HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/ejbas/default.asp

Full Length Article

Preventing male infertility by marjoram and sage essential oils through modulating testicular lipid accumulation and androgens biosynthesis disruption in a rat model of dietary obesity

*Azza M. El-Wakf**, *El-Sayed M. Elhabibi*, *Eman Abd El-Ghany*

Physiology Division, Zoology Department, Faculty of Science, Mansoura University, Egypt

ARTICLE INFO

Article history:
Received 8 March 2015
Received in revised form
7 May 2015
Accepted 8 May 2015
Available online 31 May 2015

Keywords:
Obesity
Male infertility
Aromatase
Essential oils
Marjoram
Sage

ABSTRACT

Obesity has been recognized as a leading cause for male infertility. This study aimed to investigate reproductive disorders caused by obesity and the possible prevention through the use of marjoram and sage oil extracts. Obesity was achieved in adult male rats by feeding high fat diet (HFD) for 12 weeks, while marjoram (0.16 ml/kg b.wt) and sage (0.05 ml/kg b.wt) oils were given orally for the same duration. HFD-fed rats exhibited marked obesity features indicated by increased adiposity index, with higher weight gain compared to control rats. This goes with increased lipid accumulation in testis and serum of the obese rats. Increased serum levels of leptin, prolactinL (PRL) and estrogen (E2), with reduced serum androgens; dehydroepiandrosterone (DHEA), testosterone (T) and T/E2 ratio were also observed. Additionally, the results showed significant reduction in epididymal sperm count, as well as in steriodogenic enzymes; 3β-hydroxysteroid dehydrogenase (3β-HSD), alkaline phosphatase (ALP), and acid phosphatase (ACP), with marked elevation in aromatase activity in testis of the obese rats. Histopathological alterations, including degenerative changes in seminiferous tubules, with sloughing, vacuolization and reduction of spermatogenic cells were also detected. Oral administration of marjoram or sage oil extracts, along with HFD seemed to prevent overall mentioned alterations, as evident by reduced testicular lipid accumulation, elevated androgens and sperm count, in addition to improved testicular structure. Results thus suggested that both oils should be considered in future therapeutic approaches for controlling adverse impact of obesity on male fertility.

1. Introduction

Obesity is a growing health problem that represents a major cause for number of chronic diseases. The most common, includes cardiovascular disease, type-2 diabetes, osteoarthritis and some types of cancer [1]. Besides, a direct relation between obesity and male infertility has been postulated [2].

* *Corresponding author*. Physiology Division, Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt. Tel.: +20 1001760744 (mobile).
E-mail addresses: drazza_elwakf@yahoo.com, dr_azzaelwakf@yahoo.com (A.M. El-Wakf).

Peer review under responsibility of Mansoura University.
http://dx.doi.org/10.1016/j.ejbas.2015.05.005

This relation has merited deep investigation over the past decade owing to the concurrent trends of rising obesity with increasing male infertility [3]. Researchers have found higher prevalence of oligospermia in overweight and obese men, with a significant association between sperm count and body mass index (BMI) [4]. Obese men have been shown to exhibit reduced androgens and sex hormone-binding globulin (SHBG) levels with elevated levels of circulating estradiol [5]. Obesity was also found to affect the GnRH-LH/FSH pulse that may impair Lyedig and Sertoli cell functions with subsequent effects on sperm maturation [6].

In recent years, a dramatic increase has occurred in the use of natural plants for maintaining health and preventing diseases. Of these, aromatic plants are characterized by the presence of volatile compounds with pleasant odor known as essential oils (EOs). Aromatic plants are particularly cultivated for the use in food processing, flavoring and other culinary purposes. However, they were identified also for their high curative activities [7]. One of the most familiar aromatic plants is marjoram (*Origanum majorana*, family Lamiaceae) which is particularly native to the Mediterranean region [8]. In folk medicine, marjoram extracts are used for coughs, cramps, depression, dizziness, gastrointestinal disorders, migraine and nervous headaches [9]. Besides, marjoram has been used as analgesic, antiseptic, antiviral, bactericidal and laxative agent [10]. Marjoram or its EOs seemed also to be effective in enhancing metabolism and maintaining healthy weight [11].

Sage (*Salvia officinalis*) is another aromatic plant belonging to family Lamiaceae. It is commonly used as a spice and condiment in food preparation, particularly in the Mediterranean cuisine [12]. Sage and its isolated oils are largely responsible for various therapeutic effects mainly indicated in the treatment of muscle pain and digestive disorders [13], as well as in promoting energy expenditure and fat oxidation, which may aid in body weight reduction [12]. Sage has shown also to possess antispasmodic, antidepressant and sedative activities [14].

Although much research supporting medicinal activities of both marjoram and sage oil extracts, little is known regarding their effects on obesity and related diseases, particularly male infertility. Therefore, the present study was carried out to investigate the therapeutic effectiveness of administrating marjoram and sage oil extracts in reducing negative impact of obesity on male fertility. This was achieved in terms of evaluating number of reproductive indices, including lipid profile, reproductive hormones, testicular enzymes and histopathological changes in testicular tissue.

2. Materials and methods

2.1. Experimental animals

This study was performed on male Wister albino rats initially weighing 170–180 g. Rats were permitted adequate standard rodent diet (purchased from Meladco fed Company, Aubor city, Cairo, Egypt) and given water *ad libitum* for one week of acclimation period before the experimental work. The animals care and experiments were complied with "Research Ethics Committee" Mansoura University, Egypt, in accordance with principles of the Institute of Laboratory Animal Resources, National Research Council "NRC" (NRC 1995).

2.2. Plant oils

Marjoram (*O. majorana*) oil extract was obtained from the Agriculture Research Center, Cairo, Egypt, while sage (*S. officinalis*) oil extract was obtained from "Nature's Alchemy" distributed by Lotus Brands, USA.

2.3. Chemicals

Dehydroepiandrosterone, NAD, glycerol and bovine serum albumin were purchased from Sigma Company for Chemicals, Cairo, Egypt. All other reagents are of analytical grade and purchased from local suppliers.

2.4. Experimental design

After the acclimation period, rats were randomly divided into seven groups (6 animals/each): group 1, rats were fed standard diet all over the period of the experiment; group 2, rats were fed standard diet and received sunflower oil as vehicle orally at a dose 1 ml/kg b.wt; group 3, rats were fed standard diet and received marjoram oil orally (0.16 ml/kg b.wt) diluted in sunflower oil (1:2) [9]; group 4, rats were fed standard diet and received sage oil (0.05 ml/kg b.wt) diluted in sunflower oil (1:2) [15]; group 5, rats were fed HFD consisted of normal laboratory diet in powder form mixed with melted animal abdominal fat (30%) and extra pure cholesterol (2%) [16,17]; groups 6 and 7, rats were fed HFD and received marjoram and sage oils at the same way and doses as described in the above groups. All animals were received their respective treatments daily for 12 weeks. Animal's weights were recorded at the start and at the end of the experiment in order to obtain the body weight gain.

2.5. Blood and tissue sampling

At the end of the experimental period, overnight fasted rats were sacrificed under ether anesthesia. Blood samples were collected, centrifuged at 855 × g for 10 min and sera were separated for further biochemical analysis. Immediately after collecting blood, the two testes, epididymes, vas deferens and seminal vesicle from each rat were removed and weighed. The right testis was taken for biochemical measurements, while the left testis was fixed in 10% neutral formaldehyde for histopathological examination. Adiposity index was determined by the sum of visceral, epididymal and retroperitoneal fat weights divided by body weight × 100 and expressed as adiposity percentage [18].

2.6. Preparation of tissue homogenate

One portion of the right testis was weighed, homogenized in cold distilled water and centrifuged at 855 × g for 10 min. Supernatant was used for analyzing biochemical parameters, except for 3β-hydroxysteroid dehydrogenase (3β-HSD). A second portion from the testis was weighed and homogenized at 4 °C in 20% spectroscopic grade glycerol, containing 5 mmol potassium phosphate and 1 mmol EDTA. Resulting