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histological and immunohistochemical studies of the effect germinating fenugreek (trigonella arabica) and barley (hordeum vulgare) grains on spinal cord of 21-day-old offspring rats maternally fed on high fat diet.

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Abstract: This research studies the effect of feeding on high fat diet (HFD) on the structure and function of the rat offspring spinal cord and the good effect of feeding on germinating fenugreek (*Trigonella arabica*) and barley (*Hordeum vulgare*) grains. After mating, pregnant rats were divided four groups (n=10); control, HFD (20% soft animal fat with standard nutritional diet), fenugreek & HFD (20% soft animal fat with germinated fenugreek grains) and barley & HFD (20% soft animal fat with germinated barley grains) were experimentally tested using Hematoxylin and Eosin stain to detect their histological changes and immunohistochemical analysis to detect tissue damage using P53 and synaptophysin. HFD group offspring showed adverse effects on spinal cord. HFD induced inflammatory cells and degenerated spinal cord with accumulated fat droplets and atrophied nucleus. Phytochemical analysis was performed on extracted samples from Fenugreek (*Trigonella arabica*) and Barley (*Hordeum vulgare*) grains and revealed high antioxidant content. This explained why dietary supplementation of either germinating barley or fenugreek grains showed good effects and improvement in the structure and function of spinal cord.

keywords: Maternal high fat diet, Germinating fenugreek (*Trigonella arabica*) and barley (*Hordeum vulgare*) grains, Dietary supplementation.

1.Introduction

The spinal cord is a part of the central nervous system (CNS). It extends caudally and is protected by the vertebral column. It is covered by the three membranes: the dura mater, arachnoid and the innermost pia mater. It has a long bundle of nerves and cells involved in the transmission of the nerve impulses from the brain and various motor and sensory neurons to the various appendicular regions [1].

High fat diet was found to induce the damage of spinal cord which is associated with altered gene expression such as IGF1 [2]. Increased consumption of high fat diet led to the development of neurotoxicity [3].

A study by Urbonaite et al. discovered neurodevelopmental disorders in neonates from mothers consuming high-fat diets. Such changes can have implications for learning and memory [4]. Bordeleau et al. found increased markers of neuroinflammation in offspring maternally fed on high-fat diets, which could lead to neurological disorders [5].

Fenugreek (*Trigonella arabica*) is an important ingredient which is rich in bioactive antioxidants [6]. It is known for its beneficial usage in medicine as it could be used as antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant, and immunological activities. Also, it is used as food stabilizer, adhesive, and emulsifying agent [7].

Barley (*Hordeum vulgare*) is one of the world's oldest cultivated grains, with a history rooted in the diets and traditional medical practices of various cultures. Its diverse range of nutrients and bioactive compounds make it a

valuable grain both from culinary and health perspectives. It is considered as a very important plant which contains some good nutrients and has so many health benefits. Barley helps to lose weight and decrease blood pressure and blood cholesterol because it contains ß-glucan [8]. It also reduces blood glucose in Type 2 diabetes and prevents colon cancer. It contains both soluble and insoluble fiber, protein, vitamins B and E, minerals selenium, magnesium and iron, copper, flavonoids and anthocynins [9]. One of the good reasons why we used barley in this study is it can be obtained easily being cheap and available.

2. Materials and methods:

2.1. Ethical approval:

The ethical Committee for Animal Experimentation at Faculty of Science, Mansoura University, Egypt gave its approval to the study. Approval number is (Sci-Z-M-2020-13).

2.2. Barley and fenugreek analysis:

The presence and quantification of various phytochemicals in barley and fenugreek, such as phenolic compounds, flavonoids, and tannins, were assessed using spectrophotometric methods. Extracts were prepared from the ground samples using appropriate solvents, and absorbance was measured at specific wavelengths to determine phytochemical concentrations. The antioxidant activity of barley and fenugreek extracts was evaluated using established assays such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the FRAP (Ferric Reducing Antioxidant Power) assay. These assays provided insights into the ability of these samples to neutralize free radicals.

2.3. Experimental Work:

12 male and 40 virgin female rats (*Rattus norvegicus*) were used in this study weighing approximately 100g. They were obtained from Egypt's Ministry of Health's Helwan Breeding Farm. After mating, pregnant rats were divided into four groups (n=10); control, HFD (20% soft animal fat with standard nutritional diet), fenugreek & HFD (20% soft animal fat with germinated fenugreek grains) and barley & HFD (20% soft animal fat with germinated barley grains) treated groups.

2.4. Light Microscopy:

At 21 days post-partum, offspring slaughtered by cervical dislocation after anesthesia with phenobarbitone. Cervical spinal cord of 21-day-old offspring were separated and fixed in 10% phosphate-buffered formalin (pH 7.4), and prepared for histological examinations and stained serial 5µm thick sections with Hematoxylin and Eosin.

2.5. Immunohistochemical staining:

Five µm histological sections of formalin-fixed, paraffin-embedded spinal cord sections of 21-day-old offspring were mounted on polylysine-coated glass slides and stored at room temperature. Spinal cord sections were treated with antibodies against P53 & Synaptophysin.

2.6. Statistical analysis:

One way ANOVA in SPSS (version 23) were used. The data is presented as means ± standard error (SE). The t-test was calculated and considered statistically significant at $p \leq 0.05$.

3. Results and Discussion

The phytochemical analysis of the extracted samples from Fenugreek (*Trigonella arabica*) and Barley (*Hordeum vulgare*) grains revealed high antioxidant content, including total phenolics, flavonoids, and tannins fig. (1). Both plants exhibited significant amounts of these compounds, which are known for their potential health benefits.

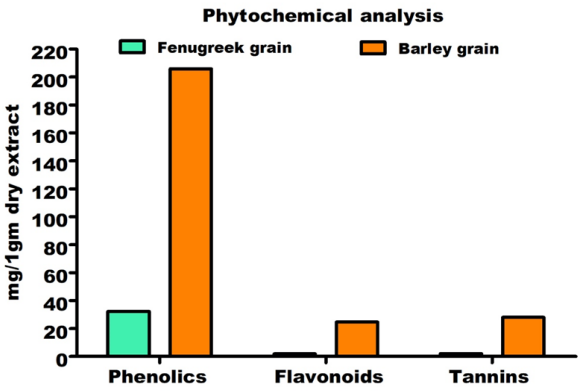

Fig. (1) Comparison between phytochemical contents of the investigated extracts of Barley grain and Fenugreek

The evaluated the antioxidant activity of Fenugreek and Barley extracts in comparison to ascorbic acid. Barley grains extract exhibited the highest antioxidant activity with the lowest IC50 value of 0.2083 mg/mL, comparable to ascorbic acid (IC50=0.0222 mg/mL). The scavenging activity of both extracts was concentration-dependent Fig. (2).

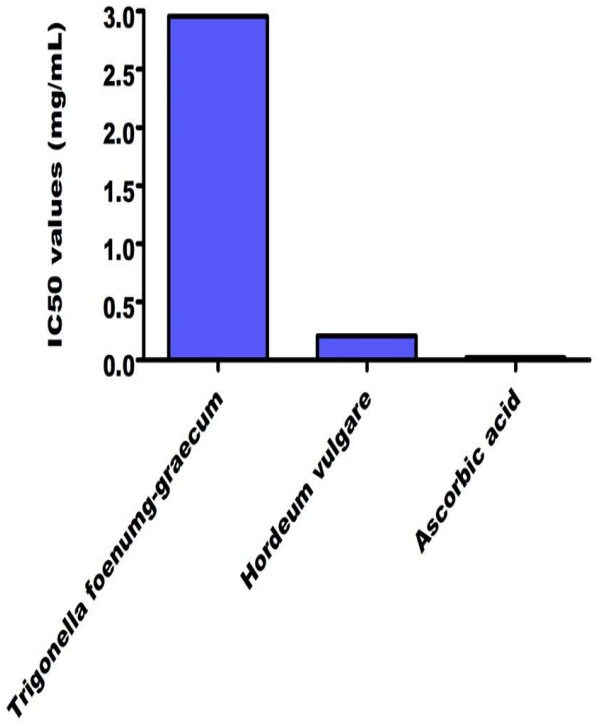

Fig. (2) Comparison between IC50 (mg/mL) values of the investigated extracts of Barley and Fenugreek grains.

The phytochemical analysis and DPPH assay demonstrated the rich antioxidant composition of fenugreek and barley extracts, highlighting their potential health-promoting properties.

Hematoxylin and eosin (H&E) staining showed Control showing normal axons surrounded by myelin space and normal blood vessel lined with endothelial cells as shown in Fig. (3A, B&C). HFD offspring had damaged spinal cord with abnormal vacuoles, demyelinated axons, pyknotic nucleus and degenerated central canal as shown in Fig. (3D&E).

Supplementation of barley shown in Fig. (5F&G) and fenugreek shown in Fig. (3H&I). attenuated the degenerative effects of HFD, as they showed improved cell body with normal nucleus and myelinated axons.

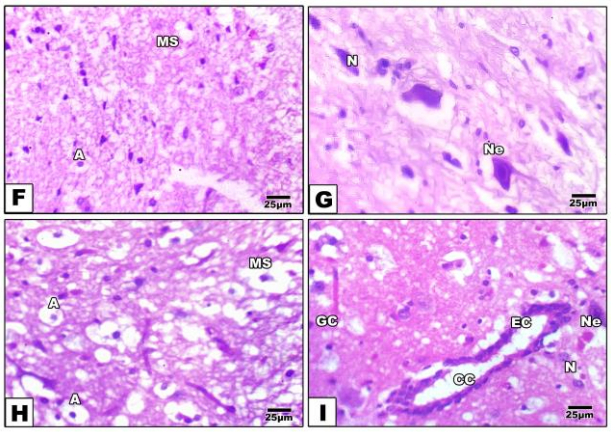

Fig. (3) Photomicrographs of histological sections of spinal cord in 21-day-old rat. A, B & C. Control showing axons surrounded with myelin space and normal blood vessel lined with endothelial cells. D & E. Neonates of hyperlipidemic mother showing vacuoles, demyelinated axons, pyknotic nucleus and degenerated central canal. H & E. Abbreviation: BV, blood vessel; A, axon; MS, myelin space; Ne, neuron; GC, glial cell; CC, central canal; EC, ependymal cells; N, nucleus; PN, pyknotic nucleus; arrow, degenerated epithelial cells; plus arrow, vacuoles.

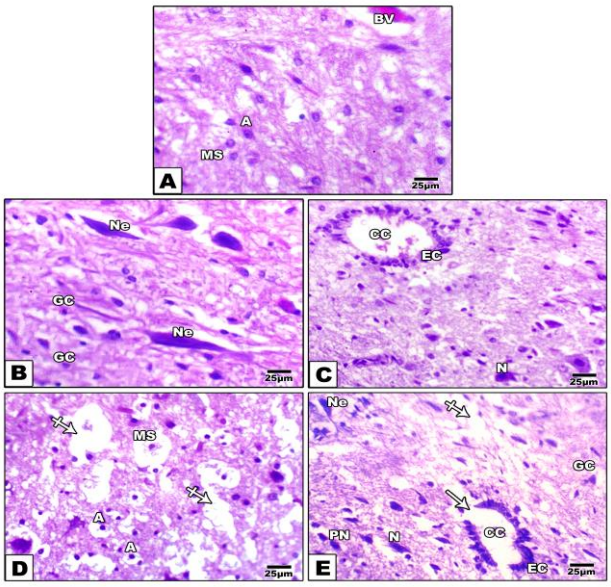

Fig. (4) Photomicrographs of histological sections of spinal cord in 21-day-old rat maternally fed on high fat diet. F & G. High fat diet plus germinating barley grains supplementation showing cell body with normal nucleus and myelinated axons. H & I. High fat diet plus germinating fenugreek grains supplementation of hyperlipidemic mother showing improvement. H & E. Abbreviation: MS, myelin space; A, axon; N, nucleus; Ne, neuron; GC, glial cell; CC, central canal; EC, ependymal cells.

Immunohistochemical staining of spinal cord sections revealed that maternal HFD induced over expression of p53 Fig. (6&7) and synaptophysin Fig. (8&9) compared to control group. Barley and fenugreek grains supplementation showed decreased reaction of these proteins.

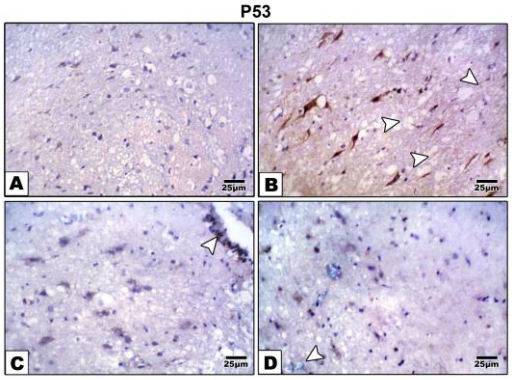

Fig. (5) Photomicrographs of formalin fixed histological sections of spinal cord immunohistochemical stained with P53 of 21-day-old rat. (A) Control revealed low immunohistochemical reaction of P53. (B) High fat diet revealed increased dark brown P53 immunohistochemical reaction in spinal cord fibers manifesting cell death. High fat diet with (C) barley and (D) fenugreek supplementation revealed decreased immunohistochemical reaction of P53. Arrow head indicating immunohistochemical reaction of P53.

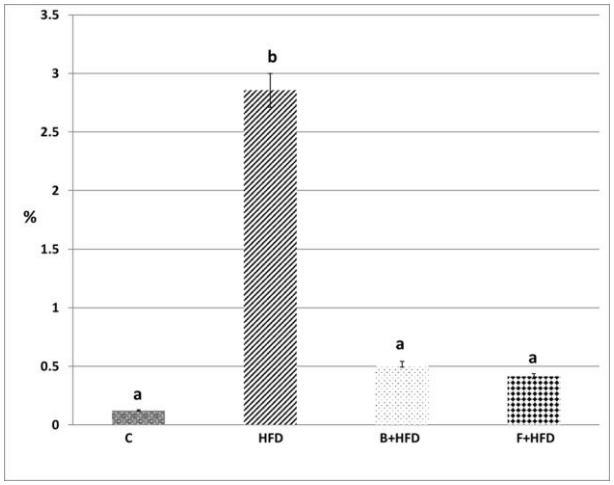

Fig. (6) Histogram illustrating the percentages of immunohistochemical reaction of P53 in spinal cord of 21-day-old rats fed on diet rich in fat with or without fenugreek or barley grains supplementation. Note over expression of P53 in spinal cord of 21-day-old rats fed on diet rich in fat and improved in that received either fenugreek or barley grains plus a high fat diet. Statistical analysis showing that B+HFD and F+HFD groups are non-significant with control (P< 0.05) and HFD group is significant to all other groups(P≤0.05).

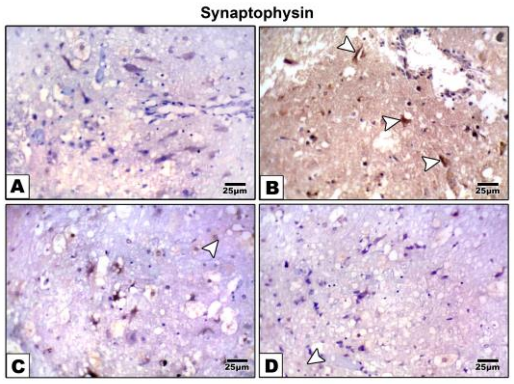

Fig. (7) Photomicrographs of formalin fixed histological sections of spinal cord fibers immunohistochemical stained with synaptophysin of 21-day-old rat. (A) Control revealed negative reaction of synaptophysin. (B) High fat diet revealed increased dark brown synaptophysin immunohistochemical reaction in spinal cord fibers manifesting cell death. High fat diet with (C) barley and (D) fenugreek supplementation revealed decreased reaction of P53. Arrow head indicating immunohistochemical reaction of synaptophysin.

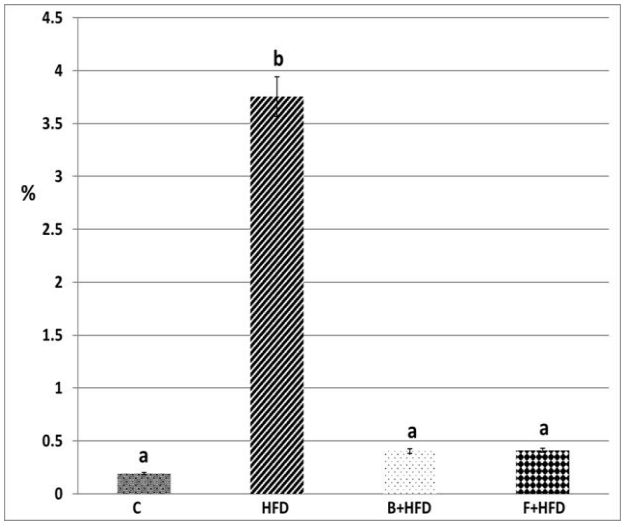

Fig. (8) Histogram illustrating the percentages of immunohistochemical reaction of synaptophysin in spinal cord of 21-day-old rats fed on diet rich in fat with or without fenugreek or barley grains supplementation. Note over expression of synaptophysin in spinal cord of 21-day-old rats fed on diet rich in fat and improved in that received either fenugreek or barley grains plus a high fat diet. Statistical analysis showing that B+HFD and F+HFD groups are non-significant with control (P< 0.05) and HFD group is significant to all other groups(P≤0.05).

Phytochemical analysis unveiled the rich antioxidant potential inherent in Fenugreek (*Trigonella arabica*) and Barley (*Hordeum vulgare)* extracts. This outcome resonates strongly with the findings of esteemed researchers [10] who have previously documented the substantial phenolic content and robust free radical scavenging capabilities of these botanical sources. Furthermore, our results are in harmony with a comprehensive meta-analysis by Koteb which highlighted the prevalence of phenolic compounds in Fenugreek and Barley extracts, emphasizing their pivotal role in antioxidant activity. These consistent findings corroborate the assertion that these natural extracts harbor immense potential for a spectrum of applications, ranging from functional food fortification to pharmaceutical formulations [11].

The assessment of free radical scavenging activity, as exemplified by the DPPH assay, underscores the robust antioxidant efficacy of Barley grain extract. Evidenced by the lowest IC50 value compared to Fenugreek extract and the standard ascorbic acid, this observation aligns with the extensive body of literature [12] emphasizing the potent antioxidant capacity resident within Barley grains. Nevertheless, it is imperative to highlight that Fenugreek extract, although demonstrating a higher IC50 value compared to Barley, exhibited commendable scavenging activity at elevated concentrations. This nuanced result underscores the importance of considering both the potency and dose-dependent dynamics of antioxidants, acknowledging that their efficacy may fluctuate under varying conditions and dosages.

Histological analysis revealed that maternal HFD induced significant degenerative changes in the spinal cord of offspring. These changes included inflammatory cell infiltration, and alterations in nuclear structure. HFD group showed mitochondrial dysfunction, oxidative stress and a loss of myelinating cells [13].

However, supplementation with Barley and Fenugreek grains mitigated these degenerative aspects, leading to improvements in spinal cord histology. Fenugreek has a good impact on nervous system disorders and improves nerve fibers function [14]. Barley showed protective effects against oxidative damage of nervous system [15].

Cell death-related proteins P53, immunohistochemical marker of mitochondrial apoptosis [16] and synaptophysin, immunohistochemical marker of axonal damage in demyelination and neuroinflammations and used in diagnosis of tumor [17], were over-expressed in HFD group. However, supplementation of fenugreek and barley grains had weak reaction with those proteins, giving an evidence of their potential effects against cell damage.

4. Conclusion

Fenugreek and barley grains have therapeutic effects on spinal cord of maternal HFD offspring due to their antioxidant capacity. The improvement appeared in histological improvement and reduction of P53 & synaptophysin immunostaining.

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