Dietary Malted Barley Grain Improved Structure and Function of Spinal Cord of Mother Rats fed on a High Cholesterol Diet

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High cholesterol diet is associated cardiovascular disorder and other metabolic diseases represent the public health problem. However, little detail information about its effects on spinal cord structure and function. Barley is one of the cereal grains rich of nutrients and shows medicinal importance. The present study assessed its role on hypercholesterolemia induced toxicological aspects on spinal cord. In the present study, sixty virgin female and twenty adult male albino rats (Rattus norvegicus) weighing approximately 100±10gm body weight, at ratio of 1 male/3 females were used for experimental work. Virgin females were fed 4 months before matting on a diet containing 3 % cholesterol. The virgin females of both control and hypercholesterolemic females were mated with fertile males (1male/3 females) for overnight and observing sperm in vaginal smears in the next morning for determining onset of gestation. The pregnant were arranged into 4 groups (n=15) ; control (C), barley supplemented group (B) (10%), hypercholesterolemic-group (H) (3%) and experimental hypercholesterolemic & malted barley group (H+B). Dams of both control and experimental groups were sacrificed post 21 weeks post-partum and their cervical spinal cord were removed. The spinal tissues were processed for histological, immunohistochemical of caspase 3 and glial fibrillary acidic protein (GFAP) and transmission electron microscopy. In addition, biochemical assessments of antioxidant enzymes (SOD, GSH), malondialdhyde, phospholipids, iron, caspase 4, 8-hdG, neutrotransmitters (DA-5-HT and 7ABA),cytokines(IL6717), leptin, homocysteine and amyloid–β. The present findings clarified that dietary supplementation on malted barley for mothers fed on a high cholesterol diet throughout pregnancy and lactation period improved the picture of neurons at light and ultrastructural level, restored myelination and reduced caspase 3 and GFAP immunohistochemistry. At the levels of the assessed biomarkers, the malted barley restored the levels of the assayed of both the antioxidant enzymes and neurotransmitters and phospholipids and reduced the high level of caspase 3, 8-hdG, cytokines,leptin, homocysteine, amyloid–β and iron.Thus , the malted barley can be used in combination with other pharmaceutical drugs in improvement or prevention the neurotoxicity due to elevated blood cholesterol level.

Keywords: Spinal cord; High Cholesterol Diet; Immunohistochemistry; Barley; Toxicological Aspects.
found to cause irregular changes in biosynthesis of cholesterol contributing to spine injury (Spann et al., 2017). Higher risk of Alzheimer’s disease is present in patients with spinal cord injury (Yeh et al., 2018). It is known that the intracellular cholesterol is important in biological function of endolysosomes and mitochondria via its passage across their membranes. Abnormal passage resulted in its accumulation in mitochondria and lysosomes and leading to the development of neurodegenerative diseases (Arenas et al., 2017). Increased extracellular cholesterol levels was associated with neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease (Zhang and Liu, 2015). Also, increased LDL cholesterol level predicted the development of breast cancer (Cedó et al., 2019). Consequently promising research studies based on chelating the increased cholesterol level by methyl-ß-cyclodextrin as well as sensitizing either tamoxifen (Mohammad et al., 2014) or doxorubicin (Mohammad et al., 2015) to overcome the development of cancer cells in breast and skin.

Barley is one of the most cereal crops belong to Magnoliophyta (Class Liliopsida (Monocotyledons), order : Cyperales, Family : Poaceae (Grass), Genus : Hordeum vulgare) widely cultivated in the Middle East (Newman and Newman, 2008). The protein content attained to 10-17% of its total mass especially hordeins (prolamins) comprising 40% to 50% (Anderson, 2013; Magliano et al., 2014). Barley is rich in phenolic (benzoic and cinnamic acid derivates, flavonoids, chalcones, tannins, quinones, proanthocyanidins) (Dvorakova et al., 2008, Carvalho et al., 2015), hydroxyl cinnamic acid derivates such as p-coumaric, caffeic, ferulic and sinapic acids (Liu et al., 2007; Kim et al., 2007), flavonoids, lignans, tocols, phytosterols, and folate ((Idehen et al., 2017) which exhibited strong antioxidant, antiproliferative, and cholesterol lowering activities (Idehen et al., 2017). ß-glucans showed hypolepidemia especially of cholesterol and triglyceride levels in animal model (Kalra and Jood, 2000) and human (Wilson et al., 2004; AbuMweis et al., 2010; McRorie and McKeown, 2017) through activation of cholesterol excretion cholesterol 7a-hydroxylase (Abumweis et al., 2010; Wang et al., 2017).

There is a little of work illustrated the hypercholesterolemia associated the development of histopathology and cytological lesions of the spinal cord coincides with the biochemical markers of lesions. At the same time illustrating the phytomedicinal importance of dietary inclusion of malted barley in improving high cholesterol diet associated the neurotoxicity.

**MATERIALS AND METHODS**

**Composition of a high cholesterol diet**

The high cholesterol diet is composed of 3% cholesterol plus 7% animal fat plus 2% cholic acid and 2% thiouracil in conjugation with other components of standard diet according to Enkhmaa et al. (2005). Rats were fed on it for 4 months prior to conception and throughout pregnancy and lactation period.

**Diets Containing Malted Barley**

Malted barley was prepared three days before mixing with normal diet by wettingbarley grains with water at a ratio of 20 percent. Throughout pregnancy and lactation time the hypercholesterolemic pregnant groups were fed on a diet that included fermented barley.

**Experimental Animal**

This research and all procedures had been authorised by the Egyptian Committee’s Animal Care and Bioethics. Sixty virgin female and twenty adult male albino rats (Rattus norvegicus) weighing approximately 100±10gm body weight, obtained from Helwan Breading Farm, Ministry of Health, Egypt were used for experimentation.

They were allowed in good aerated room with nearly 12 hours of light and dark cycle for acclimatization. Free access of food and water were allowed ad libitum. Pregnancy was carried out by keeping each normal fertile male with three females for overnight and examining vaginal smears in the next morning for observing sperm and zero date of pregnancy was determined. The pregnant were arranged into 4 groups (n=15) as follows: Control (C), barley supplemented group (B) (10%), experimental hypercholesterolemic-group (H) (3%) and experimental hypercholesterolemic & fermented barley group (H+B). Dams of both control and experimental groups were sacrificed at 1, 2 and 3 weeks post-partum.
The Following Parameters Were Investigated

**Histological Investigation**

Cervical spinal cord of mother rats of the studied groups were separated and fixed immediately in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending series of ethyl alcohol, cleared in xylene and mounted in molten paraplast at 58-62°C. Five µm histological sections were cut, stained with Hematoxylin& eosin (Weesner, 1968) and examined under a bright field light microscope.

**Transmission electron microscopy (TEM)**

Cervical spinal cord of the studied groups were fixed in 2.5% glutaraldehyde buffered in 0.1M cacodylate buffer (pH 7.4). These was followed by post-fixed in 1% osmium tetraoxide at 4°C. dehydration in ascending concentrations of ethyl alcohol, cleared in propylene oxide and embedded in epoxy–resin. Ultrathin sections were cut on a LKB Ultratome IV (LKB Instruments, Bromma, Sweden) and mounted on grids, stained with uranyl acetate and lead citrate, and examined under a Joel 100CX transmission electron microscope at Mansoura University Lab, Egypt.

**Immunohistochemistry for PCNA and GFAP**

Five µm histological sections of formalin-fixed, paraffin-embedded cerebrum tissue were placed on polylysine-coated glass slides. After overnight packing at 65°C, tissue sections were deparaffinized in xylene and rehydrated in descending grades of alcohol. Endogenous peroxidase activity was removed by incubation of tissue sections in 3% H2O2 for 10 minutes at room temperature. The tissue sections were placed in digested media composed of 0.05% trypsin (pH 7.8) for 15 minutes at 37°C and incubated with the primary monoclonal mouse antibody of glial fibrillary acidic protein GFAP (DAKO, clone MIB5, 1:50, mouse) and primary antibody against proliferating cell nuclear antigen PCNA (DAKO, clone MIB5, 1:50, mouse) at 1:50 overnight at 4°C. After washing, the slides were incubated with a secondary biotin linked anti-mouse antibody for 50 minutes at room temperature; and with the streptavidin-peroxidase complex for 50 minutes. Sections were then washed and incubated with developing solution (diaminobenzidine-hydrogen peroxide; DAKO), and counterstained with hematoxylin. The immune reaction was visualized as brown nuclear or cytoplasmic labeling counterstained with hematoxylin. Sections incubated with 1% nonimmune serum phosphate buffer solution (PBS) solution served as negative controls. Finally, the sections were examined under bright field light Olympus microscope with a digital canon camera.

**Biochemical Assays**

Cervical spinal cord tissue of both the control and experimental groups were homogenized in Tris buffer at pH 7.5 and separated, their supernatant stored in a deep freeze.

**Determination of Superoxide Dismutase and Glutathione-S-Reducate Activities**

It was determined according to Niskikimi et al. (1972) and based on the inhibition of nitroblue tetrazolium (NBT). The reduction of NBT by superoxide radicals to blue colored formazan was assayed at 560 nm. Glutathione S-transferases (GSH) is determined according to For glutathione S-transferases (GST), the conjugation of 1-chloro-2,4- dinitrobenzene with reduced glutathione is accompanied by an measuring the absorbance at 340 nm according to Prins and loose (23). GST Activity: Tissue (U/g tissue) = A340 / min x 2.812/g.

Where

O.D. of sample = optical density of sample – optical density of blank.10 = dilution during extraction.

4.7/2 = dilution during color development. Σ 412 = molar extension coefficient equal 13600. 30.7 = molecular weight of glutathione.

**Determination of Lipid Peroxidation end Product Malonaldehyde (Thiobarbituric Acid Reactive Substances, TBARS) Level**

It is carried according to Ohkawa et al. (1979) based on the developed reddish pink color and estimated at 532nm which indicates the extent of peroxidation and expressed as nmol/ mg protein.

**Determination of 8-Hydroxy-2-Deoxy Guanosine (8-hdG)**

The amount of 8-HdG was determined using the Bioxytech 8-HdG -ELISA Kit (OXIS Health Products, Portland, OR, USA, Catalog. No. KOG-200S/E) according to the manufacturer’s instructions. The reaction was terminated and the absorbance was measured using a FLUO star OMEGA microplate reader (BMG LABTECH Ltd., Germany) at a wavelength of 450nm (Attia,
Values of 8-OHdG are expressed as mg/ml. **Determination of Caspase-3**

It is determined colorimetrically by using a Stressgen kit (catalog No. 907-013). The cleavage of the peptide can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

**Neurotransmitters**

?-amino-butyric acid, dopamine and serotonin were measured by high-performance liquid chromatography (HPLC) using the pre-column PTC derivatization technique according to the method of Heinrikson and Meredith (1984). The assay conditions were as follows: Temperature: 46oC; wave-length: 254 nm; flow rate: 1ml/min. Dopamine and serotonin were assayed HPLC according to the method described by Pagel et al.

**Fig. 1.** (A-C1). Photomicrographs of cross histological section of cervical spinal cord of mother rat. Note degenerated ependymal lining canal (DELC) and degenerated multipolar neuronal cells (DMNC) (Fig. B &B1) compared to normal structure of control (Fig. A1&A2).C&C1. Showing improved ependymal canal and neuronal cells of mother fed on a high cholesterol diet with barley. HE Abbreviations; DELC, degenerated ependymal lining canal; DMNC, degenerated multipolar neuronal cell; EC, ependymal canal; ELC, ependymal lining canal; MNC, multipolar neuronal cell
(2000). It is based on removal of trace element and lipid from the tested samples by solid phase extraction CHROMABOND column NH2 phase Cat. No. 730031 and injected directly into an AQUA column 150 54.6 mm (Phenomenex, USA).

**Determination of Interleukin Level**

Interleukin 6 (catalogue No. SEA079Ra), 10 (catalogue No. SEA056Ra) and interleukin 17 (Catalogue No. SEA063Ra) were estimated in cerebrum tissue by using Enzyme-linked Immunosorbent Assay Kit of Cloud-Clone Corp. **Assessments of Phosphatidylethanolamine, Sphingomyelin and Polyenylphosphatidylcholine**

**Phosphatidylethanolamine (PE), Sphingomyelin (SM) and** polyenylphosphatidylcholine (PPC) were estimated in spinal cord tissues by Parker and Peterson (1965).

**Fig. 2.** Transmission electron micrographs of cervical spinal cord of mother rat. A-C Control. A&B. Showing normal neuronal cell with characteristic nuclei (N) and cytoplasm rich in mitochondria (M) and rough endoplasmic reticulum (RER). C. Showing normal myelinated axons containing mitochondria (M) in their neuropil. Arrow head indicate myelinated axons. Star illustrating mitochondria. A1-C1. Mother fed on a high cholesterol diet. A1&B1. Showing neuron cells with pyknotic nuclei (PN) and cytoplasm containing damaged mitochondria (DM) and vesiculated rough endoplasmic reticulum. C1. Showing demyelinated axons with damaged mitochondria (DM) in the neuropil. Arrowhead showing demyelinated axons Star showing damaged mitochondria. A2-C2. Mother fed on a high cholesterol diet with barley. A2. Showing improved neuronal cell with characterized nuclei (N) surrounding by nuclear envelope (NE) and cytoplasm containing few vacuoles (V) and intact mitochondria (M). B2. Showing less improved neuronal cell containing nuclei (N) with convoluted nuclear envelope (NE) and normal myelinated axons (MA) through the neuropil. C2. Showing improved myelinated axons (MA) containing mitochondria (M). Star showing normal mitochondria in the inner compartment of myelinated axon. Arrow head showing myelinated axon. Abbreviations; M, mitochondria; MA, myelinated axons; N, nucleus; NE, nuclear envelope; V, vacuoles. Star showing normal mitochondria in the inner compartment of myelinated axon. Arrowhead showing myelinated axon. Stars means myelinated axons.
**Determination of Leptin, Homocysteine and Amyloid–β**

Leptin (LEP, catalogue no. SEA084Ra), Homocysteine (Hcy, catalogue no. CSB-E13376r) and amyloid-B42 (Ab1-42, catalogue no. CEA946Ra) were estimated by using Enzyme-linked Immunosorbent Assay Kit of Cloud-Clone Corp.

**Determination of Iron Concentration**

Cervical spinal cord samples of the studied groups were digested by 1mL of nitric acid at highest purity and diluted with 4mL bi-distilled water and their iron concentration was measured by atomic absorption spectrometry (Scancat et al., 2000).

**Statistical Analysis**

The means value obtained in the different groups were compared by one-way post-hoc analysis of variance (ANOVA) test. All results were expressed as mean±standard error (SE) and significance was recorded at p <0.05.

**RESULTS**

**Light and Ultra-Structural of Mother**

At light microscopic level, the cervical spinal cord of mother rat fed on a high cholesterol diet showed damaged epithelial cells lining the ependymal canal as compared to the control (Fig.1A&A1). Necrotic spots have been identified. The multipolar neuronal cells become shrinked and enclosed in a halo spaces. The ground grey matter become fibrous and fragile (Fig. 1 B&B1. Nevertheless, there was a detected improvement and restoration of nearly the normal pattern structure of ependymal canal, multipolar neuronal cells, and gray elements (Fig. 1C&C1).

At transmission electron microscopy, mother rat fed on a high cholesterol diet showed neurons with abnormal pyknotic nuclei. In the cytoplasm, vesicuolated rough endoplasmic reticulum and atrophied mitochondria were detected. Many of the nerve axons become demyelinated (Fig.2 A1-C1). On the other

<table>
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<th>Table 1.</th>
<th>Cervical spinal cord content of antioxidant, phospholipids, MDA, casp3, 8 hdG and iron of mother rat fed on a high cholesterol diet with or without barley-supplementation</th>
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<tbody>
<tr>
<td></td>
<td>GSH (μg/g)</td>
</tr>
<tr>
<td>C</td>
<td>65.9±3.9</td>
</tr>
<tr>
<td>B</td>
<td>66.2±4.1</td>
</tr>
<tr>
<td>H</td>
<td>34.5±5.6*</td>
</tr>
<tr>
<td>HB</td>
<td>61.4±7.7</td>
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</tbody>
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Each result represents the mean±SE of n=5. One star means significant at P < 0.05; double stars mean high significant at P < 0.01. Abbreviations; B, barley supplemented; C, control; Casp3, caspase-3; GSH, glutathione-s-reductase; H, high cholesterol diet; HB, high cholesterol diet containing barley; 8-HdG,8-hydroxydeoxyguanosine; MDA, malondialdehyde; PE, phosphatidylethanolamine; PPC, polyenylphosphatidylcholine; SM, sphingomyelin; SOD, superoxide dismutase

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<th>Table 2.</th>
<th>Cervical spinal cord neurotransmitters, amyloid â, homocysteine and cytokine contents of mother rat fed on a high cholesterol diet with or without barley-supplementation</th>
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<tbody>
<tr>
<td></td>
<td>DA (ng/g)</td>
</tr>
<tr>
<td>C</td>
<td>52.6±2.5</td>
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<tr>
<td>B</td>
<td>61.5±3.1</td>
</tr>
<tr>
<td>H</td>
<td>41.8±2.1</td>
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<tr>
<td>HB</td>
<td>47.2±2.1</td>
</tr>
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</table>

Each result represents the mean±SE (n=5). One star means significant at P < 0.05; double stars mean high significant at P < 0.01. Abbreviations; B, barley supplemented; C, control; DA, dopamine;GABA, α-aminobutyric acid; H, high cholesterol diet; HB, high cholesterol diet containing barley; HCY, Homocysteine; 5-HT, serotonin; IL6, interleukin 6; IL10, interleukin 10; IL17, interleukin 17
hand, mother fed on a high cholesterol diet containing barley possessed a detected comparative improvement of the neuronal cells. Rough endoplasmic reticulum and mitochondria restored their normal level. The neuronal axons become myelinated and their neuropil appeared rich of mitochondria (Fig. 2 A2-C2). Regard the control (Fig. 1 A-C ).

A-C Control. A&B. Showing normal neuronal cell with characteristic nuclei (N) and cytoplasm rich in mitochondria (M) and rough endoplasmic reticulum (RER). C. Showing normal myelinated axons containing mitochondria (M) in their neuropil. Arrow head indicate myelinated axons. Star illustrating mitochondria. A1-C1. Mother fed on a high cholesterol diet. A1&B1. Showing neuron cells with pyknotic nuclei (PN) and cytoplasm containing damaged mitochondria (DM) and vesiculated rough endoplasmic reticulum. C1. Showing demyelinated axons with damaged mitochondria (DM) in the neuropil. Arrowhead showing demyelinated axons Star showing damaged mitochondria. A2-C2. Mother fed on a high cholesterol diet with barley. A2. Showing improved neuronal cell with characterized nuclei (N) surrounding by nuclear envelope (NE) and cytoplasm containing few vacuoles (V) and intact mitochondria (M). B2. Showing less improved neuronal cell containing nuclei (N) with convoluted nuclear envelope (NE) and normal myelinated axons (MA) through the neuropil. C2. Showing improved myelinated axons (MA) containing mitochondria (M). Star showing normal mitochondria in the inner compartment of myelinated axon. Arrow head showing myelinated axon. Abbreviations; M, mitochondria; MA, myelinated axons; N, nucleus; NE, nuclear envelope; V, vacuoles. Star showing normal mitochondria in the inner compartment of myelinated axon. Arrowhead showing myelinated axon.

**Immunohistochemistry Observations of Mother**

In mother fed on hypercholesterolemic diet, there was a marked reduction of PCNA immunohistochemical reaction in neuronal cells (Fig. 3 B). However, in those fed on a high cholesterol diet containing barley, there was a comparatively improved immunohistochemical reaction expressed by a moderate dark-brown immunohistochemical reaction (Fig. 3C). Regard the control (Fig. 3 A). Image analysis revealed decreased expression of PCNA in cervical cord of experimental hypercholesterolemic mothers compared to moderate reaction in those fed on a high cholesterol diet containing barley and control (Fig. 4).

Note decreased staining in mother fed on a high cholesterol diet (B) compared to control (A) and improved in mother fed on a high fat diet containing barley (C).A1-C1. Spinal cord of mother rats immunohistochemically stained with GFAP- antibody. Note decreased staining in mother fed on...
Fig. 4. Chart illustrating image analysis of immunohistochemical reactive regions of PCNA and GFAP of cervical spinal cord of different studied group. Abbreviations; B, barley supplemented mother; C, control mother; H, mother fed on a high cholesterol diet; HB, mother fed on a high cholesterol diet containing barley. Star indicates a marked depletion of the immunoreaction.

A high cholesterol diet (B1) compared to control (A1) and improved in mother fed on a high fat diet containing barley (C1). Arrow head indicates the increased immunohistochemical reaction.

On the other hand, mothers fed on a high cholesterol diet group possessed decreased expression of GFAP in the neuronal cells (Fig. 3 A1) compared to control (Fig. 3 B1). However, in those fed on a high cholesterol diet containing barley, there was a detected moderate dark-brown immunohistochemical reaction (Fig. 3 C1). Image analysis revealed appeared increased of the mentioned immunostaining in mother fed on a high cholesterol diet compared to the other studied groups (Fig. 4).

Biochemical Observations

Mother rats fed on a high cholesterol diet, the cervical spinal cord contents of both glutathione-s-reductase (GSH) and superoxide dismutase (SOD) activities were markedly decreased. On the other hand, the cervical spinal cord contents of MDA, caspase 3 (Casp-3) and 8-hydroxy-deoxyguanosine (8-HdG) were markedly increased. The mentioned dramatic alterations were improved in those of mothers fed on a high cholesterol diet containing barley, but were still varied from the control values (Table 1).

Each result represents the mean±SE of n=5. One star means significant at P < 0.05; double stars mean high significant at P < 0.01. Abbreviations; B, barley supplemented; C, control; Casp3, caspase-3; GSH, glutathione-s-reductase; H, high cholesterol diet; HB, high cholesterol diet containing barley; 8-HdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; PE, phosphatidylethanolamine; PPC, polyenylphosphatidylycholine; SM, sphingomyelin; SOD, superoxide dismutase.

In addition, there was a detected depletion of dopamine, serotonin and γ-aminobutyric acid in cervical spinal cord of mother fed on a high cholesterol diet compared to the control. These measurements were parallel with marked depletion of interleukin 10 and marked increased the content of interleukin 6. At the same time, the cervical spinal cord contents of the assayed neurotransmitters and interleukin 10 were markedly increased as well as improved the level of interleukin 6 in those of mothers fed on a high cholesterol diet containing barley. However, their level were not matched with the control values (Table 2).

Each result represents the mean±SE (n=5). One star means significant at P < 0.05; double stars mean high significant at P < 0.01. Abbreviations; B, barley supplemented; C, control;
DA, dopamine; GABA, γ-aminobutyric acid; H, high cholesterol diet; HB, high cholesterol diet containing barley; HCY, Homocystine; 5-HT, serotonin; IL6, interleukin 6; IL10, interleukin 10; IL17, interleukin 17.

**DISCUSSION**

From the present findings, mother rats fed on a high cholesterol diet interfered with the antioxidative defense leading to depletion of glutathione reductase and superoxide dismutase and increased of MDA parallel with widespread of neuronal cell damage, degeneration of ependymal lining cells, clumping chromatin materials of many of the neuronal cells, fragmentation of RER and atrophy of mitochondria.

It is known that in biological systems there is a balance between the production and neutralization of reactive oxygen species (ROS). This balance is maintained by the presence of the assayed antioxidant enzymes superoxide dismutase and glutathione s reductase, SOD converts the highly reactive radical O2− to the less reactive radical H2O2, meanwhile glutathione s reductase reduced the variety of hydroperoxides (Taylor et al., 1993).

The enhancement of lipid peroxidation or a decrease in antioxidant protection can frequently induce the reaction with the nucleophilic centers of the DNA, RNA and proteins leading to irreversible damage such as cytotoxicity, mutagenicity and carcinogenicity.

The observed neuronal damage was assessed by increased neuronal casp3,8-hdG. Like other neuronal tissues, the spinal cord is considered to be metabolic active organ containing reactive oxygen species. The free radicals and antioxidant enzymes maintained the balance of the antioxidant defense (Shim and Kim, 2013). The abnormal imbalance of antioxidants associated with cholesterol toxicities in neuronal tissues led to increased oxidative stress and breakdown of neuronal tissues. The present findings are consistent with Lim et al. (2014) who reported increased oxidative stress in neurodegenerative disorders. At the same time, the observed damage of mitochondria and vesiculated rough endoplasmic reticulum marked the increased oxidative stress. The present findings are consistent with El-Sayyad et al. (2017) and Paul et al. (2017) following assessment the involvement of hypercholesterolemia in oxidative stress of neuronal tissues.

From the present findings, a high cholesterol diet was found to alter the metabolism of phospholipids assessed by reduction of phosphatidylethanolamine, polyenylphosphatidylcholine and sphingomyelin.

It is known that phospholipids may involve in transmission and relay signals from the membrane to intracellular compartments or to other cells. Also, it is the main components of myelin (Grigletto et al., 2017). Depletion of membrane lipids may contribute to the pathogenesis of depression and anxiety disorders (Müller et al., 2015). The assayed phospholipid fractions (phosphatidylethanolamine, polyenylphosphatidylcholine and sphingomyelin) were markedly depleted in spinal cord tissue of hypercholesterolemia and retained nearly normal level after barley supplementation added in diet.

Also, the increased brain content of lipid enhanced lipid peroxidation and cholesterol oxidation and the subsequent development of metabolites such as 4-hydroxynonenal and oxysterols, respectively from the two processes. The chronic inflammatory events observed in the AD which activates inflammatory molecule and free radical release. Oxidative stress is closely associated to neuroinflammation, which re intern activated oxidative stress leading to progress neuronal damage. Taking in consideration the higher activity of oxysterol to cross the blood brain barrier, it is the main contributor of the neuronal damage (Gamba et al., 2015).

The increased oxidative stress associated with hypercholesterolemia were assessed by the upregulation of the inflammatory cytokines IL6 and 17 and a decrease of IL10. Similar findings have been reported by Yi et al. (2012) in hypothalamus post a higher fat diet.

Beside Structural and functional disorganization, there was a detected decrease of VEGF immunohistochemistry and demyelination of nerve axons which was confirmed by depletion of neurotransmitters of dopamine, serotonin and a-aminobutyric acid this may led to impair cognitive function and delayed the function of synapses.
It is known that DA and 5-HT, play an a great role in managing the sensory, motor and autonomic functions in the spinal cord (Zhang, 2016; Wei et al., 2014). Neurodegenerative lesions can be resulted from depletion of DA and 5-HT, neuronal loss, inflammation and caspase activation within the spinal cord at both post- and pre-synaptic sites (Malcangio and Bowery, 1996). ?ABA was found to be increased in the hyperglycemic stroke (Guyot et al., 2001). The observed demyelinated nerve axons confirmed the work of Saher et al. (2005) who mentioned that cholesterol is the main elements of myelin membranes and decreased metabolism of cholesterol is involved for demyelination (Raddatz et al., 2016).

Wang and Zheng (2015) reported that rabbit fed on a high cholesterol diet. The authors showed impaired of synaptic function characterized by increased mushroom spine density and decreased thin spine density.

Also, the neurodegenerative lesions of the spinal cord were confirmed by Increased level of homocysteine and amyloid-β.

Similar findings were reported in in Alzheimer’s disease model mice (Matsumura et al., 2015) and rabbit (pan et al. 2018). The mentioned authors stated that hypercholesterolemia increased the burden of intraneuronal Aß oligomers and synapses loss. At the same time increased level of homocysteine was found to initiate oxidative stress leading to inflammation and endoplasmic reticulum stress (Moretti and Caruso, 2019) as well as activated N-methyl-d-aspartate receptor leading to accumulation of amyloid and tau protein, apoptosis, and neuronal death (Obeid and Herrmann, 2006). Also, homocysteine levels are elevated in patients with spinal cord injury (SCI) due to fat distribution changes and sympathetic dysfunction (Latifi et al., 2013; Hao et al., 2014).

The observed finding revealed that the damaged spinal cord was confirmed by increased level of iron. Iron accumulation was found to increase oxidative stress associated spinal cord injury (Meng et al., 2017). Also increased iron level led neurodegenerative diseases such as Alzheimer’s disease through binding to transferrin, and crossing the brain blood barrier (Moos and Morgan, 2000). It is known that monocytes is the main cell components of the central nervous system. The iron-containing monocytes facilitate migration and transform into macrophages, and degenerate and consequently phagocytosis of this cell debris including iron may transmit it to another neuronal region promoting the formation of free radicals and induced neurodegenerative disorders (Andersen et al., 2014).

On the other hand, mother rats fed on a high fat diet plus barley possessed marked improvement of their spinal tissues.

These seemed to be resulted from the hypolipidemic activity of barley β-glucans in animal model (Kalra and Jood, 2000) and human (Wilson et al., 2004; AbuMweis et al., 2010; McRorie and McKeown, 2017). It is known that bile acids are derivatives of cholesterol, and their excretion activates cholesterol 7a-hydroxylase which increase the transport of LDL cholesterol into hepatocytes for conversion into bile acids thus lowering the serum cholesterol and LDL cholesterol levels in the body (Maki et al., 2010; Wang et al., 2017). Also, barley β-glucans was influenced in the production of short chain fatty acids via either its fermentation in the human intestinal by microflora (Barsanti et al., 2011; Othman et al., 2011) or through improving gut microflora especially Lactobacilli for synthesis it (Morrison and Preston, 2016), leading to inhibition of hepatic cholesterol biosynthesis (Hara et al., 1999) and depleting plasma triacylglycerol and cholesterol from week 3 to 7 (Mikkelsen et al., 2017).

Also, barley β-glucan was found to improve the brain and sciatic nerve of the streptozotocin-induced diabetic rat (Alp et al., 2012) and reduced the number of degenerated neurons in the ovariectomized rats (Selli et al., 2016). Ferulic acid and Coumaric acids, the main components of barley grain showed high antioxidant activity (Kováčová and Malínová, 2007) and consequently improve neuronal injuries (Szwajgier et al., 2017). Barley seeds exhibited the presence of 3, 4-Dihydroxybenzaldehyde, which inhibits oxidative DNA damage and apoptosis via scavenging H2O2 (Jeong et al., 2009; Aggarwal et al., 2011).

Tocotrienol (member of vitamin E) – treatment was found to reduce oxidative stress in Alzheimer disease through reducing free-radicals and promote mitochondrial function and cellular repair. It also inhibited glutamate-induced
neurotoxicity in the neuronal cells (Chin and Tay, 2018).

Finally, the present study concluded that high cholesterol diet is involved in the development inflammatory reactions and oxidative stress associated impairment of function. Barley supplementation exerted improvement of the assessed histopathological, immunohistochemical and biochemical pictures. This findings clarifying that the high barley contents of antioxidants and nutrients are of great importance in scavenging the free radicals and decreasing the oxidative stress and consequently restore the spinal cord structure and function.

REFERENCES


47. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid


