

RESEARCH ARTICLE

Marwa A.E. Abd El-Maksoud
Doaa S. Ibrahim

Strawberry (*Fragaria x ananassa*) leaf extract reduces brain damage in diabetic male rats

ABSTRACT:

Brain damage is one of the consequences of diabetes. The main cause of this damage is the overproduction of free radicals resulted from hyperglycaemia. This study aims to address the effect of administration of strawberry leaf extract as an antioxidant agent to reduce brain damage in diabetic male rats. Five groups of rats (N = 30) were divided into control rats group, diabetic rats group, and three diabetic rat groups treated with strawberry leaf extract as 50, 100 and 200 mg/ kg body weight for 30 days, respectively. Diabetic rat brains showed significant increases in malondialdehyde (MDA), tumour necrosis factor-alpha (TNF- α), interleukin (IL)-6 and caspase-3 and significant decreases in catalase (CAT), superoxide dismutase (SOD), and vascular endothelial growth factor (VEGF)-A. Plasma dopamine and melatonin levels were decreased significantly in diabetic rats. After treating the diabetic rats with the three different doses of strawberry leaf extract, the brain and plasma biochemical parameters were improved significantly. The study finally concluded that strawberry leaf extract might be used as an antioxidant, anti-inflammatory, and anti-apoptosis to improve brain damage caused by diabetes.

KEY WORDS:

Diabetic neuropathy, strawberry leaf extract, oxidative stress, proinflammatory cytokines, apoptosis.

CORRESPONDENCE:

Doaa S. Ibrahim
Department of Zoology, Faculty of Science,
Benha University, Egypt.
E-mail: Doaa.mohamed@fsc.bu.edu.eg

Marwa A.E. Abd El-Maksoud
Department of Zoology, Faculty of Science,
Benha University, Egypt.

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INTRODUCTION:

Diabetes mellitus is characterized by hyperglycaemia that increases reactive oxygen species (ROS) production (Ullah *et al.*, 2016). The overproduction of ROS is responsible for the progression of diabetic complications (Volpe *et al.*, 2018). One of the most common diabetic complications that affect the nervous system is diabetic neuropathy (Pop-Busui *et al.*, 2017). High levels of ROS caused impairing nerve blood flow, peripheral nerve degeneration, endoneurial hypoxia, increased vibration, thermal perception, sensory loss, axonal atrophy of large myelinated fibres and neuropathic pain (Oyenihi *et al.*, 2015). Not only that but it also activates proinflammatory cytokines secretions that lead to apoptosis (Volpe *et al.*, 2018).

Many studies suggest that antioxidants have a neuroprotective effect against oxidative damage caused by ROS in diabetic rats (Thaifa *et al.*, 2017). Strawberry (*Fragaria x ananassa*) leaf are rich with various bioactive compounds such as tannins, flavonoids, ascorbic acid, and essential oil (Ibrahim and Abd El-Maksoud, 2015). They also contain high amounts of phenolic compounds that are known for their antioxidant and anti-inflammatory activities (Kårlund *et al.*, 2014; Giampieri *et al.*, 2014). This study was conducted to address the potential impact of the strawberry leaf extract as an antioxidant to reduce the overproduction of ROS, proinflammatory cytokines secretions, and apoptosis in diabetic neuropathy rat model.

MATERIAL AND METHODS:

Extract Preparation:

Strawberry leaves were collected from Benha market, Egypt. Only mature fresh leaves were allowed to air-dry for one week under 20°C in the dark. The aqueous extract of strawberry leaves was prepared as described by Ibrahim and Abd El-Maksoud (2015).

Animals:

Thirty male albino rats (180 \pm 10 g) were obtained from the VSVRI (Veterinary

Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt). Rats were allowed to acclimatize for 10 days with the laboratory conditions ($23 \pm 2^\circ\text{C}$, 12 hours light/dark cycle).

After 12 h fasting, twenty-four rats were injected by a single intraperitoneal dose (45 mg/kg bw, 0.01 M citrate buffer, pH 4.5) of streptozotocin (STZ, Sigma Co., USA). On the third day of STZ injection, blood glucose concentrations were examined for each rat and rats having glucose concentration up 180 mg/dl were considered as diabetic.

Rats were divided into five groups. The control group (Gp I) consists of six normal rats. While the twenty-four diabetic rats were divided into four groups; a non-treated diabetic group (Gp II), and three-treated diabetic groups (Gp III, Gp IV, and Gp V). Gp III, Gp IV, and Gp V were given strawberry leaf extract (50, 100, and 200 mg/kg body weight, respectively) at the fourth day of STZ injection once a day for 30 days by the gastric tube.

At the end of the experiment, blood samples were collected from overnight-fasted rats and plasma samples were obtained after centrifugation of blood. Plasma samples were stored at -20°C immediately. Rat brains were removed and washed in ice-cold phosphate buffer saline (PBS, pH 7.2) to remove excess blood. Then, brain tissues were homogenized in ice-cold PBS and centrifuged ($5000 \times g / 5 \text{ min}$). The supernatants were stored at -20°C until used for analysis.

Biochemical assays:

MDA content and CAT activity were determined in rat brains by chemical colorimetric methods using kits purchased from BioVision (USA). While, SOD activity in rat brains were determined by enzymatic colorimetric methods using kits purchased from BioVision (USA).

TNF- α , IL-6, VEGF-A, and caspase-3 levels were measured in rat brains by rat ELISA kit purchased from the Cloud-Clone Corporation (USA).

Plasma melatonin and dopamine levels were determined by ELISA kit purchased from Uscn Life Science Inc. (USA) and mouse/rat dopamine ELISA assay kit purchased from EAGLE Biosciences Inc. (USA), respectively.

Statistical analysis:

Data were expressed as mean \pm SD for six readings. The computer program Statistical Package for Social Science (SPSS, Version 20.00, Chicago, USA) was used for performing one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS:

Oxidative stress parameters:

The present results showed a significant increase in MDA content and significant decreases in CAT and SOD levels in diabetic brain rats compared to normal rats. After treatment of the different diabetic rat groups (Gps III, IV, and V) with the three different doses of strawberry leaf extract, MDA content decreased while CAT and SOD levels increased in their brains (Table 1)

Table 1. Oxidative stress parameters in normal (Gp I), diabetic (Gp II) and diabetic rats treated with three doses (50,100 and 200 mg/ kg) of strawberry leaf extract (Gps III, IV, and V).

Parameters	Groups				
	Gp I	Gp II	Gp III	Gp IV	Gp V
MDA (nmol/mg protein)	0.91 \pm 0.02 ^e	2.64 \pm 0.11 ^a	2.45 \pm 0.04 ^b	2.19 \pm 0.12 ^c	1.96 \pm 0.09 ^d
CAT (nmol/mg protein)	2.47 \pm 0.04 ^a	0.87 \pm 0.18 ^d	1.05 \pm 0.10 ^{bd}	1.21 \pm 0.03 ^{bc}	1.41 \pm 0.04 ^b
SOD (U/mg protein)	3.63 \pm 0.32 ^a	1.37 \pm 0.39 ^c	2.04 \pm 0.11 ^b	2.14 \pm 0.08 ^b	2.33 \pm 0.04 ^b

Each value is mean \pm SD for six rats in each group.

abcde = values in the same raw with different small letters are significantly different at $p < 0.05$.

Proinflammatory cytokines parameters:

Data also revealed significant increases in TNF- α and IL-6 levels in diabetic rat brains. Only the two diabetic groups (Gp IV and V) that treated with 100 and 200 mg/ kg of the

extract showed low levels of TNF- α in their brains. Whereas IL-6 levels were decreased in diabetic rat brains that treated with 50,100 and 200 mg/ kg of the extract (Table 2).

Table 2. Proinflammatory cytokines parameters in normal rats (Gp I), diabetic rats (Gp II) and diabetic rats treated with three doses (50,100, and 200 mg/ kg) of strawberry leaf extract (Gps III, IV, and V).

Parameters	Groups				
	Gp I	Gp II	Gp III	Gp IV	Gp V
TNF- α (ng/ml homogenate)	27.04 \pm 01.18 ^c	38.12 \pm 02.44 ^a	37.83 \pm 02.48 ^a	34.30 \pm 01.41 ^b	33.61 \pm 00.85 ^b
IL-6 (Pg/mg protein)	11.32 \pm 00.91 ^d	33.85 \pm 02.03 ^a	31.76 \pm 01.48 ^b	27.91 \pm 01.01 ^c	27.35 \pm 01.03 ^c

Each value is mean \pm SD for six rats in each group.

abcd = values in the same raw with different small letters are significantly different at $p < 0.05$.

Caspase-3 and vascular endothelial growth factor-A levels:

From the table 3, caspase-3 activity was high and VEGF-A level was low in diabetic rat brains. Diabetic rats treated with the different

doses of the extract showed a significant decrease in caspase-3 activity in their brains. Only the diabetic group that treated with 200 mg/kg of the extract showed a significant increase in VEGF-A level in their brains.

Table 3. Caspase-3 and vascular endothelial growth factor A levels in normal rats (Gp I), diabetic rats (Gp II) and diabetic rats treated with three doses (50,100 and 200 mg/ kg) of strawberry leaf extract (Gps III, IV, and V).

Parameters	Groups				
	Gp I	Gp II	Gp III	Gp IV	Gp V
CASP-3 (Pg/mg protein)	19.53 ± 00.69 ^e	44.79 ± 01.59 ^a	41.01 ± 00.45 ^b	38.14 ± 00.75 ^c	34.65 ± 01.30 ^d
VEGF-A (Pg/mg protein)	72.27 ± 02.44 ^a	60.54 ± 00.72 ^c	61.39 ± 00.81 ^c	61.23 ± 01.12 ^c	63.29 ± 00.78 ^b

Each value is mean ± SD for six rats in each group.

abcde = values in the same raw with different small letters are significantly different at $p < 0.05$.

Plasma dopamine and melatonin levels:

The plasma dopamine and melatonin levels were significantly low in diabetic rats compared to normal rats. Diabetic rats that treated with two 100 and 200 mg/ kg of the

extract have high plasma dopamine levels compared to the diabetic group. Whereas the diabetic groups that treated with the different three doses of the extract showed significant increases in plasma melatonin levels (Table 4).

Table 4. Plasma dopamine and melatonin levels in for normal rats (Gp I), diabetic rats (Gp II) and diabetic rats treated with three doses (50,100 and 200 mg/ kg) of strawberry leaf extract (Gps III, IV, and V).

Parameters	Groups				
	Gp I	Gp II	Gp III	Gp IV	Gp V
Dopamine (mg/ml)	584.65 ± 18.62 ^a	129.57 ± 02.52 ^d	133.94 ± 03.42 ^d	208.66 ± 04.3 ^c	303.07 ± 07.38 ^b
Melatonin (Pg/ml)	120.61 ± 03.27 ^a	37.47 ± 06.21 ^d	47.65 ± 03.70 ^c	50.24 ± 03.88 ^c	61.72 ± 03.44 ^b

Each value is mean ± SD for six rats in each group.

abcd = values in the same raw with different small letters are significantly different at $p < 0.05$.

DISCUSSION:

The overproduction ROS in cells and tissues is known by oxidative stress (Yasuyoshi *et al.*, 2017). Oxidative stress is the main reason for diabetic neuropathy progression (Babizhayev *et al.*, 2015). The high MDA content and the low CAT and SOD activities confirm the presence of oxidative stress in diabetic rat brains. The present findings supported the work of Montilla *et al.* (2005) who reported that diabetes associated with brain oxidative stress.

The reduction in MDA content and the increase in antioxidant enzymes activities in treated diabetic rat brains is evidence of the ability of strawberry leaf extract to reduce diabetic neuropathy progression. The antioxidant ability of strawberry leaf extract may be related to the presence of polyphenolic compounds like tannins, flavonoids and ascorbic acid (Ibrahim and Abd El-Maksoud, 2015).

Also, there were significant increases in proinflammatory cytokines (TNF- α and IL-6) levels in diabetic rat brains. Overexpression of proinflammatory cytokines induced by high levels of ROS production has an important role in the development of diabetic neuropathy (Kumar *et al.*, 2017).

The treated diabetic groups showed significant decreases in proinflammatory cytokines levels. The reduction in ROS production might be the reason behind the anti-inflammatory action of strawberry leaf extract. It might be also due to the high amounts of phenolic compounds found in strawberry leaf extract that are known for their antioxidant and anti-inflammatory activities (Kärlund *et al.*, 2014).

Caspase-3, a member of caspases family, is an indicator for apoptosis in neuronal cells (D'Amelio *et al.*, 2010). While the increase in VEGF-expression is an indicator for the anti-apoptotic (Adini *et al.*, 2002). Not only that but it also promotes many neuronal functions (Ibrahim, 2017). Our data showed a high level of caspase-3 and a low level of VEGF-A levels in diabetic rats, which might be evidence of the apoptosis in diabetic rat brains. The study showed that strawberry leaf extract can reduce apoptosis in diabetic rats by decreasing the caspase-3 level and increasing the VEGF-A level in their brains.

Hyperglycaemia is the reason behind reducing dopamine synthesis that causes neuronal dysfunction (Kim *et al.*, 2018). Espino *et al.* (2011) mentioned that the melatonin level

reduced in diabetic hamsters. We observed that plasma dopamine and melatonin levels showed significant decreases in diabetic rats.

The high levels of plasma dopamine and melatonin resulting from the administration of strawberry leaf extract to diabetic rats might depress diabetic neuropathy progression. Strawberry contains higher levels of melatonin

(Stürtz *et al.*, 2011). Melatonin prevents neuropathy in diabetic rats (Zangiabadi *et al.*, 2011) as it acts as an antioxidant, anti-inflammatory and anti-apoptotic agents (Ahmadias *et al.*, 2014).

Finally, it seems that strawberry leaf extract has more than one way to depress diabetic neuropathy progression.

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المعالجة بخلاصة أوراق الفراولة تقلل من تلف الدماغ في ذكور الجرذان المصابة بداء السكري

مروه عاطف عليوة ودعاء صبري ابراهيم

قسم علم الحيوان، كلية العلوم، جامعة بنها، مصر

معنوية في المالونداي ألدهيد (MDA)، وعامل نخر الورم ألفا (TNF- α)، و الإنترلوكين-6 (IL-6) والكاسباز-3 (caspase-3) وانخفاض معنويًا في الكتاليز (CAT) والسوبر أوكسيد ديسميوتاز (SOD) ، وعامل نمو بطانة الأوعية الدموية (VEGF -A). وانخفضت مستويات بلازما الدوبامين و الميلاتونين بشكل معنوي في الجرذان المصابة بداء السكري. بعد معالجة الجرذان المصابة بداء السكري بثلاث جرعات مختلفة من مستخلص أوراق الفراولة، تحسنت جميع الدلائل البيوكيميائية للدماغ والبالزما بشكل معنوي. وأظهرت الدراسة فائدة استخدام مستخلص أوراق الفراولة كمضاد للأكسدة ومضاد للالتهابات ومكافحة الموت المبرمج لتحسين تلف المخ الذي يسببه مرض السكري.

إن تلف الدماغ هو واحد من مضاعفات مرض البول السكري. السبب الرئيسي لهذا الضرر هو الإفراط في إنتاج الجذور(الشوارد) الحرة الناتجة عن ارتفاع سكر الدم. تهدف الدراسة إلى اختبار تأثير تناول مستخلص أوراق الفراولة كمضاد للأكسدة لتقليل من تلف الدماغ في ذكور الجرذان المصابة بداء السكري. تم تقسيم خمس مجموعات من الجرذان (N = 30) إلى مجموعة تحكم الجرذان، ومجموعة الجرذان المصابة بداء السكري، وثلاث مجموعات من الجرذان المصابة بداء السكري والتي عولجت بمستخلص أوراق الفراولة (50 و 100 و 200 ملجم / كجم من وزن الجسم) لمدة 30 يومًا، على التوالي. أظهرت الدراسة ان في أدمغة الجرذان المصابة بالسكري قد حدثت زيادة