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Functional Benefits of *Ziziphus jujuba* Fruits: Anti-Fatigue Activity and Antioxidant Enzyme Activities in Experimental Animal Models

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Abstract

Ziziphus jujuba Lam. (Rhamnaceae) is commonly known as "*Ber* (*Jujube*)". In India, fruits of the plant consume fresh, dried and processed forms. This study evaluates the nutritional composition and physical endurance of jujube fruits in two forms: (1) lyophilised jujube pulp (ZJP) and (2) hydro-alcoholic extract of jujube pulp (ZJE). Free amino acids, vitamins, carbohydrates, proximate analysis and anti-fatigue activity were investigated by an experimental animal model. High concentrations of vitamin C were observed in ZJP (13.8 %) and ZJE (21.4 %). Glucose and fructose were significantly higher in ZJP compared to ZJE ($p < 0.05$). In the weight loaded forced swim test (WFST), exercise performance (swimming time) was significantly increased by the supplementation of ZJE and ZJP compared to the control group after 10 days. Tissue biochemical parameters and antioxidant enzyme studies confirms that both ZJE and ZJP had strong physical endurance capacity. The results showed in the study suggesting that *Z. jujuba* i.e. ZJP and ZJE affords anti-fatigue activity by improving swimming time in weight loaded forced swim test on experimental animal models. Information provided in this study will be helpful for developing applications of jujube in food technology as functional and nutraceutical ingredient.

Keywords: *Ziziphus jujuba; Jujube Fruits; Physical-Endurance Capacity; Anti-Fatigue Activity; Nutritional Composition*

Introduction

Ziziphus jujuba Lam. (Rhamnaceae) is commonly known as "*Ber* (*Jujuba*)", an evergreen thorny shrub with reddish-brown fruits and is mainly found in Southeast Asia [1]. Fruits of jujube are edible, sweet-sour to taste and widely grown in all over India. Jujube fruits consumes fresh (raw, juice), dried (powder) and processed form (pickle, jam, jelly) [2]. It is high in sugar, edible cellulose, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), vitamins and minerals [3, 4]. It contains many phytochemicals such as cyclopeptide alkaloids, triterpenoides, flavonoids, saponins (jujuboside A, jujuboside B), lauric acid and tannins [5]. It also contains folk which is used as an analgesic, anti-fertility, anti-diabetic, tranquilizer, anti-anxiety and anti-convulsant agent in India [6]. Jujube fruits contain health benefits in Ayurvedic and Chinese traditional systems of medicine for treating anaemia, hypertonia, nephritis and nervous diseases [7].

The public becomes health-conscious and exercise routinely to prevent lifestyle-related diseases and to make themselves physically fit [8]. Moderate exercises prevent mental stress and lifestyle-related diseases [9]. Exhaustive exercise induces oxidative stress and cell damage by over production of reactive oxygen species (ROS) [9], which may leads to muscle weakness, DNA mutations, lipid peroxidation, mitochondrial dysfunction and physical fatigue. Previous studies have shown that physical fatigue leads to chronic ACTH hypersecretion and adrenal hyperfunction [10]. Consumption of some fruits offer protection against free radicals and significantly reduces the incidence of chronic degenerative diseases that related to oxidative stress such as cancer, cardiovascular diseases, age-related pathologies, etc [11]. In the food industry, there is urgent need for new sources of safe and inexpensive antioxidants of natural origin to replace some synthetic antioxidants due to their potential health risks and toxicity [12]. Physical-performance-enhancement activity (anti-fatigue activity) of

Z. jujuba fruits has not been studied. Therefore, the present study aims to evaluate jujube fruit pulp and its hydro-alcoholic extract for physical-performance-enhancement activity (anti-fatigue activity) subjects to weight-loaded forced swim test (WFST) in experimental rat models.

Methods and Methods

Fruit material and extraction

Ziziphus jujuba fruits were purchased from the local market in Mysore, India and authenticated at the Department of Dravyaguna, Government Ayurveda Medical College. Fresh fruits were cleaned, sliced into small pieces, seeds were removed and lyophilized (ZJP). Lyophilized jujube powder was further macerated with 50% ethanol for 24h, evaporated under reduced pressure and then again lyophilized (ZJE). Both ZJP and ZJE were used for further studies.

Proximate composition

Proximate composition of ZJP and ZJE was determined by procedures of Association of Official Analytical Chemists (AOAC) [13].

HPLC analysis

Jujube samples were analysed for vitamins, carbohydrates and essential amino acids by high-performance liquid chromatography (HPLC). Columns, solvent systems, detectors and other conditions of HPLC are given in table 1.

Conditions	Vitamins	Amino acids	Carbohydrates
Column	Atlantis dC18 (4.6 X 150 mm)	AA column (200 × 2.1 mm)	Zorbax column (4.6 X 250 mm)
Solvent system	A: 0.1% TFA in water B: 0.1% TFA in ACN Gradient system: Start with 100% A, at 5 min 99.4% A, at 7 min 94% A, at 12 min 70% A, at 17, min 40% A, at 22 min 99.4% A.	A: 20 mM NaAc + 0.018 % TEA (pH 7.2) B: 20 % of 100 mM NaAc (pH 7.2) + 40 % ACN and 40 % MeOH Gradient system: Start with 100% A, at 17 min 60% B, at 18 min 100% B, at 18.1 min flow 0.45, at 18.5 min flow 0.8, at 23.9 min flow 0.8, at 24 min 100% B and flow 0.45, at 25 min 0% B.	Acetonitrile – Water (75:25) Isocratic system
Run time	30 minutes	30 minutes	25 minutes
Flow rate	1.4 ml min^{-1}	0.45 -0.8 ml min^{-1} (variable)	1.4 ml min^{-1}
Injection Program		Mix the reagents in the following order, borate buffer (5 µl), OPA reagent (1 µl), sample (1 µl), air (8 µl; six times), FMOC (1µl) followed by air 9 µl three times and inject	15 µl of sample
Oven Temp.	30 ºC	40 ºC	30˚C
Detector	PDA; Absorbance at 268 nm	FD; Excitation - 340 nm; Emission - 450 nm; PTM gain 12	RID, 30ºC

Table 1: *Conditions for high performance liquid chromatography.*

Note: TFA: Trifluro Acetic Acid; CAN: Acetonitrile; NaAc: Sodium Acetate; MeOH: Methanol; PDA: Photodiode Detector; FD: Fluorescence Detector; RID: Refractive Index Detector

Determination of *in vitro* antioxidant activities

DPPH radical-scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of the jujube samples was determined by [14]. DPPH in methanol (0.1 mM) was added to different concentrations of ZJP and ZJE. The mixture was incubated at room temperature for 30 min and the absorbance was recorded at 520 nm against methanol as blank. The percentage of inhibition was calculated as follows:

$$[A_{cont} - A_{sample}]/A_{cont} \times 100,$$

Where A_{cont} is the absorbance of the control and A_{sample} is the absorbance of jujube samples. The antioxidant activity of the extract was expressed as inhibitory concentration at 50% (IC_{50}), where the concentration of DPPH radical is reduced by half.

Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging activity was analysed and modified from the method of [15]. The reaction was initiated by the addition of EDTA (0.1 ml; 1 mM), $FeCl_3$ (0.01 ml; 10 mM), H_2O_2 (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 ZJP and ZJE (50 - 300 mg/ml) separately. The sample mixture was incubated at 37ºC for 1h and followed by the addition of equal amounts of trichloroacetic acid (TCA; 10%; 1 ml) and thiobarbituric acid (TBA; 0.05%; 1 ml). Absorbance was recorded at 532 nm and the percentage of inhibition was calculated as follows:

$$[A_{cont} - A_{sample}]/A_{cont} \times 100,$$

Where A_{cont} is the absorbance of the control and A_{sample} is the absorbance of jujube samples.

Metal-chelating activity

Metal-chelating activity of jujube samples was measured with the percentage of inhibition of ferrozine-Fe^{2+} complex formation [16]. Briefly, the extract (25 - 100 mg/ml, ZJP and ZJE separately) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and shaken for 10 min at room temperature. Spectrophotometric readings were recorded at 562 nm and the percentage of inhibition was calculated as follows:

$$[A_{cont} - A_{sample}]/A_{cont} \times 100,$$

Where A_{cont} is the absorbance of the control and A_{sample} is the absorbance of jujube samples.

Quantification of total polyphenols and flavonoids

Total polyphenols and flavonoids were determined by Folin-Ciocalteu reagent and ethanolic aluminium chloride solution (1.2%), respectively [17]. The amount of total polyphenols and flavonoids were calculated from calibration curves of gallic acid and quercetin standard solutions, respectively.

In vivo physical endurance capacity of jujube fruit

Animals and experimental design

Male Wistar rats weighing 120 - 140g were obtained from the stock colony of Defence Food Research Laboratory, Mysore, India, and housed in an acryl fibre cage in a temperature-controlled room (25ºC ± 2ºC) and was maintained in 12h light/dark cycle. Rats were fed with commercial pellet diet and watered *ad libitum*. Animals were randomly divided into 4 groups as follows: sedentary, control and two treatment groups. Rats in the treatment groups were administered orally with jujube pulp lyophilized powder (ZJP; 2 g/kg body weight per day) and jujube pulp hydro-alcoholic extract (ZJE; 100 mg/kg body weight per day) for a period of 2 weeks. Sedentary and control rats were orally administered with equal amount of water. Animal studies were conducted according to the regulations of institutional animal ethical committee, approved for the purpose of the control and supervision of experiments on animals (CPCSEA).

Weight-loaded forced swim test (WFST)

The rats of the ZJP, ZJE and control groups were allowed to swim with constant tail loads corresponding to 5 % of their body weight. The swim test was carried out in a small water tank at 25 ± 2ºC. Exhaustion time was recorded by observing the loss of coordinated movements and failed returning to the surface within 10s [18]. WFST was carried for 17 days (a total of 6 swimming tests for a period of 17 days) to monitor gradual increase of swimming time.

Biochemical analysis

Animals were quickly decapitated under mild anaesthesia immediately after the last swimming exercise. Blood was collected by cardiac puncture using a heparinised-syringe into centrifuge tubes. Liver and muscle samples were removed, cleaned with ice-cold saline and stored at −80ºC until further analysis.

Determination of glycogen, lactic acid and lipid peroxidation

Liver and muscle tissues were used to determine glycogen, lactic acid and lipid peroxidation. To measure glycogen, samples were digested with 30% KOH and then saturated with Na_2SO_4 solution. DNS (dinitrosalicylic acid) method was followed to determine hydrolysed product of tissue glycogen [19]. To determine lactic acid, tissue samples were homogenised in phosphate buffer (pH 7.2) and deproteinised with TCA (10%). 20% $CuSO_4$ solution and H_2SO_4 (conc.) were added to the supernatant of deproteinised tissue sample. p-Hydroxydiphenyl reagent was added to the sample mixture, incubated for a period of 30 min and the absorbance was measured at 560 nm [20].

Tissue samples were homogenised in phosphate buffer (pH 7.0) to determine thiobarbituric acid-reactive substances (TBARS). 10% TCA and TBA mixture [(TBA (0.35%), SDS (0.2%), $FeCl_3$ (0.05 mM) and BHT in glycine-HCl buffer (100 mM, pH 3.6)] were added to the above mentioned homogenised tissue sample and boiled for 30 minutes at 100°C. The supernatant of the mixture was measured at 532 nm and expressed as malondialdehyde content (MDA mmol/cm/g) [21].

Determination of *in vivo* antioxidant activities

Liver tissues were homogenized in 50 mM phosphate buffer saline (pH 7.4). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined with commercially available kits (Randox, Canada; Cat no. SD 125 and RS 504, respectively). The catalase (CAT) was determined by the method of [32]. Briefly, 0.1 ml of liver tissue homogenate was mixed with phosphate buffer (0.05 M, pH 7; 1.9 ml) and added with H_2O_2 (6 mM; 1 ml). Decay of H_2O_2 solution was measured by spectrophotometric reading at 240 nm.

Western blot analysis

The tissue was washed with PBS (pH 7.4) and lysed in an ice-cold lysis buffer (10 mM HEPES; 42 mM KCl; 50 mM $MgCl_2$; 0.1 mM EDTA; 0.1 mM EGTA; 5 mM DTT; 2 mM PMSF; protease inhibitor cocktail, Sigma Aldrich, India) for 30 minutes, followed by centrifugation at 10,000 rpm for 30 minutes at 4˚C. The total proteins were measured by the method of [22]. The samples (50 µg of protein) were subjected to 10% SDS-polyacrylamide mini gels at a constant current of 20 mA. The gel was transferred onto a PVDF membrane and was blocked with 5% defatted milk (blocking solution) at 37˚C for a period of 1h and immune-blotted with primary antibodies including HSP 70 (heat-shock protein-70), SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), GR (glutathione reductase) and GAPDH (glyceraldehyde 3-phosphodehydrogenase (Santa Cruz, India) at 4˚C overnight. Blots were rinsed for three times with PBST buffer (phosphate buffer saline tween 20) for 10 minutes each. Then it was incubated with 1:10,000 dilution of the horseradish peroxidase-conjugated secondary antibody (Sigma Aldrich, India) and washed again for three times with PBST buffer. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (ECL; Bio-Rad, India) [23].

Statistical analysis

Results were expressed in mean value ± standard deviation (n = 6). Statistical significance was evaluated with t-test and $p < 0.05$ was considered significantly.

Results and Discussion

Nutritional and proximate composition of jujube fruit

Fruits in the daily diet have been associated with reduced risk of several chronic diseases [24]. The proximate composition of ZJP and ZJE are given in table 2. Proteins (1.53 ± 0.11 % and 2.56 ± 0.02 %), fats (0.56 ± 0.05 % and 1.22 ± 0.01 %), carbohydrates (44.42 ± 2.2 % and 42.62 ± 2.5 %) were recorded in ZJP and ZJE, respectively. There was no significant difference found in the moisture and ash content between ZJP and ZJE. Vitamin C (13.8 ± 1.2 % and 20.4 ± 1.9 %) and vitamin B1 (10.55 ± 0.9 % and 5.27 ± 0.4 %) were detected in ZJP and ZJE, respectively. Variation of vitamin C content has been reported in different varieties, cultivars, ripening period and freeze drying [25]. Minerals such as calcium and iron were present at higher content in both samples (Table 3). Content of various essential amino acids in ZJP and ZJE is shown in table 3. Glucose and fructose were significantly higher in ZJP than ZJE ($p < 0.05$); however maltose was detected only in ZJE (Table 3). The concentrations of calcium and iron contents were observed significantly higher in ZJP (5.45 ± 0.5 % and 14.77 ± 1.2 %, respectively) when compared to ZJE (4.36 ± 0.3 % and 11.7 ± 1.0 %, respectively).

Parameters (%)	ZJP	ZJE
Protein	1.53 ± 0.11	2.56 ± 0.02
Fat	0.56 ± 0.05	1.22 ± 0.01
Moisture	0.05 ± 0.001	0.10 ± 0.001
Crude fibre	0.65 ± 0.02	ND
Total sugars	44.42 ± 2.2	42.62 ± 2.5
Reducing sugars	22.6 ± 1.2	30.66 ± 2.0
Non-reducing sugars	23.8 ± 1.1	11.96 ± 0.4
Acid insoluble ash	0.53 ± 0.02	0.08 ± 0.001
Total ash	6.41 ± 0.3	6.98 ± 0.4
Energy (Kcal/100g)	188.84 ± 9.0	191.71 ± 11.0

Table 2: *Proximate composition of ZJP and ZJE.*

	ZJP	ZJE		ZJP	ZJE
Free amino acids (mg/100 g)					
Asp	0.08 ± 0.001	ND	Leu	1.59 ± 0.05	12.83 ± 0.9
Glu	0.13 ± 0.02	660.20 ± 54.0	Lys	21.94 ± 1.9	118.27 ± 10.0
Ser	2.94 ± 0.3	ND	Pro	1.78 ± 0.05	26.47 ± 0.2
Gly	0.24 ± 0.02	ND	**Carbohydrates (mg/100 g)**		
Thr	0.71 ±0.05	21.69 ± 3.0	Fru	122.3 ± 4.0	66.30 ± 2.2
Ala	0.45 ± 0.03	4.52 ± 0.5	Glu	225.56 ± 9.1	103.36 ± 5.6
Arg	3.11 ± 0.09	7.77 ± 0.09	Mal	ND	125.98 ± 11.0
Tyr	9.33 ± 1.0	225.55 ± 15	**Vitamins and minerals (mg/100g)**		
Val	ND	35.0 ± 3.0	Vit 'C'	13.8 ± 1.2	20.4 ± 1.9
Met	ND	60.0 ± 5.5	Vit 'B1'	10.55 ± 0.9	5.27 ± 0.4
Phe	1.51 ± 0.09	7.99 ± 0.4	Calcium	5.45 ± 0.5	4.36 ± 0.3
Ile	3.60 ± 0.2	20.56 ± 0.15	Iron	14.77 ± 1.2	11.7 ± 1.0

Table 3: *Analysis of free amino acids, sugars, vitamins and minerals from jujube.*

In vitro free radical-scavenging activity of jujube fruits

Phenolics and flavonoids are the secondary metabolites present in fruits and vegetables, known to act as antioxidant activity due to their redox properties [26]. Li., *et al*. [27] studied total phenolic content in jujube fruits and observed in the range from 5.18 to 8.53 mg/g. In the present study, jujube fruits were found with polyphenols at a range of 31.6 ± 2.2 mg/g and 38.4 ± 3.1 mg/g (ZJE and ZJP, respectively; Table 4). Previous studies on jujube indicated that the content of polyphenols and flavonoids are affected by time of harvest, physicochemical properties of soil, cultivars and other horticultural factors [28,29]. In terms of antioxidant activity of jujube, the results are shown in table 3. ZJE exhibits strong antioxidant activities based on DPPH radical-scavenging activity (1.0 ± 0.07 mg/ml), ABTS radical-scavenging activity (0.8 ± 0.01 mg/ml), NO radical-scavenging activity (1.8 ± 0.14 mg/ml) and metal-chelating activity (1.4 ± 0.07 mg/ml). Moreover, the antioxidant activities of ZJE were significantly higher than ZJP ($p < 0.05$) (Table 4). Previous reports on antioxidant activity of jujube fruits are in line with the present study [30,31].

Parameter	ZJP	ZJE
DPPH radical scavenging activity[a]	1.0 ± 0.07	0.7 ± 0.03
ABTS radical scavenging activity[a]	0.8 ± 0.01	0.6 ± 0.02
Nitric oxide scavenging activity[a]	1.8 ± 0.14	1.02 ± 0.03
Metal chelating activity[a]	1.4 ± 0.07	0.85 ± 0.04
Poly phenols[b]	31.6 ± 2.2	38.4 ± 3.1
Flavonoids[c]	5.0 ± 0.2	6.8 ± 0.4

Table 4: *Free radical scavenging activity of jujube.*

[a]: Expressed IC_{50} values in mg/ml, [b]: Expressed in units of mg/g of gallic acid equivalents, [c]: Expressed in units of mg/g of rutin equivalents

In vivo anti-fatigue activity

Effect of jujube on prolonged swimming time

Perhaps swimming until exhaustion is one of the most commonly used laboratory animal models for evaluating physical performance [32]. The swimming time of rats were gradually increased from the 7th day onwards (3rd test) by supplementation of ZJP and ZJE (Figure 1). However, from 13th day onwards (5th test) there was a significant improvement in swimming time as observed from the two jujube groups when compared to control ($p < 0.05$). Maximum swimming time was recorded on the 17th (6th test) of the ZJP and ZJE supplementation, i.e. 27 ± 3.4 min and 29 ± 2.6 minutes, respectively (Figure 1). These results shows that the administration of ZJP and ZJE could extend the swimming time, indicating the jujube fruits have anti-fatigue activity and improve the exercise tolerance.

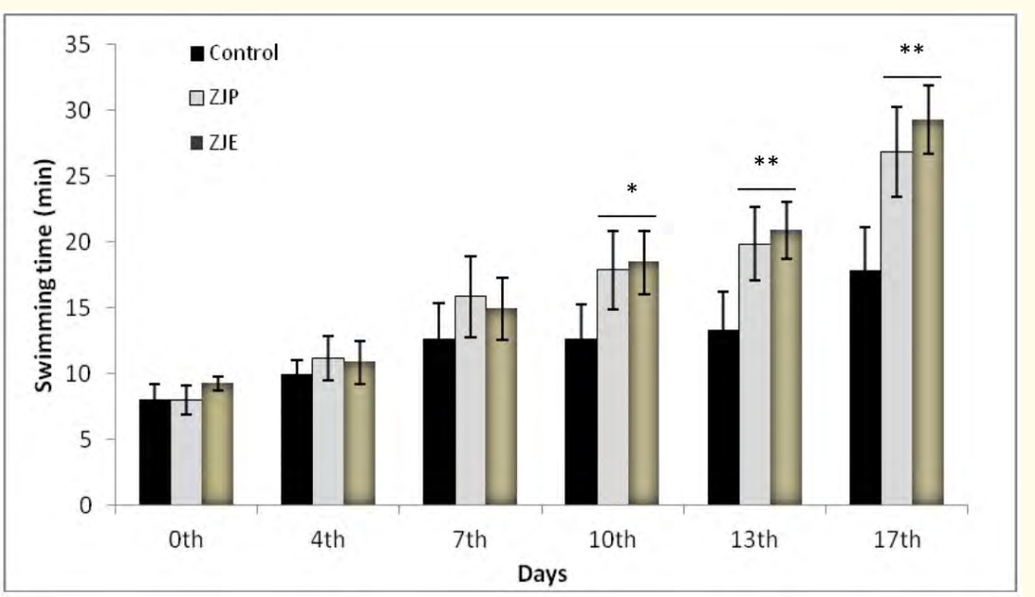

Figure 1: *Effect of jujube fruits on physical endurance capacity.*

*Data express the mean ± SD for six rats. Sedentary: rats unexposed to the WFST and treated with distilled water; Control: rats exposed to the swimming stress and treated with distilled water; ZJP and ZJE: rats exposed to the swimming stress and treated with jujube fruit pulp lyophilised powder (2 g/kg b.wt. day-1) and alcoholic extract (200 mg/kg b.wt. day-1). Different symbols indicates statistically significant differences, *p < 0.05, **p < 0.01 vs control.*

Effect of jujube on glycogen, lactic acid and lipid peroxidation after WFST

Exhaustion of glycogen levels in liver and muscle is a regular pathophysiology of physical exercises, which causes hypoglycaemia [33]. In the present study, swimming exercises reduces the levels of liver and muscle glycogen compared to that of sedentary group ($p < 0.05$) (Table 5). However, the jujube treatment groups (ZJP and ZJE) significantly up-regulated the glycogen contents in liver and muscle tissues when compared to the control group ($p < 0.05$). Anaerobic breakdown of glycogen induces accumulation of inorganic acids at cellular level, of which lactic acid is an important. Lactic acid dissociates into lactate and H+ and further causes skeletal muscle fatigue, therefore increased content of lactic acid considered as one of the biomarkers for judging the fatigue [34]. In the present study, ZJP and ZJE supplementation significantly controls lactic acid accumulation in liver and muscle tissues when compared to the control group ($p < 0.01$). Improvement of glycogen levels and depletion of lactic acid accumulation in liver and muscle tissues proves the enhancing property of muscle strength [35]. Therefore, the results of the present study confirm jujube fruits with anti-fatigue activity. Fatty acids, mitochondrial membrane glycolipids and other essential lipids are prone to peroxidation, and produces toxic malonaldehyde in stress conditions [36]. Exhaustive swimming exercise raises lipid peroxidation levels in liver and muscle tissues (Table 5). However, the supplementation of ZJP and ZJE significantly reduces the levels in both the tissues ($p < 0.01$), which indicates anti-fatigue activity of jujube fruit.

Treatment Groups	Serum parameters		Lactic acid (mg/g)		Glycogen (mg/g)		Lipid peroxidation (MDA µmol/cm/g)		Liver antioxidant enzymes (U/mg protein)		
	BUN	CK	Liver	Muscle	Liver	Muscle	Liver	Muscle	SOD	CAT	GPx
Sedentary	43 ± 5	79 ± 10	3.45 ± 0.28	3.19 ± 0.13	77.07 ± 2.6	66.18 ± 1.9	48.12 ± 3.5	20.71 ± 1.6	3.79 ± 0.5	13.87 ± 0.77	158.90 ± 7.2
Control	62 ± 5^{a}	150 ± 13^{a}	4.98 ± 0.61^{a}	5.23 ± 0.10^{a}	47.26 ± 1.4^{a}	47.06 ± 1.5^{a}	105.34 ± 8.3^{a}	40.73 ± 1.5^{a}	1.70 ±0.7^{a}	7.95 ± 0.31^{a}	82.56 ± 5.6^{a}
ZJP	54 ± 6	105 ± 18^{B}	3.18 ± 0.25^{B}	4.40 ± 0.09^{B}	59.68 ± 5.9^{A}	61.65 ± 0.4^{B}	61.20 ± 3.8^{B}	24.83 ± 2.9^{B}	1.84 ± 0.9	8.93 ± 0.29^{A}	116.83 ± 6.6^{B}
ZJE	52 ± 6^{A}	118 ± 15^{A}	3.55 ± 0.15^{B}	3.56 ± 0.06^{B}	52.58 ± 1.5^{B}	52.66 ± 2.2^{B}	53.85 ± 6.3^{B}	18.61 ±2.4^{B}	1.77 ± 0.3	11.07 ± 0.40^{B}	97.36 ± 6.8^{A}

Table 5: *Effect of jujube fruit on biochemical parameters and antioxidant enzymes.*

Data express the mean SD for six rats. Sedentary, rats unexposed to the WFST and treated with distilled water; Control, rats exposed to the swimming stress and treated with distilled water; ZJP and ZJE, rats exposed to the swimming stress and treated with jujube fruit pulp lyophilised powder (2 g/kg b.wt. day^{-1}) and alcoholic extract (200 mg/kg b.wt. day^{-1}). Different symbols indicates statistically significant differences, $^{a}p<0.01$ vs sedentary and $^{A}p<0.05$, $^{B}p<0.01$ vs control.

Effect of jujube on *in vivo* antioxidant enzymes after WFST

Free radicals such as hydroxyl, superoxide, H_2O_2, nitric oxide and peroxynitrite are the major sources of oxidative stress, which may contribute to tissue damage and various diseases [37]. Hence, free radical-scavenging activity is a significant property of any functional product for the management of oxidative stress [38]. The role of oxidative stress in exhaustive exercises or physical stress conditions is well established [39]. The antioxidant enzymes SOD, GPx, GR and CAT are the primary defence against free radicals generated during exhaustive exercise [40]. The levels of SOD, GPx, and CAT were up-regulated with the supplementation of ZJP and ZJE when compared to exercised control rats ($p < 0.05$; Figure 2). These results also suggest that *in vitro* antioxidant capacity of jujube fruits may act directly or interact with endogenous antioxidants to improve anti-fatigue activity. Previous reports on antioxidant potential of jujube fruits are in line with the findings of this study [41-43]. The results of immune-blotting analysis showed that the ZJE treated rats had significantly reduced the HSP-70 expression when compared to the control rats. The expression levels of HSP 70 was up-regulated significantly ($p < 0.05$) when compared to the control group (Figure 2). The HSP-70 family represents the most highly conserved and the best studied class of HSP [44]. The increasing level of HSP-70 expression in control rats may be attributed to the oxidative effect of fatigue which enhances the expression of HSP-70 as a cellular defence mechanism [45].

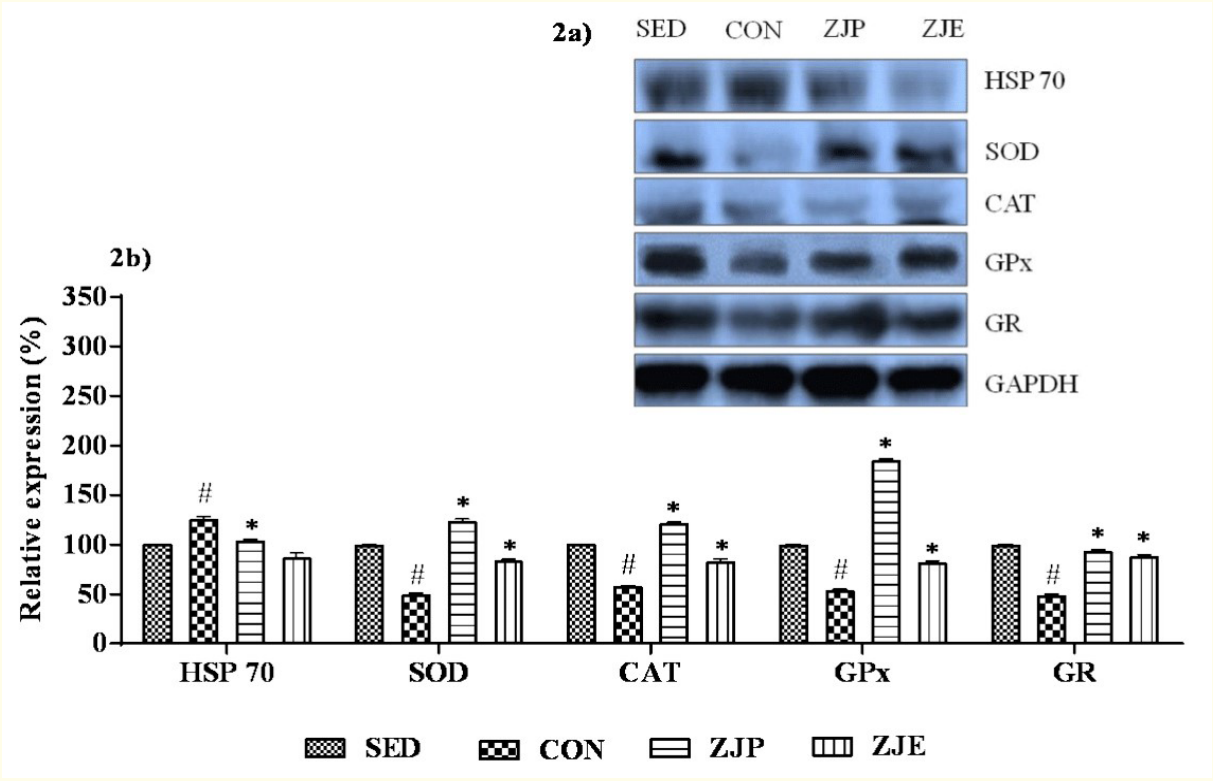

Figure 2: *Involvement of HSP 70 and antioxidant system in jujube fruits induced physical-endurance capacity by western blotting analysis*

2a) Blot images; 2b) Densitometry analysis

*Data express in mean ± SD. Sedentary: rats unexposed to the WFST and treated with distilled water; Control: rats exposed to the swimming stress and treated with distilled water; ZJP and ZJE: rats exposed to the swimming stress and treated with jujube fruit pulp lyophilised powder (2 g/kg b.wt. day-1) and alcoholic extract (200 mg/kg b.wt. day-1). Different symbols indicates statistically significant differences, #p < 0.05 vs sedentary and *p < 0.05 vs control.*

Conclusion

The fruits of *Z. jujuba* are valuable horticultural products, based on their rich nutrient composition and antioxidant activity. The results presented in this report suggest possible applications of *Z. jujuba* fruit as potential anti-fatigue agent by prolonging swimming time, modulating biochemical markers related to stress and up-regulating antioxidant enzymes. The study has demonstrated that ZJE has anti-stress property in experimental animal models. However, further issues related to safety and toxicity of the drug need to be addressed.

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Conflict of Interest

We hereby declare that all authors have no conflict of interest.

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