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Anti-hypercholesterolemic impacts of barley and date palm fruits on the ovary of Wistar albino rats and their offspring

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ABSTRACT

A high cholesterol diet is related to ovarian dysfunction and infertility which has been increased among young ages consuming processed food products. The present study was conducted to evaluate the role of a high cholesterol diet on the ovaries of young female rats via assessments of histopathology, immunohistochemistry, oxidative stress and apoptic markers. Also, mating of hypercholesterolemic female rats was carried out to measure the fertility and numbers of their offspring. At the same time, phytotherapy was carried out through supplementing the diet with barley and/ or date palm fruits (10%) during the experiment to assess the phytotherapeutic impacts in attenuation of drastic hypercholesterolemic effects.

Hypercholesterolemic diet-fed rats exhibited damage of the ovarian follicles and increased follicular atresia. Furthermore, expression of cleaved caspase-3 was upregulated, while PCNA was downregulated in granulosa, theca and stroma cells. Hypercholesterolemic female rats showed marked depletion of antioxidative enzymes, increased lipid peroxidation and apoptotic markers. Alterations to the female serum hormones were detected. Offspring maternally fed on hypercholesterolemic diet showed a significant decrease of body weight and altered sex ratio. However, concomitant supplementation of barley and or date fruits to hypercholesterolemic groups revealed marked improvement of ovarian structure and function.

On the basis of these evidences, it is believed that the enhanced synergistic effects of barley and/or date palm fruits in the amelioration of ovarian structure and functions were elicited by the potential antioxidant activity of their phytomicronutrients, polyphenols, β-glucan and trace elements. These materials scavenge free radicals from inflamed cells that can be used to establish an effective and novel therapeutic strategy for activating ovarian cell regeneration.

1. Introduction

The mammalian ovary is important for growth, maturation of ovarian follicles and production of 17β-estradiol (E2) and progesterone (P4) which in turn represents essential hormones for structural and functional integrity of reproductive system as well as of female secondary sex characteristics [1].

Cholesterol is important for steroidogenesis, especially in the theca and granulosa cells which maintain follicular maturation in the ovary [2]. Cholesterol can be delivered to the ovarian cells from plasma low-density lipoproteins (LDL) through the (LDL)-receptor mediated endocytic pathway and scavenger receptor (SR-BI)-mediated pathway [3].

Luteinizing hormone (LH) is secreted from the anterior pituitary gland with specific receptors on ovarian granulosa, theca and cumulus cells which have a regulatory role in follicular development [4]. The ovarian granulosa and luteal cells mainly synthesize and secrete progesterone and estradiol [5]. Binding of luteinizing hormone from the anterior pituitary to the theca cells increased the transcription of genes encoding the enzymes necessary for conversion of cholesterol to androgens (androstenedione and testosterone). Similarly, follicle stimulating hormone (FSH) binds to the FSH receptors in the granulosa cells promoting the transcription of genes encoding the enzymes necessary for conversion of theca-derived androgens into estrogens (17β-estradiol (E2) and estrone). Thus, FSH controls the progesterone and estrogen synthesis in the ovarian granulosa cells, whereas luteinizing hormone (LH) regulates the progesterone synthesis in the luteinized ovarian granulosa-luteal cells and androgen production in the ovarian theca-interstitial cells [6].

Hyperlipidic and hypercholesterolemic diet in our rat models was closely similar to high cholesterol diet in Ossabaw minipigs [7] and

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high fat diet of both murine [8] and rabbit models [9] that induce obesity and atherosclerosis. Also, it was reported that hypercholesterolemic [10,11] and high fat diet-fed animals [12,13] have type- 2 diabetes associated with atherosclerotic phenotype [14,15].

Previous studies have demonstrated that high fat high cholesterol diet showed a significantly luteinizing hormone surge and reduced LH response to gonadotropin releasing hormone (GnRH) in addition to increased number of atretic follicles and relatively decreased antral follicles [9,16]. In the same vein, a study of Lin et al. [17] reported decreased number of small follicular populations in the ovaries of high fat-fed dams, but there were no significant changes of the antral follicles and corpora lutea numbers.

The main culprit of hypercholesterolemic, hyperleptinemic hyperinsulinemic complications and elevated estrogen level were attributed to feeding on a high fat diet (20 weeks) in A/J and C57BL/6 J mice [18]. Another study on ob/ob mouse model has elucidated the ovarian dysfunction via excess lipid storage. This hyperlipidemia, as a consequence, induced follicular atresia, oxidative stress, apoptosis, impaired steroidogenesis and dramatically elevated caspase-3 expression [19].

The implication of high-fat diet in ovarian dysfunction was attributed to the over expression of genes involved in inflammatory response (Tnfα, IL1b, IL6) [20] or apoptosis (forkhead transcription factor subfamily 3, (Foxo3a)) and xenobiotic biotransformation (microsomal epoxide hydrolase (Ephx1), Cytochrome P450 isoform 2E1 (Cyp2e1), Glutathione S-transferase (Gst)) [21]

Many epidemiological studies in adult obese and overweight women have been reported an increased body mass index (BMI) associated with reduced LH levels [22,23] and longer follicular phase [24]. Recently, Newell-Fugate et al. [25] reported that Ossabaw minipigs fed on high cholesterol diet showed elevated levels of total cholesterol, triglyceride, and leptin levels coincides with abnormal reproductive function. Furthermore, many studies highlighted the relationship between obesity and ovulatory dysfunction [26] and gestational complications [27].

It was hypothesized that obese women demonstrated a sustained cortisol elevation and activated hypothalamic-pituitary-adrenal (HPA) axis [28] which represents a definite predictor of pathogenesis and impairment of ovarian functions [29]. Also, the higher dietary fat intake impairs hypothalamic-pituitary-ovarian (HPO) axis functionality and fertility. The reproductive dysfunction has been addressed through the increased signaling pathways of both leptin and insulin at the various levels of the HPO axis, as well as over expression of peroxisome proliferator-activated receptor gamma (PPARγ) and increased inflammation [30].

Phytonutrients of barley and date-palm fruits represent a promising natural sources for reducing the severity of hypercholesterolemia [31]. Barley (*Hordeum vulgare*) is one of the most economic cereal crops of Magnoliophyta widespread in the Middle East [32]. Its dry matter mainly composed of protein (14.31%), carbohydrate (54.70%), fat (8.70%), ash (17.45%) and crude fiber (4.84%). Dietary fibers such as β-glucans, arabinoxylans, and cellulose represents (11–34%) with about (3–20%) soluble dietary forms such as β-glucan (4–9%) [33]. β-glucans exerted hypolepidemic potentials especially for cholesterol and triglyceride levels in animal model [34] and human [35,36]. Another study of Behall et al. [37] reported potentials of barely in lowering total and LDL cholesterol by administration of 3–6 gm β-glucan/d from barely.

Also, seeds are rich in amino acids, including arginine, histidine, lysine, tyrosine, tryptophan, phenylalanine, cysteine, threonine, leucine, isoleucine, valine and glycine [38]. Furthermore, it is rich in antioxidants and phenolic compounds [39,40] such as benzoic and cinnamic acid derivatives, flavonoids, chalcones, tannins, quinones and proanthocyanidins as well as amino phenolic compounds [41,42].

Date- palm fruit (*Phoenix dactylifera*) is also one of the most native cultivated plants in Egypt, Iran and Saudi Arabia for more than 6000 years [43,44]. Date pollen ingredients and chemical composition contains mainly carbohydrate (44–88%), fat (0.2-0.5%), trace minerals (0.10–916 mg/100 g dry weight), protein (2.3–5.6%), vitamins and a high percentage of dietary soluble and insoluble fibers (6.4–11.5%) [45–48]. Moreover, it contains phenolic antioxidants (1–2%) especially tannin pigments based on (−)-epicatechin oligomers [49] which exerted an effective roles in alleviation of hypercholesterolemia [50].

Many studies have revealed the hypocholesterolemic impacts of dates and its ameliorative role in ovarian function. The oral administration of date pollen extract showed a sginificant increase of follicular growth and maturation of *in vitro* cultured mouse oocytes [51,52]. Also, date palm pollen contains estrogen-like compounds, sterols, estrone-like compounds and steroidal saponin glycoside which represent key factors in increasing female fertility [53].

The aim of the present work was to assess the impacts of high cholesterol diet in altering the ovarian structure and function as well as the susceptibility of ovarian cells for apoptosis. In addition to illustrating the phytochemical constituents of barley and date palm fruits; highlighting their ameliorative role in restoration of the ovarian activity and attenuation of cellular damage.

2. Materials and methods

2.1. Induction of hypercholesterolemia

Induction of hypercholesterolemia was carried out according to Enkhmaa et al. [54]. The standard diet is conjugated with 3% cholesterol, 8% cocoa butter, 2% cholic acid, 1% thiouracil and 1% starch. They were fed on it for 4 months. The control group was fed on a standard diet free from atherogenic components (Table 1). The animal handling and experimental work was carried out according to the guidelines of Animal Care and bioethics of the Faculty of Science, Mansoura University (Approval no: ZD18001).

2.2. Diets containing barley and/or dates

Standard diet containing all the nutrients requirements were prepared and used for feeding. Other diet components were prepared for negative control. It is composed of 10% barley grains or date palm fruit or both (10% dates plus 10% barley). Also, the hypercholesterolemic groups used for feeding barley and dates group contained 10% barley grains and or 10% of date palm fruits (Table 1).

2.3. GC–MS analysis of barley and date-palm fruit

Fresh date palm and barley were collected from local markets in Mansoura, Dakahlia, Egypt. Date-palm pollens and hulless barley grains were crushed out to small pieces and dried. About 100 g for each sample were soaked in 100 ml of highest purity chloroform in individual conical flask for 7 days. The extracts were then filtered using Whatman No.1 filter paper, condensed in rotary vacuum evaporator, and subjected to GC–MS analysis according to Glorybai et al. [55].

2.4. Animals and experimental design

Ninety six virgin female adult albino rats (*Rattus rattus*) weighing approximately 100 ± 10 gm body weight, obtained from Helwan Breading Farm, Ministry of Health, Egypt. They were housed in plastic cages with free access of food and water were *ad libitum*. The housing room is good aerated and had 12 h light and dark cycle. They were arranged into eight groups (n = 12) including; control (C), barley group (B) (Supplemented standard diet containing 10% barley grains), date-palm fruit group (D) (supplemented standard diet containing 10% fruit), barley & dates group (BD), high cholesterol diet-group (feeding on standard diet containing 3% cholesterol) (H), high cholesterol diet and barley grains(HB), high cholesterol diet and date palm fruit (HD), high cholesterol diet and both barley and date-palm fruit (HBD) (Table 1, Fig. 1).