THE EFFECTS OF STREPTOZOTOCIN INDUCED DIABETES ON THE HIPPOCAMPUS OF ADULT MALE ALBINO RATS AND THE POSSIBLE NEUROPROTECTIVE ROLE OF PANAX GINSENG

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ABSTRACT:

Background: *Learning and memory impairment are common consequences of uncontrolled diabetes mellitus (DM). Panax ginseng is one of the most famous natural herbs used in controlling metabolic disorders as DM.*

Aim of the work: *To study the histological changes of streptozotocin (STZ) induced DM on rat's hippocampus and the possible neuroprotective effect of panax ginseng.*

Material and methods: *Thirty-six adult male albino rats, aging 4-6 months and weighing 200-250 gm, were used in this study. Animals were divided into four groups.* ***Group I:*** *included 18 rats and was equally subdivided into three subgroups; 6 rats each.* ***Group II:*** *included 6 rats which received panax ginseng by gastric gavage daily.* ***Group III:*** *included 6 rats which received single intraperitoneal injection of STZ to induce DM.* ***Group IV:*** *included 6 rats in which DM was induced then rats were given panax ginseng daily. After 2 weeks, animals were sacrificed, and brains were dissected out. Paraffin blocks of hippocampus were prepared, and some sections were stained with hematoxylin and eosin and cresyl violet while others were immunohistochemically treated to detect GFAP and synaptophysin. Statistical analysis was done for some measured parameters.*

Results: *The present study revealed that STZ induced DM led to marked structural alteration of the rat's hippocampus. Diabetic rats treated with panax ginseng revealed evident improvement of these changes both histologically and immunohistochemically.*

Conclusion: *Panax ginseng exerted protective role against histoarchitectural and immunoreactivity changes in the hippocampus of diabetic rats. Thus, it could be a promising neuroprotective agent against DM induced memory impairment.*

Keywords*: Diabetes, Ginseng, Hippocampus, CA1, CA3, Dentate gyrus.*

INTRODUCTION:

Diabetes mellitus (DM) is one of the commonest serious chronic diseases among humans [1,2]. Its prevalence is increasing, where 422 million worldwide were living suffering from DM in 2014[3].

Unfortunately, several systemic secondary complications are associated with DM, such as atherosclerosis, nephropathy, neuropathy, and retinopathy [4]. Regarding neuropathy, most studies were concerned with the diabetic disorders of the peripheral nervous system however, relatively fewer

ones focused on the effects on the central nervous system (CNS) [5].

The hippocampus has a vital role in adaptive, reproductive, and emotional behaviors, as well as memory formation. It is also one of the most vulnerable parts of the CNS to metabolic disorders including DM [6]. It sends memories to suitable areas in the cerebral hemispheres to be stored as long-term memory and recalled once needed [7].

Memory and learning impairment during middle age and dementia and Alzheimer's disease (AD) in elderly are proved to be serious consequences of uncontrolled DM [8,9]. Thus, it is demanding to find an inexpensive and reliable way to avoid or delay the advancement of symptoms of such complications. However, the current anti-diabetic drugs have several side effects and may be faced with insulin resistance or hypoglycemic conditions [10,11].

Natural substances may represent alternative treatment for DM and a promising remedy for AD due to their easy availability, efficacy, and fewer adverse effects, as well as their antiapoptotic, anti-inflammatory and antioxidant role [11,12].

Panax ginseng was proved to play a significant role among all the other natural agents applied in human healthcare [13,14].

It is a traditional Chinese medication that has been extensively used for thousand years [15]. In recent decades, panax ginseng has drawn the attention of numerous researchers due to its anti-inflammatory, anti-oxidative, and anti-stress effects [16]. It has a contributary role in controlling many disorders of cardiovascular diseases [17], acute menopausal symptoms [18], and acute pancreatitis [19]. Moreover, it has been used as immune activity stimulator [20].

Also, panax ginseng has become one of the commonest herbs used in metabolic disorders including DM [21]. It has been reported that panax ginseng decreased the level of fasting blood glucose in diabetic cases. Moreover, it improved glucose intake, attenuated insulin resistance and reduced the mass of fat in obese mice with high fat diet [22]. However, clinical trials are needed to be conducted to ensure the safety as well as the efficacy of panax ginseng introduction among various subjects [23].

By reviewing the literature and, up to our knowledge, few studies were concerned with studying the impact of panax ginseng on the hippocampal structure of rats with STZ-induced diabetes.

AIM OF THE WORK:

Thus, the aim of the current work was to focus on the histopathological changes affecting the hippocampus of diabetic male albino rats, and to study the potential neuroprotective role of panax ginseng against these changes.

MATERIAL AND METHODS:

Drugs:

1. Streptozotocin (STZ): it was purchased from Sigma (St. Louis, Mo, USA) in the form of powder as 1 gm vial. Each rat was given a single dose of 55 mg/kg body weight freshly dissolved in 1 ml citrate buffer (pH 4.5) via intraperitoneal injection. Streptozotocin-induced DM in rats is considered as a model of type1 DM [24].

2. Panax ginseng (Trade name Ginsana®): it was purchased in the form of capsules 100 mg (Egyptian Int. Pharma. INO. Co. A.R.E.) under license of pharmaton SA Lugano. It was given by gastric gavage in a dose equivalent to 100 mg/kg body weight dissolved in 1ml distilled water [25].

Animals:

Thirty-six healthy adult (Wistar strain) male albino rats aging from 4-6 months and weighing 200-250 gm were obtained and locally bred at the animal house of the Faculty

of Medicine Ain Shams Research Institute (MASRI). Animals were kept in cages made of stainless steel, 30 x 35 cm, two rats in each cage. Before any intervention, rats were left for 7 days to accommodate to the experimental circumstances. They were exposed to 12 hours dark/light cycles and permitted free access of water and daily diet with good ventilation and suitable environmental conditions.

Ethical Consideration:

Experimental design and protocols were conducted following the guidelines of the Committee of Animal Research Ethics - Faculty of Medicine - Ain Shams University.

Ethical committee approval number: FMASU R49/2023.

Experimental protocol:

At the beginning of the present study, blood sample was taken from each rat via the dorsal vein of the tail to measure the level of blood glucose using Accu-chek Active (Roche Diagnostics - Germany) to ensure that all rats were nondiabetic.

Random division of rats was then done into four groups as follows:

Group I (Control group): included 18 rats that were further equally subdivided into:

- ***Subgroup IA:*** the rats were not subjected to any intervention.
- ***Subgroup IB:*** the rats received a single dose of 1ml citrate buffer which was the vehicle used for STZ via intraperitoneal injection.
- ***Subgroup IC:*** the rats received a daily dose of 1ml distilled water for 2 weeks by gastric gavage which was the vehicle used for panax ginseng.

Group II (Panax ginseng group): included 6 rats where a dose of 100 mg/kg body weight of panax ginseng was given daily for 2 weeks via oral route by gastric gavage [25].

Group III (STZ-induced diabetic group): included 6 rats in which induction of DM was performed. Following 12 hours of fasting, a single dose of 55 mg/kg body weight of STZ was administered via intraperitoneal injection to each rat. Blood samples were obtained via rats' tail veins 48 hours following STZ injection. Rats with blood glucose levels of 250 mg/dl or higher were counted to be diabetic [24].

Group IV (Panax ginseng treated diabetic group): included 6 rats in which DM was induced as in group III. These diabetic rats received a daily dose of panax ginseng via oral route as in subgroup II for two weeks.

Retrieval of the hippocampi:

After 2 weeks, all rats were anaesthetized using thiopental sodium 7mg/kg body weight via intraperitoneal injection, then the chest of each rat was opened by a median incision and perfusion with paraformaldehyde 2% and phosphate buffer of PH 7.4 via the left ventricle was done. Scarification of rats was done by decapitation, followed by immediate careful dissection of the brains after opening the skulls. The brains were kept in 10% formalin for at least ten days. Parasagittal sections were then excised from the brains exposing the hippocampi to be subjected to the following techniques:

Histological techniques:

Tissue samples from the hippocampi of all animals were processed then embedded in paraffin blocks. Cutting of serial five microns thickness parasagittal sections was performed. Some sections were stained with hematoxylin & eosin (Hx and E) [26] and others with cresyl violet to detect the Nissl substance that appeared dark blue in neurons [27].

Immunohistochemical techniques:

To detect the astrocytes' reactivity and distribution, immunohistochemical staining for glial fibrillary acidic protein (GFAP) was

done. Positive reaction was defined as brownish cytoplasmic coloration [28].

To detect the synaptic function, immunohistochemical staining for synaptophysin (SYN) was done. The intensity of brown coloration reflected the degree of abundancy of the synaptophysin protein [29].

All histological and immunohisto-chemical sections were examined, then photographed using Olympus 268M light microscope provided with an automatic photomicrographic camera system.

Morphometric analysis and statistics:

Examination of six different fields, at x400 magnification, from six different stained slides of six different rats in each group was done to measure the mean thickness of the pyramidal cell layer in Cornu Ammonis 1 (CA1) and Cornu Ammonis 3 (CA3) regions as well as the mean thickness of granular cell layer in the dentate gyrus (DG). The distribution of astrocytes was also estimated by measuring the mean area percentage of GFAP immunoreactivity and the synaptic function was assessed by measuring the mean area percentage of SYN immunoreactivity. These parameters were done using Image J software on RGB stacks of the photomicrographs. Threshold adjust-ment has been used in overlapping a binary mask on the areas of immunoreactivity.

Analysis of morphometric data was performed using the statistical package for the social sciences (SPSS) program - version 17 - USA, where mean, standard deviation (SD), analyses of variance (ANOVA test), and T-test were done. The P value < 0.001 indicates high significance, < 0.05 indicates significance and > 0.05 was considered insignificant.

To exclude bias, images were studied by an examiner who did not know the coding of the different study groups.

RESULTS:

Light microscopic examination of group I (control group with its three subgroups IA, IB and IC) and of group II (panax ginseng group) showed nearly similar histological and immunohistochemical results.

Histological results:

I- Hematoxylin and eosin stain:

Examination of group I (control group) sections showed the characteristic curved shape (C-shaped) of the hippocampus. It was composed of the Cornu Ammonis (CA) with its four regions (CA1, CA2, CA3 and CA4) and the DG which appeared as a V or U-shaped structure enclosing CA4 (Fig.1). To standardize, CA1, CA3, in addition to DG were examined and described in all groups.

Both CA1 and CA3 regions were composed of three well-defined layers namely, the polymorphic layer, the pyramidal cell layer and the molecular layer. The polymorphic layer displayed glial cells and blood capillaries, the pyramidal cell layer represented the pyramidal cells whose almost parallel processes (apical dendrites) appeared in the molecular layer which showed also some intervening glial cells and blood capillaries (Figs. 2A and 2B). The pyramidal cells in CA1 were almost rounded, closely packed and arranged in two to three rows with a characteristic palisade appearance (Fig. 2A). In CA3, they appeared larger, almost triangular, more loosely packed and arranged in three to four rows (Fig. 2B). However, pyramidal cells of both regions had pale basophilic cytoplasm enclosing central large vesicular nuclei with prominent nucleoli (Figs. 2A and 2B).

The DG was composed of three layers, molecular layer, granular cell layer, and polymorphic layer. The granular cell layer was formed mainly of aggregations of small rounded to oval granule cells having large vesicular nuclei with a scanty surrounding cytoplasmic rim. Small immature nerve cells with oval deeply stained nuclei were also

seen in the sub-granular zone. Blood capillaries and glial cells were seen in the polymorphic and the molecular layers (Fig. 2C).

Examination of sections of group III (STZ-induced diabetic group) revealed marked disorganization and dispersion of the pyramidal cells in both CA1 and CA3 (Figs. 3A and 3B). CA1 showed loss of the palisade appearance and most of the pyramidal cells appeared shrunken and having deeply stained nuclei with large pericellular spaces (Fig. 3A). In CA3, bizarre shaped pyramidal cells with dark nuclei and deep basophilic cytoplasm in addition to intervening ghost-like cells were seen (Fig. 3B). The molecular layer revealed loss of the parallel arrangement of cell processes. Both polymorphic and molecular layers showed many dilated blood capillaries and glial cells (Figs. 3A and 3B) with some ghost-like cells in the molecular as well as the polymorphic layers of CA3 (Fig. 3B).

The DG showed marked distorted architecture. The granule cells appeared shrunken and having pyknotic nuclei with large pericellular spaces. Widespread glial cells were observed. Areas of vacuolations were frequently detected in the sub-granular zone (Fig.3C).

Examination of sections of group IV (panax ginseng treated diabetic group); showed almost preserved structure of the three layers in both CA1 and CA3 regions. Most of pyramidal cells had pale basophilic cytoplasm surrounding large vesicular nuclei with prominent nucleoli. However, few irregular cells with dark and shrunken nuclei were observed. Blood capillaries and glial cells were dispersed in both the polymorphic and molecular layers (Figs. 4A and 4B). Preservation of the parallel arrangement of pyramidal cell processes was noted in the molecular layer (Fig. 4A).

The DG showed almost preserved architecture. Most granule cells were rounded with vesicular nuclei with a scanty cytoplasmic rim. However, few distorted cells with darkly stained nuclei were seen intermingled among them. Areas of vacuolation in the sub-granular zone in addition to some blood capillaries were also observed (Fig. 4C).

II- Cresyl violet stain:

Examination of sections of group I (control group) revealed regularly arranged cells with rounded vesicular nuclei surrounded by darkly stained cytoplasmic rim representing Nissl substance. These cells were large rounded to oval in the CA1 pyramidal cell layer (Fig. 5A), almost triangular in the CA3 pyramidal cell layer (Fig. 5B) and small rounded in the DG granular cell layer (Fig. 5C).

Examination of sections of group III (STZ-induced diabetic group), revealed marked disorganization in CA1 and CA3 pyramidal cells together with DG granule cells. Many cells were irregular with dark shrunken nuclei and indistinct cytoplasmic rim, while others had regular vesicular nuclei but with pale stained cytoplasmic rim (Figs. 6A, 6B and 6C).

Examination of sections of group IV (panax ginseng treated diabetic group); revealed almost regular cell arrangement in the three examined regions with preservation of the darkly stained cytoplasmic rim representing Nissl substance. However, few cells with pale stained cytoplasmic rim were also seen, especially in the DG region (Figs. 7A, 7B and 7C).

III- GFAP immunohistochemical reaction:

Examination of sections of group I (control group) showed positive GFAP immunoreactivity of astrocytes which were seen as few small star shaped cells with short thin ramifying processes mostly in both the polymorphic and the molecular layers in the

three examined regions, CA1, CA3 and DG (Figs. 8A, 8B and 8C).

Examination of sections of group III (STZ-induced diabetic group) revealed apparent increase in the intensity of GFAP immunoreactivity and distribution of astrocytes, which appeared as many large star-shaped cells with thick extensive processes invading all the layers of the three regions (Figs. 9A, 9B and 9C).

Examination of sections of group IV (panax ginseng treated diabetic group), revealed an evident decrease in the intensity of GFAP immunoreactivity and distribution of astrocytes in the molecular and polymorphic layers of the three regions. However, few positively stained astrocytes were still seen among the pyramidal cells of CA1 and CA3 regions in addition to the granule cells of the DG region (Figs. 10A, 10B and 10C).

IV- Synaptophysin immunohistochemical reaction:

Examination of sections of group I showed obvious strong synaptophysin immunoreactivity throughout the three layers of the three examined regions, CA1, CA3 and DG (Figs. 11A, 11B and 11C)

Examination of sections of group III (diabetic group), revealed less intense synaptophysin immunoreactivity in all the layers of CA1, CA3 and DG (Figs. 12A, 12B and 12C).

Examination of sections of group IV (panax ginseng treated diabetic group), revealed intense synaptophysin immunoreactivity in all the layers of CA1, CA3 and DG (Figs. 13A, 13B and 13C).

Morphometric Results:

Statistical analysis revealed no significant differences in the subgroups IB and IC when compared to subgroup IA in all examined parameters. Also, no significant difference was noted in the panax ginseng group (II) in comparison with the control group in all examined parameters.

I. The mean thickness of pyramidal cell layer of CA1 and CA3 and granular cell layer of dentate gyrus:

The mean thickness of CA1 and CA3 pyramidal cell layer as well as DG granular cell layer of group III revealed a highly significant decrease when compared with those of group I. However, the mean thickness of the three regions in group IV revealed a highly significant increase when compared to those of group III and a significant decrease when compared to the same regions of group I (Table 1 and Histogram 1).

II. The mean area percentage of GFAP staining per microscopic field:

The mean area percentage of GFAP of the hippocampus of group III showed a highly significant increase in CA1, CA3 and DG regions in comparison with those of group I. However, the mean area percentage of these three regions in group IV revealed a highly significant decrease when compared to the same regions of group III, in addition to a significant increase in CA1 and a highly significant increase in both CA3 and DG when compared to those of group I (Table 2 and Histogram 2).

III. The mean area percentage of synaptophysin staining per microscopic field:

The mean area percentage of synaptophysin of the hippocampus of group III showed a highly significant decrease in CA1, CA3 and DG in comparison with the same regions of group I. However, the mean area percentage of the three regions in group IV revealed a highly significant increase in comparison to those of group III and a highly significant decrease in comparison to the same regions of group I (Table 3 and Histogram 3).

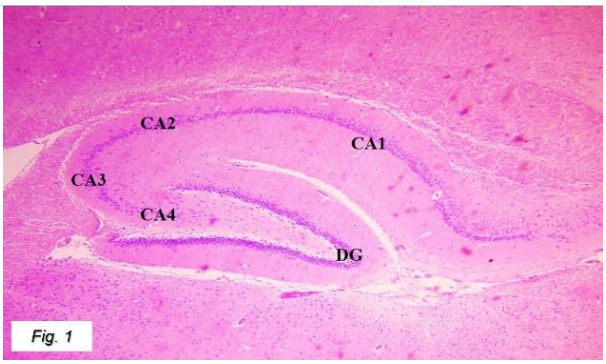


Figure 1: A photomicrograph of a parasagittal section of rat's hippocampus of group I (control group) showing C-shaped hippocampal formation with the four regions of Cornu Ammonis (CA1, CA2, CA3 and CA4). Notice the U-shaped dentate gyrus (DG) enclosing CA4. (Hx.&E. X100)

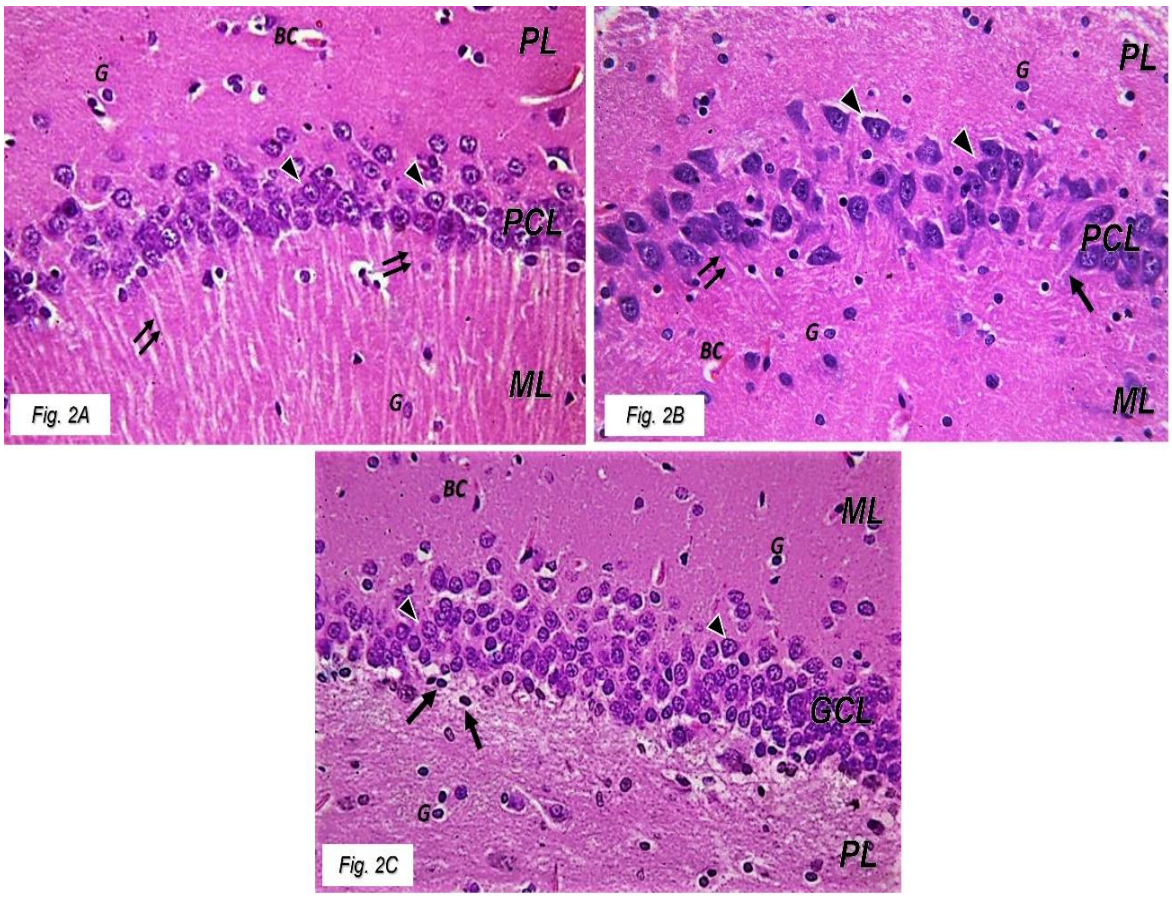


Figure 2: Photomicrographs of sections of the rat's hippocampus of group I (control group) showing CA1 (Fig. 2A) and CA3 (Fig. 2B) regions with well-defined three layers; polymorphic layer (PL), pyramidal cell layer (PCL), and molecular layer (ML). Pyramidal cells (Δ) appear rounded to oval and arranged in palisade arrangement in CA1 (Fig. 2A) and larger, almost triangular and more loosely packed in CA3 (Fig. 2B) with large vesicular nuclei, prominent nucleoli and pale basophilic cytoplasm and their almost parallel cell processes (↑↑) appear in the molecular layer. Notice the glial cells (G) and blood capillaries (BC) in molecular and polymorphic layers (Figs. 2A & 2B). Note also the three layers of the dentate gyrus, molecular layer (ML), granular cell layer (GCR), and polymorphic layer (PL), with small rounded to oval granule cells (Δ) with large vesicular nuclei and scanty cytoplasm in the granular cell layer and immature neurons with small oval deeply stained nuclei (↑) in the sub-granular zone in addition to the glial cells (G) and blood capillaries (BC) in the molecular and polymorphic layers (Fig. 2C). (Hx.&E. X400)

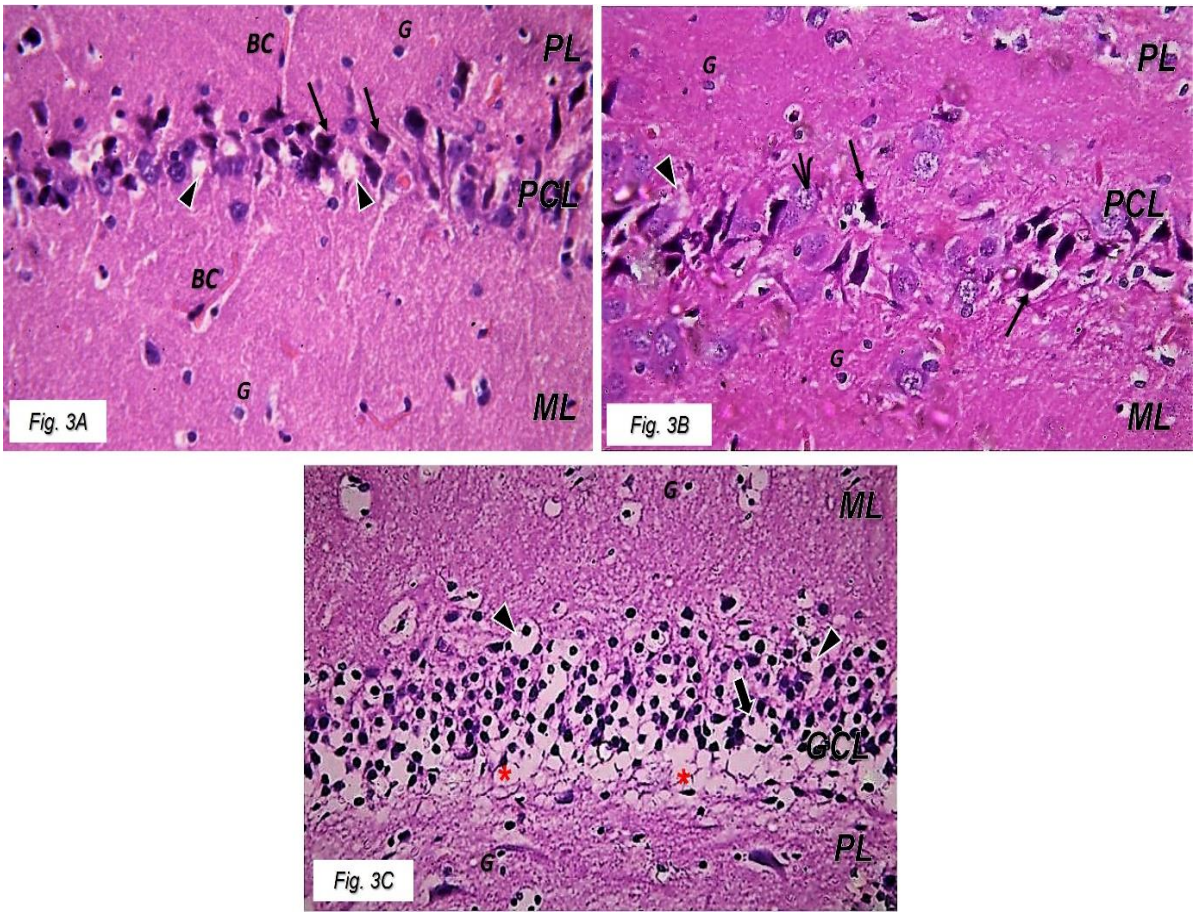


Figure 3: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (STZ-Induced diabetic group) showing marked disorganization of the pyramidal cell layer (PCL) of both CA1 (Fig. 3A) and CA3 (Fig. 3B) where many pyramidal cells appear shrunken with dark deeply stained nuclei (↑) and some intervening large pericellular spaces (Δ) in addition to some ghost like cells (⇑) in CA3 (Fig. 3B). Notice the loss of parallel arrangement of the pyramidal cell processes in the molecular layer (ML) in addition to the dilated blood capillaries (BC) and glial cells (G) seen in both the molecular (ML) and polymorphic (PL) layers in CA1 (Fig. 3A) and CA3 (Fig. 3B). Also note the distorted architecture of DG containing dark shrunken granule cells with pyknotic nuclei (↑) with large pericellular spaces (Δ), areas of vacuolation (*) in the in the sub-granular zone and the widespread glial cells (G) in the molecular layer (ML) and the polymorphic layer (PL) (Fig. 3C). (Hx.&E. X400)

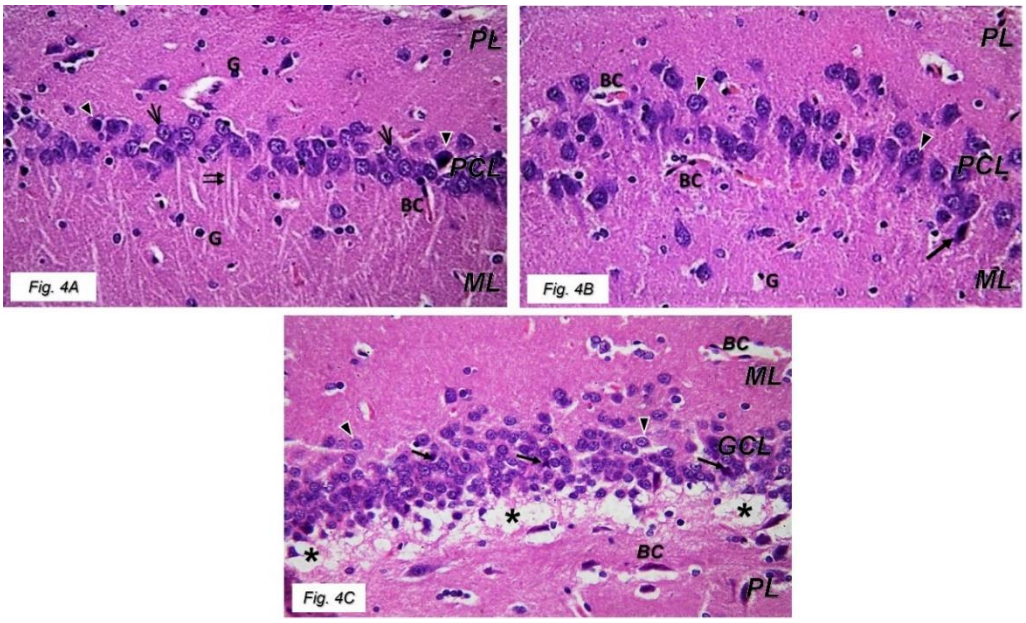


Figure 4: Photomicrographs of parasagittal sections of the rat's hippocampus of group IV (panax ginseng treated diabetic group) showing preserved architecture of CA1 and CA3 where pyramidal cell layer (PCL) contains pyramidal cells with large vesicular nuclei and pale basophilic cytoplasm (Δ) with some intervening cells with dark, shrunken nuclei (⋔). Notice the abundant glial cells (G) and the dilated blood capillaries (BC) in the molecular layer (ML) and polymorphic layer (PL) of CA1 (Fig. 4A) and CA3 (Fig. 4B) and the preserved parallel arrangement of the pyramidal cell processes (↑↑) in the molecular layer (ML) of CA1 (Fig. 4A). Note also the almost preserved architecture of DG containing rounded granule cells with vesicular nuclei and scanty rim of cytoplasm (Δ) with few intermingled distorted cells with darkly stained nuclei (↑), areas of vacuolation (*) in the sub-granular zone in addition to some blood capillaries (BC) (Fig. 4C). (Hx.&E. X400)

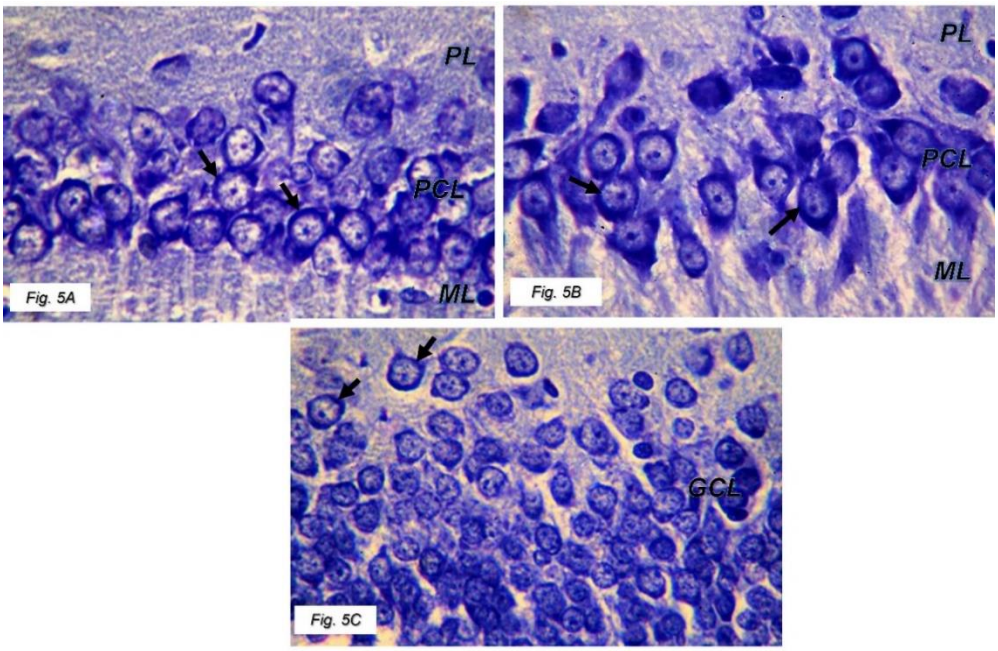


Figure 5: Photomicrographs of parasagittal sections of the rat's hippocampus of group I (control group) showing cells with darkly stained nuclei, surrounded by darkly stained cytoplasmic rim (↑) representing Nissl substance. These cells appear; large rounded to oval in the pyramidal cell layer of CA1 region (Fig. 5A), almost triangular in the pyramidal cell layer of CA3 (Fig. 5B) and small rounded in the granular cell layer of DG (Fig. 5C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer)
(Cresyl violet stain X1000)

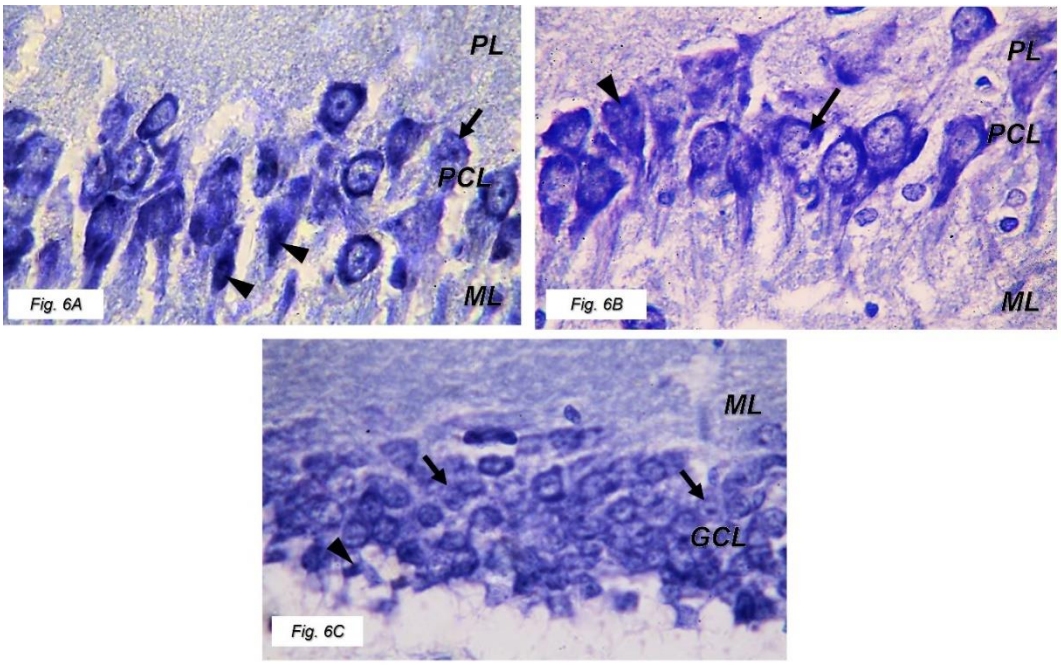


Figure 6: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (STZ-induced diabetic group) showing disorganization of pyramidal cells in CA1 (Fig. 6A) and CA3 (Fig. 6B) and granular cells in DG (Fig. 6C). Many cells appear irregular with darkly stained shrunken nuclei and indistinct cytoplasmic rim (Δ) together with other regular ones but with pale stained cytoplasmic rim (↑).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer)
(Cresyl violet stain X1000)

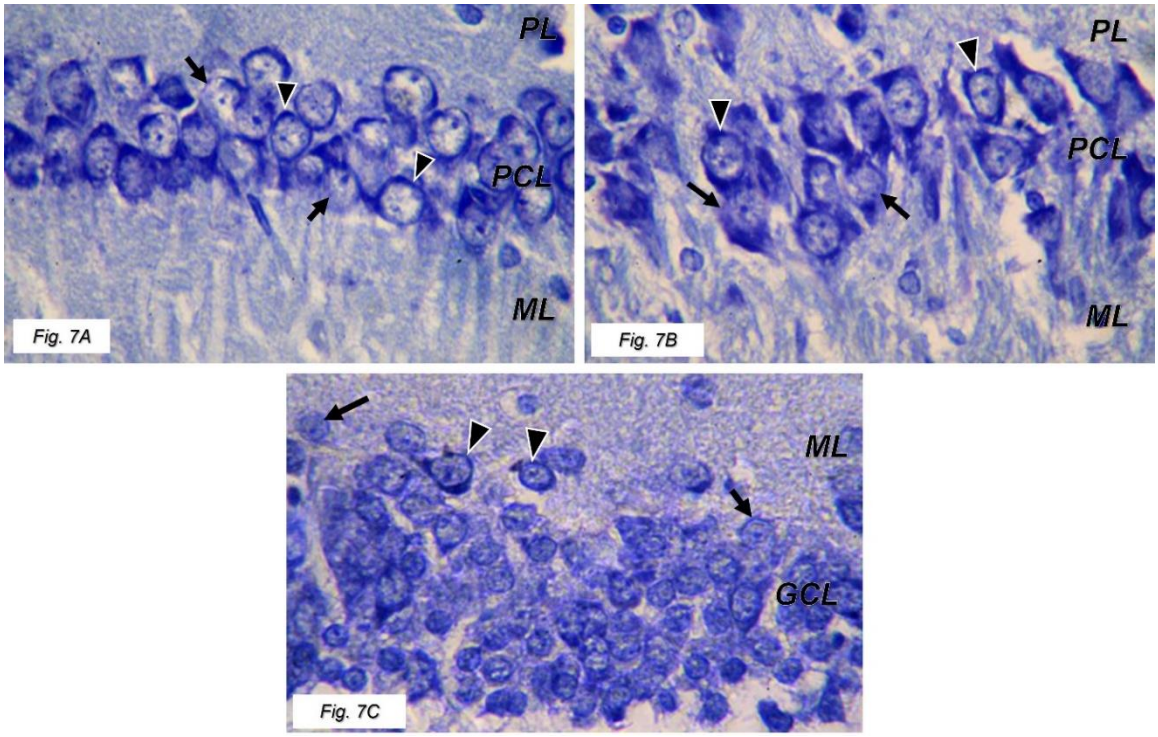


Figure 7: Photomicrographs of parasagittal sections of the rat's hippocampus of group IV (Panax ginseng treated diabetic group) showing almost regular arrangement of cells in CA1 (Fig. 7A), CA3 (Fig. 7B) and DG (Fig. 7C) where most cells have darkly stained cytoplasmic rim (Δ) with few intervening cells with pale stained rim (↑).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) Cresyl violet stain X1000).

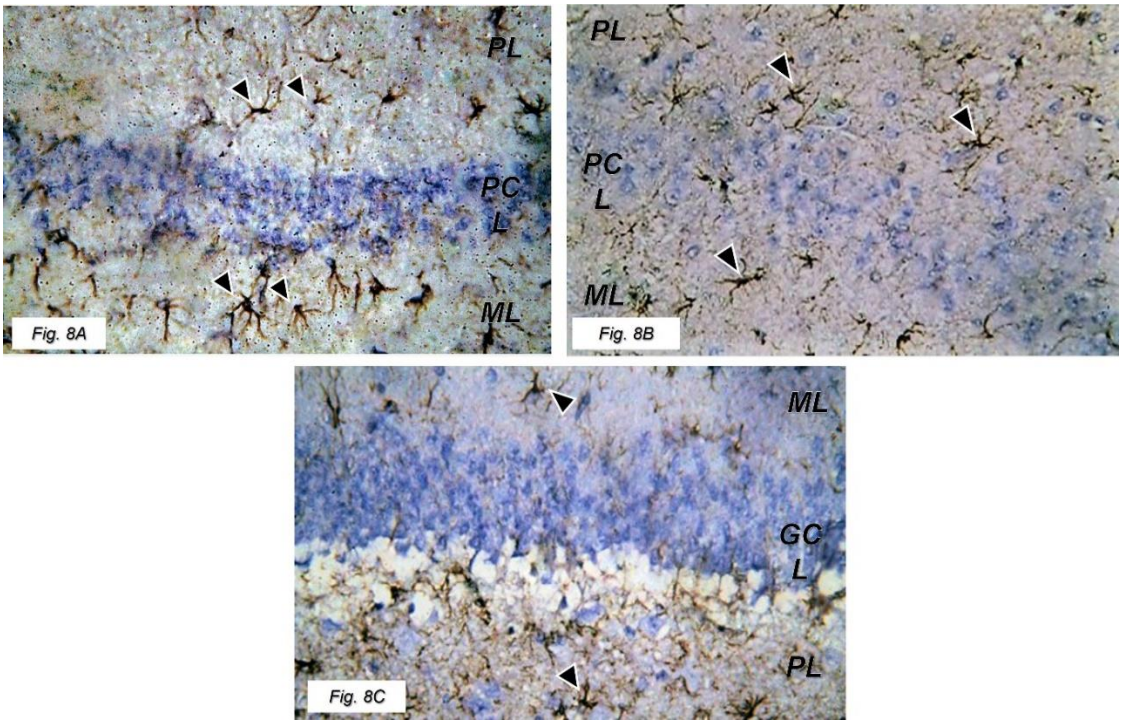


Figure 8: Photomicrographs of parasagittal sections of the rat's hippocampus of group I (control group) showing few small star shaped astrocytes with short thin ramifying processes (Δ) mostly in the polymorphic and molecular layers of CA1 (Fig. 8A), CA3 (Fig. 8B) and DG (Fig. 8C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (GFAP Immunostaining, X400)

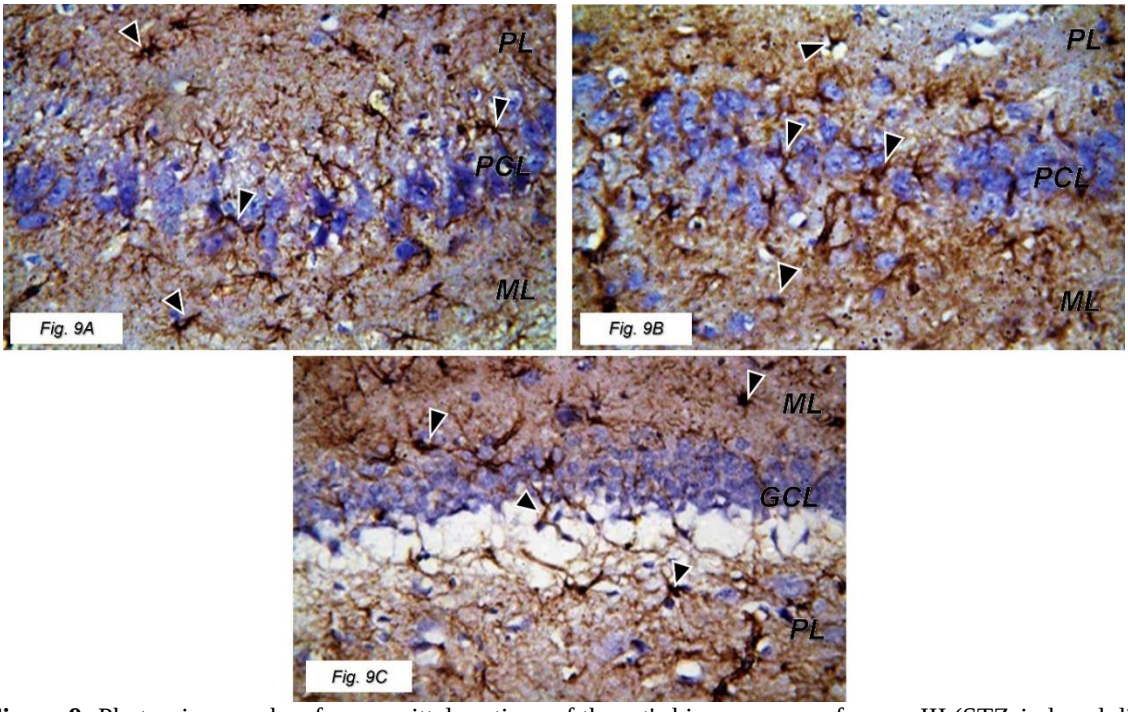


Figure 9: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (STZ-induced diabetic group) showing many large intensely stained star shaped astrocytes with thick extensive processes (Δ) in the polymorphic layer, pyramidal cell layer and molecular layer of both CA1 (Fig. 9A) and CA3 (Fig. 9B) and in the molecular layer, granular cell layer and polymorphic layer of DG (Fig. 9C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (GFAP Immunostaining, X400)

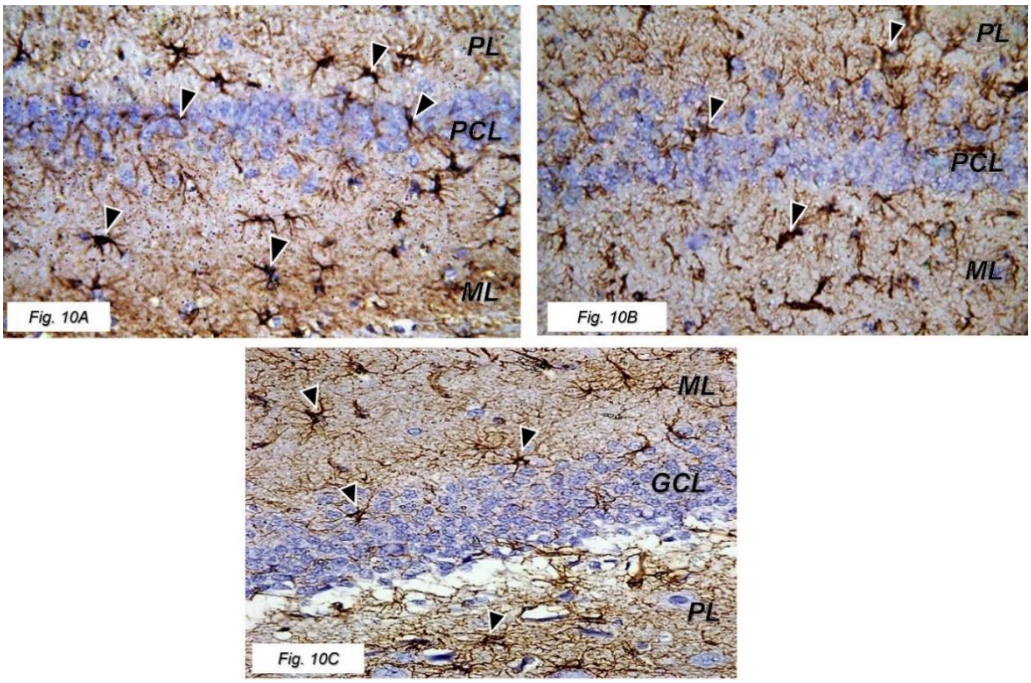


Figure 10: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (Panax ginseng treated diabetic group) showing evident decrease in the intensity of immunoreactivity and distribution of astrocytes in the molecular and polymorphic layers, however, few positively stained astrocytes (Δ) are seen among the pyramidal cells of CA1 (Fig. 10A) and CA3 (Fig. 10B) regions as well as the granule cells of the DG region (Fig. 10C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (GFAP Immunostaining, X400)

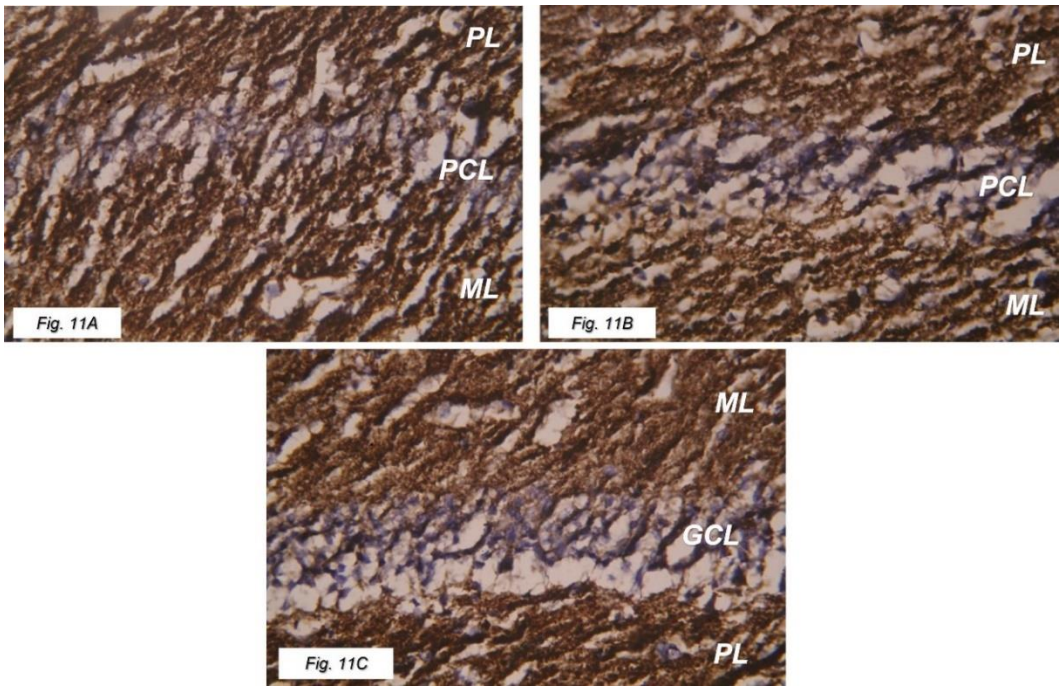


Figure 11: Photomicrographs of parasagittal sections of the rat's hippocampus of group I (control group) showing strong synaptophysin immunoreactivity throughout the three layers of the three examined regions, CA1 (Fig. 11A), CA3 (Fig. 11B) and DG (Fig. 11C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (Synaptophysin Immunostaining, X400)

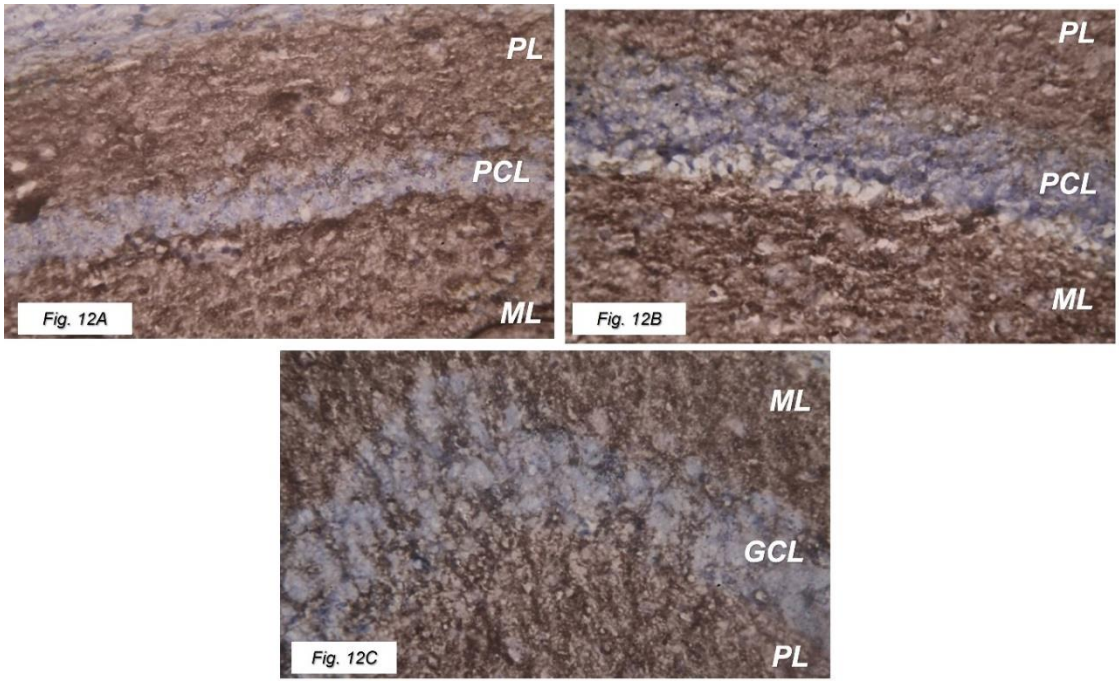


Figure 12: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (STZ-induced diabetic group) showing less intense synaptophysin immunoreactivity in all the layers of CA1 (Fig. 12A), CA3 (Fig. 12B) and DG (Fig. 12C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (Synaptophysin Immunostaining, X400)

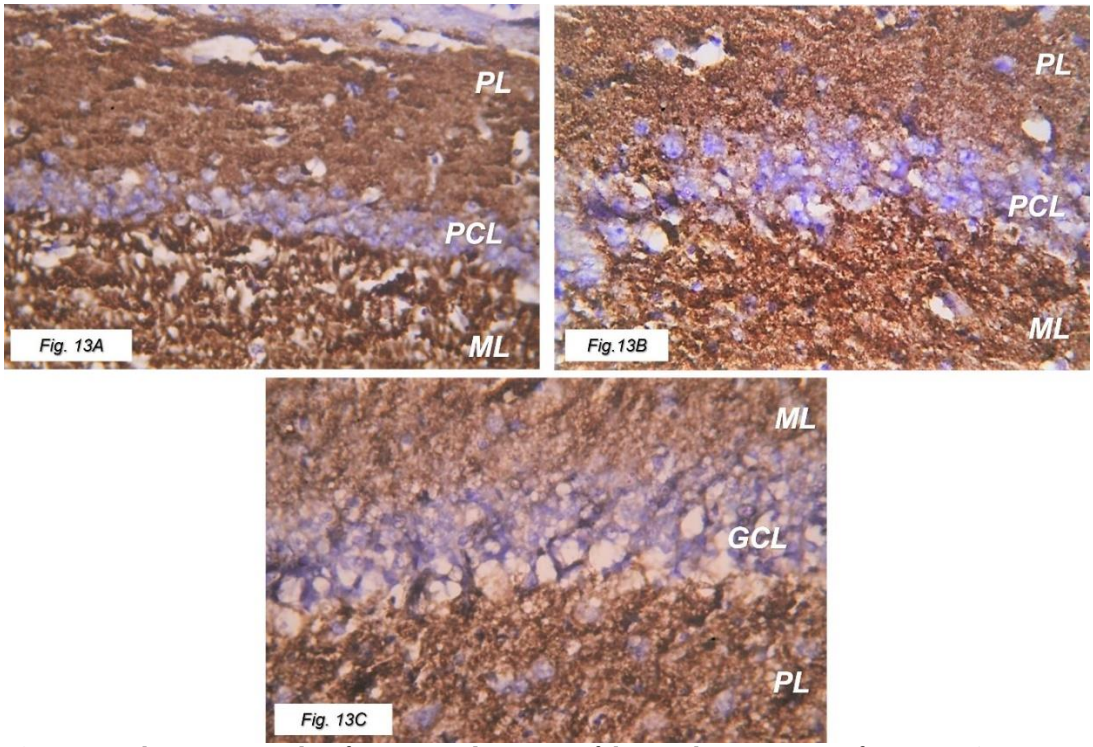
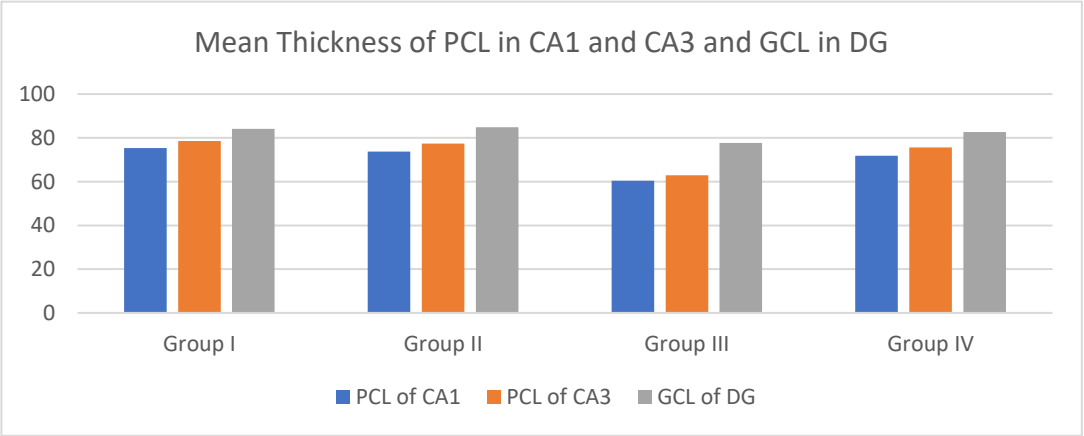


Figure 13: Photomicrographs of parasagittal sections of the rat's hippocampus of group III (Panax ginseng treated diabetic group) showing intense synaptophysin immunoreactivity in all the layers of CA1 (Fig. 13A), CA3 (Fig. 13B) and DG (Fig. 13C).
(PL=Polymorphic layer, PCL= Pyramidal cell layer, ML= Molecular layer and GCL= Granular cell layer) (Synaptophysin Immunostaining, X400)

Table 1: Showing the thickness of pyramidal cell layer of CA1 and CA3 and granular cell layer of dentate gyrus in µm ± SD among the experimental groups:

	Group I (Control group)	Group II (Panax ginseng group)	Group III (STZ-Induced Diabetic Group)	Group IV (Panax ginseng treated diabetic group)
PCL in CA1	75.36 ± 1.01	73.77 ± 1.91	60.45 ± 2.22 (P < 0.001) [a]	71.8 ± 2.17 (P < 0.001) [b] (P < 0.05) [c]
PCL in CA3	78.49 ± 0.65	77.33 ± 0.82	62.91 ± 3.93 (P < 0.001) [a]	75.62 ± 1.56 (P = < 0.001) [b] (P < 0.05) [c]
GCL in DG	84.1 ± 0.7	84.78 ± 0.74	77.6 ± 1.34 (P < 0.001) [a]	82.65 ± 3.31 (P < 0.001) [b] (P < 0.05) [c]

a) Highly significant decrease in comparison with group I.
b) Highly significant increase in comparison with group III.
c) Significant decrease in comparison with group I.

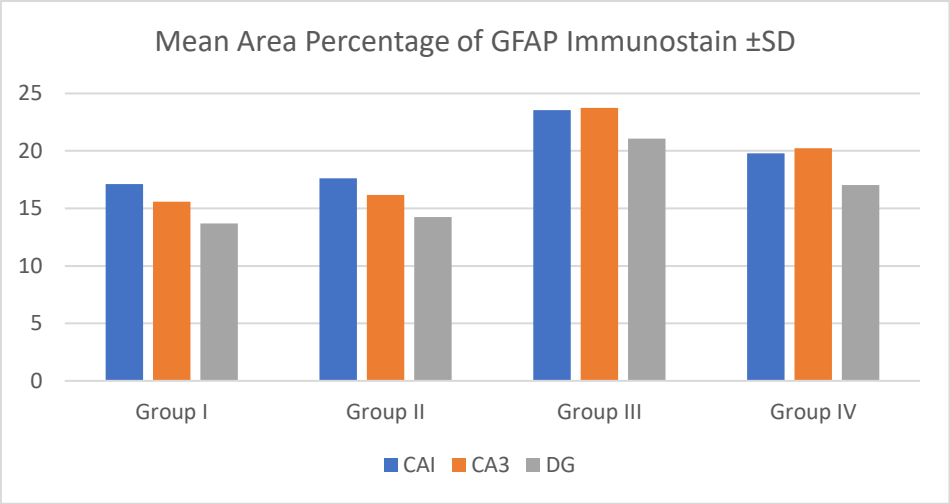


Histogram 1: Mean thickness of pyramidal cell layer of CA1 and CA3 and granular cell layer of dentate gyrus among the experimental groups

Table 2: Showing the mean area percentage of GFAP staining per microscopic field at magnification (x400) ± SD among the experimental groups:

	Group I (Control group)	**Group II (Panax ginseng group)**	**Group III (STZ-Induced Diabetic Group)**	**Group IV (Panax ginseng treated diabetic group)**
CA1	17.11 ± 0.83	17.62 ± 0.56	23.55 ± 1.22 (P < 0.001) [a]	19.28± 1.48 (P < 0.001) [b] (P < 0.05) [c]
CA3	15.6 ± 0.76	16.16 ± 1.08	23.75 ± 0.76 (P < 0.001) [a]	20.25 ± 1.49 (P = < 0.001) [b] (P < 0.001) [d]
DG	13.69 ± 0.65	14.24 ± 0.89	21.08 ± 1.52 (P < 0.001) [a]	17.03 ± 1.18 (P < 0.001) [b] (P < 0.001) [d]

a) Highly significant increase in comparison with group I.
b) Highly significant decrease in comparison with group III.
c) Significant increase in comparison with group I.
d) Highly significant increase in comparison with group I.

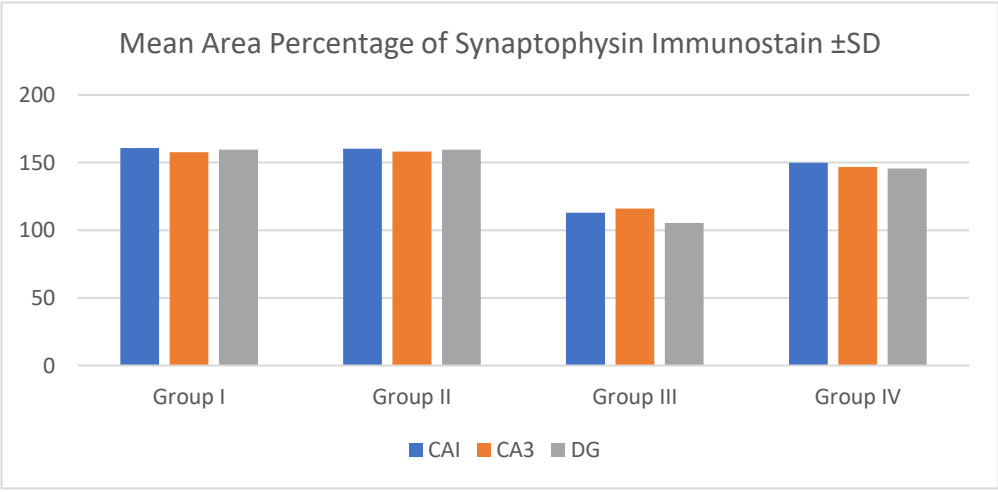


Histogram 2: Mean area percentage of GFAP staining per microscopic field among the experimental groups.

Table 3: Showing the mean area percentage synaptophysin staining per microscopic field at magnification (x400) ± SD among the experimental groups:

	Group I (Control group)	Group II (Panax ginseng group)	Group III (STZ-Induced Diabetic Group)	Group IV (Panax ginseng treated diabetic group)
CA1	160.64 ± 0.86	160.38 ± 0.86	112.86 ± 2.18 (P < 0.001) [a]	149.89 ± 1.06 (P < 0.001) [b] (P < 0.001) [c]
CA3	157.6 ± 0.81	158.09 ± 0.75	115.95 ± 2.41 (P < 0.001) [a]	146.68 ± 1.48 (P < 0.001) [b] (P < 0.001) [c]
DG	159.46 ± 0.74	159.51 ± 0.99	105.47 ± 3.24 (P < 0.001) [a]	145.58 ± 1.67 (P < 0.001) [b] (P < 0.001) [c]

a) Highly significant decrease in comparison with group I.
b) Highly significant increase in comparison with group III.
c) Highly significant decrease in comparison with group I.



Histogram 3: Mean area percentage of synaptophysin staining per microscopic field among the experimental groups

DISCUSSION:

The hippocampus is a particularly susceptible brain tissue to chronic changes in glucose metabolism; thus, it is greatly affected in diabetic patients leading to impairment of their cognitive functions and increasing their risk of dementia and AD [30,31].

Aiming to further study these diabetic hippocampal changes and their possible neuroprotection, the current work was concerned with the hippocampal regions most related to memory namely, CA1 and CA3 of the hippocampus proper as well as the DG. The hippocampal CA1 plays a fundamental role in maintaining short-term memory [32] while CA3 is responsible for fast construction of circumstantial memory [33]. As for the DG, it has a crucial role in long term memory potentiation [34]. That is why we focused on those three regions.

In the current study, examination of the hippocampal sections of the STZ-induced diabetic group after 2 weeks from the onset of induction revealed that DM caused marked neurodegenerative alterations in those three regions.

These, degenerative changes were manifested in CA1 by the appearance of darkly stained pyramidal cells with pyknotic nuclei. Additionally, those of CA3 were bizarre shaped with dark shrunken nuclei and deep basophilic cytoplasm with dispersed ghost like cells. Similarly, the DG showed distorted histoarchitecture with degenerative changes in its granular cell layer where it appeared to be formed of darkly stained cells having pyknotic nuclei, and large pericellular spaces in addition to obvious areas of vacuolations in the sub-granular zone. These degenerative changes observed upon histological examination were further reinforced by the morphometric study and statistical analysis which revealed highly significant decrease in the mean thickness of the pyramidal cell layer in both CA1 and CA3, as well as the granular cell layer of the DG.

Regarding these neurodegenerative changes, it has been previously reported that uncontrolled DM in rats can lead to hippocampal affection within two weeks or even before and further stated that longstanding uncontrolled DM can end up with severe neurodegenerative hippocampal changes [35]. This was also in accordance with **Zhou et al.** [36] who recorded that four-week following STZ-induced DM in rats the hippocampus revealed neuronal apoptosis specifically in the CA3 and DG regions [36]. The vacuolation obviously seen in the present study in the sub-granular zone of DG further denotes the neurodegenerative effect of DM on hippocampus. This zone is known for its neurogenic function throughout adulthood where division of neural precursor cells occur to develop the newborn neurons that synaptically integrate into hippocampal connections throughout life [37]. Therefore, affection of the sub-granular zone might greatly affect this neurogenic function.

Several studies focused on explaining the mechanism by which DM leads to neurodegeneration and among which was the oxidative stress mechanism. It has been stated that the DM induced hyperglycemic state which increases the reactive oxygen species (ROS) formation [38]. This state of oxidative stress causes the damage of lipids, nucleic acids, and proteins [39]. Recently, the DM induced neurobehavioral impairments, reduction in memory function, learning capacity and memory have been also attributed to the significant upregulation of proinflammatory cytokines, nitrite and acetylcholinesterase leading to marked brain insult which was manifested by obvious cortical and hippocampal histopathological changes [39,40].

These changes could explain the obvious decrease in the Nissl substance in hippocampal neurons of diabetic rats stained by Cresyl violet reported in the present work which was manifested by the marked decrease in the deep basophilic cytoplasmic rim. It has been recorded that Nissl substance is characteristic cornerstone of neuronal structure and function, and loss of such bodies clearly reflect neuronal damage [41].

It has been stated that the hyperglycemic state leads to collection of advanced glycation end products which are formed by combination of excess glucose and amino acids and cause damage to the cells, extracellular matrix, as well as the blood vessels [42]. This could explain the dilated blood capillaries manifested in the molecular as well as the polymorphic layers of the hippocampus in Hx. and E.-stained sections of diabetic group in the current study.

The present study also revealed that DM induced reactive gliosis, where GFAP stained sections revealed marked increase in the immunoreactivity and distribution of astrocytes, in both polymorphic and molecular layers of the three regions. Moreover, positively stained glial cells were frequently seen intermingled among the pyramidal cells of CA1 and CA3 in addition to the granular cells of the DG. This was further reinforced by the morphometric analysis of the GFAP immune staining which revealed statistically highly significant increase in the mean area percentage of the immunoreactivity of the diabetic group (group III) in comparison to the control group (group I). This increase in the glial activity could further explain the markedly distorted histoarchitecture noted in the Hx. and E.-stained sections of this group with subsequent disappearance of the characteristic palisade arrangement of CA1 pyramidal cell together with loss of the parallel arrangement of their cell processes that was clearly noted in the control group.

These findings were in accordance with other researchers who described similar histoarchitectural alterations in the hippocampus of diabetic rats [30,43]. Additionally, it has been noted that GFAP immunoreactivity was raised 3-folds in hippocampi of mice with DM when compared to that of non-diabetic ones [44]. Worth mentioning that GFAP is among the markers reflecting altered glial reactivity as its expressional amount, phosphorylation status and molecular size are proved to be altered in several pathological conditions including inflammatory state [45].

Inflammation is considered to be an immune reaction to various conditions such as infections and diseases. Acute inflammatory actions resolve efficiently, and the levels of inflammation go back to the normal in physiological conditions. On the contrary, in chronic inflammatory conditions as in DM, the recovery phase is not completed due to extensive pro-inflammatory signaling that induce relevant injurious actions [46]. Inflammation in the CNS is complexly regulated by blood inflammatory cells and astrocytes. In addition, the neurons seem to have a role in inflammatory mediation in the affected brain [47]. Astrocytes were previously known to be primitive cells in the CNS which however turned up to be cells playing crucial roles in balancing the neuronal excitation – inhibition status, maintaining the integrity of the blood–brain barrier as well as development of memory and learning [48]. In most neurodegenerative diseases, the primary function of glial cells is to surround and hold neurons, keeping them in place, and to insulate one neuron from another, nourish the neurons, and to destroy and eliminate dead neurons. This is considered an attempt to maintain neuronal stability and resisting any possible network dysfunction that could occurs in the early stages of disease. However, damaged nerve cells may stimulate glial cells, which unfortunately could end up with neuronal destruction [49,50]. Therefore, glial cells could

elicit either neuroprotective or deleterious effects on the hippocampal neurons of diabetic patients with subsequent deterioration of their cognitive function. Thus, targeting astrocytes could have a crucial role in future research in ameliorating these diabetes-induced neurodegenerative effects [51].

On the other hand, it has been proved that the glial cells found around the synapses play vital roles in postsynaptic and presynaptic function and remodeling as well as controlling the number of synapses throughout the CNS. Therefore, these cells are crucial in maintaining synaptic stability [52].

The present study revealed obvious decrease in the synaptophysin immunoreactivity in the molecular and polymorphic layers of the three studied regions compared to those in the control non-diabetic group. This was further clarified by the morphometric analysis of the mean area percent of synaptophysin immunoreactivity which revealed statistically highly significant decrease in the immune reaction of the diabetic group (group III) in comparison with the control group (group I).

Synaptophysin is a highly abundant presynaptic integral vesicle membrane localized on small synaptic-like micro vesicles and has a crucial role in neurotransmission and its loss would probably negatively affects the synaptic functions. Thus, it is used as a quantitative measurement for the presynaptic terminals function because of its discrete appearance in the immunostained sections [53,54]. Therefore, in the current work the decrease in synaptophysin noted in the diabetic group could reveal impairment in the hippocampal synaptic function of these rats. It has been postulated that the DM is frequently associated with defective synaptogenesis and synaptic function which was attributed to the existing insulin deficiency and aberrant lipid metabolism [55]. This could be attributed to reduction in presynaptic terminals neurotransmitter vesicles' density, decrease in the neurotransmitter release and impairment in synaptic plasticity and electrophysiology [51].

Long-term DM leads to impairment in synaptic plasticity with synaptic delay in signal integration and prolongation in the time it takes to reach the target brain receptors [56] which could explain the decline in the DM induced hippocampal neurogenesis, and atrophy of the hippocampus [57,10]. These changes are reflected in the middle-aged with moderate learning and memory impairment, while in elderly with increased risk of dementia or AD [58].

On the other hand, Hx. and E.-stained sections of diabetic rats' hippocampi given panax ginseng revealed evident improvement with restoration of their normal architecture. The pyramidal cells of CA1 and CA3 appeared with preserved morphology and so did the granule cells of the DG apart from the persistent vacuolation in the sub-granular zone. The cresyl violet stain further revealed that these cells regained their darkly stained cytoplasmic rim. However, some cells which appeared with dark shrunken or pyknotic nuclei in Hx. and E.-stained sections or with pale stained cytoplasmic rim in Cresyl violet stained sections, were still seen intermingled among those almost normal ones.

This improvement could be attributed to the recorded ability of ginsenosides, the main active ingredient in ginseng, to improve blood glucose level. It has been reported that ginseng extracts significantly improved plasma insulin and glucose levels with marked improvement in glucose tolerance [59]. This could be attributed to its ability to regulate glucose absorption [60], interference in the glucose disposal and/or glucose transport [61], and the alteration of insulin binding and secretion [62].

Regarding the neuroprotective role of ginseng, it has been noted that fermented ginseng extract administration in Alzheimer disease led to marked reduction in the

formation of Aβ amyloid in the brain and improved memory in mice [63]. Recent studies proved that ginseng components, such as gintonin and ginsenosides, do not only have potent anti-oxidative and anti-inflammatory effects, but also a boosting effect on the cholinergic systems and the hippocampal neurogenesis [64].

This could explain the ginseng induced immunohistochemical improvement noted in the current study. GFAP immune-stained sections in the ginseng treated diabetic group (group IV) showed evident decrease in the reaction and the distribution of astrocytes in all layers of CA1, CA3 and DG regions with less frequently encountered positively stained glial cells among the pyramidal cells as well as the granule cells of CA1, CA3 and DG respectively. Statistical analysis revealed a highly significant decrease in GFAP immune reaction in group IV compared to the untreated diabetic one (group III). However, a persistent significant increase was still noted in group IV when compared to the control group.

Synaptophysin in the ginseng treated diabetic group (group IV) showed an evident increase in the immunoreaction in polymorphic and molecular layers, in comparison with the diabetic group reflecting restoration in the synaptic activity of the hippocampus of these diabetic rats. This was further clarified by the statistical analysis of the morphometric study which revealed a highly significant increase in synaptophysin immune reaction in group IV compared to the untreated diabetic one (group III). However, a persistent significant decrease was still noted in the panax ginseng treated diabetic group in comparison to the control group. It has been also recorded that ginseng had the ability to restore the reduced synaptophysin and choline acetyltransferase activity of mice hippocampus following amyloid-beta oligomer toxicity [65]. This ginseng induced immunoreactive improvement could be attributed to its antioxidant, anti-inflamma tory, immune-stimulatory, and anti-apoptotic effects [66]. It inhibits neuronal apoptosis, promotes nerve cell proliferation, and has an anti-AD effect [67,68,69].

Recently, genetic bases of ginseng-mediated neuroprotective effects have been postulated, where **Lee et al., [13]** reported that gintonin-enriched fraction isolated from ginseng and gintonin greatly affected the expression of some mice hippocampal genes. It led to upregulation of ChAT gene responsible for improving the cognitive function by increasing acetylcholine synthesis in the brain, which decreases in AD. It also causes upregulation of Crh and Adrb3 genes which lower depression and anxiety-like behavior in any stressful environment. Moreover, it causes downregulation of Tdo2 which is known to induce neurodegenerative effects [70].

Conclusion:

Streptozotocin-induced DM in rats led to marked histoarchitectural and immunohistochemical hippocampal changes. However, panax ginseng greatly improved such changes. Thus, it could be considered as a highly promising neuroprotective agent for ameliorating DM induced memory and learning problems, dementia, and AD with subsequent improvement of quality of life for diabetic patients. Further research on the effect of ginseng on other neurological diabetic complications is strongly recommended.

Conflict of interest:

No conflict of interest.

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تأثير داء السكري المستحث بالستربتوزوتوسين على قرن آمون لدى ذكور الجرذان البيضاء البالغة والدور الوقائي العصبي المحتمل لباناكس الچينسنج

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المقدمة: يعد ضعف التعلم والذاكرة من النتائج الشائعة لداء السكري غير المنضبط. يعتبر باناكس الجينسنج من أشهر الأعشاب الطبيعية المستخدمة في السيطرة على الاضطرابات الأيضية التى تشتمل علي داء السكري ومضاعفاته.

الهدف من البحث: دراسة التغيرات النسيجية لداء السكري المستحث بالستربتوزوتوسين على قرن آمون فى ذكور الجرذان البيضاء البالغة وتقييم الدور الوقائي العصبي المحتمل لباناكس الجينسنج

المواد والطرق المستخدمة: تم استخدام ستة وثلاثون من ذكور الجرذان البيضاء البالغة ، التى تتراوح أوزان كل منهم 200-250 جم. و قد تم تقسيم الحيوانات إلى أربع مجموعات كالآتى:

- ❑ المجموعة الأولى: تضمنت 18 جرذًا وقد تم تقسيمهم بالتساوي إلى ثلاث مجموعات فرعية كل منها يحتوى على 6 جرذان.
- ❑ المجموعة الثانية: اشتملت على 6 جرذان تم اعطائهم باناكس الجينسنج يوميا عن طريق التزقيم المعدي.
- ❑ المجموعة الثالثة: تضمنت 6 جرذان و قد تلقي كل منهم حقنة واحدة داخل الصفاق من الستربتوزوتوسين لاستحداث داء السكرى.
- ❑ المجموعة الرابعة: اشتملت على 6 جرذان تم فيها استحداث داء السكرى ثم أعطيت الجرذان باناكس الجينسنج يوميا.

وبعد أسبوعين، تمت التضحية بالحيوانات، وتم تشريح المخ و تحضير كتل البارافين من قرن امون، وصبغت بعض المقاطع بالهيماتوكسيلين والأيوسين والكريسيل البنفسجي بينما تمت معالجة البعض الآخر بكيميائيات مناعية للكشف عن GFAP وsynaptophysin. كما تم إجراء التحليل الإحصائي لبعض القياسات.

النتائج: كشفت الدراسة الحالية أن داء السكري المستحث بالستربتوزوتوسين قد أدى إلى تغيير هيكلي ملحوظ في قرن آمون في الجرذان. بينما اظهرت الجرذان المصابة بداء السكري و التي عولجت بالجينسنج تحسنًا واضحًا في هذه التغيرات من الناحيتين النسيجية والهستوكيميائية المناعية ، وهو ما أكدته الدراسات المورفومترية.

الخلاصة: لقد أدى باناكس الجينسنج دورًا وقائيًا ضد التغيرات النسيجية والهستوكيميائية المناعية في قرن آمون في الجرذان المصابة بداء السكري. وبالتالي، يمكن أن يعتبر عاملًا وقائيًا عصبياً واعدًا ضد ضعف الذاكرة الناجم عن داء السكري.