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A study on the possible role of date fruit on the electrophysiology and neuropathic changes in diabetic polyneuropathy

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disease with many neurovascular complications particularly diabetic peripheral neuropathy (DPN) that is mostly developed secondary to much release of reactive oxygen species (ROS, the potential mediators of DM-induced oxidative stress). The aim of the present study is to evaluate the possible neuroprotective role of Date Fruit Extract (DFE), an antioxidant nutrient, in ameliorating the DPN in streptozotocin-induced diabetic rats. Forty adult male rats were used in this study. They were divided into 4 equal groups. Group I (Control group). Group II (DEF group), supplemented orally with 250mg/kg DFE daily for 6 weeks, Group III (Diabetic group): The rats were rendered diabetic through their injection with 50 mg/kg streptozotocin (STZ) intraperitoneally and Group IV (Diabetic-DFE group). At the end of 6 weeks-experimental period; fasting serum glucose, serum insulin, glycosylated hemoglobin (Hb A1ic), tumor necrosis factor alpha (TNF-α) (as an index of inflammation), glutathione peroxidase enzyme (GPX) (as an index of the tissue antioxidant enzyme defense activity) and malondialdehyde (MDA) (a tissue marker of oxidative stress and lipid peroxidation) were measured. In addition, to ensure the development of DPN in the rats, the thermal pain threshold of the rats was assessed through a paw withdrawal latency (PWL) using the hot plate analgesia meter. In addition, Nerve Conduction Velocity (NCV) of the isolated sciatic nerves of the rats was assessed. The results revealed that the untreated diabetic group exhibited a significant increase of the serum glucose, Hb A1ic, serum MDA and serum TNF-α together with a significant decrease of serum insulin and GPX. In addition, the PWL and NCV were significantly lower in the diabetic rats relative to the control group. Interestingly, when the diabetic rats were supplemented with DEF, a significant improvement of all the altered parameters was obtained. In conclusion, DEF has a beneficial effect in ameliorating the altered biochemical parameters and NCV in experimentally-induced DPN, probably through its antioxidant and its anti-inflammatory effects.

***Key words*:** Diabetic peripheral neuropathy, Date Fruit Extract, NCV, rats.

1 Introduction

In accordance to the International Diabetes Federation (IDF), the prevalence of DM is approaching 5% of the global population, about 387 millions individuals, and it has been suspected to rise at an alarming rate both in developed and in the developing countries to reach 592 millions by the year 2035 (IDF, 2014). Diabetic polyneuropathy (DPN) is one of the most troublesome complications of DM (Wooten 2009). It is characterized by progressive functional and structural changes of the peripheral sensory, motor, and autonomic nerves, and it predominantly involves the distal extremities, thus it is usually referred to as peripheral neuropathy (Babaei-Jadidi et al., 2003).

Oxidative stress and production of reactive oxygen species (ROS) are strongly suggested mediators in the pathophysiology of DPN (Zychowska et al., 2013). Based on this suggestion, considerable attention was directed to find a suitable

nutrient having an antioxidant property in the management of DPN. Date palm (Phoenix dactylifera L. Arecaceae) is a unique nutrient that constitutes a vital component of the diet and a staple food in most of Arabian countries specially Saudi Arabia. It is one of the dietary supplements which has a great antioxidant property (Vayalil, 2002). It has a great advantage over the other used antioxidant nutrients not only from the nutritional and economic points of view, but also it is rich in many antioxidant compounds like polyphenolic compounds (flavonoids, anthocyanins and phenolic acids), trace elements (selenium, copper, zinc and manganese), and vitamin C (Al-Farsi et al., 2005; Mansouri et al., 2005; Hong et al., 2006 and Saafi et al., 2009). Moreover, the date palm has been recently advocated as a promising neuroprotective agent in a neuronal damage induced by cerebral ischemia in rats (Asadi-Shekaariet al., 2008). This neuroprotective effect, besides the antioxidant property, of the date palm fruit was a motive in this study to use the Date Fruit Extract (DFE) as a dietary antioxidant supplement in preventing DPN in an animal model involving adult male rats.

2 Material and methods

Animals

In the current study, forty adult male albino rats, 160-180 g each, were used. The rats were obtained from the animal house unit in the Faculty of medicine, Menoufia University, Egypt. The rats were housed in plastic cages, fed the standard laboratory pellet diet and allowed tap water ad libitum. The rats were left to acclimatize for one week prior to the experimental procedures that were performed in accordance to the internationally accepted guidelines with an approval from the Institutional Committee for ethical care and use of laboratory animals of Menoufia University, Egypt.

Chemicals

Streptozotocin (STZ) was purchased from Egypt batch of Sigma chemical company, St. Louis, USA.

Preparation of aqueous Date Fruit Extract (DFE)

Dried date palm fruits (Phoenix dactylifera L. Arecaceae) were purchased from a local market in Jeddah, Saudi Arabia. The fleshes of date were manually separated from the pits and soaked in cold distilled water (1:3 ratio, weight to volume, 1000grams date to 3000ml distilled water) and kept for 24 h at a temperature of 4°C. Then, they were grinded with a mortar and pestle. Thereafter, the grind was centrifuged at 4°C at 4000 g for 20 min. and the supernatant was collected, lyophilized and stored at -20°C (Vayalil, 2002). An aqueous extract was selected because most of the antioxidant components in dates are extracted in water. During the experiment, the aqueous DFE was daily prepared.

Experimental design:

The experimental study period was 6 weeks after which all rats were subjected to overnight fasting, then they were anesthetized and blood samples were obtained from the retro-orbital veins and the serum was prepared and frozen at -20 °C until further biochemical analysis, Afterwards, the rats were subsequently subjected to the hot plate analgesia meter test. Then, the sciatic nerves were dissected free and isolated from the rat's body and NCV was assessed.

Induction of experimental DM

DM was induced in the rats through a single I.P injection of freshly prepared STZ solution at a dose of 50 mg/kg BW dissolved in 0.5 ml of sodium citrate buffer solution, (50mM, pH 4.5). Twenty four hours after STZ injection, fasting blood samples were taken from the rat tail veins for estimation of serum glucose level. Rats with fasting serum glucose more than 200 mg/dl were considered diabetic and hence they were selected and included in the study (Modi et al., 2006).

Animal grouping:

The rats were divided into 4 equal groups (10 rats each) as follows:

Group I (Control group): The rats were supplemented orally with 2mL distilled water, daily for 6 weeks.

Group II (DFE group): The rats were supplemented orally with 250mg/kg DFE in 2ml distilled water, daily for 6 weeks (Asadi-Shekaari et al., 2008).

Group III (Diabetic group): In this group, the rats were rendered diabetic via a single I.P injection of STZ at a dose of 50 mg/kg BW dissolved in 0.5 ml of sodium citrate buffer solution (50mM, pH 4.5) (Modi et al., 2006).

Group IV (Diabetic-DFE group): The rats of this group were rendered diabetic as mentioned in the third group, then they were immediately supplemented orally with 250mg/kg DFE in 2ml distilled water, daily for 6 weeks.

Experimental procedures

Biochemical analysis

1-Fasting serum glucose (Trinder, 1969).

2. Serum insulin was determined by enzyme-linked immunosorbent assay (ELISA) (Mullner et al., 1991).

3. HbA1C (Nayak and Pattabiraman, 1982).

4.Glutathione peroxidase enzyme (GPX) (Rotruck et al., 1984).

5. Serum malondialdehyde (MDA) (Ozaras et al., 2003).

6- Serum Tumor necrosis factor alpha (TNF-α) by ELISA (Niture et al., 2014).

Hot plate test (thermal hyperalgesia test)

To ensure the development of DPN, thermal pain threshold of the rats of different groups was assessed via measuring the paw withdrawal latency

(PWL) in sec. using the hot plate method. Briefly, in this method, the rats were individually, one animal at a time, placed in a hot plate analgesia meter apparatus that is formed of Plexiglas wall with a height of 50cm and a glass plate at its bottom with a diameter of 19cm coupled to a thermal stimulator (11 TC model Woodland Hills, C.A., USA). The stimulator was designed to produce electrically-controlled radiant heat that is focused below the plate onto the plantar surface of the hind paws of the rats. The temperature of the hot plate was adjusted and maintained at 55.5 ± 0.5°C, with a cut off time of 15 seconds to avoid tissue damage (Bachhav et al., 2009). PWL (time in seconds elapsed between the application of heat to the rats and the beginning of licking the hind paws or jumping from the hot plate) was recorded before the start of and at the end of the experiments (Rutledge et al., 2002).

Measurement of NCV

Each rat was anesthetized by I.P injection of sodium Phenobarbital (40mg/kg BW) then a longitudinal incision was done on the back of the thigh then the sciatic nerve was carefully dissected and excised outside the rat's body. Briefly, for determination NCV, about 2 cm of the sciatic nerve was isolated and mounted in a nerve chamber containing stainless wires and designed for recording an action potential of the nerves. The nerve was positioned over these wires. The proximal part of the nerve was stimulated by platinum stimulating electrode. The recording electrode was placed 1-2 cm apart from the stimulating one. The experiments were performed at room temperature. Measurements were performed using AD Instruments Power Lab 4/25 Stimulator and Bio AMP Amplifier (Castle Hill, Australia) followed by computer assisted data analysis. Sciatic nerve was stimulated with square wave pulses of 200 msec. duration at 5 volts. Finally, NCV (meter/sec.) was measured by dividing the distance (in meter) between the stimulating and the recording electrodes by the latent period which is the time (in seconds) elapsed between the application of the stimulus until the peak of the maximum compound action potential (Leal-Cardoso et al., 2004).

Statistical analysis

The obtained results of the present study were presented as Mean±Standard error of means (SEM). Paired and unpaired student-T test were done to determine the statistical difference among different groups using the Statistical Package for Social Sciences (SPSS, software version 15.0, Chicago, IL, USA). P value of less than 0.05 ($p<0.05$) was considered statistically significant (Petrie and Sabin, 2005).

3 Results

Results of biochemical analysis

In the present study, compared with control group, the DFE-group exhibited a statistical non-significant change ($p>0.05$) in the mean values of fasting serum glucose (83.32±6.11 Vs 81.67±5.81 mg/dl), serum insulin (23.35±2.19 Vs 21.41±2.82 µU/ml), HbA_{1C} (0.26±0.01 Vs 0.23±0.01 mg/g Hb), serum GPx (2.24±0.05 Vs 2.62±0.05 U/ml), serum MDA (0.89± 0.03 Vs 0.82±0.01 nmol/ml) and TNF-α (71.55± 4.29 Vs 61.47±4.43 pg/ml) (Table 1 and Fig. 1-6). On the other hand, the diabetic group exhibited a statistical significant rise ($p<0.05$) of serum glucose (299.64±16.77mg/dl), HbA_{1C} (0.93 ± 0.05 mg/g Hb), MDA (9.45 ± 0.16 nmol/ml) and TNF-α (243.55±34.49 pg/ml) compared with the control group (Table 2and Fig. 1,3,5 and 6). While, serum insulin was (8.98±0.75 µU/ml) and GPx (0.41 ± 0.01 U/ml) in the diabetic group were significantly lower ($p<0.05$) than that of control group (Table 1 and Fig. 2 & 4). Interestingly in Diabetic-DFE group, the fasting serum glucose 133.22±7.252 mg/dl), serum insulin (9.01±0.21 µU/ml), HbA_{1C} (0.53 ± 0.02 mg/g Hb), serum GPx (1.15 ± 0.01 U/ml), serum MDA (3.87±0.06 nmol/ml) and TNF-α (167.62 ±15.47 pg/ml) were significantly improved compared to the corresponding values of the diabetic group (Table 1 and Fig. 1-6).

Results of the hot plate test

The diabetic rats have got a statistical significant decrease ($p<0.05$) of the mean value of the PWL compared with the control and DFE groups (0.36±0.01 1.68±0.09 and 1.61±0.11sec. respectively). Interestingly, Diabetic-DFE group experienced a statistical significant increase of the PWL (1.26±0.03 sec.) compared with that of the diabetic group (table 2).

Results of NCV

Regarding NCV, the mean value in the Diabetic group was 0.11±0.01 meter/sec. which was significantly lower than both control group and DFE group (0.38±0.02 meter/sec. and 0.33±0.04 meter/sec.). On supplementation of the diabetic group with DFE, the NCV was improved to be 0.18±0.04 meter/sec. (table 2).

4 Discussion

DM refers to a metabolic disorder characterized by relative or absolute deficiency of insulin secretion and/or insulin resistance that presents a major health problem (Zatalia and Sanusi, 2013). DM is known to be one of the foremost causes of mortality and morbidity in the world (Can et al., 2004). It affects the quality of patient's life with a variety of symptoms which include pain,

Table (1): Fasting serum glucose, serum insulin, HbA_{1C}, GPX and MDA in the studied groups at the end 6 weeks-experimental period

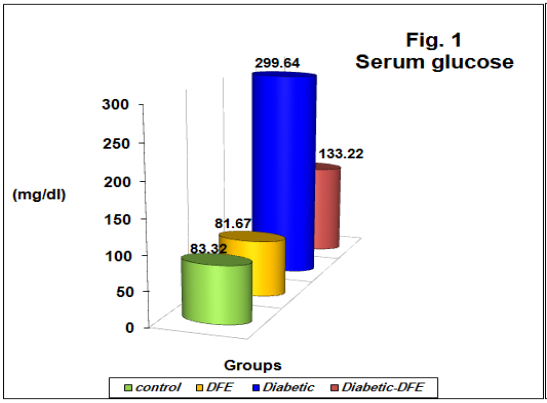
GROUPS / PARAMETERS	CONTROL	DFE	DIABETIC	DIABETIC-DFE
SERUM GLUCOSE (MG/DL)	**83.32±6.11**	**81.67±5.81**	**299.64±16.77***	**133.22±7.252*#**
SERUM INSULIN (µU/ML)	**23.35±2.19**	**21.41±2.82**	**8.98±0.75***	**9.01±0.21***
HBA_{1C} (MG/G HB)	**0.26 ± 0.01**	**0.23 ± 0.01**	**0.93 ± 0.05***	**0.53 ± 0.02*#**
PLASMA GPX (U/ML)	**2.24 ± 0.05**	**2.62 ± 0.05**	**0.41 ± 0.01***	**1.15 ± 0.01*#**
PLASMA MDA (NMOL/ML)	**0.89 ± 0.03**	**0.82±0.01***	**9.45 ± 0.16***	**3.87 ± 0.06*#**
TNF-α (PG/ML)	**71.55 ± 04.29**	**61.47 ± 4.43**	**243.55±34.49***	**167.62 ±15.47*#**

Values are represented as mean ±SEM of 10 rats for each group.
DFE: Date fruit extract, GPx: Glutathione peroxidase enzyme (GPX) and MDA: Malondialdehyde.
* significant ($p < 0.05$) when compared with the control group.
# significant ($p < 0.05$) when compared with the diabetic group.

Table (2): Paw withdrawal latency (PWL) and nerve conduction velocity (NCV) in the studied groups at the end 6 weeks-experimental period

GROUPS / PARAMETERS	CONTROL	DFE	DIABETIC	DIABETIC-DFE
PWL (IN SEC.)	**1.68±0.09**	**1.61±0.11**	**0.36±0.01** *	**1.26±0.03** *#
NCV (METER/SEC.)	**0.38±0.02**	**0.33±0.04**	**0.11±0.01***	**0.18±0.04*#**

Values are represented as mean ±SEM of 10 rats for each group.
PWL: Paw withdrawal latency and DFE: Date fruit extract.
* means significant ($p < 0.05$) when compared with the control group.
# means significant ($p < 0.05$) when compared with the diabetic group.



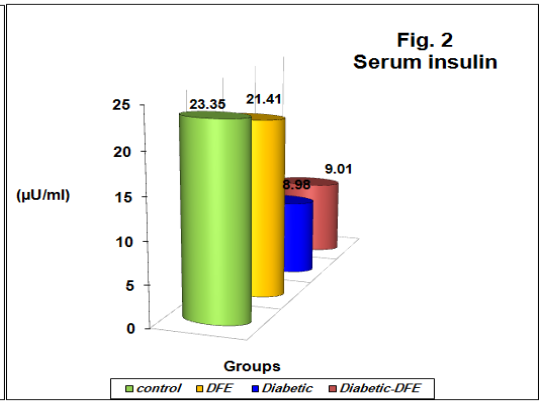
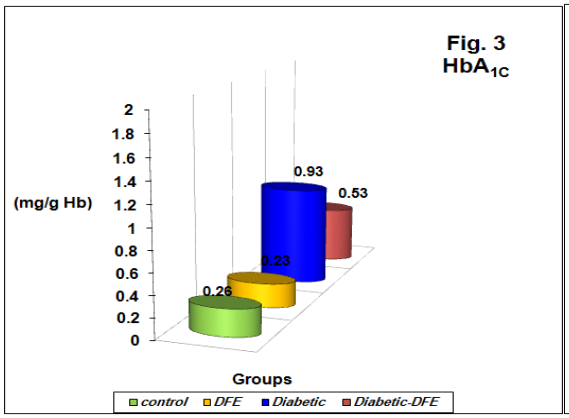


Fig.1. Change in glucose in different animal groups. Fig.2. Change in insulin in different animal groups.



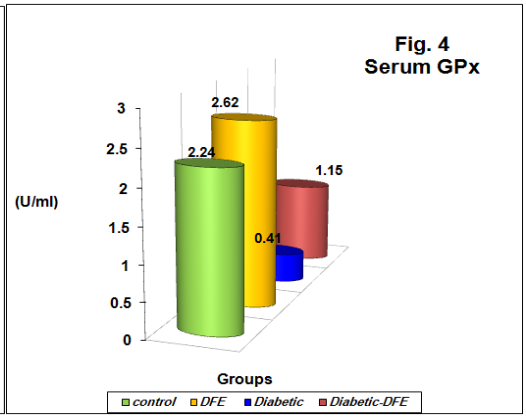
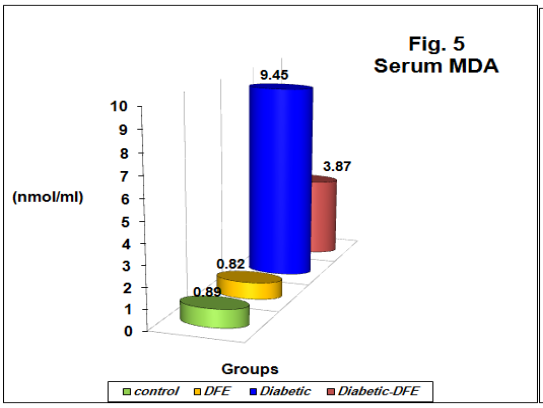


Fig.3. Change in HbA_{1c} in different animal groups.

Fig.4. Change in GPx in different animal groups.



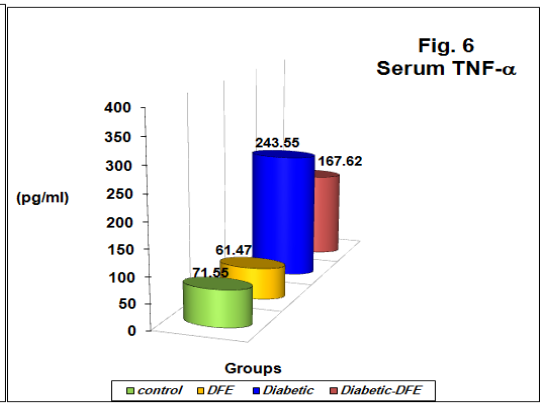


Fig.5. Change in MDA in different animal groups.

Fig.6. Change in TNF-α in different animal groups

weakness, ataxia, impotence, and sensory loss (Algaidi, 2011). It is a complex and progressive disease that results in multiple complications which include retinopathy, nephropathy, cardiomyopathy, hepatopathy, and neuropathy (Aljabri et al., 2010). The characteristic feature of DM is chronic hyperglycemia that predispose to many diabetic complications of which diabetic peripheral neuropathy (DPN) is the commonest (Tesfaye and Selvarajah 2012). DPN implies the presence of symptoms and/or signs which rages from numbness, tingling foot ulcers to gangrene with subsequent limb amputation (Tesfaye et al., 2010).

In the preset study, STZ was injected in the rats to induce an animal model of type-I DM because STZ specifically destroys most insulin-producing β cells of pancreas through formation of cytotoxic ROS inside these islet cells (Fazan et al., 2010). It was noted that these experimentally-induced diabetic rats were encountered with a highly elevated serum glucose and HbAlC and decreased serum insulin compared to the corresponding values of the control group. Interestingly, DFE supplementation to the diabetic rats resulted in a significant decreased of the serum glucose level and HbAIC and an insignificant change in the serum insulin level. The underlying mechanism by which DFE has decreased serum glucose is mostly through its antioxidant activity that could protect the β cells of pancreas and the neuronal tissue from cytotoxic effects of ROS (Allaith, 2007).

It was reported that DFE had many antioxidat compounds like polyphenolic compounds (flavonoids, anthocyanins and phenolic acids), (Mansouri et al., 2005). These antioxidant compounds are able to detoxify the free radicals and to inhibit lipid peroxidation in the peripheral nerves. This effect was evident in the preset work, where the high encountered serum MDA (a tissue marker of oxidative stress) in the diabetic rats was significantly decreased when the diabetic rats were supplemented orally with DFE. MDA is well known as an index of lipid peroxidation process that is widely considered as a key participant in the development of DPN (Maritim et al., 2003). Actually, chronic hyperglycemia was reported to enhance over production of reactive oxygen species (ROS) that are known to damage the blood-nerve barriers, an

immune modulating process, making the neurons more vulnerable to attacking and invasion by circulating macrophages (Gundogdu, 2006). The invading macrophages were known to secrete pro-inflammatory mediators such as tumour necrosis alpha (TNF-α) which impart cytotoxic effects on the peripheral neurons leading to their degeneration (Said et al., 2003). In the present work, the serum level of TNF-α was significantly higher in diabetic rats than the corresponding value in the control group. This rise of TNF-α implies an ongoing inflammatory reaction in the diabetic rats. Obviously, when the diabetic rats were supplemented with DFE, serum TNF-α was significantly decreased. This beneficial effect of DFE implied an anti-inflammatory property of this DFE which is secondary to its high contents of polyphenolic compounds (Saafi et al., 2009). Actually, preclinical studies have shown that the date fruits not only have a free radical scavenging and antioxidant properties, but also it has anti-inflammatory activity (Al-Qarawi et al., 2005). Actually, the inflammatory mediators have been considered the link between inflammation and neuropathic pain development in DPN (Niture et al., 2014). The inflammatory mediators were suggested to be responsible for the modified response of the diabetic rats to the hot plate test. In the present work, PWL of diabetic rats was lower compared to the control group. This indicated a development of analgesia in these diabetic rats. This was supported in the preset work upon measuring NCV of the diabetic rats, where the velocity of nerve conduction was significantly decreased in these rats, however there was a partial improvement in NCV upon DFE supplementation. These findings were in line with those of Watson et al. 2003 who found that the peripheral nerve fibers were degenerated in patients having DPN. Also, it was reported that, in long standing cases of DM, the peripheral nerve fibres encountered a considerable loss of their nerve terminals (Chung et al., 2003).

Definitely, the pathophysiology of DPN is a complex and a multifactorial entity, however chronic hyperglycemia has been advocated and criticized as a common denominator for almost all speculated mechanisms (Harati, 2007). It was reported that several molecular and signaling cascades are triggered and activated by chronic hyperglycemia and in turn implicated in glucotoxicity of peripheral nerves such as glucose autoxidation, excess conversion of glucose into sorbitol, non-enzymatic glycation of proteins and over production of AGEs (Maritim et al., 2003). While each of the above reactions is injurious alone, collectively all of them coalesce together in excessive production of ROS which are the potential molecular signatures of oxidative stress and lead to neuronal dysfunction and nerve damage (Calcutt et al., 2009). These generated ROS are known to increase the oxidative modification of neuronal proteins (Brownlee, 2005). Also, they decrease the ability of peripheral neurons and Schwann cells to produce nerve growth factors which are responsible for local neurotrophic support of these neurons (Zychowska et al., 2013). Therefore, the possible neuroprotective effect of DFE against DPN is mostly through its free radical scavenging ability and inhibition of oxidative damage displayed by the generated ROS because DFE is rich in many antioxidant compounds (Biglari et al., 2008).

Pathologically, DPN is reported to be ascribed to the decline of the circulating and neuronal antioxidant enzyme defensive activities in cases of DM that indirectly increase the susceptibility of the neurons to cellular oxidative stress and promote the development of DPN (Brownlee, 2005). This was obvious in the present work, where the serum antioxidant enzyme GPX of the diabetic rats was significantly lower than that of control group. However, DFE supplementation to the diabetic rats increased serum GPX, a good result that indicating the capability of DFE to increase the biosynthesis of antioxidant enzymes secondary to quenching ROS.

Conclusion: The present study demonstrated the ability of the date palm fruit as a neuroprotective nutrient in the experimentally induced DPN. Despite, the actual mechanism is not yet clear, yet its high contents of the antioxidant compounds are mostly responsible for this beneficial effect.

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