti fatigue activity of FHE was investigated in *in vivo* rat model subjected to weight loaded forced swim test (WFST) at a dosage of 10 mg/kg body weight. *In vitro* antioxidant activities were carried out by spectrophotometric methods.

**Results:** Exhaustive swimming time in WFST was increased by more than 2-fold in FHE supplemented group to that of control group on day 13. The FHE treatment lowered malondialdehyde and lactic acid levels in liver and muscle tissues compared with control exercised group ( $p < 0.05$ ). FHE also reduced serum lactic acid, blood urea nitrogen and creatine phosphokinase activities significantly to that of control. Administration of FHE significantly protected the depletion of serum glucose, liver and muscle glycogen, and activities of antioxidant enzymes i.e. SOD, CAT and GPx ( $6.05 \pm 0.5$ ;  $22.0 \pm 1.1$  and  $2084 \pm 158$  U/mg protein, respectively).

**Conclusion:** In the present study we report that FHE ameliorates various impairments associated with physical fatigue.

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### 1. Introduction

Fatigue is a physiological phenomenon that appears with physical stress or exhaustive exercises, which reduces the physical endurance capacity.<sup>1</sup> It has been demonstrated that exercise leads to increase in free radical formations thus causing oxidative damage to membranes. Chronic fatigue sometimes causes various neurological, psychiatric and systemic diseases and is often associated with aging, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis and depression.<sup>2,3</sup> The supplementation of antioxidants has a major role in reducing the degree of fatigue by the oxidation of inter or intra cellular oxidizable substrates.<sup>4</sup>

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. This crop is native to an area extending from Iran to Asia, but is now widely cultivated in China, north and east Africa, Ukraine and Greece.<sup>5</sup> In Asia, the fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses. Fenugreek seed is reported to have antidiabetic,<sup>6</sup> hypocholesterolaemic,<sup>7</sup> anticancer,<sup>8</sup> antifertility,

antimicrobial, antiparasitic, and anti viral.<sup>9</sup> The seeds of fenugreek contain several beneficial phytochemicals including polyphenols, steroidal saponin, fiber galactomannans, antioxidants, rare amino acids such as 4-hydroxyisoleucine and proteins.<sup>10</sup>

In the present study, we have evaluated the antioxidant and anti-fatigue activity of fenugreek seed extract in *in vitro* and *in vivo* rat model by weight loaded forced swim test. Biochemical changes on serum, liver and muscle tissues were observed to prove endurance capacity of fenugreek seed hydro-alcoholic extract (FHE). Thus, the evaluation of anti-fatigue properties of drugs or herbal preparations requires understanding of its potential to scavenge reactive oxygen species (ROS) and enhance antioxidant defense in the body.

### 2. Materials and methods

#### 2.1. Seed material and extraction

Fenugreek seed samples were collected from the local market of Mysore, India. Dry seeds (100 g) were cleaned and imbibed in distilled water for 12 h and macerated with 90% ethanol to get all the bioactive components. Both water and 90% ethanol fractions were combined together, evaporated the solvent and lyophilized for further use.

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## 2.2. Quantification of total polyphenols, flavonoids and amino acids

Total polyphenols were determined by the Folin–Ciocalteu procedure.<sup>11</sup> The amount of total polyphenols was calculated from the calibration curve of gallic acid standard solutions, concentration of total phenols was expressed as mg/g of dry extract. Total flavonoid contents were determined by the method of Ordon et al.,<sup>11</sup> Flavonoid concentration of the extract was expressed as mg/g equivalent of quercetin. Amino acid content was estimated by ninhydrin reagent to produce purple color and measured absorbance at 570 nm. A calibration graph of glycine was plotted and amino acid concentration of the extract was expressed as mg/g equivalent of glycine.<sup>12</sup>

## 2.3. Determination of *in vitro* antioxidant activities of FHE

### 2.3.1. DPPH radical scavenging activity

The free radical scavenging activity of the fenugreek extract was determined *in vitro* by DPPH (1, 1 diphenyl 2, picryl hydrazyl) assay.<sup>13</sup> DPPH in methanol (0.1 mM) was prepared and 3.0 ml of this solution was added to 40  $\mu$ l of extract solution in water at different concentrations. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 515 nm against corresponding blank solution. Percentage inhibition of DPPH free radical was calculated based on the control reading. The antioxidant activity of the extract was expressed as IC<sub>50</sub>, which the concentration ( $\mu$ g/ml) of extract inhibits formation of DPPH radicals by 50%.

### 2.3.2. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was measured using modified method as described previously by Halliwell and Gutteridge.<sup>14</sup> The assay was performed by adding EDTA (0.1 ml; 1 Mm), FeCl<sub>3</sub> (0.01 ml; 10 mM), H<sub>2</sub>O<sub>2</sub> (0.1 ml; 10 mM), deoxyribose (0.36 ml; 10 mM), phosphate buffer (0.33 ml; 50 mM, pH 7.4), ascorbic acid (0.1 ml) and 1.0 ml of extract (50–300  $\mu$ g/ml). The mixture was then incubated at 37 °C for 1 h and followed by addition of equal amounts of trichloroacetic acid (10%) and thio-barbituric acid (0.05%) to develop the pink chromogen, which was measured at 532 nm and the activity of the extract was reported as the percentage of inhibition of deoxyribose degradation.

### 2.3.3. ABTS radical scavenging activity

The ABTS radical scavenging activity [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical] assay was followed by the method of Re et al.<sup>15</sup> The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working reagent was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. Sample extract was allowed to react with 1 ml of the working reagent and the absorbance was taken at 734 nm after 7 min using the spectrophotometer against corresponding blank solution.

### 2.3.4. Metal chelating activity

Metal chelating activity was observed with the percentage inhibition of ferrozine-Fe<sup>2+</sup> complex formation.<sup>16</sup> Briefly, the extract (25–100  $\mu$ g/ml) was added to a solution of 2 mM FeCl<sub>2</sub> (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine – Fe<sup>2+</sup> complex formation was calculated with the absorbance of the extract or Na<sub>2</sub>EDTA (positive control).

## 2.4. *In vivo* physical endurance capacity of FHE

### 2.4.1. Animal experiment

Animal studies were conducted according to the institute animal ethical committee regulations approved by the committee for the purpose of the control and supervision of experiments on animals (CPCSEA). Eighteen male albino rats of Wistar strain weighing 120–140 g (3–4 week old) were selected from the stock colony of Defence Food Research Laboratory, Mysore, India, housed in an acryl fiber cage in a temperature controlled room (25  $\pm$  2 °C) and was maintained in 12 h light/dark cycle. The rats were randomly divided into the following 3 groups: sedentary, control and treatment groups (FHE). The treatment group rats were administered orally with FHE (10 mg/kg of body weight/per day) for a period of two weeks. Sedentary and control rats were orally administered with equal amount of distilled water. The animals were fed with a commercial pellet diet (Sri Venkateswara Enterprises, Bangalore, India) and water *ad libitum*.

### 2.4.2. Weight loaded forced swim test (WFST)

The weight loaded forced swim test (WFST) was performed as described previously with some minor modifications.<sup>17</sup> The rats of FHE administered group and control group were allowed to swim with constant loads (tagged to the tail base) corresponding to 5% of their body weight. The swimming exercise was carried out in small tank with 30 cm deep with water maintained at 25  $\pm$  2 °C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 s.<sup>18</sup> This experiment was repeated every alternate day for a period of three weeks. Animals were sacrificed under mild anesthesia immediately after the last exercise. Blood was collected from the heart using a heparinized syringe into centrifuge tubes. Separated plasma and tissue samples of brain, liver and muscle were stored at –80 °C until further analysis.

### 2.4.3. Measuring blood biochemical parameters

Serum BUN, CK, lactic acid and plasma glucose were determined using commercially available kits from Agappe Company, India.

### 2.4.4. Determination of glycogen and lactic acid

Liver and muscle tissues were digested with 2 ml of KOH (30%) and boiled in water bath for 30 min with occasional shaking and then allowed to cool at room temperature. Saturated Na<sub>2</sub>SO<sub>4</sub> solution was added to the mixture and stirred well. Glycogen was precipitated by adding 5 ml of ice cold ethanol to the sample mixture and centrifuged at 10,000 rpm for 10 min. One ml of HCl (1.2 N) was added to the supernatant (1: 1 v/v) and incubated at 90 °C for 2 h, and then allowed to cool. DNS method was followed to determine hydrolyzed product of tissue glycogen.<sup>19</sup> Lactic acid content was measured by the method of Sawhney and Singh.<sup>20</sup> Tissue samples were homogenized in phosphate buffer (100 mM, pH 7.2), and deproteinized with TCA (10%). These deproteinized samples were centrifuged at 5000 rpm for 15 min. To the supernatant, 1 ml of copper sulfate solution (20%) and 6 ml of H<sub>2</sub>SO<sub>4</sub> (conc.) were added and kept in boiling water bath for 5 min and allowed to cool. One hundred micro liters of p-hydroxy-diphenyl reagent was added to the above sample mixture and incubated at 37 °C for 30 min. The absorbance was measured at 560 nm.

### 2.4.5. Determination of SOD, CAT and GPx activities

Liver tissues were homogenized in 50 mM phosphate buffer saline (pH 7.4). SOD and GPx activities were determined with the commercially available kits (Randox, Canada; Cat no. SD 125 and RS 504, respectively). CAT was determined by measuring the decay of

6 mM H<sub>2</sub>O<sub>2</sub> solution at 240 nm by spectrophotometric degradation method.<sup>17</sup> Briefly, Liver tissue was homogenized in phosphate buffer (50 mM; pH 7.0). Centrifuged at 8000 rpm for 20 min and supernatant was diluted in phosphate buffer (1:100). To 100 µl of supernatant, 200 µl of phosphate buffer and 700 µl of H<sub>2</sub>O<sub>2</sub> (660 mM) was added and absorbance was read at 240 nm for 3 min at 15 s interval.

#### 2.4.6. Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS as malondialdehyde (MDA µmol/cm/g) was analyzed by Buege and Aust.<sup>21</sup> Brain, liver and muscle tissues (100 mg) were homogenized in 2 ml of phosphate buffer (pH 7.0). TCA (10%), 0.5 ml and 2 ml of TBA mixture were added to tissue homogenate (0.5 ml). The TBA mixture contains TBA (0.35%), SDS (0.2%), FeCl<sub>3</sub> (0.05 mM) and BHT in glycine-HCl buffer (100 mM, pH 3.6). The above reaction mixture was boiled at 100 °C for 30 min and then allowed to cool. The mixture was centrifuged at 8000 rpm for 10 min and the absorbance was measured at 532 nm.

#### 2.5. Statistical analysis

The data are expressed as mean ± standard deviation of the mean (S.D). Data was analyzed using one-way ANOVA. Differences at  $p < 0.05$  were considered to be significant.

### 3. Results and discussion

#### 3.1. In vitro antioxidant activities of FHE

Fenugreek (*T. foenum-graecum* L.) is a multi-purpose medicinal plant. It is being used as nutraceutical, functional food, as well as forage crop for livestock feed.<sup>22</sup> Phytochemistry of the fenugreek seeds reveal the presence of tannic acid, fixed and volatile oils, steroidal saponin diosgenin, alkaloids trigonelline, trigocoumarin, trigomethyl coumarin, and steroidal saponins such as gitogenin, trigogenin and vitamin A.<sup>23</sup> Phenolic compounds are a class of antioxidant agents which act as free radical scavengers and are considered as a major group of compounds that contribute to the antioxidant activities of plant materials because of their neutralizing ability on free radicals due to their hydroxyl groups.<sup>24</sup> Flavonoids are a group of polyphenolic compounds with known properties of free radical scavenging, antibacterial and anti-inflammatory action.<sup>25</sup> Different extraction protocols of fenugreek were reported in a vast range of polyphenols and flavonoids by Bukhari et al,<sup>26</sup> and the range was 1.35–6.85 mg/g GAE and 208–653 µg/g QE, respectively. However, the present extraction methodology of fenugreek had given more polyphenol ( $22 \pm 1.5$  µg/mg GAE) and flavonoid ( $16.6 \pm 1.2$  µg/mg QE) contents when compared to other extraction process by earlier researchers.<sup>26</sup> It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, flavor and also in providing health beneficial effects. The present results showed that the FHE extract is rich in amino acids contents. Moreover, fenugreek seed is rich with several amino acids like lysine, tryptophan<sup>6</sup> and 4-hydroxyisoleucine, which is reported as a major bioactive compound for hypoglycemic activity.<sup>27,28</sup> In the present study, FHE also showed high quantity of amino acid i.e.  $50.0 \pm 2.8$  µg/mg glycine equivalents. This implied that the amino acids in the FHE can enhance exercise ability.

Oxidative stress plays an important role in the pathophysiology of exhaustive exercises with fatigue condition.<sup>29</sup> Free radicals cause oxidative damage to nucleic acids, proteins, carbohydrates and lipids. The antioxidant property of an extract neutralizes the free radicals and controls the oxidative damage of tissues in the

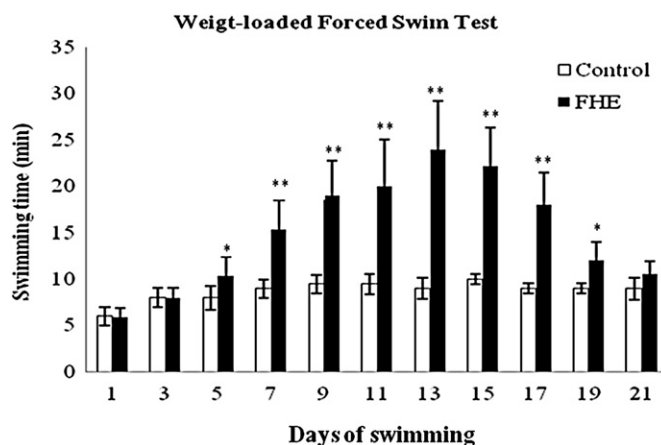
treatment of fatigue syndrome.<sup>30,31</sup> The antioxidant activity of the FHE increased with an increasing amount of extract. FHE was able to reduce the stable free radical DPPH to yellow colored diphenylpicrylhydrazine with an IC<sub>50</sub> of 395 µg/ml ( $R^2 = 0.9712$ ). In the hydroxyl radical mediated 2-deoxy-D-ribose degradation assay, IC<sub>50</sub> value were calculated using linear regression equation ( $R^2 = 0.9889$ ) and showed IC<sub>50</sub> = 188.6 µg/ml. The result of ABTS radical scavenging activity and metal chelating activity of FHE are also in dose depended manner with IC<sub>50</sub> values of 96.7 µg/ml ( $R^2 = 0.9929$ ) and 69.7 µg/ml, respectively ( $R^2 = 0.9978$ ). Moreover, in the present study, FHE is observed with excellent antioxidant activity and assume that these scavenging activities could help in delaying the fatigue or in the formation of free radicals in exercised stress conditions.

#### 3.2. In vivo endurance capacity of FHE

##### 3.2.1. Effect of FHE on prolonged swimming time

In the present study, 10 mg/kg bwt./day of FHE was orally fed to the rats for a period of two weeks to know the swimming endurance capacity. Present results showed that there was no significant change in weight gain by the rats in sedentary group, control group and FHE supplemented group (Results not shown). Muralidhara et al,<sup>32</sup> showed that the fenugreek powder, administered intra gastrically to mice and rats of both sexes failed to induce any signs of toxicity or mortality up to a maximum dosage of 2 and 5 g/kg body weight, respectively. Khalki et al,<sup>33</sup> reported with no mortality or treatment-related signs of abnormal behavioral changes at the dosage of 500 and 1000 mg/kg bwt. in the female rodents. However, Araee et al<sup>34</sup> has considered that in view of the presence of the steroidal saponin diosgenin in fenugreek seeds, in high doses adversely influences bone marrow cell proliferation. Hence, the dosage of FHE used in the study (10 mg/kg bwt./day) has been proved to be non toxic.

The weight loaded forced swim test (WFST) was carried out with 5% tail load for a period of three weeks (first two weeks with FHE treatment and then, third week without FHE treatment). The forced swim test is perhaps one of the most commonly used animal models for evaluating the physical endurance capacity.<sup>35</sup> On the first and second day of WFST, no significant difference of swimming time was recorded between control and FHE treated groups ( $p > 0.05$ ). Third day onwards, the swimming time was gradually increased with FHE treatment when compared to that of control



**Fig. 1.** Effect of FHE on physical endurance capacity by weight loaded forced swim test. Data express the mean ± S.D. for 6 rats. Different symbols above the bar indicates statistically significant differences for the same day, \* $p < 0.05$ , \*\* $p < 0.01$  vs control; FHE = fenugreek hydro-alcoholic extract.

group ( $p < 0.05$ ). Maximum swimming time was recorded on day 13 of the FHE treatment with  $24 \pm 4.2$  min (Fig. 1). The day 15 onwards it was observed in decreased swimming performance due to withdrawal of the extract. However, the swimming time of the FHE treated group was more when compared with control group. In the present study, the swimming time data showed that the administration of FHE could evidently extend swimming time for exhaustion, indicating FHE has anti-fatigue activity and could elevate the exercise tolerance.

### 3.2.2. Effect of FHE on blood biochemical parameters

The blood biochemical levels of BUN, CK, glucose and lactic acid of all studied groups are shown in Table 1. The homeostasis of blood glucose plays an important role in prolonging endurance exercise. Continuous exercises often lead to hypoglycemia and can suppress the active functioning of the brain. Therefore, the amount of blood glucose can illustrate the speed and degree of fatigue development.<sup>18</sup> Simple exercise begins with an increase in aerobic muscular activity. However, intensive exercises switch over to anaerobic metabolism, which converts LDH to lactic acid.<sup>36</sup> In the present experiment the exposure to the WFST led to decrease in plasma glucose and increase in serum lactic acid levels in control group comparison with the sedentary group ( $p < 0.05$ ). However, the FHE treatment reversed these levels (Table 1).

Blood urea nitrogen represents normally kidney function; however, there are many factors like protein breakdown, dehydration, stress, fatigue, etc., that can cause an alteration in BUN levels. This protein and amino acids are metabolized to meet the energy requirement when the body cannot derive energy from carbohydrate and fat. There may be a positive correlation between the urea nitrogen and exercise tolerance.<sup>18</sup> The present results indicate that the levels of BUN in FHE group were lower than that of control group ( $p < 0.01$ ), suggesting that FHE may reduce protein catabolism for energy. One of the functions of creatine kinase in cells is to add a phosphate group to creatine, to synthesize high-energy phosphocreatine molecule, utilized as a rapid source of energy by the cells. During the process of extreme muscle stress, the muscle cells degenerate and their contents find in the blood stream. Because most of the CK in the body normally exists in the muscle, an increase in CK in the blood indicates that muscle damage has occurred or is occurring.<sup>37</sup> The most intriguing finding of the study was the significant decreases in serum CK (a biomarker of muscle fiber damage) by FHE group, when compared to the control group ( $p < 0.05$ ). The homeostasis of blood glucose plays an important role in performance enhancement.<sup>38</sup> As shown in Table 1, the blood GLU levels of FHE were higher than that of the control group. Intensive exercise leads to the accumulation of blood lactic acid,<sup>18,37</sup> the present results confirms that the LA levels were significantly lowered by FHE treatment when compared with control group ( $p < 0.05$ ).

**Table 1**

Effect of FHE on blood biochemical parameters after the weight loaded forced swim test.

Group	BUN (mg/dL)	CK (U/L)	GLU (mmol/L)	LA (mg/dL)
Sedentary	$17.7 \pm 1.5$	$172 \pm 30.7$	$7.15 \pm 0.8$	$59 \pm 8$
Control	$21.1 \pm 2.0^{**}$	$199 \pm 25.2^{**}$	$6.62 \pm 0.5^*$	$75 \pm 11^{**}$
FHE	$18.3 \pm 1.6^{##}$	$184 \pm 19^{\#}$	$6.74 \pm 0.3$	$68 \pm 9^{\#}$

Values are expressed as mean  $\pm$  S.D.;  $n = 6$  in each group, statistically significant differences,  $*p < 0.05$ ,  $**p < 0.01$  vs sedentary; and  $^{\#}p < 0.05$ ,  $^{##}p < 0.01$  vs control. BUN = blood urea nitrogen, CK = creatine phosphokinase, GLU = glucose, LA = lactic acid; sedentary = rat unexposed to the WFST and treated with distilled water, control = rat exposed to the WFST and treated with distilled water. FHE = rat exposed to the WFST and treated with fenugreek extract.

**Table 2**

Effect of FHE on antioxidant enzymes of liver tissues after the weight loaded forced swim test.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Sedentary	$6.31 \pm 0.9$	$29.0 \pm 2.1$	$2202 \pm 181$
Control	$4.91 \pm 1.0^{**}$	$20.8 \pm 3.3^{**}$	$1689 \pm 175^{**}$
FHE	$6.05 \pm 0.5^{##}$	$22.0 \pm 1.1^{\#}$	$2084 \pm 158^{##}$

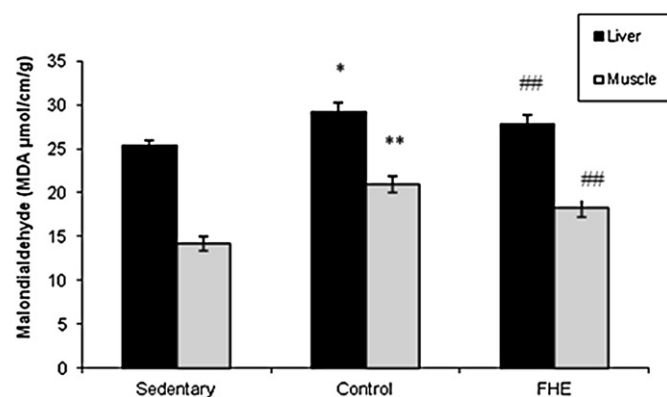
Values are expressed as mean  $\pm$  S.D.;  $n = 6$  in each group, statistically significant differences,  $**p < 0.01$  vs sedentary; and  $^{\#}p < 0.05$ ,  $^{##}p < 0.01$  vs control. SOD = Superoxide dismutase, CAT = Catalase, GPx = Glutathione peroxidase. Sedentary = rat unexposed to the WFST and treated with distilled water, control = rat exposed to the WFST and treated with distilled water. FHE = rat exposed to the WFST and treated with fenugreek extract.

### 3.2.3. Effect of FHE on antioxidant enzymes activity

Activity of liver SOD, CAT and GPx were decreased by swimming exercise when compared with sedentary group rats ( $p < 0.01$ ). However, these antioxidant activities were up regulated with supplementation of FHE by 23.2%, 5.7% and 23.3% respectively, when compared with control group (Table 2). Recent studies show that oxidative stress plays a very important role in the etiology of chronic fatigue syndrome and that antioxidants might be useful in its treatment.<sup>29</sup> The primary antioxidant enzymes include SOD, GPx and CAT.<sup>37</sup> SOD dismutates superoxide radicals to form  $H_2O_2$  and  $O_2$ . GPx is an enzyme responsible for reducing  $H_2O_2$  to water and alcohol, respectively. CAT catalyzes the breakdown of  $H_2O_2$  to form water and  $O_2$ . These antioxidant defense mechanisms become weaker during chronic fatigue and other disease conditions.<sup>37</sup> So, the improvement in the activities of these defense mechanisms can help to fight against fatigue. In the present results, the exogenous antioxidants from FHE may act directly or interact with endogenous antioxidants to form a cooperative network of cellular antioxidants to defend fatigue and strengthen the endurance capacity.

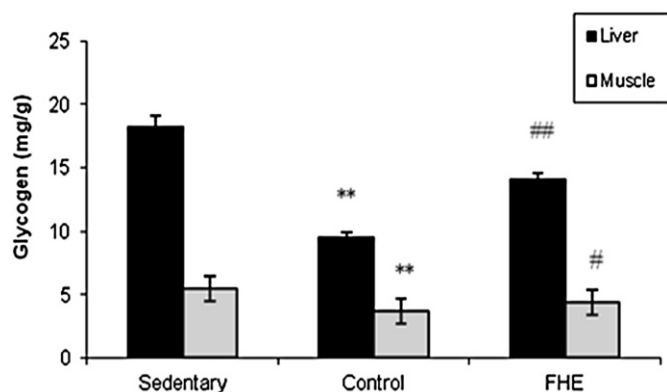
### 3.2.4. Effect of FHE on lipid peroxidation, glycogen and lactic acid

Lipid peroxidation is known to play an important role in the pathophysiology of fatigue syndrome.<sup>30</sup> Lipid peroxidation in liver and muscle tissues represented as malondialdehyde (MDA  $\mu\text{mol}/\text{cm/g}$ ) and results are shown in Fig. 2. Swimming exercise significantly increased MDA concentration in liver and muscle when compared with sedentary group ( $p < 0.05$ ). However, FHE supplemented group decreased the MDA levels in liver and muscle tissues by 4.6% and 25.1% respectively, compared with control group ( $p < 0.01$ ). Glycogen is an important source of energy during physical exercises, hence glycogen contents are sensitive

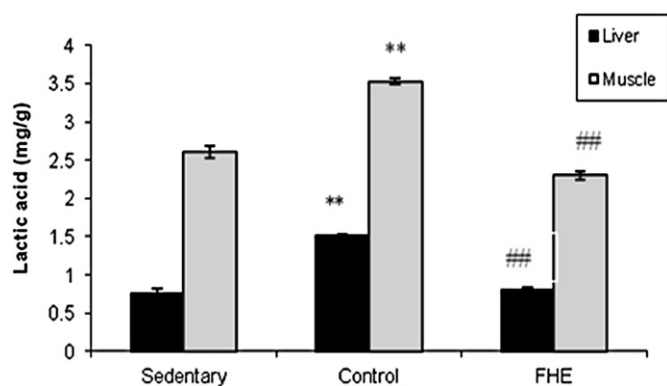


**Fig. 2.** Effect of FHE on thiobarbituric acid-reactive substances (TBARS). Data express the mean  $\pm$  S.D. for 6 rats. Different letters above the bar indicates statistically significant differences,  $*p < 0.05$ ,  $**p < 0.01$  vs sedentary; and  $^{##}p < 0.01$  vs control, FHE = fenugreek hydro-alcoholic extract.





**Fig. 3.** Effect of FHE on glycogen content in liver and muscle. Data express the mean  $\pm$  S.D. for 6 rats. Different symbols indicates statistically significant differences, \* $p < 0.05$ , \*\* $p < 0.01$  vs sedentary; and # $p < 0.05$ , ## $p < 0.01$  vs control, FHE = fenugreek hydro-alcoholic extract.



**Fig. 4.** Effect of FHE on lactic acid content in liver and muscle. Data express the mean  $\pm$  S.D. for 6 rats. Different symbols above the bar indicates statistically significant differences, \*\* $p < 0.01$  vs sedentary; and ## $p < 0.01$  vs control, FHE = fenugreek hydro-alcoholic extract.

biomarkers related to fatigue. As per the previous reports, the depletion of liver and muscle glycogen is common phenomena in physical exercises.<sup>35</sup> Depletion of liver glycogen may lead to hypoglycaemia impairing nervous function in over exercise fatigue condition.<sup>39</sup> In the study, swimming exercise reduced the levels of liver and muscle glycogen when compared to that of sedentary group ( $p < 0.01$ ). As shown in Fig. 3, the glycogen content of liver and muscle were also significantly increased by the FHE supplementation ( $p < 0.01$  and  $p < 0.05$ , respectively). Lactic acid is one of biomarkers judging the degree of fatigue.<sup>18</sup> In the present study, the lactic acid levels were significantly reduced by FHE treatment in both liver ( $p < 0.01$ ) and muscle ( $p < 0.05$ ) (Fig. 4).

#### 4. Conclusion

The results show that hydro-alcoholic extract of *T. foenum-graecum* is rich in polyphenol, flavonoid and amino acid contents. The results have also concluded that FHE is rich with *in vitro* antioxidant activities along with physical endurance capacity to delay fatigue in rat model. The present study has demonstrated the potential of FHE in reducing physical fatigue by prolonged the exhaustion swimming time and modulating several biochemical markers. Therefore, FHE can elevate the endurance capacity and

facilitate recovery from fatigue. However, further research is warranted to elucidate its mechanism.

#### Conflicts of interest

All authors have none to declare.

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