ABSTRACT:
Diabetes mellitus (DM) is well known for its serious impact on central nervous system particularly in the hippocampus. Learning and memory development have been linked to neurogenesis in the hippocampus's dentate gyrus. New neurons are created in the dentate gyrus of the hippocampus during postnatal and adult periods. This study was performed to clarify the histological and immunohistochemical changes taking place on Dentate gyrus of adult albino rat after experimental induction of diabetes and to clarify the possible protective role of Antox.

Material and Method: Thirty six adult male albino rats were classified into six groups (A1) (Negative control) they were not received medication and (A2) (positive control): were injected intraperitoneally (IP) with a single dose citrate buffer as a vehicle. group (B): (Diabetic) were injected (IP) with a single dose of STZ dissolved in citrate buffer. group (C): (Diabetic/antox) were injected (IP) with STZ then received Antox 10 mg/kg/day orally for 4 weeks. group (D): (Diabetic / insulin ) were injected STZ (IP) and received insulin 1 U/100 g m/day subcutaneous (S.C.) for 4 weeks. group E: (Diabetic / insulin / antox ) were injected STZ (IP) and received insulin (S.C.) then will be received antox orally for 4 weeks. When the experiment is finished, all animals anesthetized, cerebrum removed, the specimen subjected to histological procedures and statistical analysis was done.

Results: (DM) caused histological changes on rat dentate gyrus in the form of degenerative and apoptotic neurons which confirmed by immnuno histochemical staining of P53 and GFAP staining. Thus, diabetes mellitus had hazardous effect on dentate gyrus and that can be improved by Insulin and Antox.

Conclusion: Our experimental results proved the harmful effect of diabetes mellitus on dentate gyrus and confirm the antioxidant and the antiapoptotic properties of Antox on dentate gyrus.

KEYWORDS: Diabetes; Dentate gyrus ; Antox ; Hippocampus.

I. INTRODUCTION:
Diabetes mellitus (DM) is a disease manifested by high blood glucose levels and significant damage to the cardiovascular, urinary, and central neurological systems. Uncontrolled diabetes causes hyperglycemia, which causes substantial damage to many of the body's systems and raises the risk of dying prematurely [1]. (DM) is an endogenous stressor linked to higher levels of oxidative stress in the central nervous system, particularly in the hippocampus. The dentate gyrus (DG) is a part of the hippocampus that is involved in memory and learning. According to studies, DM inhibits neuronal proliferation while increasing neuronal death, resulting in memory impairment. [2]. (DM) has been linked to changes in the cerebral cortex in both human and animal models of the disease. Diabetes damages presynaptic and postsynaptic structures, disrupts calcium homeostasis, causes neuronal death, lowers insulin growth factor and receptor expression, and impairs neurogenesis in hippocampal neurons. A number of studies have revealed that neural progenitors in the dentate gyrus multiply, migrate, and develop into granule cells, which ultimately stretch their axons into the hippocampus. New neurons are created in dentate gyrus of the hippocampus during postnatal and adult periods. (DM) is thought to cause functional and anatomical abnormalities. Furthermore, diabetes caused by streptozotocin (STZ) has been shown to lower the amount of
proliferative cells in the dentate gyrus of rats. Adult mammals, including humans, have shown new cell birth and neurogenesis in the dentate gyrus. Learning and memory development have been linked to neurogenesis in the hippocampus's dentate gyrus. [3] Hippocampus is made up of two sections: cornu Ammonis (hippocampus proprius) and DG. A hippocampal sulcus separates the two parts, which curve into each other. [4]. Despite the DG is part of the hippocampus, it could be differentiated from the hippocampus proper by its different cellular architecture [5]. The DG is made up of three layers: The most superficial layer of DG is the polymorphic layer. Many interneurons are found in this layer, and DG cell axons travel through it on their route to CA3. The cell bodies of DG cells are contained in the granular layer. Commisural fibers from the contralateral DG and medial septum inputs terminate on the granule cells’ proximal dendrites in the molecular layer. The excitatory synapses of perforant route fibers onto the distal apical dendrites of granule cells [6]. During an animal's lifetime, specific brain zones such as the subgranular zone (SGZ) of the dentate gyrus (DG) has been found to exhibit self-renewal activity. Brain disorders and environmental factors have been shown to impact neurogenesis in some neurogenic areas, such as the hippocampus. The hippocampus, which is involved in cognitive functions such as learning and memory, is prone to disorders such as Alzheimer's. Newly generated cells in the hippocampus can immigrate to the DG's granule cell layer, where they develop into new neurons and form synaptic connections with hippocampal circuitry. [7]. Antox is a multivitamin compound formed of ascorbic acid (Vit C), vitamin A, vitamin E, and selenium. These vitamins represent strong anti-oxidants and have free radical scavenging activity [8]. Antioxidants include reactive compounds like antox as well as specialized enzymes like catalase, superoxide dismutase, and glutathione reductase. The body makes enzymatic antioxidants but not antioxidant molecules (such as antox and flavonoids), which protect body tissues that enzymatic antioxidants can't reach [9]. The limbic system includes the hippocampus. It's in charge of memory and learning. The brain is protected from some medications by the blood-brain barrier, but many chemotherapeutic agents can alter its function in direct or indirect ways. [10]. The study’s purpose is to determine whether diabetes has a neurotoxic effect on the rat dentate gyrus and whether Antox has a neuroprotective effect on adult rats.

II. MATERIAL AND METHODS:

Material

Experimental Animals

Thirty-six healthy adult male Wistar albino rats weighing 250–300 g were obtained and kept at the Faculty of Medicine's Breeding Animal House in Zagazig, Egypt. The animals were acclimatized in separate hygienic stainless-steel cages at a controlled temperature (23°±C) and humidity (60±5%) in an artificially lighted room free of chemical contamination under a 12:12 h light: dark cycle. They were fed a standard laboratory diet and had unrestricted access to the food and water. (No. ZU-IACUC/3/F/17/2018).

Chemicals:

**Streptozotocin (STZ):** obtained from Sigma Chemical Co. at Cairo, Egypt.

**Antox:** tablets was obtained from local pharmacy.

Experimental Design:

Six equal groups of thirty-six rats were created

**Group A1** (Negative control) contained 6 rats which serve as normal control and they didn't receive medication and will be left for 4 weeks. **A2** (positive control) contained 6 rats which were injected intraperitoneally (IP) with a single dose of citrate buffer as a vehicle (0.1 M, PH 4.5) and will be left for 4 weeks.

**Group B:** (Diabetic) contained 6 rats were injected (IP) with a single dose of 50 mg/kg of STZ dissolved in citrate buffer and will be left for 4 weeks [11]

**Group C:** (Diabetic/antox) contained 6 rats were injected (IP) with a single dose of 50 mg/kg of STZ dissolved in citrate buffer then receive antox orally in a dose of 10 mg/kg/day for 4 weeks [12]

**Group D:** (Diabetic / Insulin) contained 6 rats were injected (IP) with a single dose of 50 mg/kg of STZ and receive insulin 1 U/100 gm/day commercial insulin (Mixtard 30/70; Novo Nordisk) once/day subcutaneous (S.C.) for 4 weeks [13]
Group E : (Diabetic/insulin / antox ) contained 6 rats were injected (IP) with a single dose of 50 mg/kg of STZ and receive insulin 1 U/100 gm /day (S.C.) then will be received antox orally in a dose of 10 mg/kg/day for 4 weeks.

- **Induction of Type 1 DM** Type 1 DM was experimentally induced in rats through a single IP dose of 50 mg/kg BW of STZ dissolved in citrate buffer (0.1 M, PH 4.5) and will be left for 4 weeks [11]. The diagnosis of DM was confirmed on the 2nd day after STZ injection when blood glucose obtained from animals’ tail vein was 200 mg/dl or more measured using Glucometer (AccuChek, Germany).

**III. METHODS**

**Histological techniques by light microscopic examination:**

**Haematoxylin and eosin stain (H&E):**

For 24 hours, hippocampus specimens were fixed in 10% neutral buffered formalin, then washed and dehydrated with increasing degrees of alcohol, clarified in xylol, and finally embedded in paraffin. For investigating the histological structure of the rat dentate gyrus, 5 m thick slices were produced and stained with hematoxylin and eosin (H&E) according to [14].

**Immunohistochemical stains of Glial fibrillar acidic protein (GFAP)&(P53):**

Deparaffinized and hydrate deparaffinized sections were incubated for 20 min at 0.5 °c in citrate buffer (pH 6.0) for antigen retrieval of the following proteins.

1- (GFAP): is the common technique accustomed to inspecting the dissemination of astrocytes and their reaction to neural damage. The astrocytes were detected using a modified Avidin-Biotin immune peroxidase method for GFAP. Primary anti-GFAP (Ab-1 (Clone GA-5), 1:100, (Cat. #MS-280-B0), Lab Vision Corporation, Medico Co., Egypt). GFAP containing cells (astrocytes) appeared brown. The astrocytes' cell membrane and cytoplasm were brown in hue, indicating positive outcomes. Light microscopy (LEICA ICC50 W) was used to examine all stained slides in the Anatomy and Embryology department's Image Analysis Unit.

2- **P53:** tumor suppressor gene is a short-lived protein. It is the most generally mutated gene in oncogenesis. It regulates the transcription rate of several genes that regulate genomic stability, cell cycle, and apoptosis. Affixed paraffin slides were waxed in xylene, hydrated, treated with 0.3% hydrogen peroxide for endogenous blocking of peroxidase and nonspecific binding sites for antibodies. Slides were exposed to heating in a microwave at pH=6.0. Dilutions (1:80) of primary antibodies (Dako, Glostrup, Denmark) were performed at room temperature for two hours. Sections stained with immune peroxidase for evaluation of an anti-p53 expression in the nuclei (brown), which indicated positive apoptotic neurocytes.

**Morphometrical study:**

Six rats per group were used in the morphometric investigation, which was done with the help of an image analyzer. (the Image J software plugin) in Anatomy Department –Zagazig University as follow: 1-Perspective P53 immunohistochemical stained sections of each group were analyzed for calculating the immune positive cells in PCL by using point selection at the objective lens of 40x. 2. Perspective GFAP immunohistochemical stained sections of each group were analyzed to estimate the area percent of positive immunoreaction for GFAP which After image splitting, it was completed. The red stack was adjusted to the threshold and marked with a binary mask after the images were separated into RGB stacks. At a 40x objective lens, the percentage of the field's area was calculated.

**Statistical analysis:**

Because the data had normal distributions, continuous variables were reported as mean SD (parametric). The Kolmogorov Smirnov test was used to ensure that the data was normal. To find significant changes across groups, a one-way ANOVA was utilized. For multiple comparisons between groups, a post hoc Tukey's test was used. P0.05 was used to determine whether the differences were significant. The statistical tests were all two-tailed. Graph pad Prism software, version 5.0, was used to do all statistical calculations (Graph Pad Software, San Diego, CA, USA).
IV. RESULTS:

The control negative group and control positive group showed no detectable differences in histological parameters; therefore, they were considered as one group.

**Histological results:**

**Hematoxylin and eosin stain**

**Control group:**
Higher magnification of DG revealed (Molecular layer) ML: contained lightly stained glial cells nuclei, Many compactly packed granule cells with pale vesicular nuclei that are rounded were seen in (Granular cell layer) GCL. In the sub granular zone SGZ, there were a few immature neurons with oval dark nuclei and little cytoplasm (Polymporphic layer) POL contained lightly and darkly stained glial cells nuclei, Some neurons most probably mossy cells could be observed Figure 1.

**Diabetic group (B):**
Higher magnification of DG of diabetic group showed its three layers: ML had darkly and lightly stained glial cells nuclei. GCL exhibited some granule cells with shrunken deeply stained elongated nuclei with pericellular vacuolation. Some immature neurons with oval dark nuclei and paucity of cytoplasm were observed in the widening (edematous) sub granular zone. POL had darkly stained glial cells and dilated blood vessels. Figure 1

**Diabetic +Antox Group (C):**
Higher magnification of DG of diabetic +Antox group showed its three layers: ML: had lightly stained glial cells nuclei GCL: Many compactly packed granule cells with round pale vesicular nuclei were found. Few immature neurons with oval dark nuclei and paucity of cytoplasm are seen in the slightly disturbed SGZ. POL contained darkly and lightly stained glial cell nuclei with wide pericellular spaces. Figure 1

**Diabetic +Insulin: Group D**
Higher magnification of DG of diabetic+Insulin group showed its three layers, ML had lightly and darkly stained glial cells nuclei GCL:containing many compactly packed granule cells with round pale vesicular nuclei. Few immature neurons with oval dark nuclei and paucity of cytoplasm were observed in the slightly disturbed SGZ . POL contained darkly and lightly stained glial cell nuclei. Figure 1

**Diabetic +insulin +Antox: Group E**
Higher magnification of DG of diabetic+Insulin +Antox group showed its three layers, ML: had lightly stained glial cells nuclei. Many compactly packed granule cells with rounded pale vesicular nuclei were seen in GCL that appeared normal . Few immature neurons with oval dark nuclei and paucity of cytoplasm were seen in SGZ . POL displayed lightly and darkly stained glial cell nuclei. Figure 1

**Immunohistochemical results**

**P53 immunostaining results:**
On all experimental groups, the hippocampus was immunohistochemically stained with P533 antibody to clarify the location of apoptotic neurons in the dentate gyrus. Apoptotic neurons' cytoplasm was where the immunological positive reaction was found. The neurons in the control group showed no immunoreaction in the dentate gyrus of the hippocampus, whereas in group B, abundant P53 immunopositive neurons were found in the dentate gyrus of the hippocampus, and in groups C and D, moderate to mild positive reactions on granular cells in the dentate gyrus. The granular cells in the dentate gyrus in the E group, on the other hand, showed a negative reaction. Table 1 Figure 2

**GFAP immunostaining results:**
To elucidate the reaction of astrocytes to neuronal degeneration in the distinct experimental groups, the hippocampus was immunohistochemically stained with an anti-GFAP anti body. The astrocytes that developed among the granule cells in DG with small body with a few narrow, short processes in the control group were spread throughout the ML and POL. However, there was a lot of GFAP positive staining in the cytoplasm and astrocyte
processes in group B. In group C, they looked to have risen in quantity and size, with many long thick processes and group D showed astrocytes with, multiple thin long processes, with thin ramified processes in the granular cells in DG. Some astrocytes are seen dispersed among the ML and POL Whereas in E group showed astrocytes with thin ramified processes in-between the granular cells in the dentate gyrus. Few astrocytes are seen dispersed among the molecular layer and polymorphic layers. **Table 2 Figure3**

**Morphometrical results**

**P53 Immunohistochemical Staining:**
The number of P53 immune-positive neurons in DG was quantitatively analyzed, and it was discovered that group B had a higher number of immune positive neurons than group A. The number of immunopositive neurons in the control group (A) was significantly lower than in the diabetic group (B), and the number of immunopositive neurons in the diabetic group (B) was non-significantly different from the control group (A) in DG. **Figure 4**

**GFAP Immunohistochemical staining:**
When comparing diabetic group E to control group A in DG, statistical analysis of Area percent of GFAP reactivity revealed a substantial increasing the percentage of the area of reactivity concerned the microscopic field in diabetic group E (p 0.001). In the insulin + Antox (group E) group, the Area percent of GFAP reactivity in the microscopic field was significantly lower than in the diabetes (group B) group (p 0.001). In addition, there was no significant change in DG (p 0.05) when compared to control group A. **Figure 5.**

**V. DISCUSSION**

Diabetes mellitus is a significant metabolic illness that affects many people. It affects both the central and peripheral nerve systems, causing a variety of functional and anatomical issues. Because the brain is a glucose-dependent organ that can be harmed by both hyperglycemia and hypoglycemia, glucose consumption in the brain is reduced during diabetes. As a result, crucial pathogenic events are more likely to occur in the brain. The STZ-induced diabetes animal model is commonly used to research diabetes-related problems. Because of the ensuing hyperglycemia, this model is a good illustration of endogenous chronic oxidative stress. [15] In the present work, the male albino rats were used to exclude the effect of female hormone. Estrogen hormones are proposed to enhance cell proliferation [16]. The current work provided clear evidence of the harmful effect of (DM) on hippocampal neurons by inducing histological changes on the dentate gyrus. In addition, this work explored the antiapoptotic properties of Antox in hippocampal tissue. In the current work, H&E staining clarified various degenerative changes in granular cells of the dentate gyrus of diabetic rats. As in the DG, the granular cell layer had granular cells with shrunken deeply stained elongated nuclei with pericellular vacuolation and pyknotic nuclei with pericellular vacuolation. Oval-shaped neurons with darkly stained nuclei and a lack of cytoplasm were found in the sub granular zone of the dentate gyrus called immature neurons. These results were by those of [2] DNS (dark neuron) production in DG granule cells of diabetic rats is triggered by uncontrolled STZ-induced diabetes, and DNS creation can also occur in the granular layer of healthy animals. The DNS in the DG granule layer revealed morphological changes that resembled apoptotic death, such as increased electron density, shrinkage, irregularity, and apoptosis-like chromatin modifications, when examined ultra structurally. Cell growth is frequently observed in the granular layer of DG, according to studies. Memory formation necessitates this special neural renewal. Memory and learning impairment can be caused by any factor that disrupts the equilibrium between neural proliferation and neuronal loss. Correlate to our study, these results could be linked with this pyknosis which is confirmed by p53 immunohistochemical stain for apoptosis. So the major result of the current study that diabetes without treatment showed significantly increased the number of degenerated neuronal cells represented by up-regulation of the immune positive neurons for p53. Statistically, there was a significant increase in the apoptotic cells in the DG of the diabetic group in comparison to that in the control. This was in agreement with [17]. The allocation of astrocytes and their response to neuronal degeneration or injury were examined using immunohistochemical localization of GFAP. [18] Diabetic rats showed extensive GFAP positive staining of the cytoplasm and processes of astrocytes in the current investigation. In DG granular cells, the number of astrocytes rose, and they appeared bigger with many long thick processes. When compared to control rats, the area percent of GFAP in the rats increased significantly. Several investigations have discovered widespread astrogliosis, as evidenced by cellular hypertrophy and increased GFAP expression. [19]. Astrogliosis is a common response of the central nervous system to trauma, genetic abnormalities, or chemical damage. Rapid GFAP synthesis is also a feature of astrogliosis [20]. Moreover, astrocyte populations increase in or near damaged CNS regions during tissue repair (forming scars). Activated astrocytes may be evident near damaged neurons, filling the sites where neuronal
perikarya are absent and neuronal debris has been eliminated [21]. Astrogliosis is described as a typical reaction of the CNS to trauma, disease, genetic disorders, or chemical insult. Rapid GFAP synthesis is also a feature of astrogliosis. [20] Moreover, astrocyte populations increase in or near damaged CNS regions during tissue repair (forming scars). Activated astrocytes may be evident near damaged neurons, filling the sites where neuronal perikarya are absent and neuronal debris has been eliminated [21]. In this study, co-administration of antox showed that the degenerative histological changes were diminished DG showed few immature neurons with oval dark nuclei and paucity of cytoplasm are seen in the slightly edematous sub granular zone. This is in agreement with [22]. DG showed few immature neurons with oval dark nuclei and paucity of cytoplasm are seen in the slightly edematous sub granular zone. This is in agreement with [22]. Additionally, GFAP expression was slightly decreased than in the diabetic group without treatment. Which was in agreement with [15]. Also, statistically, there was a slight decrease in the apoptotic cells in the DG of the diabetic +Antox group in comparison to that in the diabetic group without treatment.

In this study administration of Insulin showed that the degenerative changes were diminished. In DG there were few immature neurons with oval dark nuclei and paucity of cytoplasm were seen in the slightly edematous sub granular zone. Additionally, GFAP expression was decreased than on diabetic +Antox group, there were decrease in the apoptotic cells in the DG of the diabetic +insulin group compared to that in the diabetic +Antox group.

These results were by those of [23] who found that treatment with insulin alone showed that preservation of small granular cells has been improved. and there was markedly decreased apoptosis of large cells. however, in GFAP expressions decreased to less than normal levels with insulin treatment alone. However, administration of both Antox and Insulin showed the degenerative histological changes greatly diminished in DG showed granule cells appeared normal and few immature neurons with oval dark nuclei and paucity of cytoplasm were seen in the slightly edematous sub granular zone. This was in agreement with [24].

Table (1): Mean ± SD of P53 in DG among five groups.

<table>
<thead>
<tr>
<th>Groups(n=5)</th>
<th>DgP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>8.8±4.14 (5-15)</td>
</tr>
<tr>
<td>B (Diabetic)</td>
<td>52.0±17.15 (42-60)</td>
</tr>
<tr>
<td>C(Diabetic+Antox)</td>
<td>24.2±5.67 (20-34)</td>
</tr>
<tr>
<td>D(Diabetic+Insulin)</td>
<td>26.6±4.7 (20-32)</td>
</tr>
<tr>
<td>E(Diabetic+Antox+ Insulin)</td>
<td>9.8±3.6 (4-14)</td>
</tr>
<tr>
<td>F test</td>
<td>56.06</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

This table shows highly significant difference between five groups as regard P53 of GFAP in DG.

Table (2): Mean ± SD of area % of GFAP in DG among five groups:

<table>
<thead>
<tr>
<th>Groups(n=5)</th>
<th>Dg GFAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>4.63±1.44 (2.77-6.45)</td>
</tr>
<tr>
<td>B (Diabetic)</td>
<td>31.22±5.12 (25.33-38.76)</td>
</tr>
<tr>
<td>C(Diabetic+Antox)</td>
<td>18.09±1.93 (15.77-20.46)</td>
</tr>
<tr>
<td>D(Diabetic+Insulin)</td>
<td>12.47±2.56 (10.54-16.87)</td>
</tr>
<tr>
<td>E(Diabetic+Antox+ Insulin)</td>
<td>5.91±1.05</td>
</tr>
</tbody>
</table>
This table shows highly significant difference between five groups as regard area % of GFAP in DG.

<table>
<thead>
<tr>
<th>F test</th>
<th>73.43</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Figure 1

Figure 2
Figure 3

Figure 4
Figure 5

Legand Figures:

**Figure 1: (A-E) group A**: A higher magnification of DG of control group with its three layers showing: (Molecular layer) ML: displays lightly stained nuclei glial cells (ls). (Granular cell layer) GCL displays many compactly arranged granule cells (G) with rounded pale vesicular nuclei. Few immature neurons with oval dark nuclei and paucity of cytoplasm (head arrow) are seen in the sub granular zone (SGZ). (Polymorphic layer) POL: displays lightly (ls) and darkly stained (ds) glial cells. Some neurons most probably mossy cells (mc) can be observed. In **group B** showing: ML has darkly stained (ds) glial cells and lightly stained (ls) glial cells nuclei GCL, granular cells with shrunken deeply stained elongated nuclei (pn) with pericellular vacuolation (v). Some immature neurons with oval dark nuclei and paucity of cytoplasm are observed in the widened (edematous) sub granular zone (SGZ). POL: displays darkly stained glial cells nuclei (ds) and dilated blood vessel (bv). In **group C** showing ML displays lightly (ls) and deeply stained (ds) glial cells nuclei GCL displays many compactly arranged granule cells (G) with rounded pale vesicular nuclei. Few immature neurons with oval dark nuclei and paucity of cytoplasm (curved arrow) are seen in the disturbed (E) sub granular zone (SGZ). POL displays darkly stained (ds) and lightly stained (ls) glial cells nuclei with wide pericellular space. In **group D**: showing: ML exhibits lightly (ls) and darkly stained (ds) glial cells nuclei GCL displays many compactly arranged granule cells (G) with rounded pale vesicular nuclei. Few immature neurons with oval dark nuclei and paucity of cytoplasm (curved arrow) are seen in the slightly disturbed sub granular zone (SGZ). POL reveals darkly stained (ds) and lightly stained (ls) glial cells nuclei. In **group E** showing: Molecular layer (ML): displays blood vessel (bv) Granular cell layer (GCL): exhibits many compactly arranged granule cells (G) with rounded pale vesicular nuclei that appear normal. Few immature neurons with oval dark nuclei and paucity of cytoplasm (head arrow) are hardly seen.

**Figure 2: group (A-E)**: A photomicrograph of immunohistochemical staining of P53 in group A showing negative reaction of the granule cells (G) in the dentat gyrus, in group B showing strong positive (arrow) in the cytoplasm of most granule cells (G) of dentate gyrus, in group C showing positive reaction (arrow) in the cytoplasm of some the granule cells (G) in group D showing positive reaction (arrow) in the cytoplasm of few granule cells (G) of DG and in group E showing negative reaction in the granule cells (G) of dentate gyrus and positive stained neurons are hardly seen (arrow).

**Figure 3: group (A-E)**: A photomicrograph of immunohistochemical staining of GFAP in the group A showing astrocytes (arrow) with thin short ramified processes among the granule cells (G) in DG. Few astrocytes are seen in the molecular layer and polymorphic layers (arrow), in group B showing many astrocytes with large ,multiple thick long processes (arrows) among granule cells (G) in DG Many astrocytes are seen dispersed among the
molecular layer and polymorphic layers (arrows), in group C showing some astrocytes with multiple thin long processes (arrows) and others with thick processes (arrow head) among the granule cells (G) in DG. Some astrocytes are seen dispersed among the nuclei of ML and POL, in group D showing astrocytes with thin ramified processes (arrow) in the granule cells (G) in DG few astrocytes having short thick processes (arrow head) astrocytes are seen dispersed among the nuclei of ML and POL, and in group E A photomicrograph of immunohistochemical staining of (diabetic+ Antox+ Insulin) group showing astrocytes (arrow) with thin short ramified processes between the granule cells (G) in DG. Few astrocytes are seen dispersed among the nuclei of molecular layer (ML) and polymorphic layers (POL).

**Figure 4:** Histograms demonstrating the quantitative analysis of the number of p53 immunopositive neurons in CA1,CA3 and DG of different experimental groups.

**Figure 5:** Histograms demonstrating the Area % of GFAP positive astrocyte in DG of different experimental groups showed Significant difference compared to the control group A, P<0.001. and there was significant difference compared to the diabetic group B ,P< 0.01.