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Effect of High Fat Diet Induced Metabolic Syndrome and Treatment with Insulin like Growth Factor 1 on Biochemical and Immunohistochemical Parameters Related to Neuronal Functions in Adult Male Albino Rats

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Abstract

The prevalence of metabolic syndrome (MetS) makes type 2 diabetes mellitus and its consequences more common in communities. Dysfunctions of the nervous system has negative implications on the lives of affected individuals and their families. This study aimed to elucidate the possible role of MetS and treatment with in insulin like growth factor 1 (IGF-1) on some biochemical and immunohistochemical parameters related to neuronal functions in adult male albino rats. For this purpose, fifty adult male albino rats were recruited and divided into five groups. (1) control group, (2) MetS group (induced by high fat diet for eight weeks), (3) MetS group treated with IGF-1 for six weeks started two weeks before induction of MetS, (4) MetS group treated with IGF-1 for six weeks started with induction of MetS, (5) MetS group treated with IGF-1 for six weeks started two weeks after starting induction of MetS. Fasting blood glucose (FBG), insulin, lipid profile, glucagon like peptide 1 (GLP1), tumor necrosis factor-α (TNF-α) and adiponectin levels were measured in the serum whereas malonaldehyde, glutathione peroxidase (GPx), brain derived neurotrophic factor (BDNF), synapsin 1 and sortilin were measured in brain tissue homogenate. Histo pathological and immunohistochemical studies for B cell lymphoma 2 (BCL2) and glial fibrillary acidic protein (GFAP) were also done. The results showed that induction of MetS caused hyperglycemia, hyperinsulinemia, dyslipidemia, elevation of the oxidant marker MDA and the proinflamatory TNF-α whereas the anti-oxidant GPx and the anti-infalimatory adiponectin were elevated. BDNF and the anti-apoptotic BCL2 were reduced whereas synapsin 1, sortilin and the astrocytes proliferation marker GFAP were increased. Treatment with IGF-1 was associated with improvement in all these parameters. It could be concluded that MetS is associated with reduction in neuronal protective factors and enhancement of factors which impair neuronal functions and structures. These effects may underlie the pathogenesis of cognitive dysfunction associating MetS. By antagonizing such effects of MetS, IGF-1 may be helpful in managing MetS and associated cognitive dysfunction.

Keywords: Metabolic syndrome, Cognitive dysfunction, Insulin like growth factor 1, Brain derived neurotrophic factor, synapsin 1, Sortilin

1- Introduction

Metabolic syndrome (MetS) is a general term for conjunction of obesity, dyslipidemia, glucose intolerance and elevated blood pressure. Indeed, it's a group of metabolic risk factors rather than being a disease itself. Those risk factors could eventually

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increase the prevalence of cardiovascular disease and diabetes. Importantly the insulin resistance is tightly associated with the pathological process of metabolic syndrome [1]. Cognitive functions confer the various mental abilities, that include perception, attention, integration, reasoning processing, thinking, learning, memory, planning as well as decision making and problem solving. Moreover, those functions can be further divided into many subcategories; for instance, memory is divided into working memory, habit, implicit and declarative memory is commonly divided into working memory (holding information in mind for a short period while it is used), implicit or habit memory (memory for skills) and declarative memory (includes memory for facts and events) [2]. MetS is not only implicated in endocrinal disturbances, but also there is emerging evidence regarding MetS being a leading cause of cognitive dysfunction. Multiple reviews and meta-analysis have introduced the increased prevalence of subtle cognitive dysfunction to extreme dementia among patients with MetS, as compared to control [3]-[4]. Dementia, which an extreme form of cognitive dysfunction, is a crucial factor of an elderly person being dependent on other family members. Such dependency has negative implications on health, psychological, social, and economic consequences for the elderly and their families. Dementia symptoms include progressive memory loss and reduction in cognitive skills, as well as language issues. The prevalence of dementia is roughly 5% in those over the age of 65, but it rises to 20-40% in people over the age of 85. Alzheimer's disease is the most prevalent kind of dementia, accounting for 60-70 % of all dementia cases. Vascular dementia, the secondary type of dementia, accounting for around 25% among dementia cases [5]. [6]. Neuroendocrine disturbances are commonly associated with cognitive dysfunction. For instances hypothyroidism, hyperthyroidisms, Cushing's disease are routinely found with significantly poor cognitive functions, even though there are no other neurological symptoms in the form of motor or sensory disturbances. Notably MetS and type 2 diabetes, the two most common endocrine disorders, have a documented deleterious impact on cognitive performance [2]. Contrarily to the old believe that the brain is an insulin independent, its currently well documented that insulin signaling is crucial for neuronal health and function of brain synapses and circuits; and thereby for cognitive functions and memory. Defective insulin signaling in the brain and the related by insulin-like growth factor 1 (IGF-1) signaling, are linked to neurological dysfunction such as Alzheimer's disease, raising the possibility that cognitive dysfunction can be related to the condition of brain insulin resistance. Interestingly the brain insulin resistance itself is attributed to mechanisms that similar to the peripheral insulin resistance in diabetes, MetS or obesity [7]. The aim of this work was to study the effect of MetS on brains of adult male albino rats particularly the biochemical parameters which may be involved in cognitive disorders.

2- Materials and Methods

2.1- Experimental design: Fifty adult male albino rats weighing 120 – 150 g were obtained, housed in suitable cages (40 x 32 x 40 cm for every 5 rats) in a room with controlled humidity, maintained at constant room temperature under suitable illumination conditions (day and night). They were kept for one week on their normal diet and free access to water for acclimatization before starting the experiment. All procedures were approved by the Animal Care Committee of Al-Azhar University. The rats were randomly divided into five equal groups as follows (ten rats for each):

- Group 1 [control]: rats fed on normal rat chow.
- Group 2 [MetS]: rats fed on high fat diet (HFD) for 12 weeks to induce MetS. They received WESTERN RD, a specialized rat diet with Atwater fuel energy of 4.6 kcal/g and comprised of 50% crude carbohydrate, 21.4% crude fat, 17.5% crude protein, 3.5% crude fiber, and 4.1% ash [8].
- Group 3 [IGF-1 – started with MetS]: rats were administered IGF-1 subcutaneously at a dose of 2 µg/100 g body weight (BW) daily for 6 weeks starting with the start of induction of MetS [9].
- Group 4 [IGF-1 – before induction of MetS]: rats were administered IGF-1 subcutaneously at a dose of 2 µg/100 g BW daily for 6 weeks started 2 weeks before induction of MetS.
- Group 5 [IGF-1 – after induction of MetS]: rats were administered IGF-1 subcutaneously at a dose of 2 µg/100 g BW daily for six weeks started after completing induction of MetS.

2.2- Biochemical measurements in serum: at the end of the experiment, overnight fasting rats were deeply anesthetized with the combination of ketamine (100 mg/kg BW) and Xylazine (10 mg/kg BW) injected intraperitoneally [10]. Blood was then collected from the tail vein. Serum was separated and

stored frozen at -80ºC until the time of analysis. The following parameters were assessed:

- ✓ Parameters assessing the metabolic syndrome:
  - FBG
  - Insulin
  - GLP-1
  - Cholesterol
  - Triacyl-glycerols (TAGs)
  - High density lipoprotein cholesterol (HDL-c)
  - Low density lipoprotein cholesterol (LDL-c)
  - Adiponectin
- ✓ Parameter assessing the inflammatory processes:
  - TNF-α

2.3- Biochemical measurements in brain tissue homogenate

After taking blood samples, animals were sacrificed by decapitation. The whole-brain was rapidly dissected and thoroughly washed with isotonic saline, dried, and then weighed. Thereafter, each brain was sagitally divided into two portions. The first portion was preserved for histological and immuno-histochemical studies. The second portion was homogenized at room temperature in distilled water (∼20-fold dilution, w/v) and the substrates were centrifuged at 10000 rpm for 5 minutes at 4 °C. The supernatants were stored frozen at −80°C until analysis [11]. Then, the following parameters were assessed:

- ✓ Parameters assessing the redox state:
  - Malondialdehyde (MDA)
  - Glutathione peroxidase (GPx)
- ✓ Parameters assessing neuronal functions
  - Brain derived neurotrophic factor (BDNF)
  - Synapsin 1
  - Sortilin

2.4- Histological examination: Fixation of brain specimens was done with 10% neutral buffered formalin. After that samples were dehydrated, embedded in paraffin wax, and cut into 5 µm thick sections, they were stained with hematoxylin and eosin (H&E), and examined by light microscope [12].

2.5- Immunohistochemistry

- ✓ Immunohistochemical staining to determine B-cell lymphoma 2 (BCL2) expression which is an anti-apoptotic factor.
- ✓ Immunohistochemical staining for revealing of glial fibrillary acidic protein (GFAP) which is considered as an indicator for astrogliosis.

2.6- Morphometrical analysis: Lieca light microscope was used to estimate the numbers of BCL2 positive cells and number of GFAP positive cells at X400 magnification. The results were expressed as cell number/high power field (HPF).

2.7- Statistical analysis: it was performed using SPSS computer program version 24. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests were used to compare means of different groups of the study. Spearman's correlation coefficient was used to evaluate the linear association between variables [13].

3- Results

3.1- Biochemical and morphometric results: These results are represented in the following tables

Table (1)
Mean ± SD of studied parameters in different groups of the study

Groups / Parameters	Group 1 [Control]	Group 2 [MetS]	Group 3 [IGF-1 – started with MetS]	Group 4 [IGF-1 – before induction of MetS]	Group 5 [IGF-1 – after induction of MetS]
FBG (mg/dl)	86 ± 8.6	234.9 ± 32.4 ‡	161.19 ± 17.4*	153.4 ± 19.7*	122.7 ± 12.3*
Insulin (µIU/L)	8.9 ± 0.95	18.9 ± 2.1 ‡	10.4 ± 1.9*	9.8 ± 1.5*	8.9 ± 0.8*
GLP 1 (ng/ml)	32.6 ± 16	11 ± 1.6 ‡	22.9 ± 4.5	23.9 ± 4.4	29.7 ± 4.5
Cholesterol (mg/dl)	131.1 ± 4	230.3 ± 7.9 ‡	181.8 ± 10.7*	177.8 ± 13*	147.1 ± 19.8*†
TAGs (mg/dl)	81.5 ± 13.4	136.8 ± 17.5 ‡	102.3 ± 13.02*	112.1 ± 9.9*	92.9 ± 7.2*
HDL (mg/dl)	70.15 ± 9.3	28.9 ± 4.9 ‡	49.02 ± 3*	53.4 ± 6.9*	53.4 ± 5.4*
LDL (mg/dl)	44.6 ± 6.9	174.1 ± 7 ‡	112.3 ± 14.6*	102.01 ± 17.1*	75.1 ± 23.9*§
Adiponectin (ng/ml)	40.8 ± 7.2	12 ± 2.4 ‡	29.5 ± 5.5*	30.03 ± 7.8*	40.9 ± 3.2*
TNF-α	61.4 ± 6.2	210.3 ± 14.7 ‡	128.6 ± 20*	126.5 ± 39.5*	113.3 ± 13.2*

(pg/ml)					
MDA (mMol/ml)	44.4 ± 12.9	133.7 ± 14.1 ‡	82 ± 10.03*	78.5 ± 19.1*	54.6 ± 11.3*
GPx (mMol/ml)	116.8 ± 6.04	41.1 ± 2.5 ‡	92.3 ± 13.5*	101.7 ± 8.7*	113.8 ± 3.9*§
BDNF (pg/ml)	232.7 ± 20.4	114.01 ± 3.7 ‡	203.8 ± 8.5*	199.6 ± 17.2*	221.9 ± 5.1*
Synapsin 1 (ng/ml)	15.03 ± 2.4	70.8 ± 10.6 ‡	24.3 ± 8.3*	22.9 ± 4.7*	19.9 ± 3.6*
Sortilin (ng/ml)	1.2 ± 0.21	7.8 ± 1.01 ‡	3.8 ± 0.3*	3.5 ± 1.1*	2.1 ± 0.63*§
BCL2 (cells/HPF)	31.7 ± 3.5	9.2 ± 1.1 ‡	12 ± 2.2	15.6 ± 0.73*	18.4 ± 2.8*§
GFAB (cells/HPF)	12.5 ± 2.5	28.3 ± 3.3 ‡	21.4 ± 2.6*	21.3 ± 2.4*	18.6 ± 2.01*

‡ = significant difference when comparing with group 1 [control group]
* = significant difference when comparing with group 2 [MetS group]
§ = significant difference when comparing with group 3 [IGF-1 – started with MetS]
† = significant difference when comparing with group 4 [IGF-1 – before induction of MetS]

The results of this study showed that induction of metabolic syndrome has altered the glycemic parameters. FBG and insulin levels were significantly higher whereas GLP 1 was significantly lower in MetS group as compared with control group. As regard lipid profile and the lipid related adipokine; cholesterol, TAGS and LDL elevated significantly in MetS group than control group whereas HDL and adiponectin were significantly lower. The significantly elevated TNF-α in MetS group as compared to control group points to MetS associated inflammation. Oxidative stress in MetS is evidenced by significant elevation in the lipid peroxidation marker MDA and significant reduction in the antioxidant GPx in MetS group as compared to control. BDNF was significantly lower whereas synapsin 1 and sortilin were significantly higher in MetS group compared to control group. Treatment with IGF-1 showed significant improvement in all these parameters whether started with, or before, or after induction of MetS as compared to the non-treated MetS group. An exception is the GLP 1 where its levels in treated groups were higher than MetS group, but this elevation was not significant.

In-between IGF-1 treated groups; cholesterol level was significantly lower in group 5 [treatment with ILGF1 started after induction of MetS] compared to group 4 [treatment with ILGF1 started before induction of MetS]. Also, LDL and sortilin were significantly lower whereas GPx was significantly higher in group 5 compared to group 3 [treatment with ILGF1 started with induction of MetS].

The results revealed significant decrease in in number of BCL2 positive cells in MetS group as compared with control group whereas treatment with IGF-1 showed significant increase in number of BCL2 positive cells whether started before or after induction of MetS as compared to the non-treated MetS group. Also, there was a significant increase in in number of GFAP positive cells in MetS group as compared with control group whereas Treatment with IGF-1 showed significant decrease in number of GFAP positive cells whether started with, or before, or after induction of MetS as compared to the non-treated MetS group.

Table (2)
Correlation factors of glycemic parameters & lipid profile with other measured parameters

	GLP 1	Adiponectin	TNF-α	MDA	GPx	BDNF	Synapsin1	Sortilin	BCL2	GFAB
FBG	-0.705*	-0.728*	0.822*	0.898*	-0.895*	-0.903*	0.819*	0.926*	-0.890*	0.851*
Insulin	-0.500	-0.494*	0.484*	0.460*	-0.515*	-0.699*	0.595*	0.491*	-0.653*	0.572*
Cholesterol	-0.575*	-0.716*	0.764*	0.884*	-0.790*	-0.708*	0.779*	0.917*	-0.783*	0.816*
TAGs	-0.621*	-0.761*	0.829*	0.786*	-0.840*	-0.808*	0.702*	0.806*	-0.755*	0.808*
HDL	0.484*	0.661*	-0.878*	-0.842*	0.724*	0.656*	-0.708*	-0.902*	0.803*	-0.764*
LDL	-0.513*	-0.700*	0.815*	0.899*	-0.803*	-0.725*	0.731	0.934	-0.812*	0.818*

* = significant correlation ($P \leq 0.05$)
Study of linear correlation showed that glycemic parameters (FBG &insulin) and lipid profile parameters (cholesterol, TAGs & LDL) were significantly positively correlated with the inflammatory marker TNF-α, the oxidation marker MDA and the neuro-biochemical factors synapsin 1, sortilin & GFAP. MetS was associated with upregulation of these parameters

On the other hand, glycemic parameters and lipid profile parameters (apart of HDL) were significantly negatively correlated with GLP 1, adiponectin, the antioxidant enzyme GPx, the neurotrophic factor BDNF and number of BCL2 positive cells. MetS was associated with downregulation of these parameters.

HDL was significantly positively correlated with GLP 1, adiponectin, the antioxidant enzyme GPx, the neurotrophic factor BDNF and number of BCL2 positive cells. While it was significantly negatively correlated with other parameters.

3.2- Light microscopic results

Histopathological changes observed in group 2 [MetS] (Fig. 2 E – J), included separation of pia matter with mononuclear cellular infiltrations. Disorganization in cerebral layers with darkly stained neuronal cells (apoptotic cells) characterized by neuronal shrinkage and chromatin condensation, wide intercellular space, in addition multiple areas of vacuolization, brown pigment deposit and swollen edematous neurons. Area of hippocampus showed many elongated darkly stained pyramidal cell, and wide intracellular space. Mild improvement in histological pictures were observed in the group 3 [IGF-1 – started with MetS] (Fig.3) and group 4 [IGF-1 – before induction of MetS] (Fig.4). They showed cerebral cortex with organized layers, small area of mononuclear cellular infiltrations. Number of darkly stained neuronal cells and normal neuronal cells were also observed.

Significant improvements in histological pictures were observed in group 5 [IGF-1 – after induction of MetS] (Fig.5). There was normal pattern cerebral cortex but showing area of loss of pia matter, near normal histo-architecture in the hippocampus, little number of darkly stained pyramidal cells, normal neuronal cells and normal neuroglia cells.

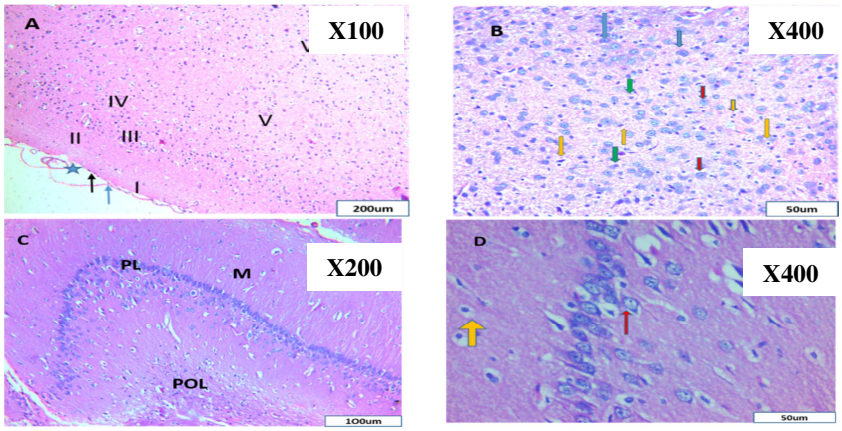

Figure 1: Photomicrographs of cerebral cortex and hippocampus of group1 (control). **A:** cortex showing normal histo-architecture of the cerebral cortex with its six layers I, II, III, IV, V and V. Also, showing layers of meninges the arachnoid layer (blue arrow), pia matter (black arrow) in intermediate lamella (star). **B:** cortex showing many pyramidal cells (blue arrow) multipolar shape with rounded nuclei and basophilic cytoplasm and its dendrites. Granular cells (red arrows) with round open face nuclei, prominent nucleolus and little cytoplasm. Neuroglia cells with its elongated nuclei some of them are in close contact with neurons (yellow arrows). Many interneurons (green arrows). **C:** hippocampus showing molecular cell layer (M), pyramidal cell (PL) and polymorphic cell (POL) layers. **D**: showing pyramidal layer (PL) contained normal pyramidal cell, granular flask shaped cell (red arrow), polymorphic layer (POL) contained neuroglia cells (yellow arrows)

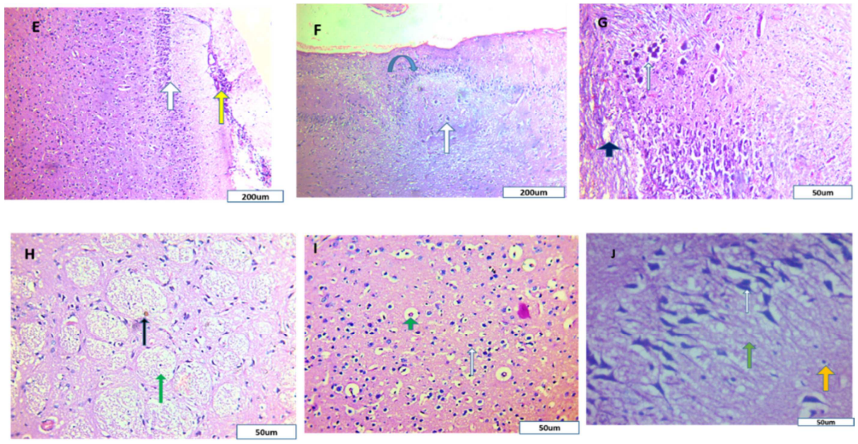

Figure 2: Photomicrographs of cerebral cortex and hippocampus of group 2 (fed on HFD). **E:** cortex showing separation of pia matter with mononuclear cellular infiltrations (yellow arrows), darkly stained neuronal cells (white arrow). **F:** cortex with disorganization in cerebral layers (curved arrow) with darkly stained neuronal cells (white arrow). **G:** cortex showing apoptotic cells characterized by neuronal shrinkage and chromatin condensation (white arrow), degenerative vacuolization, wide intercellular space (thick arrow). **H:** cortex showing multiple area of vacuolization (green arrow), brown pigment deposit (black arrow). **I:** cerebral cortex showing swollen edematous neurons (green arrows) darkly stained shrunken neuronal cells (white arrow). **J:** hippocampus showing many elongated darkly stained pyramidal cell, (white arrow), neuroglia cells (orang arrows) wide intracellular space (green arrow).

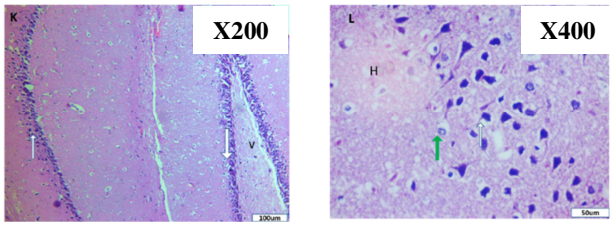

Figure 3: photomicrographs of cerebral cortex and hippocampus of group 3 (IGF-1 – before induction of MetS). **K:** hippocampus showing vacuolated cellular cells (v) and darkly stained shrunken neurons (white arrows) **L:** swollen edematous neuron (green arrow) tiny area of hemorrhage (H)

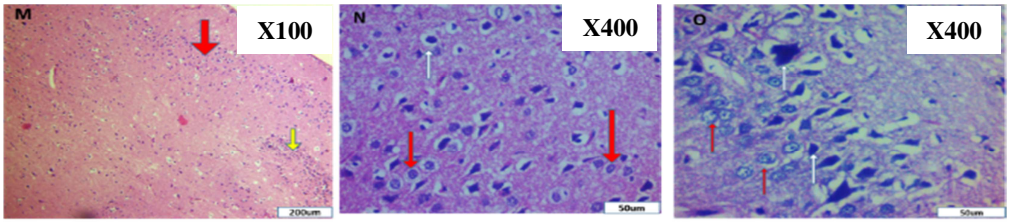

Figure 4: photomicrographs of cerebral cortex and hippocampus of group 4 (IGF-1 – with induction of MetS). **M:** cerebral cortex with organized layers, small area of mononuclear cellular infiltrations (yellow arrows). **N:** cortex and **O:** hippocampus both showing number of darkly stained neuronal cells (white arrow) and normal neuronal cells (red arrow)

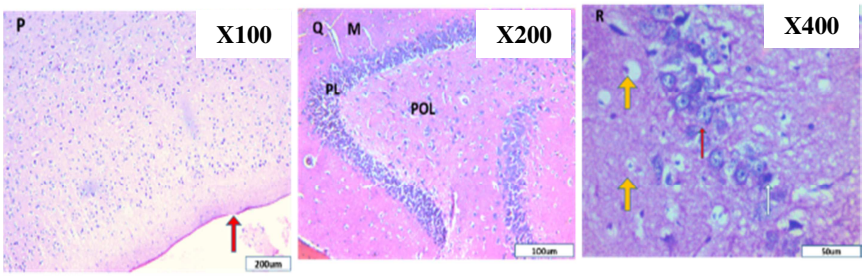

Figure 5: photomicrographs of cerebral cortex and hippocampus of group 5 (IGF-1 – after induction of MetS) **P:** showing normal pattern cerebral cortex with area of loss of pia matter (red arrow). **Q:** near normal histo-architecture in the hippocampus showing molecular cell layer (M), thick pyramidal layer (PL) and polymorphic cell (POL) layers. **R:** little number of darkly stained pyramidal cells (white arrow), normal neuronal cells (red arrow) normal neuroglia cells (orang arrows)

3.3- immunohistochemical results:

Immunohistochemically stained sections for BCL2 showed strong cytoplasmic reactions (red arrow) in group 1 and weak cytoplasmic reactions groups 2 and3 whereas the reaction was moderate in groups 4 and 5.

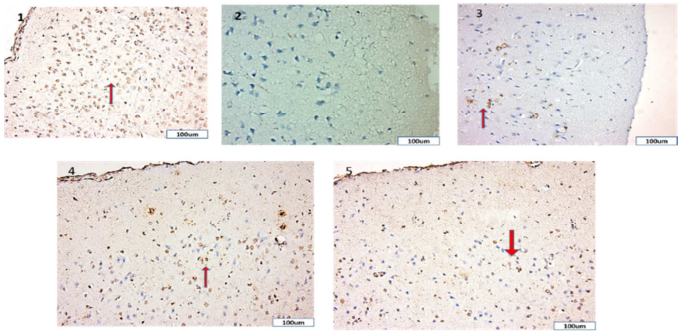

Figure 6: Photomicrographs of immunohistochemical staining of BCL2 (red arrows) in nervous tissue of different groups of the study (X200)

Immunohistochemically stained sections for GFAP showing the distribution and activation of astrocytes in the cerebral cortex of different groups. In group 1, there was a faint positive GFAP immunoreaction-stained area in cytoplasm and processes of astrocytes as they appear small. In groups 2 and 3, there was a strong positive cytoplasmic reaction as astrocytes cytoplasm and processes appeared enlarged. Whereas group 4 showed moderate positive cytoplasmic reaction and group 5 showed weak positive GFAP immunoreaction-stained areas.

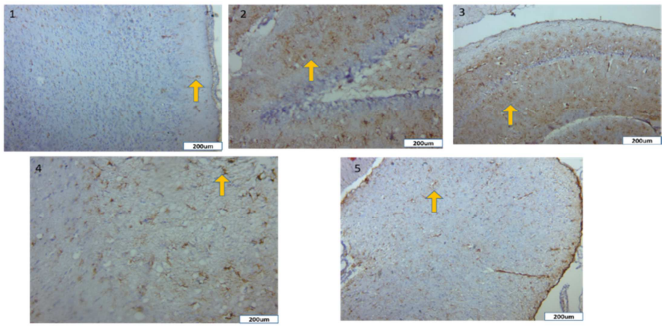

Figure 7: Photomicrographs of immunohistochemical staining of GFAP (yellow arrows) in nervous tissue of different groups of the study (X100)

4- Discussion

The current study showed that administration of HFD to induce MetS led to hyperglycemia, hyperinsulinemia and dyslipidemia. These metabolic derangements were significantly associated with pro-inflammatory state, as evident by significant elevation in TNF-α, and increased oxidative stress, as evident by elevated oxidant marker MDA and reduced anti-oxidant enzyme, GPX. Previous studies found that animals fed HFD exhibited an increase in both glucose and insulin levels, which is related to insulin resistance, and disturbances in lipid metabolism. These metabolic derangements are associated with an increase in oxidative stress and inflammation, making the CNS vulnerable to persistent degenerative process [14].

As a general concept, oxidative stress can potentiate neurotoxicity. reactive oxygen species (ROS) generated by the accelerated oxidative metabolism oxidizes cellular proteins, cell membranes, lipids, DNA, and causing cellular degeneration. The antioxidant enzyme superoxide dismutase catalyzes the reaction of superoxide radicals forming H2O2 which is cleared by GPx and catalase (CAT). Hence CAT activity in the brain is not sufficient and restricted to peroxisomes, GPx is essential in scavenging H2O2 in the brain. Because of its lipid content and high oxygen consumption, the brain is very vulnerable to oxidative stress which subsequently can impair normal CNS function [15] [16]

GLP1 was significantly lower in MetS group as compared to control. This is in agreement with Muscelli et. al. [17] and with Toft-Nielsen et. al. [18] who concluded that the response to glucagon-like peptide-1 is reduced in type 2 diabetes mellitus (T2DM), which lead to the reduced incretin effect. [18].

Incretin is a secretory hormone that is produced from the gut minutes after eating and stimulates pancreatic β-cells to release insulin, resulting in postprandial glucose stability. Glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-like peptide 1 (GLP-1) are two major incretin hormones released by L-cells in the ileum and K-cells in the duodenum and jejunum. Those incretins are the most crucial for 50% to 70% of the postprandial insulin secretion in normal situations. However, the incretin effect may drop to < 20% in T2DM, a situation closely related to MetS. In a majority of studies meal-related GLP-1 secretion was reported to be insufficient in T2DM patients as compared to the normal condition. Nevertheless, other studies demonstrated a lower fasting level of GLP-1 in T2DM patients when compared to normal [19].

Concerning adiponectin, the current study showed a significant decrease in its level in MetS group compared to normal group, a finding that is consistent with Albarracin and Torres [20] who stated that many studies have demonstrated that low plasma concentrations of adiponectin associate closely with MetS and related disorders. Adiponectin is the most abundant adipokine in plasma and is mostly released by white adipose tissue. Many studies have shown that it has insulin-sensitizing, anti-atherogenic, anti-inflammatory properties and anti-oxidant. Furthermore, low adiponectin levels in the blood are associated to MetS-related disorders [21] [22].

The concentration of adiponectin in plasma has an inversely proportional relationship

with MetS characteristics, particularly visceral obesity and insulin resistance. Pro-inflammatory cytokines can reduce adiponectin secretion in cultured adipocytes, indicating that inflammation may play a role in hypoadiponectinemia associated with obesity and insulin resistance. Conversely, physical activity increases the circulating quantity of adiponectin and its receptor expression in muscle, resulting in increased insulin sensitivity and the absence or reduction of MetS components [20].

Brain-derived neurotrophic factor (BDNF) is a protein that belongs to the neurotrophin family, which contributes to neuroprotection and neuroregeneration. Neurotrophins are mostly generated in the CNS, although they are also synthesized in non-neuronal peripheral tissues including B and T lymphocytes, vascular endothelial cells, smooth and skeletal muscle [23]. This neurotrophic factor is important in the modulation of higher cognitive function and memory. The BDNF gene is expressed in the cortex, basal forebrain areas, and hippocampus, which are critical for memory, learning, and higher cognitive performances. BDNF increases synaptic development and plasticity, as well as neurogenesis and neurotransmission across synapses. BDNF is necessary for memory formation as it causes long-term potentiation in the hippocampus nucleus [24]. Importantly, neurodegenerative and neuropsychiatric disorders are frequently associated with inadequate neuronal supply of BDNF and other neurotrophic elements [23]**.** The current study showed that induction of MetS by HFD was associated by significant decrease in BDNF. A finding which is consistent with Molteni et. al. [25], Singh et. al. [26] and Motamedi et. al. [27]. A high-fat diet and greater physical activity decrease or increase BDNF levels, respectively. Pre-inflammatory signals originating from adipose tissue influence many other bodily organs and may even induce neuro-degenerative diseases [27]. Low BDNF concentrations have been documented in patients with insulin resistance, metabolic syndrome, and type 2 diabetes; all of which are related with an increased risk of cognitive dysfunction and dementia. A change in lifestyle, such as calorie restriction and fitness training, can boost BDNF levels [28].

The current study showed an elevated synapsin 1 level in rats with MetS compared to control ones. The subclinical inflammatory state and increased oxidative stress associating MetS have negative impact on cognitive functions [29]. Synapsin 1, a highly abundant synaptic protein, plays important roles in both neuronal development and synaptic physiology. It is important for synaptic communication and neuronal plasticity. At synapses, neurotransmitter-containing vesicles constitute dense clusters; during prolonged activity, these clusters serve as a reservoir from which vesicles are pulled for exocytosis. Synapsin 1, among other components, helps forming such clusters [30] [31]**.** The recorded increase in synapsin 1 level in the current study may be compensatory mechanism attempting to limit the negative impact of MetS on cognitive function. However, Gao et. al. [32] reported a decrease in synapsin 1 level in HFD induced MetS. On the other hand, Wei et. al. [33] reported no significant change in synapsin 1 level in rats injected by streptozotocin in their hippocampi. Like MetS, streptozotocin induces oxidative stress and inflammation. This controversy may be attributed to the duration and the severity of MetS. The onset and duration of MetS and related derangements may have some influence on synaptic plasticity. However, short-term acute changes may not, unless extremely severe [34].

The current study showed significant elevation in sortilin level in MetS group compared to control group. This is consistent with Su et. al. [35] who reported that sortilin is highly suggestive to be correlated with the development and severity of MetS and its complications. Also, this result conicoid with El-Khodary et. al. [36] who stated that sortilin has been linked with dyslipidemia and with the risk of developing atherosclerosis as well as the possibility of acquiring atherosclerosis. Sortilin have a critical role in hepatic and muscular insulin response, indicating that it may be associated with insulin resistance and MetS [36]. Sortilin, among multiple factors which regulate the development of cardio metabolic syndrome, has been found in multiple cell types, such as adipocyte, hepatocyte, macrophage, and brain cells [35].

Sortilin can be detected in the circulation and within cellular compartments at important sites such as the trans-Golgi network and cell membrane. It is essential in numerous biological processes, such as lipid or glucose metabolism, as well as atherosclerosis [36]. It is also essential for membrane signal transduction, intracellular trafficking and protein sorting [37] [38].

Being related to the development of atherosclerosis and to intracellular protein sorting & trafficking, sortilin can share in the pathogenesis of cognitive disorders. Atherosclerosis is thought to play a role in the evolution of dementia, primarily through the development of Alzheimer's disease and vascular dementia [39].

Data from recent studies indicate that the vacuolar protein sorting 10p (Vps10p) family, particularly sortilin, is involved in the development

of cognitive disorders and dementia. Sortilin has a critical role in the generation of amyloid plaques, aberrant protein sorting, tau phosphorylation, and cellular apoptosis [37].

Sortilin is primarily found in the cerebral cortex, the hippocampal formation, subcortical dopaminergic, noradrenergic, and cholinergic neurons. Sortilin plays a role in brain ageing, mood disorders, and the etiology of Alzheimer's disease (AD) via interacting with β-amyloid precursors, β-secretase-1 and tau. Sortilin can create a unique C-terminal fragment that forms senile plaque-like lesions in the brains of patients of Alzheimer's disease. Sortilin may also plays a critical role in protein sorting and transport into the lysosome system, which is important for intracellular protein homeostasis, but may be related to the intraneuronal proteinopathies [40].

This study showed that treatment with IGF-1 showed significant improvement in glucose, insulin, lipid profile, adiponectin, TNF-α, MDA, GPx, BDNF, synapsin1 and sortilin.

Insulin-like growth factor 1 (IGF-1), anabolic hormone that is synthesized in the liver, regulates various biological functions associated with growth hormone (GH), particularly insulin metabolism and cell proliferation, differentiation, and apoptosis. A reduced blood IGF-1 level is frequently associated with MetS, T2DM, and cardiovascular disease [41]. IGF-1 increases insulin sensitivity, glucose absorption, lipid metabolism, and possess anti-inflammatory properties [42]. Lower IGF-1 levels are frequently associated with insulin resistance and metabolic syndrome, implying that IGF-1 holds therapeutic potential in the treatment of MetS. Patients with T2DM consistently responded to IGF-1 therapy with better glucose tolerance, hyperinsulinemia, and dyslipidemia. This could be because it has a similar glucose-lowering effect to insulin [43]. Evidence suggests that IGF-1 can be a viable therapy for MetS. IGF-1 has been involved in a variety of physiological effects, including anabolic, and antioxidant capabilities, tissue growth and development, proliferation, anti-aging, anti-inflammatory, as well as neuroprotection and hepatoprotective qualities. Additionally, IGF-1 protects mitochondria from oxidative damage caused by free radical generation and increased metabolism [44].

The histopathological manifestations and the decrease in immunoreactivity of BCL2 (which is antiapoptotic) in MetS group are attributed to oxidative stresses and the prevalence of the pro-infalimatory mediators associating MetS [45] [46]. It had generally been suggested that hyperglycemia enhances neuronal damage [47]. Regarding the multiple areas of neuronal vacuolations and swollen edematous neurons, it might be occurred as a result of lipid peroxidation and accumulation of sodium in the cell leading to an increase in water content in the cell with subsequent swelling [45]. The current work showed that the number of GFAP-positive astrocytes significantly increased in MetS group compared to control group. This microglial activation is linked to many factors including hyperglycemia (via the formation of advanced glycation end products or other protein glycation products) [48], oxidative stress [49] and systemic low-grade inflammation caused by MetS [46]. The current study showed that treatment with IGF1 induced a significant improvement in histological picture; as well as significant increase in number of BCL2 positive cells associated with significant decrease in number of GFAP positive cells whether the treatment with IGF1was started with, or before, or after induction of MetS.

Despite the fact that IGF-1 therapy results in significant metabolic improvement and therapeutic benefit, the long-term safety of IGF-1 remains a major clinical issue. Long-term recombinant human IGF-1 medication has been linked to a variety of side effects, including cancer development, cataract, and renal hypertrophy, according to previous of clinical trials [43]. However, it should be noted that: (1) These complications are usually rare, (2) Apart of neoplastic formation, other complications were transient, well tolerated and easy managed without treatment discontinuation, (3) These clinical trials have been evaluated by administration of high doses (80 µg – 4 mg/kg BW/day) and ranging in a long spectrum of lengths (months). On the other hand, murine studies utilized short cycles and a very low doses (20 µg/kg BW/day) were associated with no such recorded side effects, (4) Regardless of the limitations of the animal studies, in previous clinical trial for evaluating IGF-1 in liver function and cirrhosis using small dosage (20 µg/kg/day), conversely no documented side effects and liver functions were even enhanced. Yet, the use of IGF-1 still requires more depth study despite the known benefit on insulin resistance and glucose metabolism [43] [44] [50].

Furthermore, the well-known effect of exercise on rising IGF-1 concentration in plasma is significant. For many years, it has been shown that following only single session of moderate to high-intensity exercise, total IGF-1 plasma level rises by 10 to 30% and reaches the peak within 5-10 minutes after the training begins. One of the most effective IGF-1 synthesis and priming methods is to exercise. It appears plausible to designate IGF-1 as a

potential target for multifactorial therapy of metabolic syndrome [44] [51].

5- Conclusion: continuously accumulating evidences about progression of MetS to complications affecting almost all body system. It is not only the traditionally known macro- and micro-vascular complications including strokes, ischemic heart disease, retinopathy, nephropathy, neuropathy and peripheral vascular diseases, but also MetS is now implicated in the pathogenesis of cognitive related disorders including Alzheimer's disease. The underlaying pathogenic mechanisms are pleotropic including oxidative stress, subclinical inflammation and many other molecular mechanisms including, for example but not limited, the subject of the current study; BDNF & sortilin which share in the pathogenesis in MetS associated cognitive dysfunction. Being have pleotropic pathogenic mechanisms, MetS should be confronted with strategies that are pleotropic in their action. Healthy dietary habits and exercise are pivotal. IGF1 can meet many of the pathological aspects of MetS being insulin sensitizer, anti-oxidant and anti-infalimatory. However, further studies are needed to determine suitable approaches concerning dose and duration of such a promising agent.

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9- References

[1] H. H. Wang, D. K. Lee, M. Liu, P. Portincasa, and D. Q. H. Wang, "Novel insights into the pathogenesis and management of the metabolic syndrome," 2020.

[2] V. H. Taylor and G. M. MacQueen, "Cognitive dysfunction associated with metabolic syndrome," Obes. Rev., vol. 8, no. 5, pp. 409–418, 2007.

[3] Y. Y. Borshchev, Y. P. Uspensky, and M. M. Galagudza, "Pathogenetic pathways of cognitive dysfunction and dementia in metabolic syndrome," Life Sci., vol. 237, p. 116932, 2019, doi: https://doi.org/10.1016/j.lfs.2019.116932.

[4] M. Guicciardi, A. Crisafulli, A. Doneddu, D. Fadda, and R. Lecis, "Effects of metabolic syndrome on cognitive performance of adults during exercise," Front. Psychol., vol. 10, p. 1845, 2019.

[5] I. Cova, A. Markova, I. Campini, G. Grande, C. Mariani, and S. Pomati, "Worldwide trends in the prevalence of dementia," J. Neurol. Sci., vol. 379, pp. 259–260, 2017.

[6] C.-H. Perng, Y.-C. Chang, and R.-F. Tzang, "The treatment of cognitive dysfunction in dementia: a multiple treatments meta-analysis," Psychopharmacology (Berl)., vol. 235, no. 5, pp. 1571–1580, 2018.

[7] S. T. Ferreira, "Brain insulin, insulin-like growth factor 1 and glucagon-like peptide 1 signalling in Alzheimer's disease," J. Neuroendocrinol., vol. 33, no. 4, p. e12959, Apr. 2021, doi: https://doi.org/10.1111/jne.12959.

[8] N. Auberval et al., "Beneficial effects of a red wine polyphenol extract on high-fat diet-induced metabolic syndrome in rats," Eur. J. Nutr., vol. 56, no. 4, pp. 1467–1475, 2017.

[9] J. R. De Ita et al., "Altered liver expression of genes involved in lipid and glucose metabolism in mice with partial IGF-1 deficiency: an experimental approach to metabolic syndrome," J. Transl. Med., vol. 13, no. 1, pp. 1–13, 2015.

[10] C. H. Gergye, Y. Zhao, R. H. Moore, and V. K. Lee, "A comparison of ketamine or etomidate combined with xylazine for intraperitoneal anesthesia in four mouse strains," J. Am. Assoc. Lab. Anim. Sci., vol. 59, no. 5, pp. 519–530, 2020.

[11] E. Kindt et al., "Development and validation of an LC/MS/MS procedure for the quantification of endogenous myo-inositol concentrations in rat brain tissue homogenates," Anal. Chem., vol. 76, no. 16, pp. 4901–4908, 2004.

[12] J. D. Bancroft and C. Layton, "Connective and other mesenchymal tissues with their stains," Bancroft's Theory Pract. Histol. Tech. E-b., vol. 153, 2018.

[13] R. Levesque, "SPSS programming and data management," A Guid. SPSS SAS Users, 2007.

[14] G. Muñoz-Arenas et al., "Effects of metformin on recognition memory and hippocampal neuroplasticity in rats with metabolic syndrome," Synapse, vol. 74, no. 9, p. e22153, 2020.

[15] G. Sharma et al., "Glutathione peroxidase-1 and neuromodulation: Novel potentials of an old enzyme," Food Chem. Toxicol., vol. 148, p. 111945, 2021, doi: https://doi.org/10.1016/j.fct.2020.111945.

[16] S. Salim, "Oxidative stress and the central nervous system," J. Pharmacol. Exp. Ther., vol. 360, no. 1, pp. 201–205, 2017.

[17] E. Muscelli et al., "Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients," Diabetes, vol. 57, no. 5, pp. 1340–1348, 2008.

[18] M.-B. Toft-Nielsen et al., "Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients," J. Clin.

Endocrinol. Metab., vol. 86, no. 8, pp. 3717–3723, 2001.
[19] S. Yoo et al., "Impact of longitudinal changes in metabolic syndrome status over 2 years on 10-year incident diabetes mellitus," Diabetes Metab. J., vol. 43, no. 4, pp. 530–538, 2019.
[20] M. L. G. Albarracín and A. Y. F. Torres, "Adiponectin and Leptin Adipocytokines in Metabolic Syndrome: What Is Its Importance?," Dubai Diabetes Endocrinol. J., vol. 26, no. 3, pp. 93–102, 2020.
[21] H. M. Choi, H. M. Doss, and K. S. Kim, "Multifaceted physiological roles of adiponectin in inflammation and diseases," Int. J. Mol. Sci., vol. 21, no. 4, p. 1219, 2020.
[22] T. M. D. Nguyen, "Adiponectin: role in physiology and pathophysiology," Int. J. Prev. Med., vol. 11, 2020.
[23] E. Palasz, A. Wysocka, A. Gasiorowska, M. Chalimoniuk, W. Niewiadomski, and G. Niewiadomska, "BDNF as a promising therapeutic agent in Parkinson's disease," Int. J. Mol. Sci., vol. 21, no. 3, p. 1170, 2020.
[24] T. K. S. Ng, C. S. H. Ho, W. W. S. Tam, E. H. Kua, and R. C.-M. Ho, "Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): a systematic review and meta-analysis," Int. J. Mol. Sci., vol. 20, no. 2, p. 257, 2019.
[25] R. Molteni, R. J. Barnard, Z. Ying, C. K. Roberts, and F. Gomez-Pinilla, "A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning," Neuroscience, vol. 112, no. 4, pp. 803–814, 2002.
[26] R. B. Singh et al., "Effect of brain derived neurotrophic factor, in relation to diet and lifestyle factors, for prevention of neuropsychiatric and vascular diseases and diabetes," Open Nutraceuticals J., vol. 7, no. 1, 2014.
[27] S. Motamedi, I. Karimi, and F. Jafari, "The interrelationship of metabolic syndrome and neurodegenerative diseases with focus on brain-derived neurotrophic factor (BDNF): Kill two birds with one stone," Metab. Brain Dis., vol. 32, no. 3, pp. 651–665, 2017.
[28] A. Jamali, S. Shahrbanian, and S. Morteza Tayebi, "The effects of exercise training on the brain-derived neurotrophic factor (BDNF) in the patients with type 2 diabetes: A systematic review of the randomized controlled trials," J. Diabetes Metab. Disord., vol. 19, no. 1, pp. 633–643, 2020.
[29] E. Rojas-Gutierrez et al., "Alzheimer's disease and metabolic syndrome: A link from oxidative stress and inflammation to neurodegeneration," Synapse, vol. 71, no. 10, p. e21990, 2017.
[30] C. Hoffmann et al., "Synapsin Condensates Recruit alpha-Synuclein," J. Mol. Biol., vol. 433, no. 12, p. 166961, 2021, doi: https://doi.org/10.1016/j.jmb.2021.166961.
[31] I. Buenger et al., "Maternal synapsin 1 autoantibodies are associated with neurodevelopmental delay," medRxiv, 2022.
[32] X.-R. Gao, Z. Chen, K. Fang, J.-X. Xu, and J.-F. Ge, "Protective effect of quercetin against the metabolic dysfunction of glucose and lipids and its associated learning and memory impairments in NAFLD rats," Lipids Health Dis., vol. 20, no. 1, pp. 1–15, 2021.
[33] Y.-D. Wei et al., "Resveratrol ameliorates learning and memory impairments induced by bilateral hippocampal injection of streptozotocin in mice," Neurochem. Int., vol. 159, p. 105385, 2022.
[34] D. Cheng, S. Yang, X. Zhao, and G. Wang, "The Role of Glucagon-Like Peptide-1 Receptor Agonists (GLP-1 RA) in Diabetes-Related Neurodegenerative Diseases," Drug Des. Devel. Ther., vol. 16, p. 665, 2022.
[35] X. Su, L. Chen, X. Chen, C. Dai, and B. Wang, "Emerging roles of sortilin in affecting the metabolism of glucose and lipid profiles," Bosn. J. Basic Med. Sci., vol. 22, no. 3, p. 340, 2022.
[36] N. M. El-Khodary, H. Dabees, and R. H. Werida, "Folic acid effect on homocysteine, sortilin levels and glycemic control in type 2 diabetes mellitus patients," Nutr. Diabetes, vol. 12, no. 1, pp. 1–8, 2022.
[37] S.-Y. Xu, J. Jiang, A. Pan, C. Yan, and X.-X. Yan, "Sortilin: a new player in dementia and Alzheimer-type neuropathology," Biochem. Cell Biol., vol. 96, no. 5, pp. 491–497, 2018.
[38] S. Eggert, C. Thomas, S. Kins, and G. Hermey, "Trafficking in Alzheimer's disease: modulation of APP transport and processing by the transmembrane proteins LRP1, SorLA, SorCS1c, sortilin, and calsyntenin," Mol. Neurobiol., vol. 55, no. 7, pp. 5809–5829, 2018.
[39] P. Mohseni-Moghaddam, R. Ghobadian, and H. Khaleghzadeh-Ahangar, "Dementia in Diabetes mellitus and Atherosclerosis; Two Interrelated Systemic Diseases," Brain Res. Bull., 2022.
[40] S.-Y. Xu et al., "Regional and cellular mapping of sortilin immunoreactivity in adult human brain," Front. Neuroanat., vol. 13, p. 31, 2019.
[41] X. Yang et al., "The influence of metformin on IGF-1 levels in humans: a systematic review and meta-analysis," Pharmacol. Res., vol. 151, p. 104588, 2020.
[42] S. Xie et al., "The relationship between serum-free insulin-like growth factor-1 and metabolic syndrome in school adolescents of northeast China," Diabetes, Metab. Syndr. Obes. Targets Ther., vol. 12, p. 305, 2019.
[43] J. Ren and P. Anversa, "The insulin-like growth factor I system: physiological and pathophysiological implication in cardiovascular diseases associated with metabolic syndrome," Biochem. Pharmacol., vol. 93, no. 4, pp. 409–417, 2015.
[44] G. A. Aguirre, J. R. De Ita, R. G. De La Garza, and I. Castilla-Cortazar, "Insulin-like growth factor-1 deficiency and metabolic syndrome," J. Transl. Med., vol. 14, no. 1, pp. 1–23, 2016.

[45] S. Z. Naqi, "A Comparative Study of the Histological Changes in Cerebral Cortex, Hippocampus, Cerebellum, Pons & Medulla of the Albino Rat Due to Lead Toxicity.," 2015.
[46] W. Zhang, H. Liu, M. Rojas, R. W. Caldwell, and R. B. Caldwell, "Anti-inflammatory therapy for diabetic retinopathy," Immunotherapy, vol. 3, no. 5, pp. 609–628, 2011.
[47] C. Ding, Q. P. He, and P. A. Li, "Diabetes mellitus causes early ultrastructural changes to the nuclei and mitochondria of neurons and astrocytes in rats subjected to a brief period of cerebral ischemia," Microsc. Microanal., vol. 11, no. S02, pp. 1010–1011, 2005.
[48] S. F. Abcouwer, "Neural inflammation and the microglial response in diabetic retinopathy," J. Ocul. Biol. Dis. Infor., vol. 4, pp. 25–33, 2011.
[49] G. Baydas, R. J. Reiter, A. Yasar, M. Tuzcu, I. Akdemir, and V. S. Nedzvetskii, "Melatonin reduces glial reactivity in the hippocampus, cortex, and cerebellum of streptozotocin-induced diabetic rats," Free Radic. Biol. Med., vol. 35, no. 7, pp. 797–804, 2003.
[50] M. Conchillo et al., "Insulin-like growth factor I (IGF-I) replacement therapy increases albumin concentration in liver cirrhosis: results of a pilot randomized controlled clinical trial," J. Hepatol., vol. 43, no. 4, pp. 630–636, 2005.
[51] R. Agrawal and F. Gomez-Pinilla, "'Metabolic syndrome'in the brain: deficiency in omega-3 fatty acid exacerbates dysfunctions in insulin receptor signalling and cognition," J. Physiol., vol. 590, no. 10, pp. 2485–2499, 2012.