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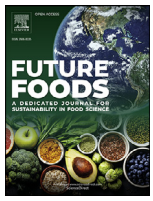

Using dates (*Phoenix dactylifera* l.) to improve energy metabolism in fatigue-induced Sprague Dawley rats

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ABSTRACT

Fatigue increases oxidative stress which damages body cells and increases the risk for the development of various chronic diseases. In this study, the anti-fatigue activity of Piyarom date extract was evaluated in fatigue-induced rats using a forced swimming test (FST). Results showed that rats treated with 500 mg/kg BW date extract exhibited enhanced energy production with a highest endurance capacity (632.9±28.7 min). The date-treated rats also had lower blood lactate, indicating efficient energy utilization with lower fatigue as compared to normal and fatigued rats. Moreover, the serum lactate dehydrogenase levels in 250 and 500 mg/kg BW date extracts and caffeine groups were significantly lower compared to the fatigued rats. Creatine kinase activity was the lowest in the group fed with 500 mg/kg BW date extract. The treated rats showed an amelioration of lipid profiles, while the histological study revealed improvement in various organs. Rats treated with 500 mg/kg BW date extract demonstrated an enhancement in energy production and improved energy metabolism which could be due to the presence of bioactive compounds in the dates. Piyarom date extract demonstrated anti-fatigue property and could be used as a functional ingredient in the development of beverages or snacks that address fatigue associated maladies.

1. Introduction

Fatigue is a common, non-specific symptom experienced by many people and demonstrated in different health conditions. Physical fatigue is associated with a reduction in performance as a result of inefficient energy utilization (Kim et al., 2002; Wan et al., 2017) and is further defined as an overwhelming sense of tiredness, lack of energy, and a feeling of exhaustion that is related to difficulty in initiating or sustaining voluntary activities (Kadum et al., 2018; Li et al., 2020). Fatigue increases oxidative stress that can damage cells and increase the risk for the development of various chronic diseases (Lee et al., 2018; Osman and Mohamed, 2018).

Ergogenic property is the ability of substances to enhance energy production and utilization (Halim et al., 2018). For example, athletes and active people benefit from ergogenic aid that helps to boost their competitive performance and to improve energy utilization (Kreider et al., 2010; Silver, 2001). Ergogenic aid helps to improve the production of energy and thus prevent fatigue by lowering oxidative stress and improving health in general (Halim et al., 2017; Osman and Mohamed, 2018). There is an increasing trend toward the use of natural products as ergogenic aid to replace and/or reduce the use of synthetic compounds in preventing oxidative stress-related fatigue.

Dates (*Phoenix dactylifera* Linn) are rich in various bioactive compounds and have been reported to be an effective part of a diet that helps to protect against chronic diseases (Al-Alawi et al., 2017). Moreover, dates are known to be rich in antioxidant compounds such as phenolics including flavonoids, phenolic acids, ascorbic acid, tocopherols and carotenoids (Al-Farsi and Lee, 2008; Shabani et al., 2016). In previous studies, we demonstrated that Piyarom and Rabbi dates exhibited the highest antioxidant, antimicrobial and anti-elastase activities among five tested varieties of dates (Kadum et al., 2019). The beneficial activities noted in the studies could be attributed to the identified metabolites in the dates which include ascorbic acid, epicatechin, citric acid

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and gallic acid. This outcome was done based on a correlation study from Partial Least Squares biplot obtained from a metabolomics study of the different date extracts (Kadum et al., 2019). The above-mentioned antioxidants are known to exhibit radical scavenging activity which is crucial in alleviating oxidative stress and associated fatigue (Zhu et al., 2021). In addition, we also showed that Piyarom dates consist of adequate levels of electrolytes and sugars that are known to have ergogenic attributes (Kadum et al., 2018). In the present study, we aimed to evaluate the anti-fatigue and ergogenic properties of Piyarom date extract and determine its effects on the energy metabolism of fatigue-induced Sprague Dawley rats.

2. Materials and methods

2.1. Plant materials and extraction

Piyarom dates were purchased from a local store in Kuala Lumpur, Malaysia and were free from defects and contaminants. The seeds were removed, and the edible parts were cut into pieces and dried at 40°C. The dried pieces were then ground into powder, aseptically dispensed into sterile plastic bags and stored at −80°C. Extraction was carried out using 80% ethanol at 25°C for 24 h as optimized in the previous study (Kadum et al., 2019). The solvent was evaporated using a rotary vacuum evaporator (EYELA A-1000S, USA) at 40°C, and the resultant extract was freeze-dried and stored in dark bottles at 4°C.

2.2. Animal experimentation

Male Sprague Dawley rats (aged 5-6 weeks, average body weight of 204.5 g) were purchased from the Animals Resource Unit (ARU), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), and approval for the study was obtained from the Animal Ethics Committee of UPM (UPM\IACUC\AUP-R037\2017). The rats were allowed to acclimatize to the standard laboratory conditions for 1 w andwere also given free access to tap water and chow diet throughout the one-week period. They were randomly divided into groups including Normal Group (NG), Normal Fatigue Group (NFG), Group subjected to 250 mg/kg BW Date Extract (LDFG) (250 Date), Group subjected to 500 mg/kg BW of date extract (500 Date) and Group subjected to 5 mg/kg BW of caffeine (5 C).

2.3. Experiment design

Male Sprague Dawley rats ($n = 60$) were randomly divided into two clusters as follows: cluster 1 (NG) ($n = 12$) which was named the non-fatigue control group; and cluster 2 ($n = 48$) which was subjected to the Forced Swimming Test (FST). Once becoming fatigued, the rats in cluster 2 were divided into four different groups. Two groups were fed with date extract at 250 mg/kg BW (low dose), and 500 mg/kg BW (high dose) as described in a previous study (Sheikh et al., 2016). One group served as a positive control and was fed 5 mg/kg BW caffeine, and a control group was subjected to the FST but did not receive any treatment. The animals were fed their diets, and dosages of the date extract were administered through gastric intubation. Date extract and caffeine were given to the respective groups continuously every day for 8 w per the study design.

2.4. Forced swimming test

The rats were subjected to the FST in order to induce fatigue and determine their endurance capacities following the method described by Prasad and Khanum (2012). Briefly, a water tank containing water 50 cm in depth and maintained at 25 ± 2°C was used. Steel washers weighing approximately 7% of each rat's body weight were tied to the tails. The animals were then carefully placed into the tank, and the time taken for each one to swim before exhaustion was recorded. Exhaustion/fatigue was determined when the rat's head failed to surface within 7 s. The experiment was repeated every 2 w over a period of 8 w of treatment.

2.5. Blood and organ collection

Fasting blood samples were collected from the rats at weeks 0 and 4 by retro-orbital vein collection, and, at the end of the treatment (week 8), by cardiac puncture under general anesthesia using ketamine and xylazine. All of the blood samples were transferred into EDTA containing tubes, centrifuged at 3500 rpm at room temperature for 15 min, and plasma was collected and stored at -80 °C until further analysis. After 8 w of treatment, the organs (liver, kidney, heart, lung, muscle) were collected for analysis.

2.6. Biochemical analyses

Various biochemical parameters related to energy metabolism were measured in this study. Serum was used to analyze glucose, serum urea nitrogen (SUN), lactate (LAC), lactate dehydrogenase (LDH), creatine kinase (CK), and creatine (Cr). All biochemical parameters were assayed based on standard procedures provided by commercially available assay kits (Span diagnostic Ltd., India). The units for SUN and Cr were mg/dL, while LDH and CK were expressed in IU/L. Serum lipid profiles were also assayed (triglycerides, TG, total cholesterol, TC, low-density lipoprotein cholesterol, LDL-C, and high-density lipoprotein cholesterol HDL-C). Liver and kidney function tests were performed, and their assayed markers were alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). The Roche/Hitachi Cobas C system procedure was followed.

2.7. Histological study of selected organs

The collection of organs of interest (liver, kidney, heart, and muscle), was followed by weighing and then storage in 10% formalin. The heart tissue was cut transversely in order to obtain ventricular sections, the four-chamber cross-sections and the liver and muscle tissues (soleus) were minced and dipped in paraffin and then hematoxylin and eosin (H&E) for staining. The tissue examination by a veterinary pathologist (Huang et al., 2015) was conducted under a light microscope equipped with a CCD camera (BX-51, Olympus, Tokyo, Japan).

2.8. Statistical analysis

One-way ANOVA followed by Tukey's HSD test was used to obtain statistical differences between the samples and the controls. Results were expressed as mean ± standard deviation (SD). A difference in the mean values with $p < 0.05$ was considered to be statistically significant, and obtained data was analyzed using MINITAB version 16 (Minitab, Inc., State College, Pennsylvania, USA).

3. Results and discussion

3.1. Food intake and water consumption

Results of the study showed no significant differences in the food and water intake among the rats (Fig. 1 A and B). However, rats fed with date extract showed a slight body weight gain throughout the experiment (Fig. 1 C).

3.2. Forced swimming test

The Forced Swimming Test (FST) represents a valid animal model for testing the anti-fatigue property of bioactive compounds (Prasad and

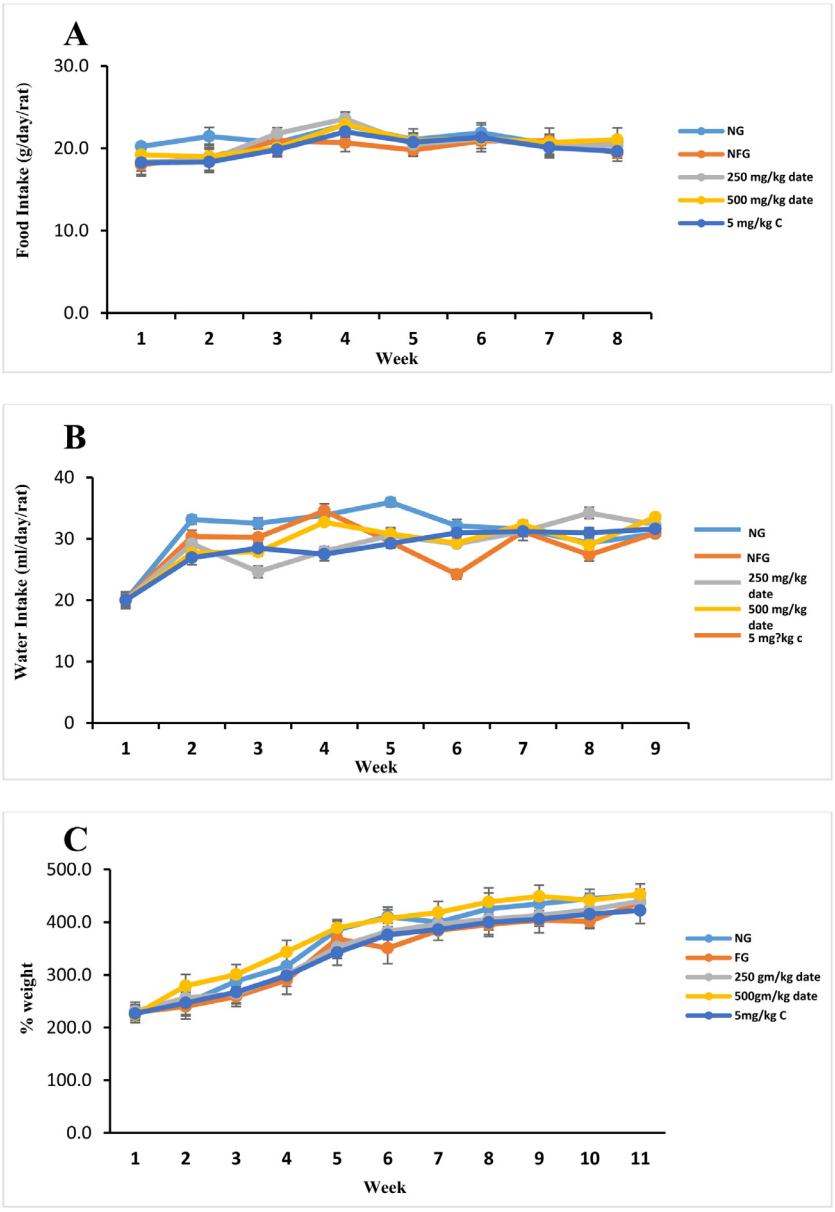

Fig. 1. Effects of date extract on (A) weekly food intake, (B) weekly water intake, and (C) body weights of the tested rat groups (n=6). NG: normal group; NFG: normal fatigue group; 250 Date: group subjected to 250 mg/kg BW date extract; 500 Date: group subjected to 500 mg/kg BW date extract; 5 C: group subjected to 5 mg/kg BW caffeine.

Khanum, 2012; Hao et al., 2014; Halim et al., 2017). The FST results clearly showed an anti-fatigue effect of the date extract on the rats during swimming (Fig. 2). The rats receiving the higher dose of date extract (500 mg/kg BW) exhibited the longest endurance capacity of approximately 632.9 ± 28.7 s, which was significantly ($p < 0.05$) higher compared to that of the groups treated with 250 mg/kg BW extract and 5 mg/kg BW caffeine. Interestingly, the enhanced swimming capacity of the rats fed with date extract was observed as early as the fourth week of the experiment. The prolonged swimming time of the rats indicated higher resistance towards fatigue during the FST (Tanaka et al., 2003; Ma et al., 2007). Similar results have been reported by several researchers who tested the anti-fatigue potential of plant extracts (Prasad and Khanum, 2012; Xu et al., 2013). For instance, in one previous study, rats fed with holy basil (*Ocimum sanctum*) exhibited better performance against fatigue and prolonged their swimming times (Singh et al., 2012b). In a recent study, Peruvian ginseng (*Lepidium meyenii*) fed to mice at three different doses for 28 d resulted in anti-fatigue effects, and the researchers suggested that *L. meyenii* was an effective ergogenic aid (Zheng et al., 2018). In another report, four different doses of moringa (*Moringa oleifera*) extracts were given to rats in order to test their endurance capacity. In that study, the extract was found to have delayed the fatigue time for the rats and enhanced their swimming capacities (Lamou et al., 2016). In the present study, dates have been shown to exhibit potential for application in energy drinks and are comparable other natural plant extracts.

3.3. Blood glucose, lactate and lactate dehydrogenase levels

The extent of fatigue in muscles after exercise can be measured by various biochemical markers including glucose, lactate, and lactate dehydrogenase. The blood glucose levels of the rats after 8 w of treatment were thus measured in order to determine the effects of the date extract. The results showed no significant differences in glucose levels among the rat groups except for the Normal Fatigue Group (NFG) which was significantly ($p < 0.05$) lower (Table 1). The results indicated protective effects of the date extract that may be attributed to the ergogenic property of dates that is ostensibly as good as that of caffeine. In skeletal muscles, glucose is the fuel for energy generation during exercise, and glycogen supplies the bulk of it through glycogenolysis in order to maintain the flow of glycolysis for energy generation. It has been shown that nutritional interventions via the consumption of dates may help in boosting or maintaining muscle glycogen concentrations both before and during

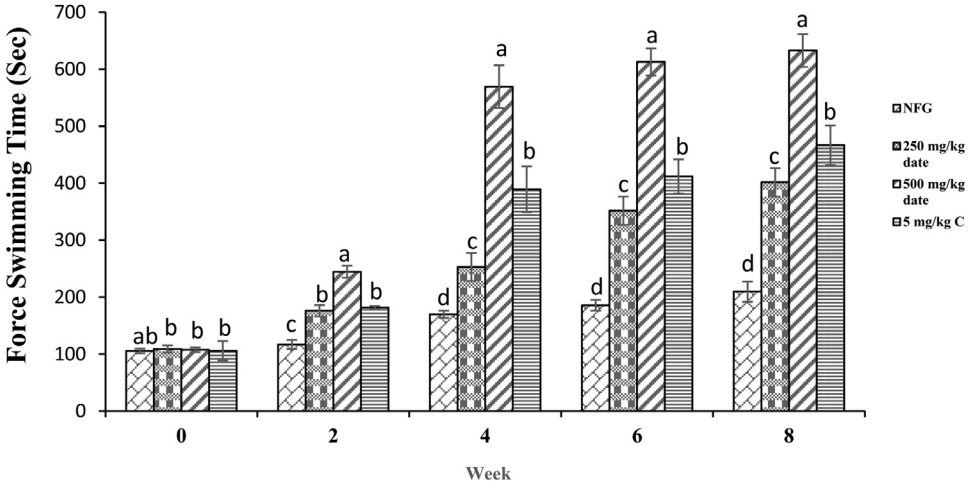

Fig. 2. Endurance capacity of the rats as determined by forced swimming test during eight weeks of treatment (n = 6). Data are mean with bars indicating standard deviation. Means with different letters within the same week are significantly different ($p < 0.05$). NG: normal group; NFG: normal fatigue group; 250 Date: group subjected to 250 mg/kg BW date extract; 500 Date: group subjected to 500 mg/kg BW date extract; 5 C: group subjected to 5 mg/kg BW caffeine.

Table 1
Effects of date extract on the levels of glucose, lactate, and LDH in the blood samples of the studied rat groups (n=6).

Group	Glucose mmol/L	Lactate mmol/L	LDH (U/L)
NG	5.64 ± 0.667[a]	14.41 ± 0.898[a]	287.83 ± 8.147[b]
NFG	3.98 ± 0.548[b]	16.35 ± 0.187[a]	588 ± 13.305[a]
250 Date	5.43 ± 0.521[a]	12.08 ± 0.457[b]	316.16 ± 27.580[b]
500 Date	5.88 ± 0.641[a]	10.73 ± 0.309[b]	297 ± 29.214[b]
5 C	5.41 ± 0.474[a]	11.06 ± 0.256[b]	305.83 ± 25.918[b]

Data are mean ± standard deviation. Means with different letters within the same column are significantly different ($p < 0.05$).
Abbreviations: LDH, Lactate dehydrogenase; ALT, alanine transferase; AST, aspartate aminotransferase, GGT, gamma-glutamyl transferase, NG, Normal Group; NFG, normal fatigue group; 250 Date, Group subjected to 250 mg/kg BW date extract; 500 Date, Group subjected to 500 mg/kg BW date extract; 5 C, Group subjected to 5 mg/kg BW caffeine

exercise (Li et al., 2018, 2017). However, it is important to keep blood glucose levels lower than 6 mol/L in order to avoid complications from diabetic conditions that may occur when hyperglycemia is allowed to persist over a period of time. In a previous study, mice fed with *Cistanche deserticola* extract for 3 w showed increased blood glucose levels which resulted in improved energy levels (Cai et al., 2010). In another study, the extract of *Radix rehmanniae* was reported to be responsible for anti-fatigue activity due to the increased glycogen stored in the liver and decreased build-up of glucose in the blood as a result of glucose consumption by muscles (Liu and Liu, 2016; Tan et al., 2012).

Blood lactate, a crucial marker for fatigue during heavy exercise, is used to measure an existing pathology in a routine stress test (Halim et al., 2017). Lactic acid is a product of glycolysis due to anaerobic condition which is the primary form of metabolism in the muscles during exercise (Fu et al., 2010; Sun et al., 2014). Lactate accumulation is considered to be a major inducer of fatigue, and thus the inhibition of lactate accumulation or the reduction of lactate levels represents an anti-fatigue effect (Blasiak et al., 2014). The results obtained in the current study demonstrated significant ($p < 0.05$) reduction in lactate levels between rat groups treated with date extract and caffeine as compared to that of normal and normal fatigue groups (Table 1). The lactate levels were 10.75 ± 0.90, 11.08 ± 0.26, and 12.09 ± 0.761 mmol/L for the rat groups treated with 500 mg/kg BW, caffeine 5 mg/kg BW, and 250 mg/kg BW date extract, respectively. The lactate levels for the normal group and fatigue groups were 14.41 ± 0.45 and 16.35 ± 0.18 mmol/L, respectively. The results indicated positive effects from the date extract that caused the reduction in lactate levels in the rat groups. The observed effects could be attributed to the improved performance of the rats during the FST. This result, in turn, could be due to the reduction in muscle damage caused by the lower lactate accumulation. In a previous study, rats fed with Amarkand extract had decreased blood lactate and increased endurance associated with fatigue (Narkhede et al., 2016). The previous report is in agreement with the findings of the present study, indicating the anti-fatigue activity of different plant extracts that were able to decrease blood lactate level in the rats, and thereby improved the animals' performance.

The presence of high levels of LDH in the blood is an indication of excessive muscle use and is thus a marker for fatigue (Halim et al., 2017). Excessive LDH is also an indication of necrosis in cells and damage to tissues (Li et al., 2017). Our results showed that FST significantly ($p <$ 0.05) increased the blood LDH levels of the normal fatigue rats (588 ± 13.30 U/L) which was significantly ($p < 0.05$) higher than that of rats fed with 500 mg/kg BW of date extract (297 ± 29.21 U/L), the lower dose (316.16 ± 27.58 U/L) of date extract and caffeine (305 ± 25.91 U/L) after 8 w treatment. It can thus be suggested that the date extract was able to enhance energy production in rats and consequently reduced damage to the rat muscle. Our results agreed with those reported previously by Narkhede et al., (2016). In that study, Amarkand extracts were given to Wistar rats, and after 2 w, there was a significant reduction in the animals' blood LDH levels. Similar results have also been reported in another study in which fatigued rats treated with water extract from fermented rice bran showed a decrease in serum LDH levels (Kim et al., 2002).

3.4. Creatine kinase activity

Creatine kinase (CK) is an established marker for muscle damage. The function of CK in the cells is to phosphorylate creatine into a high energy compound (*i.e.*, activated creatine) and phosphocreatine to be utilized as a quick source of energy by muscle cells (Abdul Majid et al., 2020). Most of the CK in the body is normally present in the muscle, and any increase in the CK levels in the blood could indicate muscle cell damage (Brancaccio et al., 2007). The results in Table 2 show a low CK level in rats treated with 500 mg/kg BW date extract, and it was significantly ($p < 0.05$) lower compared to that of all other groups (Table 2). It should be highlighted that our results revealed the protective effects of date extract against muscle damage that can be caused during heavy exercise. It was also observed that the effect of the date extract at a higher dose was better than that of caffeine. Prasad and Khanum (2012) reported that fatigued rats treated with ethanol extract of *O. sanctum* had a decrease in their serum CK level and improved performance. However, the current study showed that date extract significantly ($p < 0.05$) reduced CK levels as compared to the results from several other plant extracts. Therefore, date extract has a high potential as a natural anti-fatigue agent that can improve performance and reduce muscle damage during exercise or other physical activities.

Table 2
Creatine kinase activity following eight weeks of treatment with date extract (n=6).

Group	Creatine kinase activity (U/L)
NG	1144.17 ± 103.46[b]
NFG	1295.6 ± 114.79[a]
250 Date	1012.67 ± 155.36[bc]
500 Date	749.17 ± 139.40[d]
5 C	937.83 ± 212.85[c]

Data are mean ± standard deviation. Means with different letters indicate significant difference ($p < 0.05$).
Abbreviations: NG, Normal Group; NFG, normal fatigue group; 250 Date, Group subjected to 250 mg/kg BW date extract; 500 Date, Group subjected to 500 mg/kg BW date extract; 5 C, Group subjected to 5 mg/kg BW caffeine

3.5. Lipid profiles

Various scientific evidence has supported the positive effects of exercise on the levels of certain lipids in the blood such as HDL, LDL, TG, and TC. Interestingly, in our study, there were significant ($p < 0.05$) differences in the lipid profiles for the normal group of rats that did not undergo FST in comparison to the fatigue groups (Fig. 3). The rats fed with 500 mg/kg BW of date extract exhibited significantly ($p < 0.05$) lower cholesterol levels as compared to that of the normal and fatigue groups. However, the level was not significantly different from that fed with caffeine and the low dose of 250 mg/kg BW date extract. Similarly, total triglycerides for the rats were not significantly different from each other except for the normal group. Results of this study clearly showed that exercise resulted in a significant reduction ($p < 0.05$) of serum TC, TG and LDL (Fig. 3). This study also revealed elevation in the serum HDL levels observed for all rats that had undergone FST, with those rats that were fed higher doses of the date extract having significantly ($p < 0.05$) higher HDL levels than those fed with lower doses, although not significantly different from those fed caffeine. The decreased LDL and increased HDL levels are useful for protecting the body from cholesterol sedimentation and atherosclerosis (Yang et al., 2005). The results agree with those from a previous study that investigated the effects of Tri-Sura-Phon (TSP) extract on humans that significantly reduced serum levels of TC, TG and LDL, and increased serum levels of HDL (Kuamsub et al., 2017). In other studies, green tea extract significantly reduced total cholesterol, triglycerides, and LDL, and increased serum levels of HDL in obese women (Hsu et al., 2008; Suliburska et al., 2012). The reductions observed in the elevated serum TC, TG, and LDL and increase in the HDL levels of fatigue rats treated with date extract in the present study are suggested to be due to exercise as well as the anti-fatigue property of the date extract.

3.6. Toxicity indicator of the treatment

A large number of plant extracts have been recommended for consumption due to their ability to enhance physiological activities of the body by virtue of their antioxidant, anti-diabetic and anti-fatigue properties (Sallehuddin et al., 2020). However, cytotoxicity or safety is the main concern when considering the use of specific plant extract for human consumption. The toxic effects may cause an increase in liver and kidney enzymes such as aspartate transaminase (AST) and alanine aminotransferase (ALT). These enzymes are recognized as markers for liver and kidney damage with regard to the diagnosis, therapeutic monitoring, and evaluation of the toxicity levels and safety risk of plant extracts. The results of the 8 w of treatment with date extract at a dose of 500 mg/kg BW showed significantly ($p < 0.05$) lower levels of AST and ALT as compared to those t of normal group rats (Table 3). Duan et al., (2017) reported the reduction of AST and ALT using luteolin-6-C-neohesperidoside to determine the anti-fatigue effect and dysfunction of the liver and skeletal muscle induced by oxidative stress by regulating inflammatory and oxidative reactions.

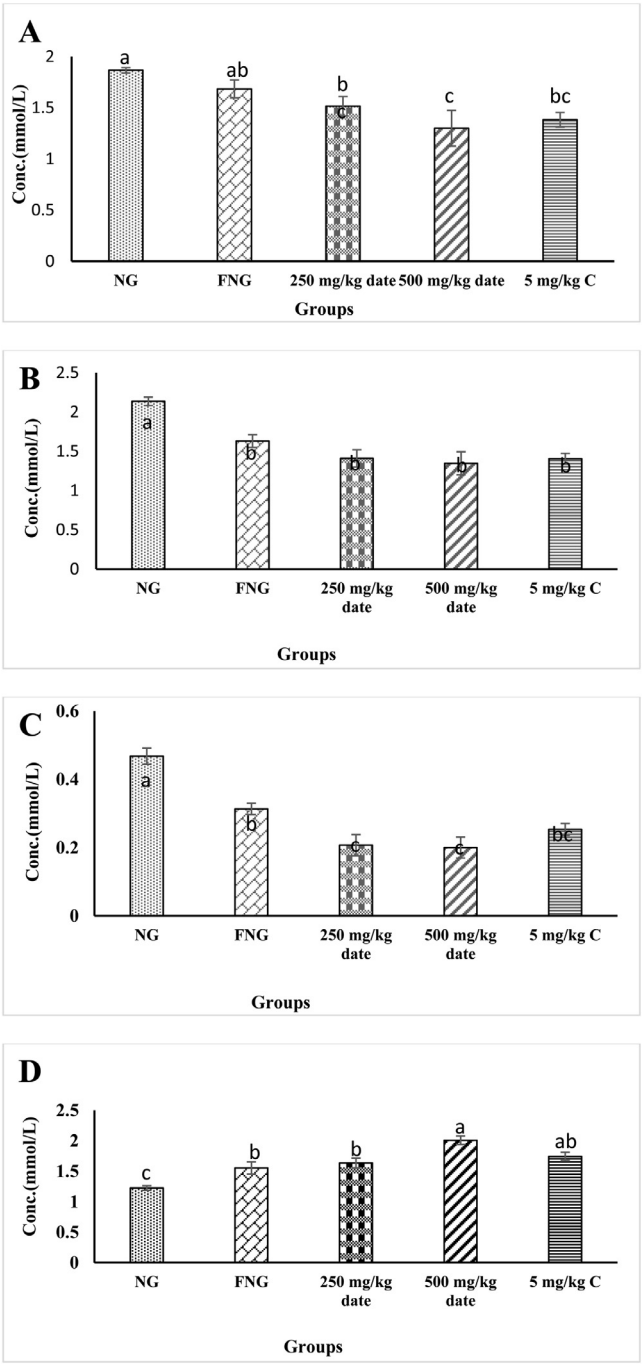

Fig. 3. Lipid profiles of the rats following eight weeks of treatment, including (A) total cholesterol, (B) triglycerides levels, (C) total LDL levels, and (D) HDL levels (n = 6). Data are mean with bars indicating standard deviation. Means with different letters are significantly different ($p < 0.05$). NG: normal group; NFG: normal fatigue group; 250 Date: group subjected to 250 mg/kg BW date extract; 500 Date: group subjected to 500 mg/kg BW date extract; 5 C: group subjected to 5 mg/kg BW caffeine.

Urea level represents renal function, and an increased level could indicate changes in stress conditions (Ang et al., 2008). Urea is the by-product of gluconeogenesis in which protein and free amino acids are catabolized during the limited access to carbohydrates and fats supplies (Lee et al., 2015). The results of the current study showed that the treatment with 500 and 250 mg/kg BW date extracts and 5 mg/kg caffeine

Table 3
Serum creatinine, urea, ALT, AST, and GGT determined from the blood samples of the tested rat groups following eight weeks of treatment with date extract.

Group	mmol/L Creatinine	Urea	U/L ALT	AST	GGT
NG	42.50 ± 1.52^{b}	10.33 ± 0.32^{bc}	65.33 ± 5.25^{ab}	246.83 ± 8.79^{ab}	< 2
NFG	57.50 ± 1.18^{a}	16.78 ± 0.26^{a}	68.33 ± 4.13^{a}	270.83 ± 15.49^{a}	< 2
250 Date	36.67 ± 1.17^{c}	11.0 ± 0.23^{b}	63.00 ± 4.83^{ab}	228.33 ± 29.00^{bc}	< 2
500 Date	29.0 ± 1.67^{e}	7.18 ± 0.21^{c}	54.33 ± 3.59^{c}	202.50 ± 9.85^{c}	< 2
5 C	32.33 ± 0.37^{d}	8.8 ± 0.31^{c}	57.17 ± 4.01^{bc}	212.17 ± 21.70^{bc}	< 2

Data are mean ± standard deviation. Means with different letters within the same column are significantly different ($p < 0.05$).
Abbreviations: ALT, alanine transferase; AST, aspartate aminotransferase, GGT, gamma-glutamyl transferase, NG, Normal Group; NFG, normal fatigue group; 250 Date, Group subjected to 250 mg/kg BW date extract; 500 Date, Group subjected to 500 mg/kg BW date extract; 5 C, Group subjected to 5 mg/kg BW caffeine

have no significant difference compared to the normal group. Thus, a significant ($p < 0.05$) reduction in the serum urea levels of the groups received the treatment was observed compared to that of the fatigue group that did not receive treatment (Table 3). Treatment with 250 mg/kg, 500 mg/kg date extracts and 5 mg/kg caffeine decreased serum urea levels (36.67%, 29.0%, and 32.33% respectively) after 8 w of treatment. Significantly ($p < 0.05$) lower creatinine levels were also observed in rats fed with date extracts and caffeine. The combination of date extract and caffeine, therefore, help to prevent kidney damage which could occur in fatigue rats. The levels of urea and creatinine increased in the blood during renal dysfunction or renal diseases (Öner-Iyidoğan et al., 2013). In a previous study, fatigue rats were observed to have high levels of urea and creatinine due to the damage caused to the liver and kidney, while the treatment with Amarkand extract reduced the presence of urea and creatinine (Narkhede et al., 2016). Findings from the study thus revealed that the treatments given were not toxic to the liver or kidney of the rats.

3.7. Histological examination of heart, kidney, liver and gastrocnemius muscle tissues

Fig. 4 (A–D) shows the histological alterations of major organs of the rats, including heart, kidney, liver and skeletal muscle tissues stained with H & E. It was observed that the treatments affected the organs of the different groups of rats in different ways. For example, Fig. 4 A1 is the longitudinal section of cardiac muscle tissue in normal rats, showing normal vascularization and inter-muscular tissue. Conversely, A2 shows the cardiac muscle tissue of fatigue rats, showing inflammation (arrow) with excessive cellular infiltration (circle) and massive damaged myocytes. Interestingly, A3 (cardiac muscle of fatigue rats fed with low dose date extract) shows visible improvement and reduction in the inflammation cellular infiltration after 8 w of treatment. As expected, A4 and A5 (cardiac muscle of fatigue rats fed with high dose 500 mg/kg BW of date extract and caffeine, respectively) showed an excellent improvement with diminished signs of inflammation and cellular infiltration.

The inflammation and cellular infiltration of the cardiac muscle tissue are signs of oxidative stress and myocyte damage, which were probably due to the fatigue caused by the FST (Halim et al., 2017; Abdul Majid et al., 2020). The treatment with low and high dose date extracts (500 mg/kg BW) significantly improved oxidative damage to the cardiac muscle dose dependently. Previously, we reported that Piyarom date extract exhibited potent radical scavenging activities that are attributed to the array of bioactive compounds present (Kadum et al., 2019). The improved cardiac muscles could thus be attributed to these bioactive compounds. This is interesting as the improvement in the muscle was on par with that demonstrated by the caffeine treated groups.

Fig. 4B shows the transverse sections of kidney tissues of normal, fatigue, low dose, high dose: 500 mg/kg BW of date extract, and caffeine treated rats. As expected, B1 (normal rats), shows normal renal

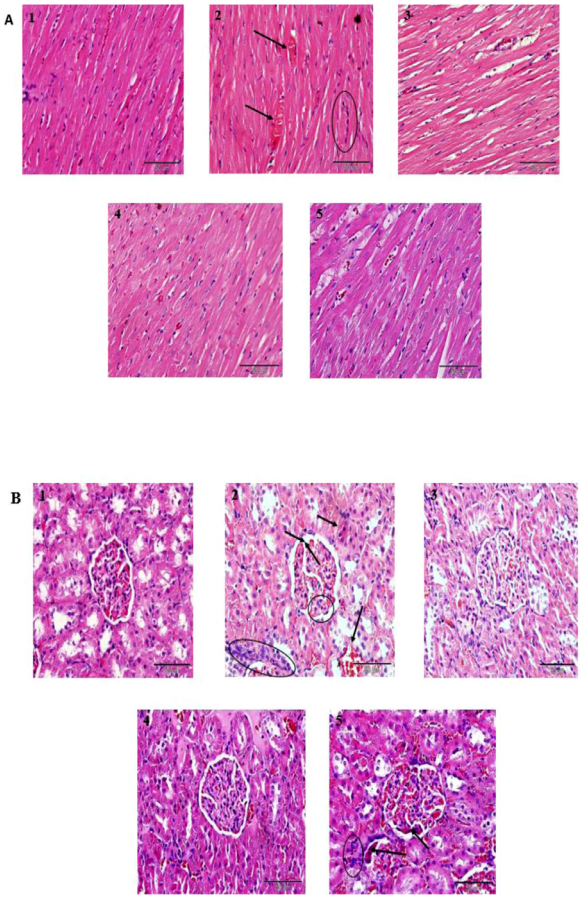

Fig. 4. Histological study of various organs: (A) heart, (B) kidney, (C) liver, and (D) Gastrocnemius muscle of the rats. Representative histological sections of the heart, kidney, liver, and Gastrocnemius muscle were stained with haematoxylin and eosin. Specimens were photographed using light microscope. (H&E stain, magnification: × 400; Scale bar, 50 μm). 1: NG (normal group); 2: NFG (normal fatigue group); 3: 250 Date (group subjected to 250 mg/kg BW date extract); 4: 500 Date (group subjected to 500 mg/kg BW date extract); 5: 5 C (group subjected to 5 mg/kg BW caffeine).

tissue and normal vascularization of the cortex and medulla. On the other hand, B2 (fatigue rat), shows acute inflammatory infiltration at the perivascular (single arrows) and periglomerular regions (circle) of the renal tissue. The photo micrograph also shows that Bowman's capsule was undergoing degeneration as indicated by widening of the Bowman's space (double arrow). The renal section of group 3 rats (fatigue + low

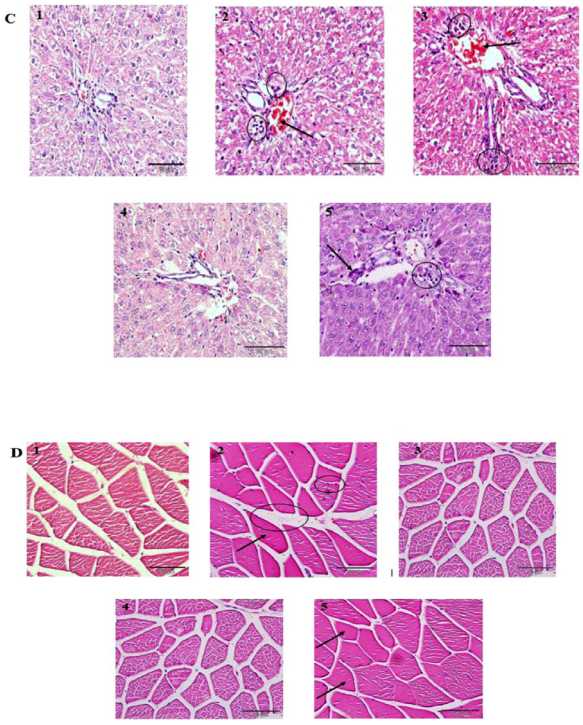

Fig. 4. Continued

dose date treatment) shows clear improvements in the inflammatory conditions after 8 w of treatments with date extract. B4 (fatigue + 500 mg/kg BW of date extract) shows better improvement with a complete absence of inflammatory conditions. However, B5 (caffeine treated rats) shows the re-emergence of inflammation in the Bowman's capsule (arrows) cellular infiltration in the cortex regions (circle) after 8 w of treatment with caffeine.

The FST caused inflammation as clearly seen in the fatigue rats. The reduction in the inflammatory conditions and closure of the wider Bowman's space were observed in the low and 500 mg/kg BW of date extract treated groups. This could also de due to the presence of bioactive compounds in the dates. The caffeine treated rats continued to have inflammation in the glomerular region (arrows) and cellular infiltration in the medulla (circle). This could be due to the fact that the administered caffeine was also having a negative effect on the renal cells in addition to its positive effect of regeneration of the Bowman's capsule (*i.e.*, closure of the Bowman's space).

Fig. 4 C shows the transverse section of hepatocytes of normal, fatigue, low dose, high dose, and caffeine treated rats. As expected, the normal rats showed normal branches of hepatic artery, portal vein, bile ducts and well-arranged hepatocytes without any sign of inflammation. However, hepatocytes from fatigue rats showed bulging of the portal vein with increased vascularization (arrow), and the portal area showed infiltration with white blood corpuscles (circles) and reversible cellular injury (signs of hepatic inflammation). Interestingly, C3, which shows the hepatocytes from fatigue rats fed with low dose date extract (250 mg/kg BW), reveals a slight improvement in cellular injury, although persistent cellular infiltration around the portal area remained. While C4 shows the hepatocytes from rats fed with high dose of date extract (500 mg/kg BW) which reveals well preserved hepatocytes and the absence of inflammatory signs after 8 w of treatment. On the other hand, liver cells from rats fed with caffeine (C5) showed remnants of cellular damage that were reversible along with cellular infiltration in the region between the bile duct and the hepatic artery. This would indicate that the treatment of 500 mg/kg BW of date extract protected the liver cells better than that with caffeine.

The observed cellular infiltration and damage could be caused by the FST that the rats endured. It could be said that the effect of date extract on liver cells following exercise was dose dependent since the low dose treatment was not able to completely clear the cellular damage and infiltration. The complete treatment of these conditions and the inflammation were seen in the high dose treated group, which showed much greater improvement than the caffeine treated group. Therefore, the high dose date treatment could be used to replace caffeine in the treatment of inflammation.

Fig. 4D is the transverse section of the gastrocnemius tissue from the normal, fatigue, low dose, high dose of date extract, and caffeine treated rats. Normal rats (D1) showed normal longitudinally arranged muscle fibers with peripheral nuclei and transverse striated muscle fibers. However, the tissue muscle of fatigue rats (D2) revealed excess vascularization of red blood corpuscles in the muscle fiber (arrows). The sarcoplasm nuclei also had significant darkening (small circle), and the myofibrils had a relatively reduced striation (big circle) when closely observed. In the D3 section, the striation of the muscle fibers had regained shape, and the extra vascularization of the paramecium diminished. These conditions completely disappeared in the D4 section (high dose treated rats) after 8 w of treatment. The caffeine treated rats (D5) also showed marked improvement in the striation of myofibrils. However, vascularization of the paramecium remained high.

These histological changes in the skeletal muscle tissue observed in the fatigue rats indicated degeneration of muscle tissues (muscle damage) caused by the FST (Sari and Das, 2013). However, the reduction in muscle damage observed in the low and high dose treated rats is a sign of regeneration of damaged muscle tissue. This could be due to the presence of antioxidant components and protein metabolites in the Piyarom date extract used in the treatment (Kadum et al., 2019). The persistent inflammation observed in the caffeine treated rats could be a side effect of caffeine despite its regenerative effect on the muscle tissues.

In general, feeding the rats with date extract and caffeine improved the muscle damage that may have been caused by excessive oxidative stress resulting from the FST. Moreover, a histological study revealed the protective effect of the date extract against oxidative stress-associated fatigue.

5. Conclusions

The findings from this study revealed that Piyarom date extract exhibited an anti-fatigue property in Sprague Dawley rats as determined by an FST. The rats treated with a high dose of 500 mg/kg BW date extract demonstrated an excellent endurance capacity that could be attributed to improved energy metabolism. Moreover, the serum levels of glucose, lactate, LDH, and CK were significantly reduced. The results thus showed that 500 mg/kg BW is the recommended dose for enhancing energy generation and lowering tissue damage caused by oxidative stress- associated fatigue. Histology results also revealed a protective effect from the date extract against oxidative stress -associated fatigue. Importantly, the date extract was not toxic to hepatocytes and kidney cells. Further studies should thus be carried out in order to explore the mechanisms involved in the reduction of fatigue by date extract in Sprague Dawley rats. Dates could then be recommended as an ergogenic aid to be used in the development of functional beverages or snacks with ergogenic properties.

CONFLICT OF INTEREST

The authors hereby declare no conflict of interest.

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REFERENCES

Al-Alawi, R.A., Al-Mashiqri, J.H., Al-Nadabi, J.S., Al-Shihi, B.I., Baqi, Y., 2017. Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options. Front. Plant Sci. 8, 845.

Abdul Majid, N., Abdul Hamid, A., Salleh, S.Z., Saari, N., Abas, F., Pak Dek, M.S., Jaafar, A.H, 2020. Metabolomics approach to investigate the ergogenic effect of Morinda citrifolia L. leaf extract on obese Sprague Dawley rats. Phytochem. Anal. 31 (2), 191–203.

Al-Farsi, M.A., Lee, C.Y., 2008. Nutritional and functional properties of dates: a review. Crit. Rev. Food Sci. Nutr. 48 (10), 877–887.

Ang, W.T., Hang, Y.Z., Ao, J.G., Ing, X.D., Ao, S.G., 2008. The anti-fatigue effect of 20 (R)-Ginsenoside Rg3 in mice by intranasally administration. Biol. Pharm. Bull. 31 (11), 2024–2027.

Blasiak, J., Petrovski, G., Veréb, Z., Facskó, A., Kaarniranta, K., 2014. Oxidative stress, hypoxia, and autophagy in the neovascular processes of age-related macular degeneration. BioMed Res. Int. 2014 768026.

Brancaccio, P., Maffulli, N., Limongelli, F.M., 2007. Creatine kinase monitoring in sport medicine. Br. Med. Bull. 81-82 (1), 209–230.

Cai, R.L., Yang, M.H., Shi, Y., Chen, J., Li, Y.C., Qi, Y., 2010. Antifatigue activity of phenylethanoid-rich extract from Cistanche deserticola. Phytother. Res. 24 (2), 313–315.

Duan, F., Guo, Y., Li, J., Yuan, K., 2017. Antifatigue effect of Luteolin-6-C-Neohesperidoside on oxidative stress injury induced by forced swimming of rats through modulation of Nrf2/ARE signaling pathways. Biol. Trace Elem. Res. 2017 3169358.

Fu, X., Ji, R., Dam, J., 2010. Antifatigue effect of Coenzyme Q10 in mice. J. Med. Food 13 (1), 211–215.

Halim, H.H., Pak-Dek, M.S., Hamid, A.A., Jaafar, A.H., 2017. Fatigue onset through oxidative stress, dehydration and lactic acid accumulation and its in vivo study using experimental animals. Int. J. Adv. Sci. Res. Rev. 35 (1), 1–12.

Halim, H.H., Williams Dee, E., Pak Dek, M.S., Hamid, A.A., Ngalim, A., Saari, N., Jaafar, A.H., 2018. Ergogenic attributes of young and mature coconut (Cocos nucifera L.) water based on physical properties, sugars and electrolytes contents. Int. J. Food Prop. 21 (1), 2378–2389.

Hao, G., Zhang, C., Cao, W., Hao, J., 2014. Effects of intragastric administration of five oyster components on endurance exercise performance in mice. Pharm. Biol. 52 (6), 723–728.

Hsu, C., Tsai, T., Kao, Y., Hwang, K., 2008. Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial. Clin. Nutr. 27 (3), 363–370.

Huang, L., Chen, J., Cao, P., Pan, H., Ding, C., Xiao, T., Su, Z., 2015. Anti-obese effect of glucosamine and chitosan oligosaccharide in high-fat diet-induced obese rats. Mar. Drugs 13 (5), 2732–2756.

Kadum, H., Hamid, A., Abas, F., Ramli, N.S., Mohammed, A.K.S., Muhialdin, B.J., 2018. Applications of date (Phoenix dactylifera L.) fruits as bioactive ingredients in functional foods. J. Pure Appl. Microbiol. 12 (3), 1101–1109.

Kadum, H., Hamid, A., Abas, F., Ramli, N.S., Mohammed, A.K.S., Muhialdin, B.J., Jaafar, A.H., 2019. Bioactive compounds responsible for antioxidant activity of different varieties of date (Phoenix dactylifera L.) elucidated by 1 H-NMR based metabolomics. Int. J. Food Prop. 22 (1), 462–476.

Kim, K.M., Yu, K.W., Kang, D.H., Suh, H.J., 2002. Anti-stress and anti-fatigue effect of fermented rice bran. Phytother. Res . 16 (7), 700–702.

Kreider, R.B., Wilborn, C.D., Taylor, L., Campbell, B., Almada, A.L., Collins, R., Antonio, J., 2010. ISSN exercise & sport nutrition review: research & recommendations. J. Int. Soc. Sport. Nutr. 7 (1), 1–43.

Kuamsub, S., Singthong, P., Chanthasri, W., Chobngam, N., Sangkaew, W., Hemdecho, S., Chusri, S., 2017. Improved lipid profile associated with daily consumption of trisura-phon in healthy overweight volunteers: An open-label, randomized controlled trial. Evid. Based Complement. Altern. Med. 27 2687173.

Lamou, B., Taiwe, G.S., Hamadou, A., Houlray, J., Atour, M.M., Tan, P.V., 2016. Antioxidant and antifatigue properties of the aqueous extract of Moringa oleifera in rats subjected to forced swimming endurance test. Oxid. Med. Cell. Longev. 2016 3517824.

Lee, J.S., Kim, H.G., Lee, D.S., Son, C.G., 2018. Oxidative stress is a convincing contributor to idiopathic chronic fatigue. Sci. Rep. 8, 12890. doi:10.1038/s41598-018-31270-3, 2018| DOI.

Lee, J.S., Kim, H.G., Han, J.M., Kim, Y.A., Son, C.G., 2015. Anti-fatigue effect of Myelophil in a chronic forced exercise mouse model. Eur. J. Pharmacol. 764, 100–108.

Li, H., Chawla, G., Hurlburt, A.J., Sterrett, M.C., Zaslaver, O., Cox, J., Tennessen, J.M., 2017. Drosophila larvae synthesize the putative oncometabolite L-2-hydroxyglutarate during normal developmental growth. Proc. Natl. Acad. Sci. 114 (6), 1353–1358.

Li, H., Hurlburt, A.J., Tennessen, J.M., 2018. A Drosophila model of combined D-2-and L-2-Hydroxyglutaric Aciduria reveals a mechanism linking mitochondrial citrate export with oncometabolite accumulation. Dis. Model. Mech. 11 (9) dmm035337.

Li, Y., Deng, Y., Li, Z., Liu, Z., Piao, M., Cui, X., 2020. Composition, physicochemical properties, and anti-fatigue activity of water-soluble okra (Abelmoschus esculentus) stem pectins. Int. J. Biol. Macromol. 165, 2630–2639.

Liu, Y., Liu, C., 2016. Antifatigue and increasing exercise performance of Actinidia arguta crude alkaloids in mice. J. Food Drug Anal. 24 (4), 738–745.

Ma, D.L., West, B.J., Su, C.X., Gao, J.H., Liu, T.Z., Liu, Y.W., 2007. Evaluation of the ergogenic potential of noni juice. Phytother. Res. 21 (11), 1100–1101.

Narkhede, A.N., Jagtap, S.D., Nirmal, P.S., Giramkar, S.A., Nagarkar, B.E., Kulkarni, O.P., Harsulkar, A.M., 2016. Anti-fatigue effect of Amarkand on endurance exercise capacity in rats. BMC Complement, Altern. Med. 16 (1), 1–7.

Öner-Iyidoğan, Y., Koçak, H., Seyidhanoğlu, M., Gürdöl, F., Gülçubuk, A, Yildirim, F., Uysal, M., 2013. Curcumin prevents liver fat accumulation and serum fetuin-a increase in rats fed a high-fat diet. J. Physiol. Biochem. 69 (4), 677–686.

Osman, W.N.W., Mohamed, S., 2018. Standardized Morinda citrifolia L. and Morinda elliptica L. leaf extracts alleviated fatigue by improving glycogen storage and lipid/carbohydrate metabolism. Phytotherapy Research 32 (10), 2078–2085.

Prasad, M.V., Khanum, F., 2012. Antifatigue activity of ethanolic extract of Ocimum sanctum in rats. J. Med. Plants Res. 6 (1), 37–46.

Sallehuddin, N.A., Abdul-Hamid, A., Salleh, S.Z., Abdul-Majid, N., Halim, H.H., Ramli, N.S., Pak-Dek, M.S., 2020. Ergogenic, anti-diabetic and antioxidant attributes of selected Malaysian herbs: characterisation of flavonoids and correlation of functional activities. Int. Food Res. J. 27 (1), 197–207.

Sari, Y., Das, S, 2013. Alterations of glial glutamate transporters and certain neurotransmitters in alcohol withdrawal syndrome using alcohol preferring rat model. Neuropsychopharmacol. Rep. 40, 585–S585.

Shabani, F., Kumar, L., Nojoumian, A.H., Esmaeili, A., Toghyani, M., 2016. Projected future distribution of date palm and its potential use in alleviating micronutrient deficiency. J. Sci. Food Agric. 96 (4), 1132–1140.

Sheikh, B.Y., Zihad, S.N.K., Sifat, N., Uddin, S.J., Shilpi, J.A., Hamdi, O.A., Jahan, I.A., 2016. Comparative study of neuropharmacological, analgesic properties and phenolic profile of Ajwah, Safawy and Sukkari cultivars of date palm (Phoenix dactylifera). Orient. Pharm. Exp. Med. 16 (3), 175–183.

Silver, M.D., 2001. Use of ergogenic aids by athletes. J. Am. Acad. Orthop. Surg. 9 (1), 61–70.

Singh, E., Sharma, S., Dwivedi, J., Sharma, S., 2012b. Diversified potentials of Ocimum sanctum Linn (Tulsi): An exhaustive survey. J. Nat. Prod. Plant Resour. 2 (1), 39–48.

Suliburska, J., Bogdanski, P., Pupek-musialik, D., Jablecka, A., 2012. Effects of green tea supplementation on elements, total antioxidants, lipids, and glucose values in the serum of obese patients. Biol. Trace Elem. Res. 149 (3), 315–322.

Sun, S., Niu, H., Yang, T., Lin, Q., Luo, F., Ma, M., 2014. Antioxidant and anti-fatigue activities of egg white peptides prepared by pepsin digestion. J. Sci. Food Agric . 94 (15), 3195–3200.

Tan, W., Yu, K., Liu, Y., Ouyang, M., Yan, M., 2012. Anti-fatigue activity of polysaccharides extract from Radix Rehmanniae Preparata. Int. J. Biol. Macromol. 50 (1), 59–62.

Tanaka, M., Nakamura, F., Mizokawa, S., Matsumura, A., Nozaki, S., Watanabe, Y., 2003. Establishment and assessment of a rat model of fatigue. Neurosci. Lett. 352 (3), 159–162.

Wan, J.J., Qin, Z., Wang, P.Y., Sun, Y., Liu, X., 2017. Muscle fatigue: general understanding and treatment. Exp. Mol. Med. 49 (10), e384.

Xu, C., Lv, J., Lo, Y.M., Cui, S.W., Hu, X., Fan, M., 2013. Effects of oat β-glucan on endurance exercise and its anti-fatigue properties in trained rats. Carbohydr. Polym. 92 (2), 1159–1165.

Yang, Y., Yan, B., Fu, M., Xu, Y., Tian, Y., 2005. Relationship between plasma lipid concentrations and HDL subclasses. Clin. Chim. Acta 354 (1-2), 49–58.

Zheng, W., Du, S., Tian, M., Xu, W., Tian, Y., Li, T., Fu, Y., Wu, S., Li, C., Jin, N., 2018. Lepidium meyenii Walp exhibits anti-inflammatory activity against ConA-induced acute hepatitis. Mediat. Inflamm. 2018 8982756.

... Zhu, S., Yang, W., Lin, Y., Du, C., Huang, D., Chen, S., Cong, X., 2021. Antioxidant and anti-fatigue activities of selenium-enriched peptides isolated from Cardamine violifolia protein hydrolysate. J. Funct. Foods 79 104412.