# Prevention and treatment of brain damage in streptozotocin induced diabetic rats with Metformin, *Nigella sativa, Zingiber officinale*, and *Punica granatum*

**Sibghatullah Muhammad Ali Sangi[1,*], Nora Abdulaziz Al Jalaud[2]**

**ABSTRACT**

**Introduction**: Diabetes mellitus (DM) is well-known metabolic disorder, which causes serious effects on human health with its complications. Many mechanisms has been postulated to cause DM related complications. One of the main complications is neuronal damage, for which no proper preventive and curative therapies are available. **Methods**: In this study the effects of Ginger, *Nigella sativa*, *Punica granatum* and Metformin were seen on the prevention and treatment of brain damage caused by diabetes mellitus in streptozotocin (STZ)- induced diabetes in rats. 50 adult Wistar albino male rats were used in the study, the rats were divided in 10 study groups. The body weight, serum glucose levels were measured, and histopathological examination was performed. **Results**: In comparison to the diabetic control rats, significant increase in weight was found in animals of all the studied groups. Serum glucose levels reduced significantly in comparison to the STZ induced diabetic rats in all the animals. Histopathological examination showed prevention from brain damage and repair of the neuronal tissues by Ginger, *Nigella sativa*, *Punica granatum* and Metformin. **Conclusion**: The studied substances were observed to possess preventive and curative effects on the brain damage caused by diabetes mellitus.

**Key words:** : Diabetes mellitus, Brain damage, Ginger, Nigella sativa, Punica granatum, Metformin

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**History**

- Received: **Mar 26, 2019**
- Accepted: **Jun 11, 2019**
- Published: **Jul 05, 2019**

**DOI :**
**https://doi.org/10.15419/bmrat.v6i7.554**



## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that is characterized by an increase in blood glucose (BG) and excretion of glucose in urine. DM is one of the major global health hazards of the recent years and also an emerging threat to public health in Kingdom of Saudi Arabia. The endemic Saudi population appears to possess a special genetic trend to develop Type II DM, that is complicated by an increment in obesity statistics, and the presence of other causes of the insulin resistance [1].

The main pathology associated with Type II DM is insulin resistance, which causes insulin deficiency in the hepatic and peripheral tissues causing hyperglycemia [2,3].

In type1 and type II diabetes mellitus, glucose uptake gets disturbed, especially in muscle and adipose tissues, resulting in hyperglycemia [4]. The neurons start taking up glucose without insulin, therefore, in hyperglycemic diabetes mellitus, hyperglycemia causes an up to four-fold increase in neuronal cell glucose uptake. Increased cytosolic glucose concentration and metabolism leads to neuron damage [2,5].

In previous human and animal studies, DM has been found to be associated with pathological changes in the central nervous system, causing cognitive function decline, behavior disorders and an increased risk of vascular abnormalities in the brain [2,6,7].

It has been suggested that long-term DM augments the risk of brain shrinkage, lacunar infarcts and white matter (WM) abrasions. The functional and behavioral consequences of diabetic brain abnormalities include cognitive decline and movement disorders. Many structural brain changes have been described such as increased hippocampal astrocyte reactivity, abnormal synaptic plasticity, vascular alterations, decreased dendritic complexity and disturbed neurotransmission [8–10].

In the pathogenesis of diabetes related brain damage, disturbed coupled electron transport systems are caused by damaged mitochondria, which is the main foci for reactive oxygen species (ROS) in the neurons. Therefore, oxidative stress is recognized as a main mediating process in the pathogenesis of diabetic complications, due to increased production of free radicals and abnormal antioxidant defenses [11,12].

Many of herbal substances have been found to possess antioxidants and protect brain from damages. *Nigella*

**Cite this article :** Ali Sangi S M, Al Jalaud N A. **Prevention and treatment of brain damage in streptozo-tocin induced diabetic rats with Metformin, *Nigella sativa, Zingiber officinale*, and *Punica granatum***. *Biomed. Res. Ther.; 7*(6):3274-3285.

*Sativa* is known to possess antioxidants and anti-hyperglycemic effects and prevents neuronal damage [13,14].

Because of the chronic symptoms, the development of new treatment strategies is requiredto improve the effectiveness in treatments [10].

Ginger has also been found to possess anti-oxidative properties and has been shown to perfrom neuroprotection in diabetic rats and protective and therapeutic effect on Alzheimer's disease [15–17].

Pomegranate or *Punica granatum,* is a small tree or shrub found in the Mediterranean region [18]. It possess several biological effects such as antitumor and antibacterial functions, which have been reported with the extracts from different parts of *P. Granatum* [19,20].

It has been shown that *Punica granatum* can alleviate brain oxidative stress in diabetic rats via the regulation of antioxidant defense mechanisms, which can ameliorate the abnormality in learning and memory performances in diabetic rats [21,22].

Metformin is a standard antidiabetic drug, belonging to the Biguanide group, which was shown to attenuate stroke-induced nitrative signaling in GK rats [23]. Previous studies showed that metformin can significantly reduce Neuro-inflammation, decrease the loss of neurons in the hippocampus of diabetic animals, and prevent diabetes-induced memory loss in rats [24,25].

The main objective of this study was to ascertain the prophylactic and therapeutic potential of *Zingeber officinale*, *Nigella sativa*, *Punica granatum*, and Metformin in diabetes mellitus associated brain damage and to discover cost effective treatment of the ailment.

## MATERIALS AND METHODS

### Plant materials

Seeds of *Nigella sativa*, Ginger (*Zingiber officinale*), and *Punica granatum* were acquired from the regional market.

### Preparation of Ginger ( *Zingiber Officinale* ) Extracts

Fresh ginger root was purchased from the local market in Rafha Municipal Council, Northern Border Region of Saudi Arabia. The roots were identified and authenticated by the Department of Pharmacognosy, in the Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia.

Preparation was done according to the modified method used by (Kebe *et al*., 2015) [26]. Cleaning of 2.5 kg of fresh ginger rhizome was done with running tap water. It was shredded into small pieces and air dried for 2 weeks, then crushed into powder form with electric blender. 2000 g of this powdered *Zingiber officinale* was soaked in 5000 ml of 99.9% ethanol and rattled vigorously. It was left for 48 hours at room temperature and was stirred at different times. The dissolved ginger in ethanol was filtered with a mesh with small pores after 48 hours. Then, it was filtered using No1 Whatmann paper (filter paper) and funnel. The percolate was assembled in a tray and was air dried for 5 days to ensure complete evaporation of the ethanol used. The ginger paste was collected from the tray with a spatula into a container and was weighed using an electric scale. 50g of ginger paste was collected and then dissolved in extra virgin olive oil 100ml (to serve as a vehicle). The extract was then kept in a dry place at 37°C.

### Preparation of Black Seed (*Nigella sativa)* Extract

This method was adopted from Shahraki *et al.* [27]. To obtain a hydroalcholic extract, powder was made from 100 g of dried *N. sativa* seeds. After that, it was macerated in a solution of 70% alcohol and 30% DW for 72 hours. To prepare the fractions, 10 g of the extract was mixed with 100 ml of ethanol and decanted by funnel. The n-hexane solvent was added to the funnel, and the n-hexane fraction was then extracted. Then, the remaining solvent in the decanter funnel was mixed with dichloromethane solvent, followed by extraction of the dichloromethane fraction. Finally, the remaining solvent from the previous steps was mixed with ethyl acetate, and the ethyl acetate fraction was taken out. The total extract, n-hexane and ethyl acetate fractions were prepared after the removal of the solvent.

### Preparation of *Punica granatum* peels extract

The preparation was performed as done by Anibal *et al.* [28]. Fresh fruit, separated into skin, coverings and seeds, pericarp, and the whole fruit was submitted to ethanolic extraction (70% ethanol) at 37°C by maceration. The extract was filtered, the solvents was eliminated under vacuum and lyophilized to get the crude extract. Crude extracts from all parts of the fruit were monitored by Thin Layer Chromatography.

### Animals

50 adults Wistar albino male rats, of 8 weeks old and weighing 250±10g, were obtained from the animal facility of faculty of pharmacy, Northern Border University. The experimental animals were kept in temperature controlled rooms (25°C), with uniform humidity (40–70%) and 12h/light-12h/dark cycle before

the experiment. All the animals were treated in consensus with the Principles of Laboratory Animal Care. The research protocol approval was taken from Deanship of Scientific Research at Northern Border University, in conformity with the guidelines for the care and use of experimental animals.

All rats were fed a proper feed and aqua. The daily intake of animal water was checked once a week before beginning of treatments to determine the amount of water needed per experimental rat.

### Induction of diabetes with STZ

DM was induced by a single intra-peritoneal injection of STZ, (Sigma-Aldrich, St Louis, MO, USA) in 0.1 M citrate buffer (pH 4.0), 55 mg/kg body weight[29]. Serum glucose concentration and alterations in body weight were checked regularly. Male Wistar rats were divided into ten groups, each group comprised of five rats as follows:

**G1**: Control rats were given only 5cc saline (0.9% NaCl).

**G2**: Control rats were given *Zingeber officianal*e (ginger) (100mg/kg/rat) daily).

**G3**: Control rats were given *Nigella sativa* (Black seed) (80 mg/kg).

**G4**: Rats were given *Punica granatum* (Pomegranate) 400 mg/kg/day.

**G5**: Rats were given Metformin 150 mg/kg/day

**G6**: Diabetic control (55 mg/kg, single I/p injection of STZ).

**G7**: Diabetic group (55 mg/kg, single I/p injection of STZ) received 100mg/kg/day ginger.

**G8**: Diabetic group (55 mg/kg, single I/p injection of STZ) received *Nigella sativa* 80 mg/kg/day.

**G9**: Diabetic group (55 mg/kg, single I/p injection of STZ) received (Pomegranate) 400 mg/rat/day.

**G10**: Diabetic group (55 mg/kg, single I/p injection of STZ) received Metformin 150 mg/kg/day.

### Histological examination

Anesthetized rats were perfused trans-cardially with normal saline and 4% paraformaldehyde in phosphate-buffered solution. The brains of the sacrificed animals were removed immediately and post fixed in the same fixative at 4 °C, until being sectioned on a cryostat (Leica, Germany). Coronal brain sections of 10 $\mu$m measurement were obtained and stored at -20 °C until used.

Assessment of neuronal damage in the cortex was done with Nissl staining. Incubation of the brain sections was done with a 5% toluidine blue solution at room temperature for fifteen minutes. The brain sections were dehydrated and mounted following rinses water.

The axons and neutrophil, morphology and integrity were assessed with Bielschowsky's sliver (BS) staining.

### Statistical Analysis

To analyze the data, Statistical Package for Social Science (SPSS) version 20 was applied. The data were expressed as means +/- standard deviation (SD). Comparison of variables between groups were performed using One Way ANOVA test (LSD). Statistical significance was considered at $P$-value $\leq 0.05$.

## RESULTS

The body weight in G4 significantly decreased compared to G1, G2, and G3 on the 1$^{st}$ day(142.83±16.36 versus 182.83±16.81, 176.50±20.34 and 174.50±5.68; P =0.001 for all groups), day 15$^{th}$ (151.17±15.47 versus 183.00±21.23, 183.33±19.97 and 177.33±5.65; P =0.005, P =0.004 and P =0.019, respectively) and on 28$^{th}$ day (158.33±15.24 versus 190.00±20.36, 195.50±16.53 and 183.83±9.62; P =0.004, P =0.001 and P =0.018, respectively). Body weight in G5 significantly increased compared to G1, G2, G3 and G4 at 1$^{st}$(205.33±6.98 versus 182.83±16.81, 176.50±20.34 174.50±5.68 and 142.83±16.36; P =0.011, P =0.001, P =0.001 and P =0.001, respectively), on day 15$^{th}$ (209.00±12.82 versus 183.00±21.23, 183.33±19.97, 177.33±5.65 and 151.17±15.47; P =0.020, P =0.021, P =0.005 and P =0.001, respectively) and on 28$^{th}$ day (218.00±16.46 versus 190.00±20.36, 195.50±16.53, 183.83±9.62 and 158.33±15.24; P =0.010, P =0.035, P =0.002 and P =0.001, respectively). In G6, on 1$^{st}$ day, body weight significantly increased compared to G4 (185.80±11.41 versus 142.83±16.36; P =0.001), but significantly decreased compared to G5 (185.80±11.41 versus 205.33±6.98; P =0.035). While on 15$^{th}$ day, BW significantly decreased compared to G5 (166.60±15.53 versus 209.00±12.82, P =0.001); on 28$^{th}$ day, BW significantly decreased compared to G1, G2, G3 and G5 (142.5±8.54 versus 190.00±20.36, 195.50±16.53, 183.83±9.62 and 218.00±16.46; P = 0.001 for all).

In G7, on 1$^{st}$ day, BW significantly decreased compared to G1, G2, G3, G5 and G6 (148.50±9.03 versus 182.83±16.81, 176.50±20.34, 174.50±5.68, 205.33±6.98, 185.80±11.41; P= 0.001. P =0.00. P =0.004, P =0.001 and P =0.001) at 15$^{th}$ day, BW significantly increased compared to G4 (181.67±9.07 versus 151.17±15.47; P =0.007) but significantly

decreased compared to G5 (181.67±9.07 versus 209.00±12.82; P =0.014); on $28^{th}$ day, BW significantly increased compared to G4 and G6 (158.33±15.24 and 194.83±8.08 versus 142.5±8.54; P =0.001 for both) but significantly decreased compared to G5 (194.83±8.08 versus 218.00±16.46; P =0.030). In G8, on $1^{st}$ day BW significantly increased than G4 and G7 (185.50±8.89 versus 142.83±16.36 and 148.50±9.03; P =0.001 for both) but significantly decreased than G5 (185.50±8.89 versus 205.33±6.98; P =0.025); on $15^{th}$ day, BW significantly increased than G1-7 (247.33±31.19 versus 183.00±21.23, 183.33±19.97, 177.33±5.65, 151.17±15.47, 209.00±12.82, 166.60±15.53, 181.67±9.07; P =0.001 for all); on $28^{th}$ day, BW significantly increased than G1, G2, G3, G4, G5, G6, and G7 (263.00±33.93 versus 190.00±20.36, 195.50±16.53, 183.83±9.62, 158.33±15.24, 218.00±16.46, 142.5±8.54, 194.83±8.08; P =0.001 for all).

In G9, on $1^{st}$ day, BW significantly decreased compared to G1, G5, G6 and G8 (160.50±28.31 versus 182.83±16.81, 205.33±6.98, 185.80±11.41, 185.50±8.89; P = 0.012, P =0.001, P =0.007 and P =0.005); on $15^{th}$ day, BW significantly increased than G4 (175.83±28.58 versus 151.17±15.47; P =0.026) but significantly decreased than G5 (175.83±28.58 versus 209.00±12.82; P =0.003); on 28 days, BW significantly increased than G4, G6 (195.33±25.56 versus 158.33±15.24 and 142.5±8.54; P =0.001 for both) but significantly decreased than G5 and G8 (195.33±25.56 versus 218.00±16.46 and 263.00±33.93; P =0.034 and P =0.001). In G10, BW increased than G4 (173.50±6.80 versus 142.83±16.36; P =0.001) but significantly decreased than G5and G7 173.50±6.80 versus 205.33±6.98 and 148.50±9.03; P =0.001 and P =0.005); on $15^{th}$ day, BW significantly increased than G4 (179.83±7.83 versus 151.17±15.47; P =0.010) but significantly decreased than G5 and G8 (179.83±7.83 versus 209.00±12.82 and 247.33±31.19; P =0.009 and P =0.001); on $28^{th}$ day, BW significantly increased than G4 and G6 (186.00±8.60 versus 158.33±15.24 and 142.5±8.54; P =0.011 and P =0.001) but significantly decreased than G5 and G8 (186.00±8.60 versus 218.00±16.46 and 263.00±33.93; P =0.003 and P =0.001)**Figures 1, 2 and 3**.

Blood glucose levels in G6 significantly increased compared to G1, G2, G3, G4 and G5 on $1^{st}$ (234.80±13.03 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33; P =0.001 for all), on $15^{th}$ (237.20±12.72 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03 and 122.67±9.37; P =0.001 for all) and on $28^{th}$ day (227.00±8.37 versus 125.17±3.76, 123.67±6.15, 122.67±2.80, 125.83±8.64 and 124.33±5.47; P =0.001 for all). Blood glucose levels in G7 significantly increased compared to G1, G2, G3, G4 and G5 but significantly decreased compared to G6 on $1^{st}$ (195.83±10.03 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33; P =0.001 for all and 195.83±10.03 versus 234.80±13.03; P =0.001), on $15^{th}$ day (157.00±8.00 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03 and 122.67±9.37 and 157.00±8.00 versus 237.20±12.72; P =0.001) and on $28^{th}$ day (155.67±7.03 versus 125.17±3.76, 123.67±6.15, 122.67±2.80, 125.83±8.64 and 124.33±5.47; P =0.001 for all; and 155.67±7.03 versus 227.00±8.37, P =0.001).

Blood glucose levels in G8 significantly increased compared to G1, G2, G3, G4 and G5 but significantly decreased compared to G6 on $1^{st}$day (190.67±5.13 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33; P =0.001 for all and 190.67±5.13 versus 234.80±13.03; P =0.001), on $15^{th}$ day (156.40±6.88 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03 and 122.67±9.37 and 156.40±6.88 versus 237.20±12.72; P =0.001) and on $28^{th}$ day 143.00±4.00 versus 125.17±3.76, 123.67±6.15, 122.67±2.80, 125.83±8.64 and 124.33±5.47; P =0.001 for all; and 143.00±4.00 versus 227.00±8.37, P =0.001) and significantly decreased compared to G7 on $28^{th}$ day (143.00±4.00 versus 155.67±7.03; P =0.009).

Blood glucose levels in G9, on $1^{st}$ day significantly increased compared to G1, G2, G3, G4, G5 and G8 (208.67±18.93 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33, 190.67±5.13; P =0.001, P =0.001, P =0.001, P =0.001, P =0.001 and P =0.008, respectively) but significantly decreased compared to G6 (208.67±18.93 versus 234.80±13.03; P =0.001), on $15^{th}$ day (174.83±12.84 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03, 122.67±9.37 and 156.40±6.88; P =0.001 for all) but significantly decreased compared to G6 (174.83±12.84 versus 237.20±12.72; P =0.001) and $28^{th}$ days blood glucose levels significantly increased compared to G1, G2, G3, G4 and G5 (144.67±4.97 versus 125.17±3.76, 123.67±6.15, 122.67±2.80, 125.83±8.64 and 124.33±5.47; P =0.001 for all) but significantly decreased compared to G6 and G7 (144.67±4.97 versus 227.00±8.37 and 155.67±7.03; P =0.001 and P =0.017).

Blood glucose levels in G10, on $1^{st}$ day significantly increased when compared to G1, G2,

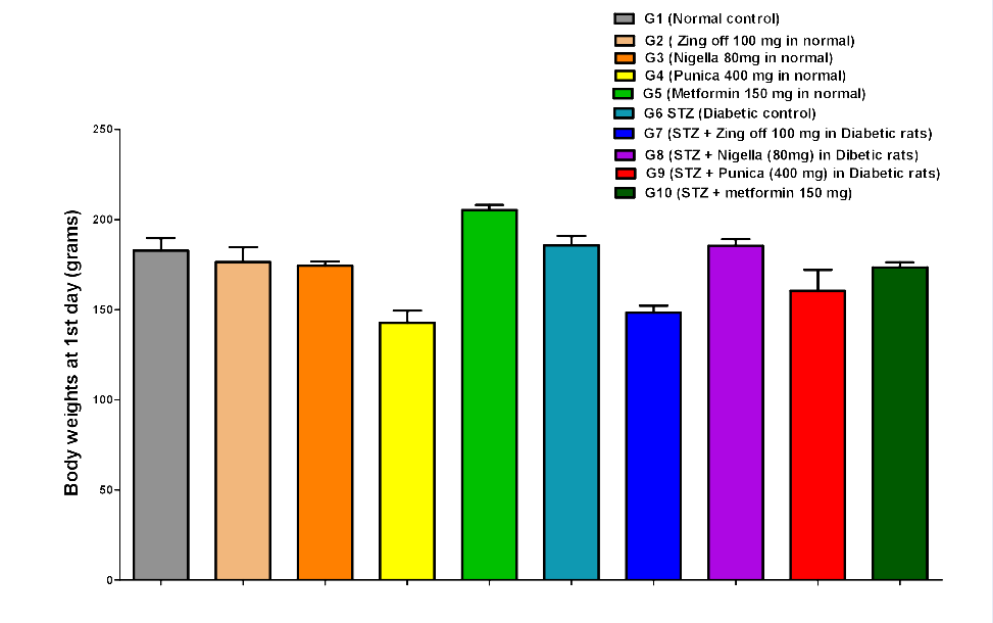


**Figure 1**: **Comparison of the body weight (grams) in different studied groups at 1$^{st}$ day**. Data are expressed as mean ± standard deviation. The body weight in G4 significantly decreased compared to G1, G2, and G3 on 1$^{st}$ (142.83±16.36 versus 182.83±16.81, 176.50±20.34 and 174.50±5.68; P =0.001 for all the studied groups. Using one-way analysis of variance test (least significant difference).

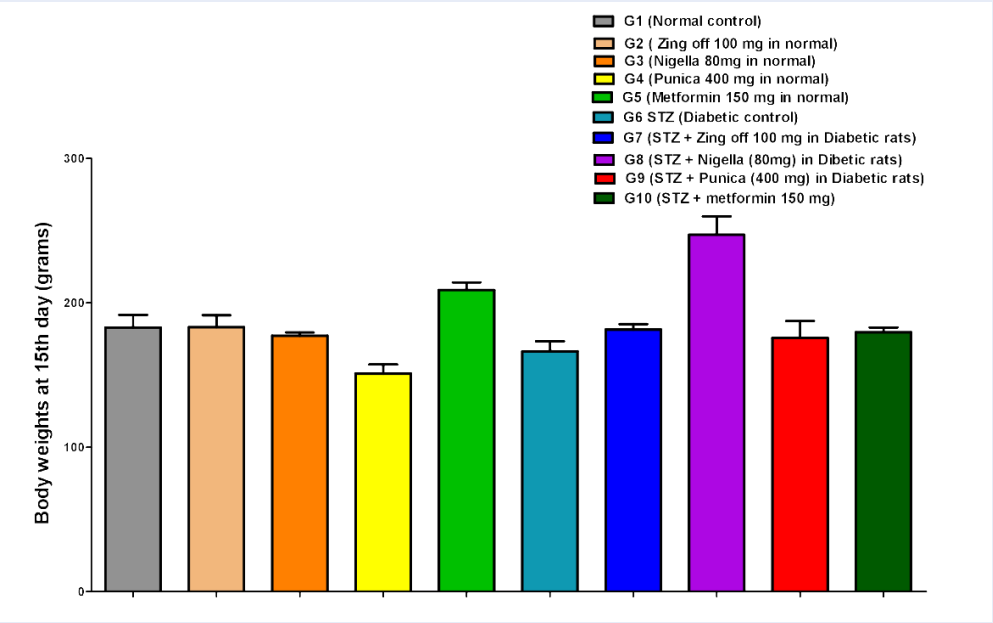


**Figure 2**: **Comparison of the body weight (grams) in different studied groups at 15$^{th}$ day**. Data are expressed as mean ± standard deviation.The body weight in G4 significantly decreased compared to G1, G2, and G3 on day 15$^{th}$(151.17±15.47 versus 183.00±21.23, 183.33±19.97 and 177.33±5.65; P =0.005, P=0.004 and P =0.019, respectively). Using one-way analysis of variance test (least significant difference).

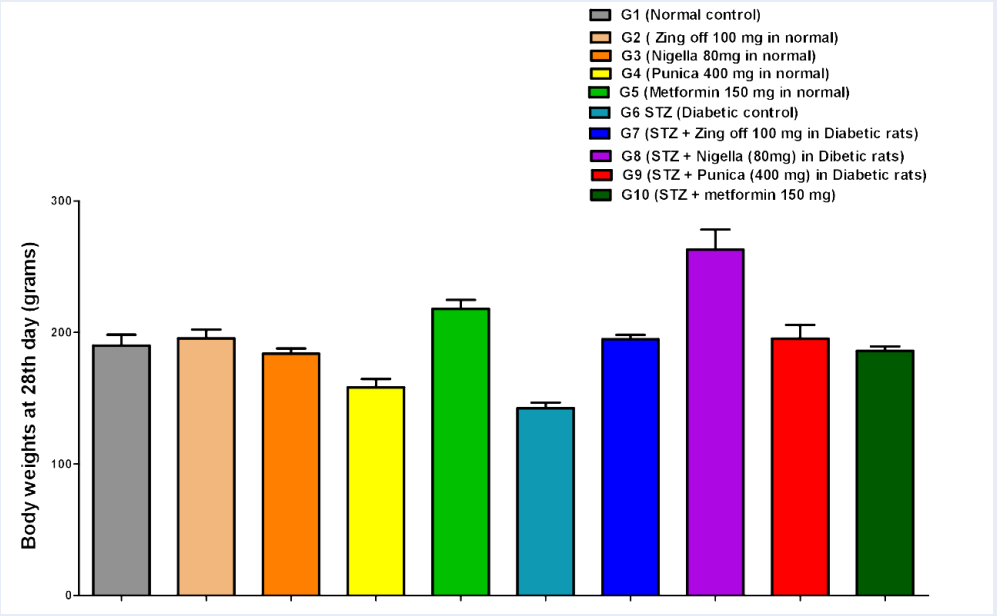


**Figure 3**: **Comparison of the body weight (grams) in different studied groups at 28$^{th}$ day**. Data are expressed as mean ± standard deviation. In G6, on 28$^{th}$ day, BW significantly decreased compared to G1, G2, G3 and G5 (142.5±8.54 versus 190.00±20.36, 195.50±16.53, 183.83±9.62 and 218.00±16.46; P = 0.001 for all). Using one-way analysis of variance test (least significant difference).

G3, G4, G5, G7 and G8 (209.17±10.26 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33, 195.83±10.03 and 190.67±5.13; P = 0.001, P =0.001, P =0.001, P =0.001, P =0.001, P =0.046 and P =0.006) but significantly decreased when compared to G6 (209.17±10.26 versus 234.80±13.03; P = 0.001); on 15$^{th}$ day, blood glucose levels significantly increased compared to G1, G2, G3, G4 and G5 (158.00±10.37 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03, 122.67±9.37; P =0.001 for all) but significantly decreased compared to G6 and G9 (158.00±10.37 versus; 237.20±12.72 and 174.83±12.84; P =0.001 for all); on 28$^{th}$ day, blood glucose levels significantly decreased compared to G6, G7, G8 and G9 (131.17±16.30 versus 227.00±8.37, 155.67±7.03, 143.00±4.00, 144.67±4.97; P= 0.001, P =0.001, P =0.015 and P =0.004, respectively)**Figures 4, 5 and 6**.

The control group showed that most vital neurons (cortical, hippocampal and cerebellar Purkinje cells) have active large vesicular lightly stained nuclei figure 7. STZ induced Type 2 diabetic rat showed an increase in dark degenerated neurons compared to cells with highly active stained nuclei, the cortex and striatum of the diabetic animals were characterized by demyelination and axonal degradation (**Figures 7 and 8**).

In all the treated groups, brain tissue sections show cerebral cortex with distinct gray and white matter areas. Cortex shows normal neuronal cells with scattered glial cells and neutrophils in background (**Figure 9**).

## DISCUSSION

Diabetes mellitus type II is a common prevailing metabolic disorder emerging as global health hazard and it is associated with various complications such as micro-angiopathy, nephropathy, retinopathy, and neuropathy. Dating back to 1922, it has been noticed that diabetes mellitus may even lead to central nervous system disorder[30].

Long-standing hyperglycemia DM affects the brain and manifests anatomical, structural, neurophysiological, and neuropsychological changes. Various pathophysiological factors are found to be involved in the development of the cerebral dysfunction in diabetes mellitus, such as the hypoglycemic bouts, cerebrovascular changes, insulin's role in the brain and associated structures, and the mechanisms of hyperglycemia induced alterations[31].

Diabetes mellitus type II is known to cause a decrease in different areas of cognitive functioning. The risk of cognitive dysfunction is higher for the patients, who suffer from diabetes mellitus, prediabetes and from

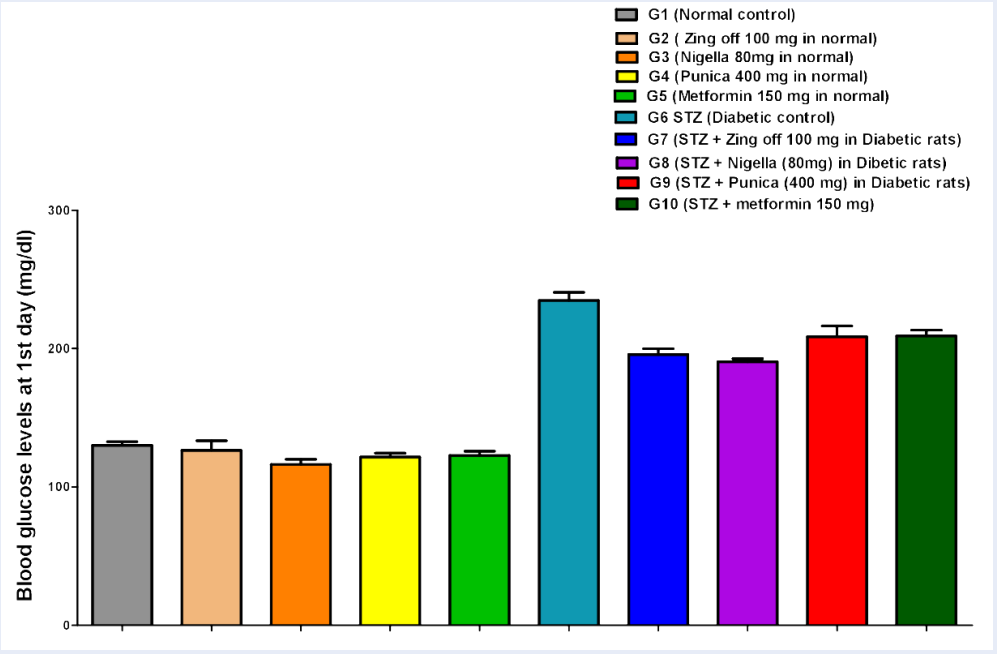


**Figure 4**: **Comparison of the blood glucose (mg/dl) in different studied groups at 1$^{st}$ day.** Data are expressed as mean ± standard deviation. Blood glucose levels in G7 significantly remained increased than G1, G2, G3, G4 and G5 but were significantly decreased than G6 at 1$^{st}$ (195.83±10.03 versus 130.17±6.46, 126.50±17.00, 116.33±9.05, 121.67±6.95, 122.83±7.33; P=0.001 for all and 195.83±10.03 versus 234.80±13.03; P =0.001). Using one-way analysis of variance test (least significant difference).

metabolic syndrome, characterized by dyslipidemia, central abdominal obesity and hyperglycemia [32,33].

Hyperglycemia decreases antioxidant levels and at the same time, causes an increase in the production of free radicals. These effects add to the deleterious effects on tissues, facilitating the complications/tissue damage in DM, leading to changes in the redox potential of the cells with consequent activation of redox-sensitive genes [34].

Neurons are especially sensitive to oxidative stress, and because of that, reactive oxygen species (ROS) cause several neural degenerative processes in diabetes [35–37].

In the current study, it was discovered that long-standing hyperglycemia in rats causes significant damage in different areas of the brain. Treatment with different substances containing antioxidants prevented the harmful effects of diabetes mellitus on neurological tissues.

In the previous studies [38–40], it has been found that metformin prevents the brain damage by reducing oxidative stress. The results of our study are in line with these studies, and significant prevention of the neuronal tissue damage was found in all areas of the brain of the animals.

Studies conducted by [41–43] revealed that *Nigella sativa,* which is rich in antioxidants, reduces neuro-inflammatory damage and improves cognitive functions. The results in this study are in conformity with the previous studies regarding these findings.

The previous research suggests that ginger shows a neuroprotective effect by speeding up the processes of brain antioxidant defense and down-regulating the *N*-MDA levels to the normal range in diabetic rats [44–46].

The current research results also verify the observations found in previous studies regarding the decrease, prevention and improvement by Ginger in the damage caused by DM in the brain.

In previous studies, Pomegranate (*Punica granatum*), has been found to be effective in reducing oxidative stress and damage caused by oxidative stress. It has also been found effective in reducing the blood glucose levels [47–49]. In the current study, it was found to possess neuro-protective effects. The results of the current study show promising preventive and repairing effects of brain damage caused by DM.

## CONCLUSION

DM is notorious to cause irreversible complications including peripheral neuropathy and brain damage, leading to the early occurrence of dementia

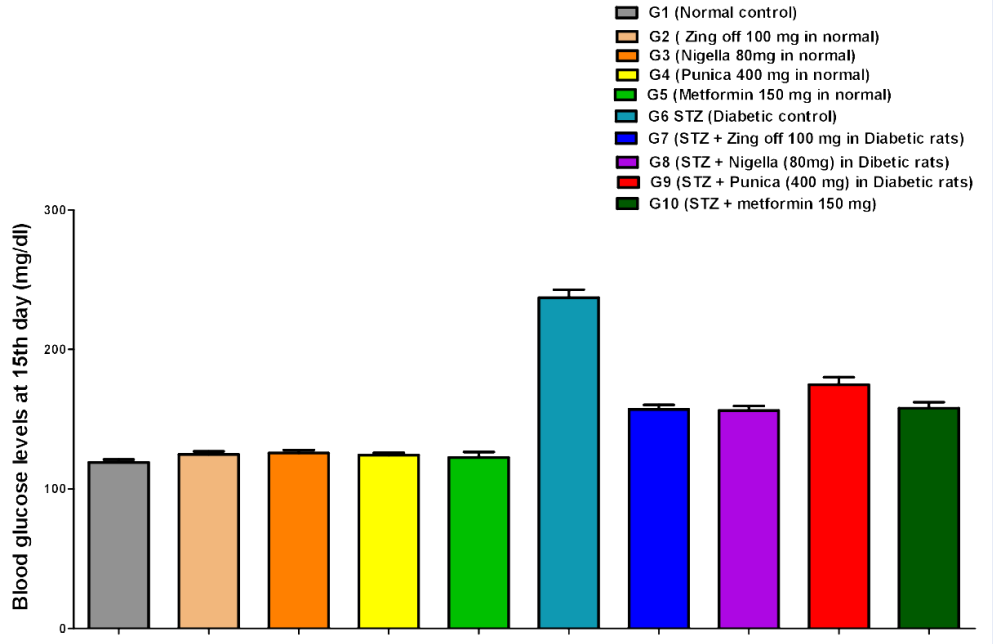


**Figure 5**: **Comparison of the blood glucose (mg/dl) in different studied groups at15$^{th}$ day**. Data are expressed as mean± standard deviation. Blood glucose levels in G8 were significantly increased than G1, G2, G3, G4 and G5 but were significantly decreased than G6 on 15$^{th}$day (156.40±6.88 versus 119.17±5.04, 124.83±5.27, 125.83±5.42, 124.33±4.03 and 122.67±9.37 and 156.40±6.88 versus 237.20±12.72; P =0.001). Using one-way analysis of variance test (least significant difference).

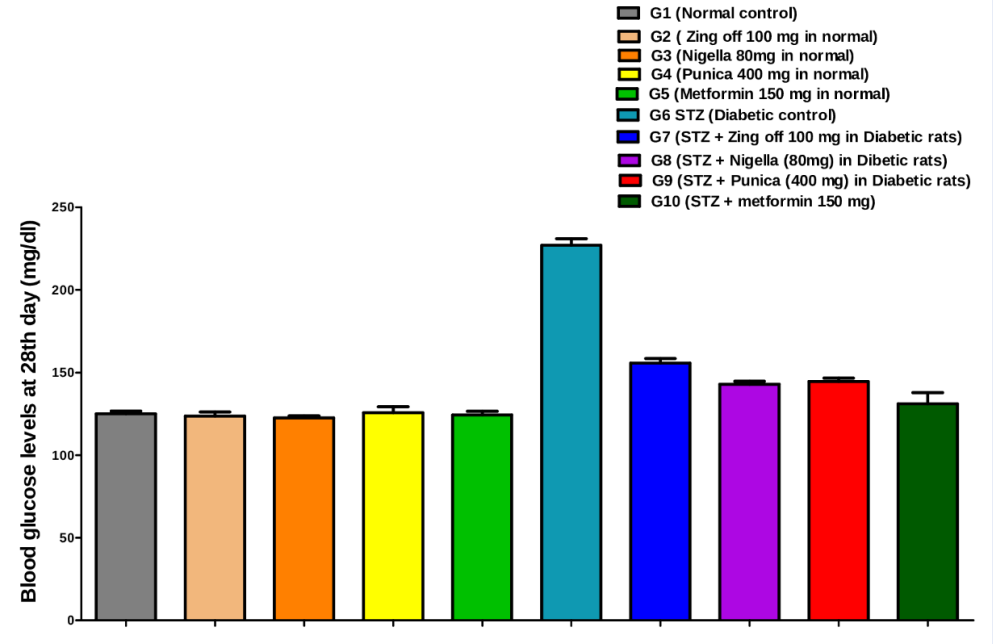


**Figure 6**: **Comparison of the blood glucose (mg/dl) in different studied groups at 28$^{th}$day.** Data are expressed as mean ± standard deviation. Blood glucose levels inG10, on 28$^{th}$ day, significantly decreased than G6, G7, G8 and G9 (131.17±16.30 versus 227.00±8.37, 155.67±7.03, 143.00±4.00, 144.67±4.97; P=0.001, P =0.001, P =0.015 and P =0.004, respectively). Using one-way analysis of variance test (least significant difference).

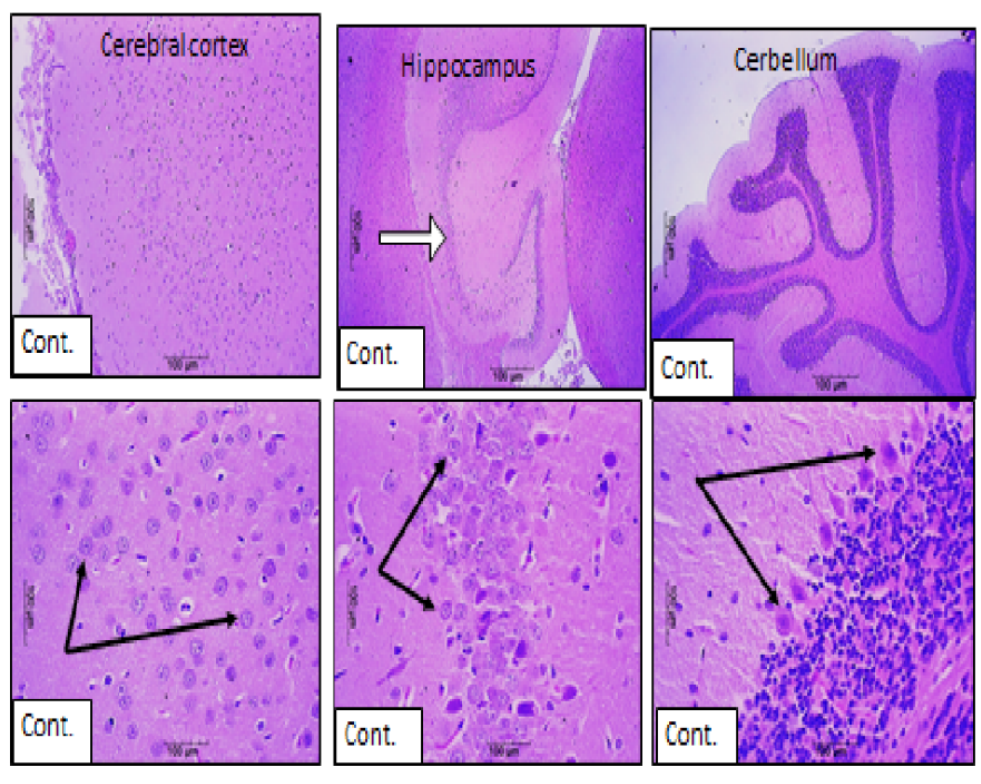


**Figure 7**: **Examination of sections from different regions of control rat brain showing the most vital neurons (cortical, hippocampal and cerebellar Purkinje cells) have active large vesicular lightly stained nuclei (arrows).** H&E stain, Magnification 100&400.

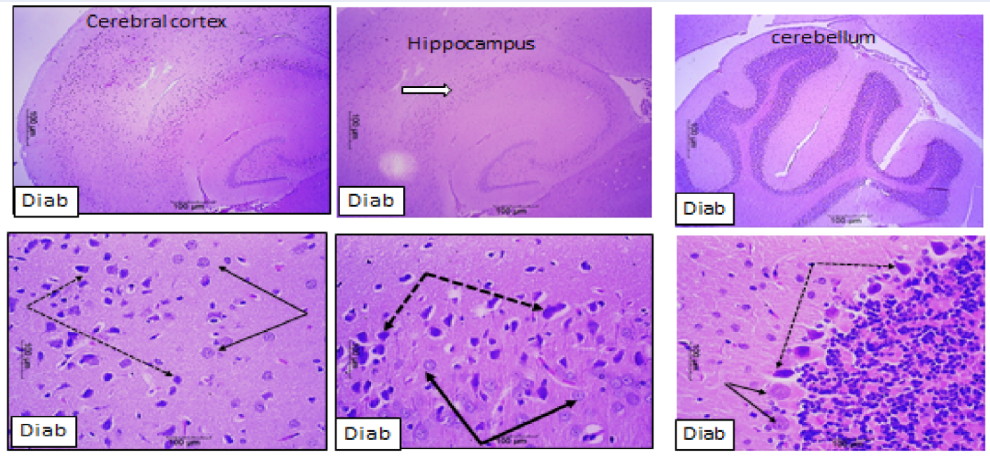


**Figure 8**: **Examination sections from different regions of STZ-type 2 diabetic rat indicates dark degenerated neurons dark degenerated neurons compared to cells with highly active stained nuclei, (black arrows) the cortex and striatum of the diabetic animals are characterized by demyelination and axonal degradation figure (dotted arrows)**. H&E stain magnification 100&400

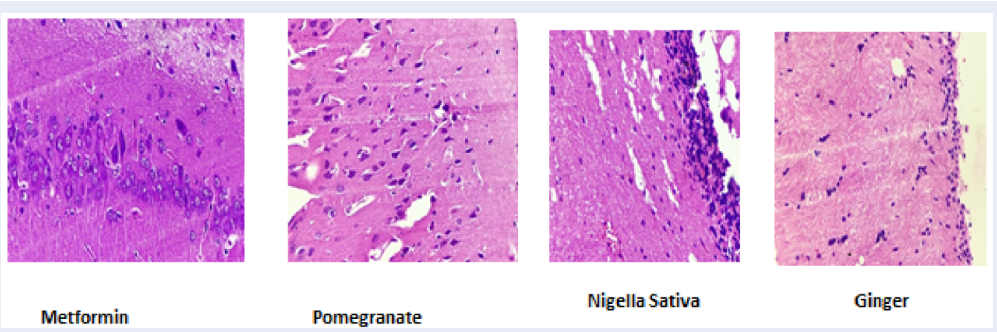


**Figure 9**: **Examination sections of brain after treatment of diabetes in groups with different substances, it shows normal neuronal cells with scattered glial cells and neutrophils in the background**. H&E stain, magnification 100&400

(Alzheimer's disease). The substances used in the study, including Metformin, *Nigella sativa*, Ginger and *Punica granatum*, effectively prevented the brain damage and repaired the damaged neurons. These substances can be used as adjuvant therapy to prevent DM related complications. It is highly suggested that the active ingredients of these substances should be studied for their effects on oxidative stress parameters, and composition to pinpoint exact neuroprotective mechanism.

## COMPETING INTERESTS

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTIONS

Author 1 responsible for research /experimental design, histopathological study and interpretation of results. Author 2 responsible for literature review, compilation of results.

## ACKNOWLEDGMENTS

The author wishes to acknowledge the approval and the support of this research study by the grant No (7267-PHM-2017-1-8-F) from the Deanship of Scientific Research in Northern Border University (N.B.U.), Arar, KSA.

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