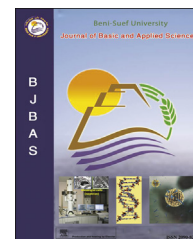


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## Full Length Article

# Effects of Orlistat and herbal mixture extract on brain, testes functions and oxidative stress biomarkers in a rat model of high fat diet

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## ABSTRACT

This study was designed to assess the effectiveness of herbal mixture extracts of pumpkin seed oil, peanuts shell and Orlistat on brain, testes functions, oxidative stress biomarkers and histopathological changes in male albino rats administered high fat diet. Fifty male rats were divided into four groups: 1st administered normal diet, 2nd administered high fat diet, 3rd administered high fat diet with Orlistat and 4th administered high fat diet with herbal mix.

A group of rats were fed with a standard control diet (1st control group was 12 rats for 22 weeks) and another group of rats were fed a diet containing 35% fat (2nd high fat diet) for 16 weeks. Then, this group of high fat diet was divided into 3 groups for the following 6 weeks: 1st group administered high fat diet only (13 rats), 2nd group administered high fat diet plus 2 mg/kg bw/day Orlistat (12 rats) and 3rd group administered high fat diet plus 5 mg/kg bw/day pumpkins and 2 mg/kg bw/day peanut shell extract (13 rats). Blood samples, brain and testes tissues were collected for biochemical assays and histopathological studies.

High fat diet group showed a high significant increase ( $P < .001$ ) in feed intake, body weight and body mass index. HFD showed a significant increasing in Nor Epinephrine, Dopamine, BCHE, Homocysteine and malondialdehyde contents in brain. In testes high fat diet increased malonaldehyde contents of testes. An improvement by the treatments with Orlistat and herbal mixture was observed. Histopathological examination of brain and testes sections of high fat diet rats supported the previous biochemical results.

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We concluded that the treatment with Orlistat and herbal mixture ameliorated the harmful effects of the high fat diet and reduce feed intake.

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## 1. Introduction

Obesity had been one of the leading public health concerns in industrialized societies for the last 40 years. It had been defined by the WHO as a body mass index above 30 kg/m<sup>2</sup> (WHO, 2006). It was reported that obesity increased the risk of neurodegenerative diseases, such as the degradation of neural membrane glycerophospholipids, the disruption of protein synthesis, degradation and the generation of reactive oxygen species (Kawarazaki et al., 2010). While treatment with Orlistat showed that alteration of the various hypothalamic neuropeptides' Central nervous system (CNS) levels, or alter the key CNS appetite monoamine neurotransmitters' levels that will suppress appetite. Also, treatment with herbal mix (PO) improved the brain from damage due to its content of omega-3 fatty acids. Goncalves et al. (2005) demonstrated that omega-3 fatty acids modulate changes in the concentrations and actions of several orexigenic and anorexigenic neuropeptides in the brain, including neuropeptide Y, alpha melanocyte stimulating hormone and the neurotransmitters serotonin and dopamine. But in case of PES, luteolin that found in peanut shell extract effectively produce a natural anti-inflammatory effect that stops the errant microglial cells from causing damage, protect cell neurons from damage and inhibited the production of neurotoxic inflammatory mediators (Benson, 2010) and is significantly increased the activities of Superoxide dismutase (SOD), Catalase (CAT) and decreased the levels of Malonaldehyde (MDA).

Also, it was reported that obesity is a cause of male infertility (Du Plessis et al., 2010). As morbidly obese males present with excess scrotal fat. The environmental toxins that accumulate in the white adipose tissue surrounding the scrotum might have a direct localized effect on spermatogenesis in the testes lipophilic contaminants are associated with decreased sperm production and thus decreased male reproductive potential. It was found that men with a body mass index greater than 25 kg/m<sup>2</sup> had a lower total sperm count than men of normal weight (Aggerholm et al., 2008). Belong to oxidative stress; it was known that obesity increased oxidative stress in reproductive system that defined as stress induced by increased numbers of molecules containing free oxygen. While treatment with Orlistat showed a high significant decrease in testes MDA level (Yesilbursa et al., 2005). Also, with treatment with herbal mix showed improvement in sperm count and testicular histology. This could be due to antioxidative properties such as tannins and vitamin A present in the oil (Nkang et al., 2003). Vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis. This study investigates effect of an obesity-associated risk factor on brain and testes oxidative stress, its

mechanism of action and biomarkers associated with the effect of Orlistat and herbal mixture on it.

## 2. Material and methods

### 2.1. Materials

#### 2.1.1. Diet

Two types of diets had been used, control rat chow diet and special High fat diet (HFD) (35%) for induction of obesity in rats:

- a **Normal rat chow diet:** It was formed according to Kim et al., 2004. The standard normal rat pellet chow consists of concentrate (350 g), corn (600 g), calcium carbonate, dicalcium phosphate, sodium chloride magnesium oxide and vitamins (50 g). Standard normal rat diet composed of 65% CHO (60% starch+5% sucrose), fat 5%, crude protein 20%, vitamins and minerals 5%, fibers 5%, metabolic energy of this diet is 2813 kcal/kg with 8% from fat.
- b **The high fat diet:** composed of 300 g concentrates, 350 corns, 300 g beef tallow, 50 g vitamins, minerals and fibers according to Kim et al., 2004. Percentage of HFD was 20% crude protein, 35% fat, 40% CHO (starch 35%, 5% sucrose) 5% vitamins, minerals and fibers. Metabolic energy of this diet is 5130 kcal/kg, 61% of this energy from fat. HFD would be lard, sunflower oil and starch for induction of obesity from local market by adding 30% lard or beef tallow and 5% sunflower to the control diet (Kim et al., 2004).

#### 2.1.2. Chemicals

Orlistat is a white to off-white crystalline powder. Orlistat is practically insoluble in water, freely soluble in chloroform, and soluble in methanol and ethanol. Orlistat is available for oral administration as a dark-blue or turquoise hard-gelatin capsule. Each capsule contains a pellet formulation consisting of 120 mg of the active ingredient, Orlistat, as well as the inactive ingredients microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, povidone, and talc (The internet drug index RxList, 2012). Orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester. Its empirical formula is C<sub>29</sub>H<sub>53</sub>NO<sub>5</sub>, and its molecular weight is 495.7. It was given orally by oral gavage with a dose 2 mg/kg bw/day (Kumar and Alagawadi, 2010).

#### 2.1.3. Herbal mixture

Mixture herbal extract of *Arachis hypogaea* nutshell extract and cucurbitamoschata plus morus alb (Moreno et al., 2006) from local market.

- 1 Pumpkin oils were purchased from El Kaptin pharmaceutical company; it is a liquid was given by oral gavage with dose 5 mg/kg bw/day (Zuhair et al., 2000).
- 2 Ethanolic extract of Nutshell was prepared by soaking 100 g of dry nutshell in 500 ml ethyl alcohol 95% with daily shaking for 5 days and kept in a refrigerator. Ethanol was evaporated using a rotatory evaporator apparatus which was attached with vacuum pump then the extract was used by dose 2 mg/kg bw/day (Moreno et al., 2006). Both herbal were mixed together (1:1).

## 2.2. Methods

### 2.2.1. Animals grouping

This study was carried out on white male albino rats weighing about  $100 \pm 20$  g which were used as experimental animals. Rats were fed with a standard control diet for 22 weeks. In the present investigation, the rats were obtained from the animal house of Research Institute of Ophthalmology, El Giza, Egypt. The rats were kept under observation for about 7 days before the onset of experiment to exclude any intercurrent infection. During the period of the research in the laboratory of Biochemistry, Faculty of Science, Beni Suef University, the chosen animals were housed in plastic container at normal atmospheric temperature ( $25 \pm 5$ ) °C. Food and water were consumed ad libitum. For manuscripts involving animal experiments, "All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

Body weights were recorded weekly and food consumption was calculated daily. Fifty male rats were divided into four groups: 1st a normal diet group (12 rats for control negative maintained on standard normal rat chow diet along the all period of experiment 22 weeks), 2nd High fat diet group (13 rats HFD along the all period of experiment 22 weeks) act as control positive, 3rd HFD with 2 mg/kg bw/day Orlistat (12 rats for 6 weeks) and 4th HFD with 5 mg/kg bw/day PO and 2 mg/kg bw/day Peanut shell extract (PSE) (13 rats for 6 weeks). Our experiment continued for 22 week and divided into two phases: Induction period and treatment period.

- a **Induction obesity period:** It began from 0 to 16th week; by feeding rats with HFD. The animals were divided into four groups; control, HFD, HFD with Orlistat and HFD with herbal mix group.
- b **Treatment period:** It began form 16–22nd week during this period, HFD group was divided into three groups, HFD group continued on fat diet, HFD with Orlistat treated group maintained on HFD to 6 weeks with administration of Orlistat treatment at dose 2 mg/kg/bw rat/day by stomach tube. While other group was HFD maintained on fatty diet to 6 weeks with administration of herbal mix treatment at dose 5 mg/kg/bw rat/day for PO and 2 mg/kg/bw rat/day for PSE by stomach tube.

Calculation of average food consumption per each rat was recorded daily by subtracting the amount of food remaining in

each day from the measured amount of food provided at the previous day (Roberts et al., 2002). It was known that metabolic energy of standard rat chow and HFD were 2813 and 5130 kcal/kg respectively, so the average energy intake was calculated by multiplying the average consumed diet by 2.813 and 5.130 respectively (Roberts et al., 2002). Body mass index (BMI) for rats was measured every week and calculated by dividing the body weight in g by the length (nose to base of tail) in cm<sup>2</sup>. Also, BW was measured.

### 2.2.2. Sampling and tissue preparation

**Blood samples** were collected from medial canthus of the eye, via glass capillaries at fasting state. The samples were collected in dry glass centrifuge tubes, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 min at room temperature for separation of serum. The clear, non-haemolysed supernatant sera were separated using clean dry disposable plastic syringes and stored at  $-20$  °C for subsequent biochemical measurements.

**Tissue sampling:** At the end of experiment (22 week), rats were sacrificed by decapitation and abdominal incision was immediately done after taking of blood sample for separation of brain and testes tissues. Brain and testes were taken and washed by saline (0.9%), dried by filter paper and weighed 0.5 g of this tissue then underwent homogenization then centrifuged at 3500 rpm for 15 min and the supernatant were kept at  $-20$  °C for the biochemical tissue analysis of SOD, MDA and Reduced glutathione (GSH). For light microscopic study, the specimens were fixed in 10% neutral-buffered formalin, dehydrated through alcohols, cleared in xylene and then embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxylin and eosin (Bancroft and Gamble, 2002).

## 2.3. Biochemical examination

Butyrylcholinesterase (BCHE) concentration was determined colorimetrically. Butyryl cholinesterase hydrolyzes butyrylthiocholine to give thiocholine and butyrate. The reaction between thiocholine and DTNB gives 2-nitro-5-mercaptobenzoate, a yellow compound which can be measured at 405 nm (Knedel and Bottger, 1967). Also, catecholamines were determined according to Alsalamou 3al-koum WAB by prepare 10% homogenate of the tissue i.e. 1 g is homogenized in 10 ml ice-cold 70% methanol. Centrifuge in cooling centrifuge at 5000 rpm for 15 min and inject 20 µl of the supernatant into the HPLC (Pagel et al., 2000). Homocysteine level was estimated according to chemiluminescent microparticle immunoassay (CMIA) (Kaul et al., 2006) and GSH level also, measured according to colorimetric method that based on the reduction of 5, 5' dithiobis 2-nitrobenzoic acid with GSH to produce a yellow compound (Beutler et al., 1963) but SOD are metalloenzyme that catalyze the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide (Nishikimi et al., 1972). MDA concentration was determined by reaction with thiobarbituric acid in acidic medium at temperature of 95 °C for 30 min to form Thiobarbituric acid (TBA) reactive product (Ohkawa et al., 1979).

## 2.4. Statistical analysis of the results

Data were presented as mean  $\pm$  SEM and analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer methods for post-hoc analysis. A value of  $P < .05$  was considered statistically significant. Graph Pad Prism 5 software (San Diego, CA, USA) was used for statistical analysis.

## 3. Results

### 3.1. Effect of HFD, Orlistat and herbal mix on feed intake

In this study, feed intake was significantly increased during the period of the experiment in HFD rats compared with that of control group while Orlistat and herbal mix treatment tended to decrease food intake Fig. 1.

Data shows, in the 18th week HFD group, a high significant increase ( $P < .001$ ) in feed intake compared to control group, while treatment with Orlistat and herbal mix exerted non-significant decrease in feed intake and weight loss. With continuous feeding of rats on HFD and treatment with Orlistat and herbal mix, continuous increasing had occurred ( $P < .001$ ) in feed intake with these rats that feed on HFD than control group. Also, a high significant decrease ( $P < .001$ ) in feed intake with these rats that treated with Orlistat and herbal mix and this occurred in 20 week. At the end of the experimental study that took 22 week, we observed a high significant increase ( $P < .001$ ) in feed intake with HFD group compared to control

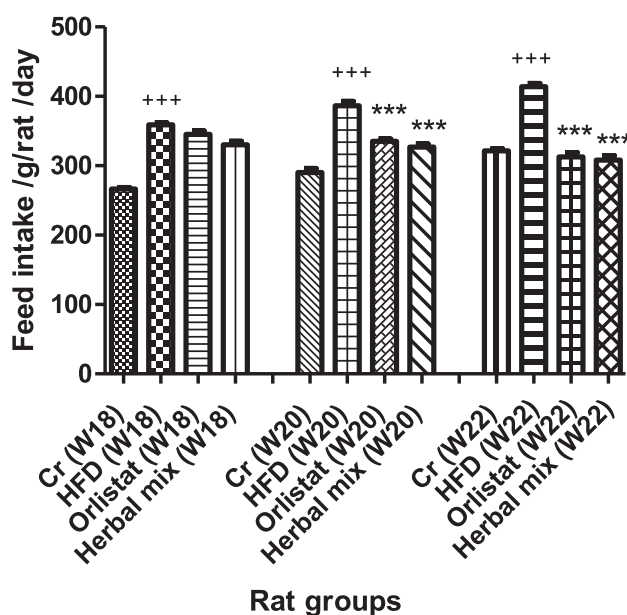


Fig. 1 – Means of feed intake (g/day/rat) in different groups of experiment (18th–22nd week) in rats. Each value is the mean  $\pm$  SEM. Values significantly different compared high fat diet to control: +++ $P < .001$ , + $P < .05$ , and values significantly different compared treatments to high fat diet: \*\*\* $P < .001$ .

group, while treatment with Orlistat and herbal mixture showed a high significant decrease ( $P < .001$ ) in feed intake.

### 3.2. Effect of diet induced obesity and treatment on body weight and body mass index (BMI)

Our data shows that there was a higher significant in BW during free diet of HFD compared to control diet. It was found that HFD caused a high significant increase ( $P < .001$ ) in BW and BMI from 0 to 16th to 18–22 wk. At the beginning of the experiments (initial weight) all weight of two groups was the same, control & HFD, and showed non-significant between them in weight. However these weights changed quickly when we feed one of them on HFD and the other was fed on normal diet. We observed that the rats that were fed on HFD had increased in BW and BMI compared to normal diet. These changes occurred 0–16th week and continued to the final 18–22 weeks, Figs. 2 & 3. But these increases in BW and BMI were changed by decreasing it with treatments of HFD with Orlistat and herbal mixture. When HFD treated with Orlistat from 18 to 22 weeks a significant ( $P < .05$ ) and a high significant decrease ( $P < .001$ ) occurred in these parameters respectively, but in case of treatment with herbal mix in the same period showed a high significant decrease ( $P < .001$ ) occurred in these parameters according to these figures. After the induction period of obesity finished (0–16 week), HFD began treated with Orlistat and herbal mix in 18–22 weeks. We observed that HFD had a high significant increase ( $P < .001$ ) in BW and BMI till the end of experiments in 16–22 weeks compared to control group. However when treatment started in 18–22 weeks, our results showed that the treatment of HFD with Orlistat and

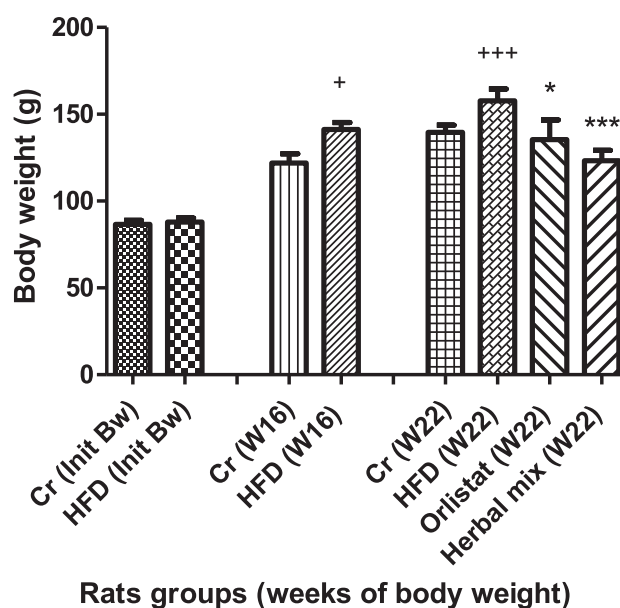
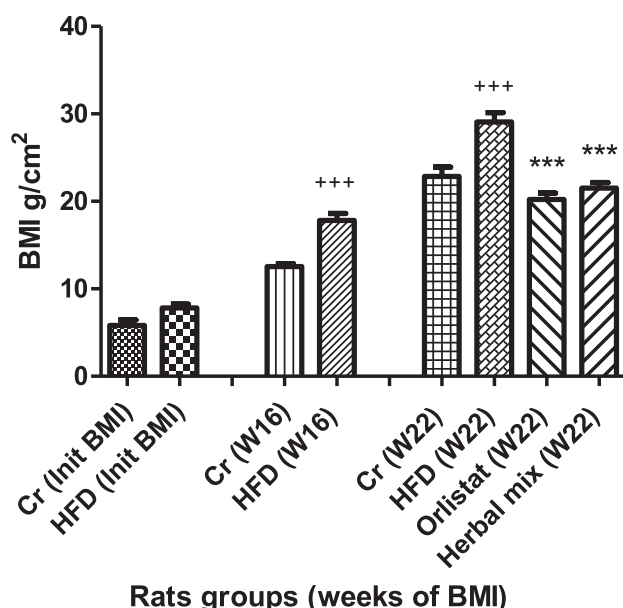


Fig. 2 – Means of BW in rats during all period of experiment (22 weeks). Each value is the mean  $\pm$  SEM. Values significantly different compared high fat diet to control: +++ $P < .001$ , + $P < .05$ , and values significantly different compared treatments to high fat diet: \*\*\* $P < .001$  and \* $P < .05$ .



**Fig. 3 – Means of BMI in rats during the period of the experiment (22 weeks). Each value is the mean ± SEM. Values significantly different compared high fat diet to control: +++P < .001, and values significantly different compared treatments to high fat diet: \*\*\*P < .001.**

herbal mix exerted a significant decrease in BW and BMI as shown in Figs. 2 & 3.

**3.3. Effect of HFD, Orlistat and herbal treatment on Epinephrine, Nor Epinephrine, Dopamine and Hydroxytryptamine (5HT)**

Data showing the effect of HFD, Orlistat and herbal mix on Epinephrine, Nor Epinephrine, Dopamine and 5HT of HFD rats. HFD rats exhibited no significant increase of Epinephrine and 5HT but it showed a high significant increase (P < .001) of Nor Epinephrine and dopamine as compared to normal rats. While treatment of HFD with Orlistat exerted no significant decrease in Epinephrine, a high significant decrease (P < .001) in Nor Epinephrine, Dopamine and 5HT as compared to high fat diet rats. Also, treatment of HFD with herbal mix exerted a significant increase (P < .01) in Epinephrine, a significant increase (P < .05) in Nor Epinephrine, a high significant decrease (P < .001) in Dopamine and a significant decrease (P < .05) in 5HT as compared to high fat diet rats. (Table 1).

**Table 2 – Effect of HFD, Orlistat and herbal treatment on BCHE and Homocysteine inflammatory markers.**

Groups	BCHE (nm/min)	Homocysteine (umol/ml)
Control	84.35 ± 7.46	73.73 ± 5.38
HFD	133.20 ± 4.93 <sup>+++</sup>	227.60 ± 19.12 <sup>+++</sup>
Orlistat	81.41 ± 7.97 <sup>***</sup>	30.34 ± 1.12 <sup>***</sup>
Herbal mix	89.97 ± 7.64 <sup>***</sup>	26.48 ± 4.72 <sup>***</sup>

Each value is the mean ± SEM. Values significantly different compared high fat diet to control: <sup>+++</sup>P < .001, and values significantly different compared treatments to high fat diet: <sup>\*\*\*</sup>P < .001.

**3.4. Effect of HFD, Orlistat and herbal treatment on BCHE and Homocysteine inflammatory markers**

Data showing the effect of HFD, Orlistat and herbal mix on BCHE and Homocysteine of high fat diet rats. HFD exhibited a high significant increase (P < .001) of BCHE and Homocysteine as compared to normal rats. While treatment of HFD with Orlistat and herbal mix exerted a high significant decrease (P < .001) in BCHE and Homocysteine as compared to high fat diet rats. (Table 2).

**3.5. Effect of HFD, Orlistat and herbal treatment on brain oxidative markers**

Data showing the effect of HFD, Orlistat and herbal mix on brain GSH, SOD and MDA of HFD. HFD exhibited a significant increase (P < .01) of brain MDA while showed no significant decrease of brain GSH and a significant decrease (P < .05) of brain SOD as compared to normal rats. While treatment of HFD with Orlistat and herbal mix exerted a high significant decrease (P < .001) in brain MDA and exerted a significant increase (P < .05) in brain SOD as compared to high fat diet rats. While exerted no significant increase in brain GSH in Orlistat but exerted a high significant increase (P < .001) in brain GSH in herbal mix. (Table 3).

**3.6. Effect of HFD, Orlistat and herbal treatment on testicular oxidative markers**

Data showing the effect of HFD, Orlistat and herbal mix on testes GSH, MDA and SOD of HFD. HFD exhibited a significant increase (P < .05) of testes MDA while no significant decrease of testes GSH and a high significant decrease (P < .001) of testes SOD as compared to normal rats. While treatment of HFD with Orlistat and herbal mix exerted a significant decrease (P < .05)

**Table 1 – Effect of HFD, Orlistat and herbal treatment on Epinephrine, Nor Epinephrine, Dopamine and 5HT.**

Groups	Epinephrine (mg/g)	Nor Epinephrine (mg/g)	Dopamine (mg/g)	5HT (mg/g)
Control	0.17 ± 0.00	0.59 ± 0.05	1.27 ± 0.09	0.06 ± 0.00
HFD	0.18 ± 0.00	1.36 ± 0.09 <sup>+++</sup>	2.51 ± 0.17 <sup>+++</sup>	0.06 ± 0.00
Orlistat	0.16 ± 0.01	0.77 ± 0.03 <sup>***</sup>	0.75 ± 0.02 <sup>***</sup>	0.04 ± 0.00 <sup>***</sup>
Herbal mix	0.24 ± 0.01 <sup>**</sup>	1.68 ± 0.05 <sup>*</sup>	1.37 ± 0.19 <sup>***</sup>	0.05 ± 0.00 <sup>*</sup>

Each value is the mean ± SEM. Values significantly different compared high fat diet to control: <sup>+++</sup>P < .001, and values significantly different compared treatments to high fat diet: <sup>\*\*\*</sup>P < .001, <sup>\*\*</sup>P < .01, and <sup>\*</sup>P < .05.

**Table 3 – Effect of HFD, Orlistat and herbal treatment on brain oxidative markers.**

Groups	Brain GSH (mg/dl)	Brain MDA (nmol/ml)	Brain SOD (u/gm)
Control	3.367 ± 0.093	20.10 ± 0.50	441.50 ± 8.92
HFD	3.055 ± 0.055	27.80 ± 0.70 <sup>++</sup>	312.90 ± 27.23 <sup>+</sup>
Orlistat	3.547 ± 0.254	16.80 ± 2.46 <sup>***</sup>	503.00 ± 38.63 <sup>*</sup>
Herbal mix	4.443 ± 0.257 <sup>***</sup>	14.57 ± 1.35 <sup>***</sup>	501.80 ± 80.17 <sup>*</sup>

Each value is the mean ± SEM. Values significantly different compared high fat diet to control: <sup>++</sup>P < .01, <sup>+</sup>P < .05, and values significantly different compared treatments to high fat diet: <sup>\*\*\*</sup>P < .001 and <sup>\*</sup>P < .05.

in testes MDA while exerted a significant increase ( $P < .05$ ) in testes GSH and a high significant increase ( $P < .001$ ) and ( $P < .01$ ) respectively in testes SOD as compared to high fat diet rats. (Table 4).

### 3.6.1. Light microscopic examination of the brain

3.6.1.1. *Group (1) the brain of control rats.* Light microscopic examination of the cerebellar cortex of the control group (Group 1) in (Fig. 4a), revealed its well-known architecture. The gray matter of the cerebellum was formed of three layers; outer molecular layer, middle purkinje cell layer and inner granular cell layer. The purkinje cell layer showed large flask shaped cells arranged typically in single row at the junction of the molecular layer with the granular layer (Fig. 4b). These cells displayed a characteristic centrally located rounded open face nucleus with prominent nucleolus surrounded by cytoplasmic Nissl's granules. They each possess numerous dendrites which arborize in the molecular layer (Fig. 4b). The next granular cell layer contained numerous compactly disposed granule nerve cells with darkly stained nuclei surrounded by very little cytoplasm.

3.6.1.2. *Group (2) rats treated with high fat diet.* Light microscopic examination of the cerebellar cortex of high fat diet (Group 2). The purkinje cells revealed disturbed normal linear organization with marked disarrangement, where some cells were displaced upwards in the molecular layer while the others were displaced downwards in the granular cell layer (Fig. 4c). In addition, purkinje cells appeared pyknosis, irregular, distorted and shrunken, were also seen (Fig. 4d). In the same group the cerebellum of some rats showed, number of purkinje cells appeared swollen with karyolysed nuclei (Fig. 4e).

**Table 4 – Effect of HFD, Orlistat and herbal treatment on testicular oxidative markers.**

Groups	Testicular GSH (mg/dl)	Testicular MDA (nmol/ml)	Testicular SOD (u/gm)
Control	4.000 ± 0.081	18.90 ± 2.34	222.0 ± 12.9
HFD	3.639 ± 0.091	32.73 ± 2.91 <sup>+</sup>	115.0 ± 13.7 <sup>+++</sup>
Orlistat	7.396 ± 1.410 <sup>*</sup>	25.60 ± 3.71 <sup>*</sup>	459.0 ± 8.1 <sup>***</sup>
Herbal mix	6.662 ± 0.744 <sup>*</sup>	21.66 ± 2.08 <sup>*</sup>	170.3 ± 3.1 <sup>**</sup>

Each value is the mean ± SEM. Values significantly different compared high fat diet to control: <sup>+++</sup>P < .001, <sup>+</sup>P < .05, and values significantly different compared treatments to high fat diet: <sup>\*\*\*</sup>P < .001, <sup>\*\*</sup>P < .01, and <sup>\*</sup>P < .05.

3.6.1.3. *Group (3) rats treated with high fat diet with Orlistat.* Light microscopic examination of the cerebellar cortex of high fat diet with Orlistat (Group3) showed the molecular layer displayed prominent spongiosis in the form of multiple vacuolated areas, in parallel with morphological alteration seen in purkinje layer, (Fig. 4f). We observed the following changes, purkinje cells exhibited obvious pyknosis and granular cell layer showed hemorrhage (Fig. 4g).

3.6.1.4. *Group (4) rats treated with high fat diet with pumpkin oil and nutshell extract.* Animals treated with high fat diet plus pumpkin oil plus nutshell extract showed histological picture more or less similar to control group (Fig. 4h).

### 3.6.2. Light microscopic examination of the testes

3.6.2.1. *Group (1) the testes of control rats.* Histological examination of the testes of the control group in (Fig. 5a & b) showed that the seminiferous tubules to be composed of spermatocytes, containing all the components of spermatogenesis (i.e. spermatogonia, primary and secondary spermatocytes and spermatozoa). The tubules were separated by intertubules connective tissue in which interstitial cells are embedded (Fig. 5 a & b).

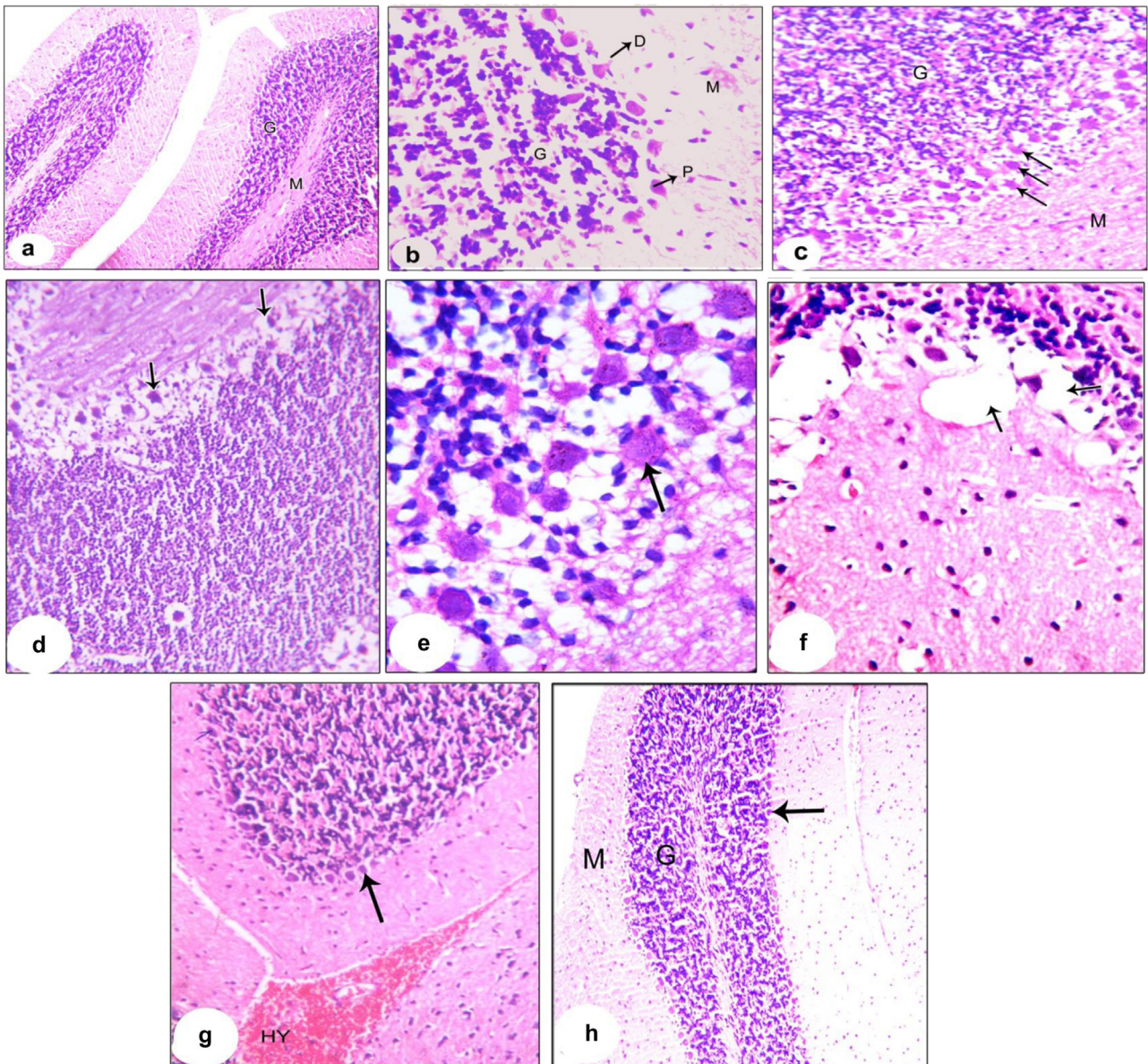
3.6.2.2. *Group (2) the testes of high fat diet rats.* Light microscopic examination of the testes of high fat diet revealed that congestion of blood vessels, dissolved parts of some seminiferous tubules and vacuolation of some tubules (Fig. 5c). In addition, seminiferous tubules with azospermia (absence of sperm) the basement membrane were ruptured and fatty changes in seminiferous tubules were also noticed (Fig. 5d). Besides, most tubules showed pyknotic nuclei in secondary spermatocytes (Fig. 5e) and vacuolation of germinal epithelium of seminiferous tubules (Fig. 5f).

3.6.2.3. *Group (3) testes of rats treated with high fat diet plus Orlistat.* (Rats treated with high fat diet plus Orlistat) resulted in severe degenerative changes in seminiferous tubules and spermatocytes exhibited signs of pyknosis (Fig. 5g). Marked dilatation and congestion of blood vessels were noticed in the interstitial spaces (Fig. 5h). Also, degeneration and azospermia in most of the seminiferous tubules.

3.6.2.4. *Group (4) testes of rats treated with high fat diet plus pumpkin oil and nutshell extract.* (Rats treated with high fat diet plus pumpkin oil and nutshell extract) showed histological picture more or less similar to control group and large numbers of spermatozoa are seen in the seminiferous tubules (Fig. 5i & j).

## 4. Discussion

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. It increases the probability of being infected with various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer and

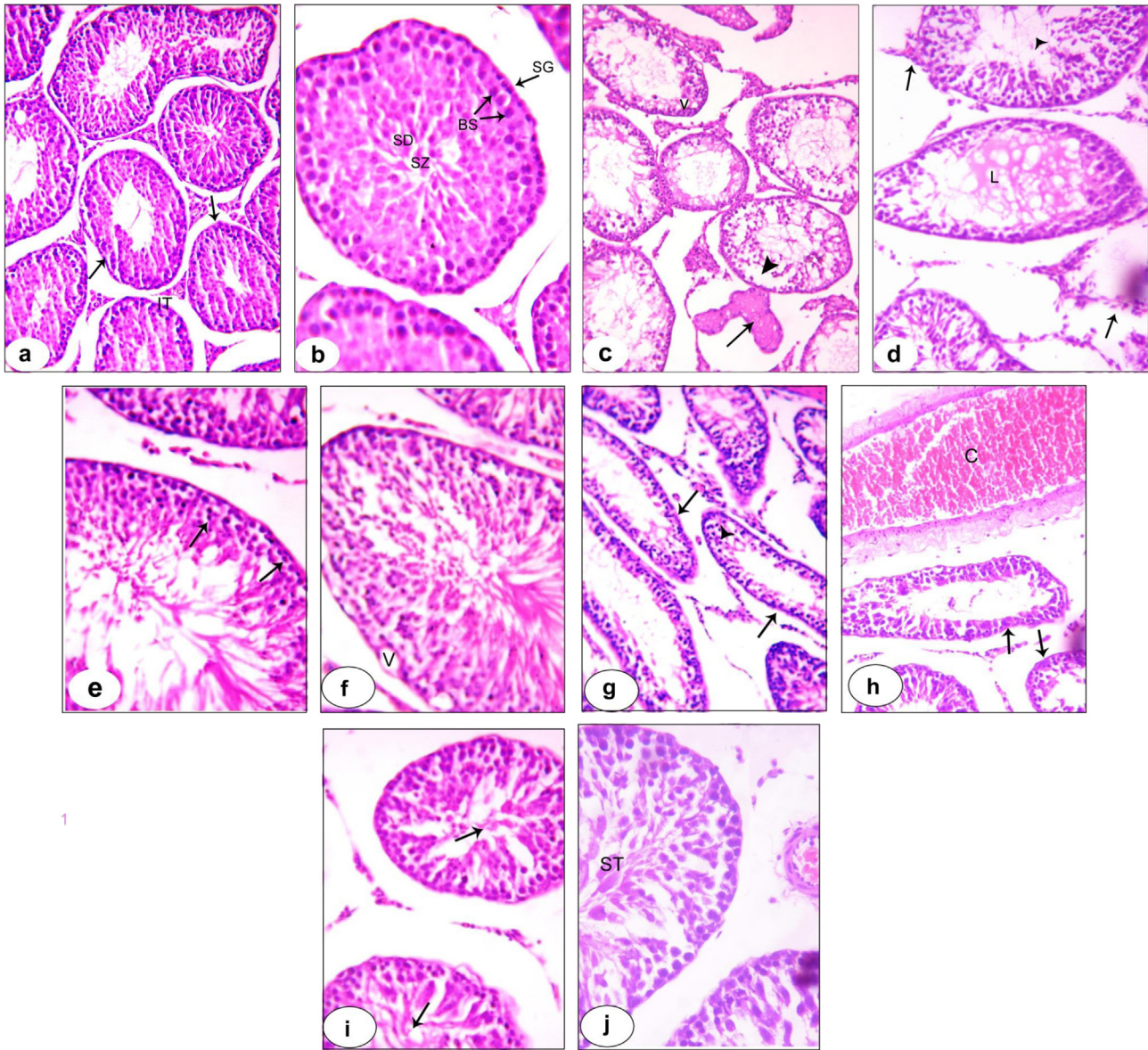


**Fig. 4 – a):** Photomicrograph of histological sections of the cerebellar cortex of control rats showing Molecular layer (M) and Granular layer (G). H & E  $\times$  100. **b):** Photomicrograph of histological sections of the cerebellar cortex of control rats showing molecular layer (M), granular layer (G), purkinje cell (P) and dendrites (D). H & E  $\times$  400. **c):** Photomicrograph of histological sections of the cerebellar cortex of rats treated with HFD showing molecular layer (M), granular layer (G) and purkinje cells ( $\uparrow$ ) revealed disturbed normal linear organization with marked disarrangement. H & E  $\times$  200. **d):** Photomicrograph of histological sections of the cerebellar cortex of rats treated with high fat diet showing pyknosis of purkinje cells ( $\uparrow$ ). H & E  $\times$  200. **e):** Photomicrograph of histological sections of the cerebellar cortex of rats treated with high fat diet (HFD) showing purkinje cells revealed swollen ( $\uparrow$ ) with karyolysed nuclei. H & E  $\times$  400. **f):** Photomicrograph of histological sections of the cerebellar cortex of rats treated with high fat diet plus Orlistat showing Molecular layer and Granular cell layer showed many vacuoles ( $\uparrow$ ). H & E  $\times$  400. **g):** Photomicrograph of histological sections of the cerebellar cortex of rats treated with high fat diet plus Orlistat showing Granular cell layer showed haemorrhage (Hy) and pyknosis of urkinje cells. H & E  $\times$  200. **h):** Photomicrographs of histological sections of the cerebellar cortex of rats treated with high fat diet plus pumpkin oil and nutshell extract showing molecular layer (M), granular layer (G) and purkinje cells ( $\uparrow$ ). H & E  $\times$  200.

osteoarthritis. Newer researches point to obesity as an important risk factor for male infertility (Du Plessis et al., 2010).

It is clear in this study that there is a high significant increase of food intake in free diet of HFD during 0th–16th week

and 18th–22nd weeks compared to the controlled diet. This is may be due to HFD causing hyperphagia which similar to human cafeteria diet. The mechanisms for how saturated fat based beverages contribute to human obesity are clear in rats on an HF choice diet, plasma leptin concentrations and



**Fig. 5 – a):** Photomicrograph of section of the testes of control rats showing the seminiferous tubules (t) as well as the interstitial tissue (IT). H & E  $\times$  100. **b):** Photomicrograph of section of the testes of control rats revealing successive stages of spermatogenesis, spermatogonia (SG), primary spermatocytes (PS), spermatids (SD) and spermatozoa (SZ). H & E  $\times$  400. **c):** Photomicrograph of section of the testes of rats treated with high fat diet showing congestion of blood vessels (arrow), dissolved parts of some seminiferous tubules (arrow head) and vacuolation of some tubules (v). H & E  $\times$  400. **d):** Photomicrograph of section of the testes of rats treated with high fat diet showing azospermia ( $\blacktriangle$ ), fat (L) and the basement membrane were ruptured (t). H & E  $\times$  100. **e):** Photomicrograph of section of the testes of rats treated with high fat diet showing nuclear pyknosis of secondary spermatocytes (t).H & E  $\times$  400. **f):** Photomicrograph of section of the testes of rats treated with high fat diet showing vacuolation of germinal epithelium (v) of seminiferous tubules. H & E  $\times$  400. **g):** Photomicrograph of the testes sections of rats treated with high fat diet plus Orlistat showing degenerated seminiferous tubules (t) with the absence of spermatogonial series and pyknotic nuclei of primary spermatocytes ( $\blacktriangle$ ).H&Ex100. **h):** Photomicrograph of the testes sections of rats treated with high fat diet plus Orlistat showing dilated congested blood vessels (C), degeneration in most of the seminiferous tubules (t). H & E  $\times$  100. **i):** Photomicrograph of the testes section of rats treated with high fat diet plus pumpkin oil and nutshell extract showing mild normal structure of spermatozoa (t). H & E  $\times$  100. **j):** Photomicrograph of the testes section of rats treated with high fat diet plus pumpkin oil and nutshell extract showing large numbers of spermatozoa are seen in the seminiferous tubules (ST). H & E  $\times$  400.



proopiomelanocortin mRNA increased and neuropeptide Y mRNA decreased (La fleur et al., 2009). Both palatability and energy density contribute to fat hyperphagia and reduced satiation signaling accompanying HFD consumption which can contribute to overconsumption and often lead to obesity. The current data shows a significant increase in BW and BMI especially in the 16th week of HFD in accordance with Akiyama et al. (1996). Increased BW and BMI may occurred due to the increase of caloric intake resulting in more adipose tissue deposition than starch diet. When we treated HFD with Orlistat and herbal mix ameliorated feed intake, significantly decreased BW and BMI. These effects of Orlistat are due to its blocking to the absorption of fat by inhibiting gastric and pancreatic lipase enzymes leading to the increased excretion of fat in faeces (Drent and van der Veen, 1995). Also, treatment with PO has its effects on feed intake, weight gain and BMI. This is because it contains a number of anti-oxidants and an excellent source of protein, zinc, magnesium, manganese and phosphorus. They also contain a high amount of tryptophan, an essential amino acid involved in the synthesis of a key brain chemical called serotonin which is involved in mood, sleep and appetite regulation (Supplementation with PSO, 2008).

Obesity increased neurotransmitters level. It caused increase in homocysteine, BCHE and catecholamine level. Butyrylcholinesterase is a serum esterase which is primarily synthesized in the liver (Silver, 1974) and released into plasma immediately following its synthesis. This enzyme is also found in the small intestine, smooth muscle, adipose tissue, brain and other tissues. It was suggested that it is a precursor of acetylcholinesterase in the nervous system, with an important role in the regulation of slow impulse conduction in the nervous system and that it is involved in the hydrolyzes of ingested esters from plant sources These results are parallel to Karatela and Sainani (2009) who found significantly raised plasma homocysteine, along with reduced vitamin B12 and folic acid levels compared to normal weight normotensives. Among the hypertensives, homocysteine was positively correlated significantly with obesity and arterial pressure levels. Leptin, the product of the obesity gene increases sympathetic nervous activity could contribute to its adverse effects on cardiovascular health by increasing total homocysteine (tHcy) levels. It was shown that higher BCHE activity is associated with higher BMI, higher triglycerides and lower High density lipoprotein (HDL). It was reported highly significant BCHE associations with serum total Low density lipoprotein (LDL) cholesterol (Valle et al., 2006). High serum lipid concentrations may induce stereoscopic alteration in the enzymatic configuration that modifies BuChE activity or altered expression of the enzyme encoding gene that determines BuChE concentration and activity. Belong to catecholamine induced lipolysis in visceral adipose tissue is increased in obesity due to increased function of beta3-adrenoceptors, decreased function of alpha2-adrenoceptors and increased ability of cyclic AMP to stimulate lipolysis. It was showed that obese rats have an increased catecholamine secretion response to all of the secretagogues that were tested in the adrenal medulla incubations. Tyrosine hydroxylase (TH) catalyzes the rate limiting step in the biosynthesis of the catecholamines dopamine, noradrenaline and

adrenaline. The increase of synthesis and activity of TH can be induced by leptin in vitro. It was reported that adult obese mice have decreased expression of the enzymes TH and dopamine beta hydroxylase (Martins et al., 2004). Despite this decrease in TH expression, catecholamine content is increased in the adrenal gland of prediabetic obese rats, which can be the result of decreased pre synaptic stimulation of adrenal medulla chromaffin cells which secretes catecholamines (primarily adrenaline).

Our results showed that treatment with Orlistat improved the level of these parameters according to Filippatos et al. (2005) who observed that after the 3-month treatment period with Orlistat showed significant reductions in gamma glutamyl transpeptidase activity and homeostasis model assessment (HOMA) index that alter the various hypothalamic neuropeptides' CNS levels, or alter the key CNS appetite monoamine neurotransmitters' levels may be suitable candidates for drugs that will suppress appetite. Sibutramine is the first new drug for treating obesity via appetite suppression (Tziomalos et al., 2009). Its main mechanism caused an increase in the feeling of satiety by controlling noradrenaline, serotonin, 5-hydroxytryptamine and dopamine. Sibutramine is a neurotransmitter reuptake inhibitor that reduces the reuptake of serotonin (by 53%), norepinephrine (by 54%) and dopamine (by 16%).

Also, our results showed that treatment with herbal mix (pumpkin oil) improve the level of these parameters due to its content of unsaturated fatty acids. Due to high omega-3 (6 and 9)-fatty acids. According to Goncalves et al. (2005) who demonstrated that omega-3 fatty acids modulate changes in the concentrations and actions of several orexigenic and anorexigenic neuropeptides in the brain, including neuropeptide Y, alpha melanocyte stimulating hormone and the neurotransmitters serotonin and dopamine. Taking a daily supplement of omega-3 fatty acids could help to lower levels of the amino acid homocysteine, high levels of which are linked to an increased risk of heart disease and dementia (Huang et al., 2011). In case of treatment with peanut shell extract it is contain effective compound called luteolin it is effectively produce a natural anti-inflammatory effect that stops the errant microglial cells from causing damage, protect cell neurons from damage and inhibited the production of neurotoxic inflammatory mediators.

Dietary cholesterol causes a significant decrease of brain GSH, brain SOD and a high significant increase of brain MDA as compared to the normal rats these results in a greement with Park et al. (2010) who found that HFD also increased the level of malondialdehyde (MDA) and decreased the level of brain derived neurotrophic factor (BDNF) in the hippocampus. The toxic effects of MDA were evaluated on neural progenitor cells (NPCs). MDA reduced the growth of neural progenitor cells (NPCs), but brain-derived neurotrophic factor (BDNF) treatment restored NPCs proliferation. The present data indicate that a HFD impairs hippocampal neurogenesis and NPCs proliferation through increased lipid peroxidation and decreased brain-derived neurotrophic factor (BDNF). While our study indicated that treatment of HFD rats with Orlistat produced a significant increase in brain GSH, a significant increase SOD and a high significant decrease in brain MDA as compared to the drug-administrated-control rats.

These results are in parallel to [Ohia et al. \(2002\)](#) who reported the action of Garcinia that is drug ameliorated feed intake and significantly decreased Body weight (BW) and BMI. These effects of hydroxy citric acid (HCA) in Garcinia achieved by increasing release/availability of 5 hydroxy tryptamine, or serotonin, also enhanced serotonin uptake in the brain. Serotonin, a neurotransmitter implicated in the regulation of eating behavior, appetite control and weight management by curbing appetite, reduction of food intake and inhibiting body fat biosynthesis. Also, treatment of HFD rats with herbal mix produced a significant increase in brain GSH, a significant increase SOD and a high significant decrease in brain MDA as compared to the drug-administrated-control rats. These results are similar to the results of [Patten et al. \(2013\)](#) who found that postnatal omega-3 supplementation was able to increase glutathione levels and reduce lipid peroxidation in prenatal ethanol exposure (PNEE) animals, partially reversing the effects of alcohol exposure, particularly in the dentate gyrus and the cerebellum. Luteolin also increases the level of Mn-SOD, (Cu/Zn)-SOD and glutathione (GSH) in the cortex and hippocampus to reduce the oxidative stress.

While in case of testes dietary cholesterol causes a significant decrease of testes GSH, testes SOD and a high significant increase of testes MDA as compared to the normal rats. These results in agreement with [Erdemir et al. \(2012\)](#) who stated that male rats fed with a high fat diet had significantly lower levels of testosterone compared with the control diet male rats. Obesity may induce oxidative stress and decrease testosterone levels. These changes may alter testicular functions and consequently it may be speculated that obesity can be important causative factor in the etiology of the male infertility. ROS damage the sperm membrane which in turn reduces the sperm's motility and ability to fuse with the oocyte and directly damage sperm DNA, compromising the paternal genomic contribution to the embryo. Our study indicated that treatment of HFD rats with Orlistat produced a significant increase in testes GSH activity, a significant increase in SOD activity and a high significant decrease in testes MDA level as compared to the high fat diet rats. These results are in agreement with [Yesilbursa et al. \(2005\)](#) who reported that MDA levels were significantly higher in obese patients than the control group. After 6 months of treatment with Orlistat in obese subjects. Plasma MDA levels were significantly reduced by weight loss with Orlistat.

While treatment of HFD rats with herbal mix produced a significant increase in testes GSH, a significant increase SOD and a high significant decrease in testes MDA as compared to the drug-administrated-control rats. These results are in agreement with that of [Modi et al. \(2007\)](#) who reported that the fluted pumpkin seed oil (FPSO) had been reported to possess some essential properties (vitamin A, tannins, linoleic acid, oleic acid and alkaloids) which suppress lipid peroxidation, hence, improving testicular function. This could be due to antioxidative properties such as tannins and vitamin A present in the oil. According to [Fukuchi et al. \(2004\)](#), vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis.

#### 4.1. Photomicrograph of the brain sections of normal, HFD, Orlistat and herbal treatment

The present work revealed that Orlistat induced morphological changes in the cerebellar cortex of adult albino rats, mostly on the purkinje and granular nerve cells which were in turn reflected on the morphological structure of all layers of the cerebellar cortex. The appearance of distorted shrunken electron dense purkinje cells was similar to the previous findings of earlier study that reported that long-term VPA administration causes considerable damage to the system associated with structural and functional biosynthesis of cell proteins manifested in markedly increased electron density in cytoplasm of purkinje cell ([Sobaniec Lotowska, 2001](#)).

Some investigators believed that presence of dark neurons situated in various regions of gray matter of the CNS is usually due to ischemia that occurs as a result of substantial abnormalities in the capillary wall of the cerebellar cortex. Subsequently, there were disorders in the transportation of sodium VPA and/or its toxic metabolites through structural elements of the blood-brain barrier to neurons and vice versa. Others suggested that the appearance of dark neurons might reflect a certain phase of apoptosis as they displayed markedly condensed cytoplasm and nucleoplasm ([Ratan et al., 1994](#)). Orlistat treated rats revealed will evidenced unstained haloes around purkinje cell perikarya and vacuolation in the nearby molecular and granular layer. This was mostly attributed to shrinkage of purkinje cells and withdrawal of their protoplasmic processes secondary to disintegration of the cytoskeletal elements of these cells. The processes of purkinje cells showed interesting morphological changes where they appeared shrunken and ischemic with electron dense cytoplasm. This was consistent with similar changes in their corresponding perikarya. Similarly [Sobaniec Lotowska \(2001\)](#) revealed the same findings and attributed them to severe damage to cytoskeletal elements within the perikaryon and protoplasmic processes of purkinje cells. As the degenerated purkinje cells failed to establish contact with the granule cells, this will lead to lack of normal synchronism between both that might minimize the regulatory role on them. This idea was supported by earlier postulations that assumed that several factors including Orlistat might be able to affect the cerebellar interneurons, glial cell appearance and proliferation in young rats ([Trabelsi et al., 2001](#)).

Dilated and congested blood capillaries observed after Orlistat treatment in this study were in agreement with other researchers who explained that sodium fluoride affected the vascular endothelial cells leading to releases of nitric oxide, which is an endothelial relaxing factor ([Jana et al., 2001](#)). In the present cases, there was a relationship between degree of purkinje cell degeneration and severity of granular cell diminution. This suggests that granular cell alteration was secondary to purkinje cell degeneration. Investigation of cerebellar mutant mice suggested that there was an interaction between purkinje cells and the external granular layer, i.e., the number of granular cells formed was appropriate to the number of purkinje cells nearby.

The protective effect of the present medicinal plants could be due to the antioxidant properties of pumpkin seed oil.

Nicolson (2007) demonstrated that administration of antioxidants can prevent excess oxidative membrane damage, restore mitochondrial and other cellular membrane functions. Pumpkin seed oil (PSO) is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, amino acids,  $\beta$ -carotenes, lutein and selenium (Zuhair et al., 2000). The seeds also contain L-tryptophan and omega (-6 and -3) fatty acids. When an antioxidant defense reaction occurs in an organism in response to toxicants, superoxide dismutase (SOD) is the first antioxidant to react with the reactive oxygen species, causing the superoxide anion radical ( $O_2^-$ ) to disproportionately form  $H_2O_2$  and  $O_2$ . Subsequently,  $H_2O_2$  is decomposed by catalase (CAT) into  $H_2O$  and  $O_2$  to decrease the accumulation of  $H_2O_2$  in organisms (Xu et al., 1995).

#### 4.2. Photomicrograph of the testes sections of normal, HFD, Orlistat and herbal treatment

The observations obtained from the present light microscopic studies, clearly demonstrated that the administration of the anti-obesity drug Orlistat to adult male rats induced variable pathological changes in the testes. The present investigation has revealed that some of seminiferous tubules appeared devoid of sperm, most probably, this is, due to alteration or inhibition of the process of spermatogenesis as a results of the drug administration and such changes may depend on the stage of spermatogenesis at which the drug exert its effect. Such observations reinforce those presented by Sakr (2010) who reported necrosis, focal disorganization, marked loss of spermatogenic cells and maturation arrest in the seminiferous epithelium of mice treated with difluorobenzuron and chlorfluazuron.

The present results also showed that the drug caused the appearance of degenerated spermatocytes at various stages of maturation partially separated from Sertoli cells or spermatogonia by vacuolated areas. The results of the present study showed many vacuoles appeared in the spermatogenic cells. These vacuoles may be formed by the fluid within the ground cytoplasm of the germinal epithelial cells especially in the spermatogonia and spermatocytes (Singh and Dominic, 1995). These features are in accordance with reports on the testes of vitamin A-deficient rats and testes of mice exposed to halogenated diamines. It was attributed this symptom to the adverse or noxious effects of treatment on the cell membranes, both structurally and functionally, causing marked disturbances in its permeability system.

On the other hand, Creasy and Foster (1991) presumed that large vacuoles may be phagocytic vacuoles remaining after the digestion of necrotic germ cells. Also, the same authors suggested that necrosis of spermatogonia and spermatocytes are very rapid so that early stages of degeneration are rarely seen. The formation of large vacuoles at the locations of spermatogonia and primary spermatocytes could be attributed to the degeneration and detachment of the spermatogenic cells (Sakr, 2010). The various vascular alternations (congestion of blood vessels and hemorrhage in the interstitial tissue) observed in the present investigation as consequence of Orlistat application are more or less in accordance with those encountered post-treatment with cadmium chloride

and X-rays, methotrexate in mice and codeine phosphate in rats. Similar features were also noticed by El-Far (1996) in the testicular tissues of mice and rats under the influence of the organophosphorous insecticide, dimethoate and the anti-inflammatory drugs, dexamethasone and diclofenac sodium, respectively. It was suggested that congestion of blood vessels as being due to the assumption that increased breakage of blood capillaries lead to further augmentation of interstitial edema and is consequent to the damaging effect on the interstitial tissue of the testes. On the other hand, Balasubramanian et al. (1980) attributed the congestion of blood vessels, in the intertubular tissue of testes of rats exposed to aspirin to the inhibition of prostaglandins synthesis, since these compounds are involved in the regulation of blood flow in the testes.

This study revealed that at dose of 5 mg/kg body weight fluted pumpkin seed oil (FPSO) improved testicular histology. This could be due to antioxidative properties such as tannins and vitamin A present in the oil. According to Fukuchi et al. (2004), vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis. Linoleic acid, a polyunsaturated fatty acid present in this oil is known to increase membrane fluidity and allows for osmosis, intracellular and extracellular gaseous exchange. Though they are easily susceptible to lipid peroxidation, the presence of vitamin A prevents it. Also, the presence of oleic acid, a monounsaturated fatty acid also reduces the susceptibility of the testis to lipid peroxidation. Foremost among organs and cell types affected by iron overload are liver, heart, kidney, pancreatic beta cells and testis (Fattahi et al., 2009). This accounts for the enlarged lumen and hypocellularity in the testis of the animals that received the highest dose. Tannins, even though they are classified as antioxidants, at a high dose, they could become pro-oxidant, increasing lipid peroxidation (Nworgu et al., 2007).

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## 5. Conclusion

From the above results we concluded that there is a significant relationship between fatty diet intake and structural changes to the brain with the elevated levels of dopamine, BCHE, homocysteine, and the brain damage was associated with increased oxidative stress monitored by increased MDA level whereas GSH and SOD production decreases. These changes may alter testicular functions and consequently it may be speculated that obesity can be an important causative factor in the etiology of the male infertility. While oral administration of Orlistat and herbal mix extracts reduced the level of circulating lipids as well as the decrease of body weights in male albino rats, these extracts improve brain and testes functions.

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## Authors' contributions

All authors carried out experimental work; biochemical analysis, statistical analysis, interpretation and discussion of the results related to their part of the work.

“Sanaa R. Galaly’ designed the study, wrote the protocol, managed the study of histopathology and wrote the first draft of the manuscript. ‘Walaa G. Hozayen’ designed the study, wrote the protocol, managed the analyses of the study and parts of biochemistry and wrote the first draft of the manuscript and ‘Kamal A. Amin’ designed the study, wrote the protocol, performed the statistical analysis and. ‘Shimaa M. Ramadan’ managed the literature searches. All authors read and approved the final manuscript.”

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