Egypt. J. Chem. Vol. 66, No 2 pp 213- 223 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of Frankincense (*Boswellia Carterii*) on Animal Reproductive Performance

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Abstract

Obesity is associated with metabolic disorders which affect male and female fertility. *Boswellia carterii* is rich in phenolic compounds and well known by its antioxidant, anti-inflammatory, hypoglycemic and hypolipidemic effect. The aim of this study was to study the potency of boswellia, if administered for complete reproductive cycle, to correct the metabolic disruption and improve the reproductive performance of animals. Boswellia oil was extracted and fractionated to identify its phenolic content. Microcapsules were prepared from the resin water extract using Arabic gum and maltodextrine. Antimicrobial activity was done for water extract. Forty *Sprague Dawely* rats were classified into 4 groups. Animals were separated according to sex and fed on normal fat diet (NFD), NFD beside boswellia microcapsules (500 mg/kg body weight), high fat diet (HFD) and HFD beside boswellia microcapsules at the same dose respectively. The experiment lasted for 60 days. Animals were sacrificed. Fecal colony count was done, the collected plasma was analyzed and epididymal spermatozoa profile was done. Histopathological examination and immune histochemistry were done to genital organs. Feeding on HFD resulted in hyperlipidemia, hyperglycemia, and low level of steroid hormones, IGF, insulin, TSH and the antioxidant enzymes. Elevated levels of leptin, lipid profile, glucose, liver enzymes and oxidative markers were also demonstrated. HFD negatively affected male and female fertility confirmed by histopathological examination. The immune staining showed strong expression of both markers (Caspase-3 and iNOS). Daily administration of boswellia microcapsules corrected all the metabolic and pathological disorders and enhanced animal fertility.

Key words: Testis; Ovary; Boswellia; Obesity; Hyperglycemia

Introduction

Both under-nutrition and over-nutrition are associated with low reproductive performance. High fat diets develop obesity and effect on fertility of both male and female. Many theories discuss the relationship between obesity and infertility. Elevated leptin level, which is produced by adipose tissue, lowers the level of testosterone in obese males as it binds receptors on Leydig cells [1]. Also, leptin at high concentration can directly inhibit hCG-stimulated testosterone production by inhibiting the conversion of 17OH-progesterone to testosterone. Leptin may have receptors on spermatocytes [2] leading to dysfunction of spermatogenesis [3].Concerning females, the condition of obesity is more complex than males due to cyclicity of the female reproductive system. Obese females have higher number of atretic follicles and increased ovarian hyalinization. It starts anovulatory cycles due to polycystic ovary. Most of these changes are reversible when body fat decreased by losing weight [4]. Fertility regulation with plant preparations had been used since ancient time. Frankincense is known as olibanum is obtained from trees of genus Boswellia. The resin is widely used as aphrodisiac and fertility promoter. It contains boswellic acid and other cyclic compounds which act as steroids and lowers lipid profile and blood sugar in obese individuals. Additionally it has strong antioxidant and antimicrobial effect and used for treatment of several inflammatory diseases [5]. The aim of the current study was to use boswellia to ameliorate the hazardous effect of obesity on male and female fertility as well as to take the elevated lipid profile and blood sugar back towards normal.

Material

Frankincense (oleogum resin of *Boswellia carterii*) was purchased from the local market of herbs and spices in Egypt. It was authenticated by comparison with a genuine sample kept in the drug museum of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Methods

Isolation of volatile compounds of *Boswellia Carterii*

The volatiles of *Boswellia Carterii* were extracted according to the hydro-distillation method using

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Receive Date: 15 May 2022, Revise Date: 28 May 2022, Accept Date: 31 May 2022

DOI: 10.21608/EJCHEM.2022.138276.6093

Clevenger's apparatus for 3 hours [6]. The yield of volatile oils was weighed and calculated in g /100 g dry plant.

Gas chromatography-mass spectrometry (GC – MS) analysis

A gas chromatography (Hewlett–Packard model 5890) coupled to a mass spectrometer (Hewlett–Packard-MS (5970) was used for analysis. Volatiles were separated using a fused silica capillary column DB-5 (60 m × 0.32 mm i.d. × 0.25 µm film thickness). The oven temperature was maintained initially at 50ºC for 5min, and then programmed from 50 to 250ºC at a rate of 4º C /min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The sample size was 2 µl, split ration 1:10, the injector temperature was 220ºC. Mass spectra in the electron impact mode (EI) were obtained at 70 eV, and scan m/z range from 39 to 400 amu. The isolated peaks were identified by matching with data from the mass spectra library (National Institute of Standard and Technology, NIST) and comparison with those of authentic compounds and published data [7].

Preparation of microcapsules

100 g Boswellia Resin was soaked in 1L boiled distilled water for 24 hours with continuous stirring. An emulsion of resin-in-water was formulated as an initial step to microencapsulation using spray drying. The emulsion was formed by hydrating gum Arabic (GA) in deionized water to prepare 15.0% w/v solution. The gum solution was left overnight at 4ºC to allow full hydration**.** Equal weight of maltodextrin (MD, 15.0 g) was dissolved in the same gum, solution to end up with total solid content 30.0% w/v (GA/MD 1:1w/w was added toTween®80 (1.0 % w/v, based on water). Intensive mixing was done followed by the addition of certain weight of resin to obtain a 1:3 w/w resin/ total solids emulsion. The prepared resin emulsion contains 10 g resin, 15.0g GA, 15.0g MD, 1.0g Tween 80 and 100 mL water. The whole mixture was magnetically stirred for 5.0 minutes to form a coarse pre-emulsion then was homogenized for 20 minutes at full sonicator power (200.0 Watt, 24kHz) using UP200S ultrasound homogenizer (IKA Hielscher GmbH, Berlin, Germany) equipped with an impulse generator 14 mm in diameter. To avoid the rise of emulsion temperature above ambient during ultrasound homogenization, the emulsion vessel was immersed in a cold water bath. The formulated emulsion was dried using spray drier during the whole process.

Spray drying process

The spraying system consisted of two-fluid nozzle composed of an internal tip with an opening of 0.7 mm in diameter and an external ring with an opening of 1.5 mm in diameter. Moreover, the constant process parameters included a drying air flow rate of 85.0% of the suction fan controller. The inlet and outlet temperatures were 160.0EC and 80.0EC (±1.0EC), respectively. Furthermore, the resulted powder was collected from the drying chamber wall and the cyclone.

Antimicrobial activity of Boswellia

Culture and maintenance of microorganisms

Pure cultures of bacteria and fungi were obtained from the Laboratory of Microbiology of the National Research Centre, (Egypt) and maintained on nutrient agar medium and potato dextrose agar (PDA) medium for bacteria and fungi, respectively. Subculture was done for all organisms on the same medium and stored at 4ºC. The used bacteria were Gram-negative bacteria (*E. coli* ATCC 6937, *Sal. Typhimurium* ATCC® 14028™ and *Klebsiella pneumoniae*) and four strains of Gram-positive bacteria (*B. cereus* ATCC 33018, *S. aureus* ATCC 20231, *Bacillus subtilis* ATCC 21332 and *Streptococcus pneumonia* ATCC 49619), and fungi (*Candida albicans* ATCC 14053and *Saccharomyces cerevisiae* ATCC9763). The cultures of bacteria were maintained in their appropriate agar slants at4°C and used as stock cultures. Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA) were used in the present study.

Media Preparation

For agar well diffusion method antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay Mueller-Hinton agar and for fungus Potato Dextrose Agar was used for developing surface colony growth. The suspension culture for bacterial cells growth was in tryptone Soya Broth (w/v), and for fungus cells growth was in Potato dextrose broth (PDB).

Agar well diffusion method

Agar well-diffusion method was done to determine the antimicrobial activity [8]. The strains were transferred to test tubes containing Mueller-Hinton medium at 37°C for 24 h. 1 mL culture of the activated indicator strain (10^5cfu /mL) was inoculated into 20 mL of Mueller-Hinton agar (Becton Dickinson, USA) and poured in sterile Petri dishes. After solidification of the agar, wells of 5 mm diameter were cut from the agar with a sterile borer and 50 µL of boswellia water extract was poured in each well. After holding the plates at room temperature for 2 h to allow diffusion of the extract into the agar, the plates were incubated at 37 °C for 24 h. The antifungal spectrum activity was performed by the agar-well diffusion method [9]. Fungal spore count was adjusted to $1x10^6$ spores / ml by making appropriate dilutions [10]. Petri plates containing SDA (Himedia) were inoculated aseptically with 100 µl spore suspension through spread plate technique and kept in the laminar flow for 30 min. Wells of 5 mm diameter were punched in the center of the petri plates by using sterile cork borer. Into each well, 10 µl of boswellia extract was dropped followed by 40 µl of dimethyl sulphoxide (DMSO) so as to make the 50 µl total volume in the well. Plates were kept in refrigerator at 4 ˚C for 3 h, to allow boswellia extract and DMSO to diffuse into the agar medium. Plates were finally incubated at 25±1 ˚C for 5 days. DMSO without boswellia extract was set as negative control. The diameter of the zone of inhibition around each well was measured in mm.

Diet formulation

Two types of diet were formulated (table 1): normal fat diet and high fat diet [11].The dry matter was added on the expense of starch.

Table 1. Diet formulation for experimental animals

Ingredients (gm)	Balanced diet (NFD)	High fat diet (HFD)
Casein	12	12
Safflower oil	10	-

Refined palm oil	-	10
Coconut oil	-	15
Sucrose	23	38
Starch	45.5	19
Vitamin mix	1	1
Mineral mix	3.5	3.5
Cellulose	5	-
Cholesterol	-	1
Bile salts	-	0.25

Biological study

Animal experiment was designed and done under the regulation of ethical committee of NRC (ethical approval no. 20170). 40 Sprague dewily rats, of both sexes, were purchased from NRC animal house, kept in stainless steel cages under controlled temperature, ventilation, feeding and drinking system. Animals were divided into 4 groups (10 each subdivided into two subgroups according to sex, 5 each). The first was control negative group received Balanced or normal fat diet (NFD). The second group received NFD and administered the boswellia microcapsules (BOS) (500 mg / kg body weight) (table 1). The third group received high fat diet (HFD); the fourth group was given HFD plus BOS at dose 500 mg/kg body weight. The experiment lasted for 60 days.

Plasma chemistry

At the end of the experiment, blood was collected from animals under anesthesia, centrifuged at 3000 rpm for 10 min. Sera were collected and stored at −20 °C until used for determination of glucose, lipids profile, total antioxidants, malondialdhyde (MDA), catalse, gamma glutamyl transferase (GGT), urea, creatinine using colorimetric kits purchased from Bio diagnostic, Egypt. Testosterone for males, estrogen for females, triidothyronine (T3) and thyroid stimulating hormone (TSH), insulin growth factor (IGF) and insulin hormone ELISA kits were purchased from Sun Long Biotech, China.

Microbial colony count of fecal samples

At the end of the experiment, rats' fecal samples were collected for microbiological evaluation by soaking in tubes containing 9 ml of serial diluted solution of sterile saline. Total bacterial counts were aerobically incubated using nutrient agar at 25°C for 48h. Coliform groups were detected using Violet Red bile Agar and the plates incubated at 35 ºC for 24 h[12]. Mold and yeast counts were determined using acidified potato dextrose agar and the plates incubated at 25 ºC for 3days [13].

Genital organs weight

The total body weight of animals before scarification and weight of the reproductive organs in both males and females were recorded to compare between groups. After collection of epididymal spermatozoa from males' evaluation of concentration, motility, live, dead and abnormal percentage was carried out.

Epididymal spermatozoa profile

Sperms Motility

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 mL with Tris buffer solution [0.3 M Tris (hydroxymethyl) amino methane, 0.027 M glucose, 0.1 M citric acid]. A warmed glass slide was placed on phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from 3 different fields in each sample. The mean of the 3 estimations was used as the final motility score. Samples for motility evaluation were kept at 37°C [14].

Sperms count

The epididymal spermatozoa concentration was determined with a hemocytometer using a modified method [15]. The right epididymis was finely minced by anatomical scissors in 1 mL of isotonic saline in a Petri dish. It was completely squashed by a tweezers for 2 min, and then allowed to incubate at room temperature for 4 h to provide the migration of all spermatozoa from epididymal tissue to fluid. After incubation, the epididymal tissue-fluid mixture was filtered via strainer to separate the supernatant from tissue particles. The supernatant fluid containing all epididymal spermatozoa was drawn into the capillary tube up to 0.5 lines of the pipette designed for counting red blood cells. The solution containing 0.595 M sodium bicarbonate, 1% formalin and 0.025% eosin was pulled into the bulb up to 101 lines of the pipette. The contents of the pipette were mixed by holding the ends of the pipette between the thumb and the index finger and shaking it vigorously in 100 back-and-forth 30 cm movements. The bulb of the pipette contains a small glass beads for mixing. Sufficient solution was then blown from the pipette to ensure that the diluents containing no sperm were flushed from the capillary. This gave a dilution rate of 1:200 in this solution. Approximately 10 µL of the diluted sperm suspension was transferred to both counting chambers of an Improved Neubauer (Deep 1/10 mm, LABART, Darmstadt, Germany) and allowed to stand for 5 min. The sperm cells in both chambers were counted with the help of a light microscope at 200× magnification.

Sperms abnormality

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared, then viewed under a light microscope at 400x magnification. Two-hundred spermatozoa were examined on each slide. The head, tail and total abnormality rates of spermatozoa were expressed as percent. Also the Live sperms were white (unstained) while, dead sperms acquired the stain. 100 sperms per rat were counted and viability percent (%) was calculated [16].

Histopathological examination

At the end of the experiment period, the total body weight of animals and genital organ weight were recorded. Specimens from ovaries and testis were collected, fixed in neutral buffered formalin 10%, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 5 micron thickness and stained with Hematoxylin and Eosin [17] for histopathological examination. Stained sections were examined by a light microscope (Olympus BX50, Japan).

Histopathological lesion scoring for testes

Histopathological alterations of testes were recorded and scored as, no changes (0), mild (1), moderate (2) and severe (3) changes, the grading was determined by percentage as follows: <30% changes (mild change), <30% – 50% (moderate change), and >50% (severe change) [18, 19].

Histomorphometric study for ovarian tissue

In female rats samples, the number of follicles from three sections of each ovary/ (n=10) were counted microscopically using TS view version 6.2.4.5 software [18].

Immunohistochemistry

The tissue sections from ovaries and testis were deparaffinized in xylene and rehydrated in different grades of alcohol. The antigen retrieval was done by pretreating the sections with citrate buffer of pH 6 for 20 min. Sections were incubated with rabbit polyclonal anti-caspase-3 antibody at a concentration of 1:1000 (ab4051; Abcam, Cambridge, UK), and rabbit polyclonal anti-iNOS antibody at a concentration of 1:100 (ab15323; Abcam, Cambridge, UK) for two hours in a humidified chamber. The sections were incubated with goat anti-rabbit IgG H&L (HRP) (ab205718; Abcam, Cambridge, UK), and 3, 3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) was used as a chromogen. Finally, the slides were counter stained with haematoxylin and mounted with DPX. The negative control slides were prepared by replacing primary antibodies using PBS [20].

Evaluation of caspase-3 and iNOS immunostaining

The quantitative immunoreactivity of caspase-3 and iNOS was evaluated in tissue sections of testis and ovaries in each group [21], five tissue sections were examined. Immuno-reactivity was analyzed in 10 microscopical fields per each section under high-power microscopic field (x 400). The percentage of positively stained cells (%) was estimated by color deconvolution image J 1.52 p software (Wayne Rasband, National Institutes of Health (U.S.A.).

Results and Discussion

GC mass of Boswellia oil

Table2:Volatile components isolated by hydrodistillation of Boswellia

Peak	RT	Name	Area %
1	17.92	Acetic acid, decyl ester	4.87
2	28.93	Nerolidol	7.27
3	41.61	geranyl-.alpha.-terpinene	0.47
4	42.651	Cembrene	1.28
5	43.624	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	4.56
6	43.83	VERTICELLOL	1.69
7	44.934	(S,E)-8,12,15,15-Tetramethyl-4-methylenebicyclo[9.3.1]pentadeca-7,11-diene	4.68
8	45.689	Butyl 4,7,10,13,16,19-docosahexaenoate	0.44
9	46.073	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	1.97
10	48.425	(3E,7E,11E)-1-Isopropyl-4,8,12-trimethylcyclotetradeca-3,7,11-trienol	1.65
11	48.499	(3E,7E,11E)-1-Isopropyl-4,8,12-trimethylcyclotetradeca-3,7,11-trienol	1.52
12	49.008	Thunbergol	36.9
13	49.592	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol	32.69

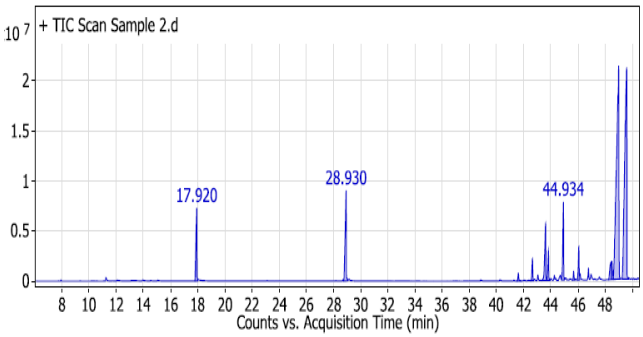

Fig 1: Gas Chromatograms of hydro-distilled *volatile compounds of Boswellia Carterii*

The Essential oil of Boswellia resin appeared as pale yellowish, fragrant oil lighter than water. The current Gas-Chromatography–mass spectrometry analysis of *Boswellia carterii* in table (2) and Figure (1) showed mainly 13 volatile compounds. The main volatile compounds were Thunbergol (36.9%) followed by Isopropyl-1, 5, 9-trimethyl-15-oxabicyclo [10.2.1] pentadeca-5, 9-dien-2-ol (32.69%). Also Nerolidol (7.27%), Acetic acid, decyl ester (4.87%)(S,E)-8,12,15,15-Tetramethyl-4- methylene bicycle [9.3.1] pentadeca -7,11-diene (4.68), (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene (4.56%) and other volatile compounds were detected as shown in table (2). The identified components were identified by their retention time and mass spectral comparison. The retention time is influenced by the number and the type of functional groups present and generally increases with increasing molecular weight of compounds **[22]**.

Earlier study mentioned that Frankincense contains 5-9% of essential oil, whose composition varies in relation to the biological source. It is a mixture of heteropolysaccharides, polysaccharides and polymeric substances. About 65-85% of resin are alcohol-soluble and about 20% are water-soluble gum **[23]**.

The essential oil of *Boswellia carterii* was previously reported to have octyl acetate as a major component beisde α-thujene , α-pinene, camphene, sabinene, β-pinene, myrcene , hexyl acetate, p-cymene , Z-β-ocimene , E-β-ocimene , limonene, 1,8-cineole, 1-octanol, linalool , α-pinene epoxide, trans verbenol, terpinene-4-ol and bornylacetate **[24]**.

Earlier studies recorded that the Pharmacokinetic activity of the gum resin of B. carterii showed inhibitory effect on CYP450 enzymes which is large and diverse group of enzymes that catalyse the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones [**25, 26**].

The antimicrobial effect of Boswellia

Table 3. Antimicrobial activity of Boswellia

No.	Strains	Diameters Inhibition zone (mm)
1	*Staphylococcus aureus*	22
2	*Bacillus cereus*	14
3	*Escherichia coli*	22
4	*Salmonella typhimurium*	18
5	*Bacillus subtilis*	12
6	*Streptococcus pneumoniae*	15
7	*Klebsiellapneumoniae*	17
8	*Candida albicans*	10
9	*Saccharomyces cerevisiae*	12

Concerning the antimicrobial activity of Boswellia, results (Table 3) showed that the greatest inhibition zone of *boswellia e*xtract was against *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Klebsiella pneumonia, Streptococcus pneumoniae, Bacillus cereus, Bacillus subtilis, Saccharomyces cerevisiae and Candida albicans* respectively. The antimicrobial effect of boswellia was confirmed by microbial count of rat fecal samples (table 4) where the groups of animals given the microcapsules showed significant lower colony count of natural inhabitant coliform bacteria, mold and yeast compared to control groups. Boswellia has high content of different phytochemicals which might inhibit bacteria by different mechanisms like enzymatic inactivation and target sites modifications. So that, administration of Boswellia can control the antibiotic resistant bacteria such as *S. aureus*, *E. coli*, *K. pneumonia* and *B. subtilis*if given in association with antibiotics and can lower the therapeutic dose of antibiotic and prevent its residue inside tissue [**27**].

Table 4. Microbial count of fecal samples of rats fed on Boswellia microcapsules

Treat	Total bacterial counts		Coliform counts		Mold & yeast	
	F	M	F	M	F	M
	Log CFU/g					
NFD	5.92	5.80	2.51	2.4	4.59	4.62
NFD+BOS	4.6	4.41	1.39	1.24	2.7	2.04
HFD	7.78	7.17	2.90	2.38	4.31	4.26
HFD+BOS	6.00	5.86	1.14	1.10	2.36	2.27

Weight of genital organs

Table 5: weight of male and female genital organs

Male organs weight	NFD	NFD+BOS	HFD	HFD+BOS
B w (g)	320±12.05^{a}	323±17.9^{a}	353± 6.65^{a}	341± 12.05^{a}
B fat (g)	13.9±0.92^{ab}	12.12±1.08^{b}	16.5±1.49^{a}	15.35±1.44^{a}
R testis (g)	2.29±0.11^{a}	2.45±0.20^{a}	2.27± 0.78^{a}	2.28±0.12^{a}
L testis (g)	2.28±0.11^{ab}	2.47±0.17^{b}	2.23± 0.06^{a}	2.36±0.93^{a}
R.epidid (g)	0.20 ±0.01^{b}	0.26±0.04^{a}	0.22± 0.12^{a}	0.28± 0.02^{a}
L. epidid (g)	0.26±0.03^{a}	0.26±0.11^{a}	0.23 ±0.016^{a}	0.30±0.01^{a}
S.vesicle (g)	1.16±0.5^{c}	1.26±0.08^{bc}	1.07±0.24^{a}	1.24±0.05^{bc}
Female organs weight				
B w(gm)	108.0 ±3.1^{a}	99.8±3.03^{ab}	120±4.3^{b}	103.0±2.8^{a}
R.ovary (g)	4.90 ± 0.06^{ac}	5.72±0.19^{ab}	4.0±0.24^{c}	6.00±1.22^{b}
L.ovary (g)	4.80 ± 0.03^{a}	5.31±0.10^{ab}	3.98±0.20^{c}	5.90±0.90^{b}

Data were presented as means ± SEM. Different superscripts within rows means significant P≤ 0.05.

Results in table (5) showed that administration of BOS microcapsules to rats fed on NFD showed higher weight of genital organs either in male and females compared to control group (NFD). The animals fed on HFD were higher in body weight and body fat and in the same time there were lower weight of testis, epididymis and seminal vesicles of males and ovaries in females compared to NFD group. Administration of boswellia microcapsules to animals fed on both NFD and HFD showed marked increase in genital organs and lower body weight in both male and female groups if compare to their control groups. Earlier studies showed that feeding male rats on frankincense for complete reproductive cycle showed increased body weight, weight of testis, epididymes, seminal vesicles and ventral prostate [**28**].

Rats fed on NFD

Table 6. Evaluation of Epididymal Spermatozoa

	NFD	NFD+BOS	HFD	HFD+BOS
Concentration x10^{6}/ml	4.6±0.61^{a}	11.8±0.54^{b}	3.6±0.5^{a}	9.0±1.7^{b}
Motility %	76.0± 1.78^{a}	86.0± 1.87^{b}	72.0±1.22^{a}	79.0± 4.0^{ab}
Motility % after1h	55.0± 2.44^{b}	66.0 ±1.88^{b}	62.0 ±3.4^{ab}	66.0±1.8^{a}
Live sperm %	83.4±1.5^{b}	84.4±1.6^{b}	90.4±1.2^{a}	89.0±0.4^{a}
Dead sperm %	14.6±3.78^{b}	16.8±4.4^{b}	10.0±1.04^{a}	9.4±0.24^{a}
Abnormal %	28.6±2.9^{a}	18.6±2.83^{b}	37.4±3.6^{a}	30.0±2.02^{a}

Data were presented as means ± SEM. Different superscripts within rows means significant P≤ 0.05.

Evaluation of Epididymal Spermatozoa showed that administration of Boswellia to NFD males group (Table 6) showed better epididymal spermatozoal profile that was clear in elevated concentration and motility and lower percentage of abnormalities compared to NFD group. These finding were confirmed by the results of analysis of plasma chemistry (table 7) where significant higher levels of testosterone, Estrogen, IGF, catalase, glucose, cholesterol and HDL in the same group (NFD plus BOS) in all animals (male and female) compared to control group (NFD). Also the histopathological finding of the males of the same group (NFD+BOS**)** showed normal histological structure of seminiferous tubules (fig 2b), the same as NFD group (fig 2 a) and there was no immune expression (fig 3 a, b) in testis for both markers (Caspase-3 and iNOS). Female rats received the same treatment (fig 3a) showed normal histological picture. The number of ovarian follicles was increased by administration of boswellia microcapsules if compared to NFD control group (table 8).

Earlier studies showed that feeding male rats on frankincense for complete spermatogenic cycle showed significant increase in sperm density and motility. The authors added that the histological and histometry study showed significant increase in nuclear diameter of leydig cell and the height of epithelial lining of epididymes and seminal vesicles.

The authors reported marked elevation in glucose, FSH and testosterone hormone levels and marked decrease in cholesterol and triglycerides levels in treated animals. Authors suggested the positive effect of boswellia on hypothalamo-pitutary axis [**28**]. Another study mentioned that addition of boswellia resin to broiler chickens feed resulted in significant increase in body weight gain, elevation of serum immunoglobulins and marked decrease in ventral body fat of carcasses [**29**]. Concerning females, earlier study mentioned that the administration of boswellia resin lowered the rate of pregnancy and the number of embryos. He referred the condition to hormonal causes where the level of estrogen kept high while pregnancy is maintained by higher level of progesterone **[30]**.

Table 7. Plasma chemistry of animals fed on high fat diets and Boswellia microcapsules

	NFD	NFD+BOS	HFD	HFD+BOS
Testosterone (pg/ml)	104.08±0.16^{a}	114.82±0.76^{b}	93.67±0.13^{c}	104.14±0.39^{ab}
Estrogen (pg//ml)	30.97± 0.24^{a}	31.97± 0.20^{c}	30.00 ± 0.32 ^{b}	32.17±0.51^{c}
IGF (ng/ml)	569.9±2.4^{a}	571.65±5.1^{b}	455.8± 8.5^{b}	509.0±7.8^{ab}
Insulin (µU/ml)	17.10± 0.33^{a}	17.00± 0.03^{a}	9.89± 0.15^{b}	12.64± 0.29^{c}
T3 (ng/ml)	11.22±0.26^{a}	11.82±0.72^{a}	10.92±0.13^{a}	11.27±0.39^{a}
TSH (ng/ml)	2.04 ± 0.04^{a}	2.14 ± 0.04^{a}	1.50 ± 0.17^{b}	1.90±0.05^{c}
Leptin ng/ml	9.67±0.1^{a}	9.5±0.2^{a}	11.17±0.1^{b}	10.9±0.2^{b}
Catalase (IU/l)	244±0.02^{a}	266±0.05^{b}	204±0.05^{c}	234±0.05^{ab}
MDA (mMol/L)	13.06±0.02^{a}	10.14±0.09^{a}	28.02±0.02^{b}	13.00±0^{a}
Tl.Antoxidant (mM/L)	2.13±0.03^{a}	2.03±0.03^{a}	1.43±0.04^{b}	2.08±0.16^{a}
Zinc (mg/dl)	281.3±6.61^{a}	278.0±9.36^{a}	283.5±11.7^{a}	292.0±10.4^{a}
Phosph (mg/dl)	8.18±1.35^{a}	8.58±0.29^{a}	10.58±0.45^{b}	8.54±0.92^{a}
Glucose (mg/dl)	81.4±0.4^{a}	90.8±0.89^{b}	149.8±3.1b^{c}	109.9±2.8^{d}
Cholesterol (mg/dl)	150.6±3.8^{a}	179.1±3.85^{b}	237.1± 4.6^{b}	237.0±3.6^{b}
HDL (mg/dl)	45.4±1.2^{a}	67.5±1.3^{c}	25.6±5.6^{b}	41.4±5.3^{a}
LDL (mg/dl)	37.7±1,09^{a}	33.1± 1.3^{a}	276.5±2.5^{b}	104.9±1.3^{c}
Triglycerides (mg/dl)	73.2±3.4^{a}	70.9 ±2.6^{a}	102.61.3^{b}	68.0±1.6^{a}
Total lipid (mg/dl)	317.3± 20.4^{a}	327.3± 20.0^{b}	548.49 ± 2.1^{c}	473.19 ±34.2^{d}
Total protein (g/dl)	5.48±0.32^{a}	5.58±0.21^{a}	5.40±0.15^{a}	5.54±0.12^{a}
Albumin (g/dl)	3.48±0.14^{a}	3.50±0.52^{a}	3.96±0.29^{a}	3.12±0.29^{a}
GGT (g/dl)	75.4 ± 1.13^{a}	70.4 ± 1.03^{a}	84.6± 9.36^{b}	64.6± 3.36^{c}
Urea (nmol/l)	8.26±0.02^{a}	8.30±0.001^{a}	8.28±0.03^{a}	8.28±0.02^{a}
Creatinine (mg/dl)	0.67±0.01^{a}	0.50±0.01^{a}	0.57±0.02^{a}	0.60±0.03^{a}

Data were presented as Means ± SEM. different superscripts in the same row means significance P≤ 0.05

Table 8. Scoring of histopathological alterations in testis of males and the number of ovarian follicles and cysts in female

Male genital organs	NFD	NFD+BOS	HFD	HFD+BOS
- Testicular degeneration	0	0	2	0
- Edema of tunica albuginea	0	0	3	1
- Tunica albuginea b v. congestion	0	0	3	1
- Interstitial edema	0	0	3	1
- Interstitial congestion	0	0	2	1
Female genital organs				
Number of ovarian follicles	19.25±2.53^{b}	21.75±3.12^{b}	4.75±1.82^{a}	15.25±2.61^{b}
Number of follicular cyst	0	0	5.6±1.32^{b}	1.3±0.94^{a}

The score system of males was designed as: score 0 = absence of the lesion in all rats of the group (n= 5), score 1= (<30%), score 2= (<30% – 50%), score 3= (>50%).

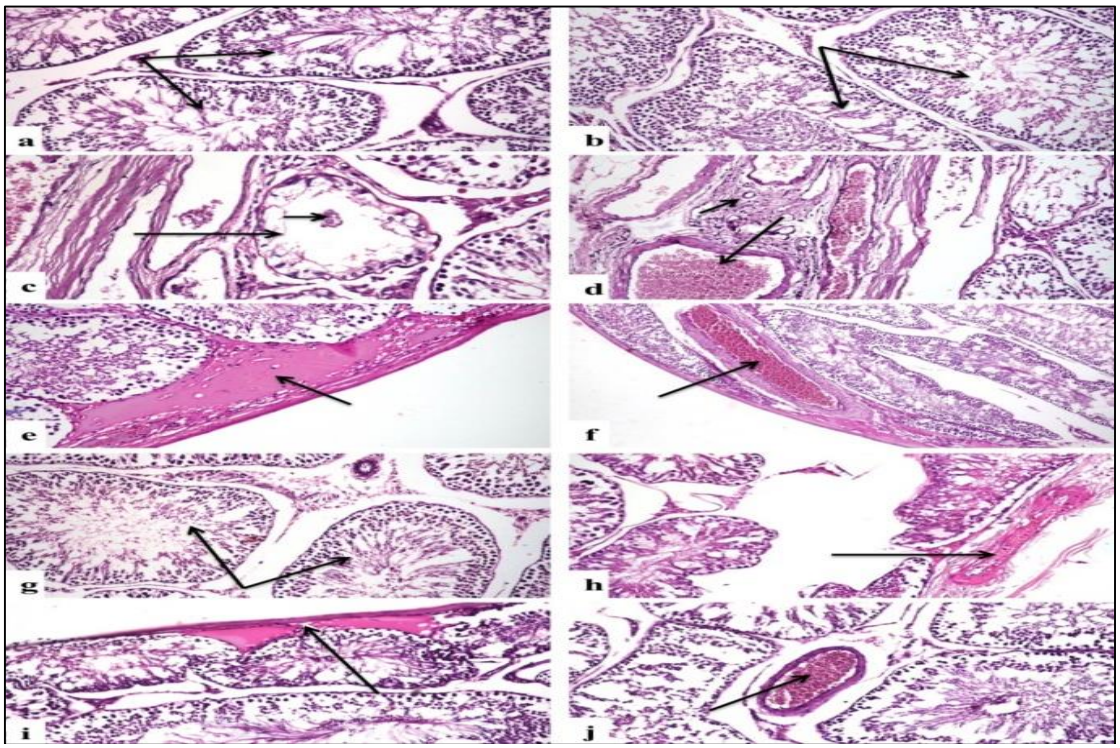

Figure 2. Photomicrograph, rat testes (a) and (b) NFD and BOS treated groups showing normal histological structure of seminiferous tubules (arrows). (c) HFD-treated group showing testicular degeneration (long arrow) with spermatid giant cells (short arrow). (d) HFD-treated group showing tunica albuginea edema (short arrow) and congestion (long arrow). (e) HFD-treated group showing interstitial edema (arrow). (f) HFD-treated group showing congestion of interstitial blood vessels arrow). (g) Group treated with HFD+ BOS showing nearly normal structure of seminiferous tubules (arrows). (h) HFD+ BOS group showing mild edema and mild congestion of tunica albuginea (arrow). (i) HFD+ BOS group showing interstitial mild edema (arrow). (j) HFD+ BOS group showing mild interstitial congestion (arrow) (H&EX400).

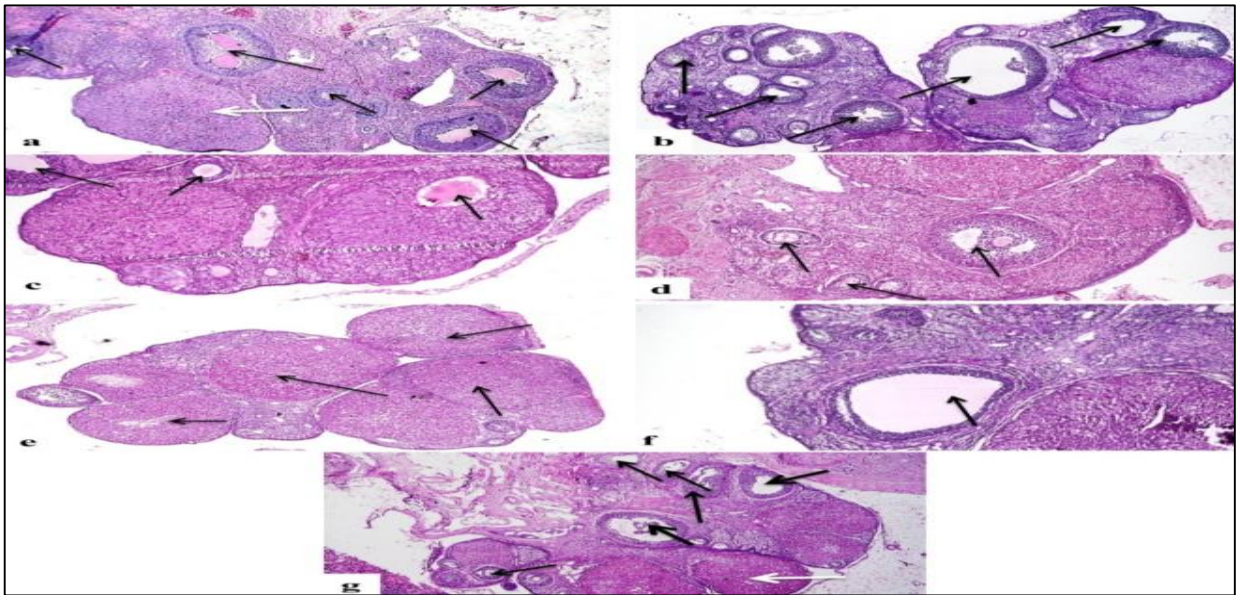

Figure 3. Photomicrograph, rat ovary. (a) and (b) NFD and NFD+BOS group respectively showing normal number of mature follicle (black arrows) and corpora lutea (white arrow) (H&EX40). (c) and (d) HFD group showing few mature follicles (arrows) (H&EX40). (e) HFD group showing increased proportion of corpora lutea (arrows) (H&EX40). (f) HFD group showing formation of follicular cyst (arrow) (H&EX100). (g) HFD+BOS treated group showing increased number of mature follicles (arrows) with absence of follicular cysts (H&EX40).

Rats fed on HFD

The current study showed (Table 6) drop in sperm concentration and motility and higher percent of abnormalities in males fed on HFD. Significant improvement in sperm motility and concentration took place in males administered the boswellia microcapsules. Analysis of serum chemistry (table 7) of the HFD group (males and females) showed drop in testosterone, estrogen, IGF, Insulin, TSH, catalase, total antioxidants and HDL and in the same time, elevated leptin, MDA, glucose, cholesterol, LDL, triglycerides and GGT. Administration of boswellia microcapsules took all the disrupted values back towards normal.

Earlier studies reported that excess of body fat is associated with increased aromatization of testosterone into estrogen with subsequent disorder in hypothalamo-hypophyseal gonadal (HPG) pathway [**31**].

Leptin acts not only as an adipostat by giving signals to the brain about the amount of body fat and the energy imbalance trying to ameliorate obesity, and achieve glucose homeostasis **[32, 33]**. The elevated leptin hormone, due to obesity, leads to disruption of HPG axis and decreased production of testosterone from leydig cells [**34**] and impair the TSH production [**35**]. In the same time hyperglycemia was associated with elevated proinflammatory cytokines that affect negatively on HPG axis [**36**] and excess of ROS specially MDA which cause sperm DNA damage (specially guanosine base) and abnormalities [**37**].

The reduced circulating IGF-1 level in HFD group was noticed in association to vascular dysfunction and elevated inflammatory response [**38**]. Consumption of HFD is known to impair endothelial function by upregulating reactive oxygen species (ROS) production in the vascular wall [**39**].

The elevated γ-GT in HFD group was closely related to hepatic steatosis [**40,41**] which strongly associated with metabolic syndrome [**42-44**] and largely reflects ectopic liver fat or secondary inflammation [**45**]. To correct this disruption, losing weight, lowering the level of lipids, blood sugar and oxidative markers should be achieved.

Daily administration of boswellia reduces fasting glucose levels by inhibition of carbohydrate digestion, glucose absorption and release of glucose from the liver [**46, 47**].

Boswellia as a high source of polyphenols was reported to have antioxidant compounds so that it took all the disrupted values back towards normal and showed better spermatozoa profile [**48-50**].

The HFD males group recorded high histopathological alteration score (table8) accompanied by edema of tunica albuginea (fig 2d), interstitial edema and congested blood vessels (fig 2, e&f). In the same time the immune staining showed strong expression of both markers (Caspase-3 and iNOS) (fig 3c). administered boswellia microcapsules showed nearly normal structures of seminiferous tubules of the testis (fig 2g) with mild interstitial edema and congestion (fig 2i&j) the score of histopathologic alterations markedly decreased (table 8) Also the tissue showed weak immune expression of both oxidative markers (caspase-3 and iNOS) (fig 4 d and e).

Female group fed on HFD showed drop in number of follicles if compared to NFD control group. Also follicular cysts were demonstrated in this group of animals. Administration of boswelia microcapsules, to HFD fed animals elevated the number of ovarian follicles and lowered the number of follicular cysts (table 8). This was confirmed by histopathological examination where the females fed on HFD showed few number of follicles (fig 3c&d) with relative increase in corpora lutea number (fig 3e), some sections revealed presence of follicular cyst that characterized by cysticaly dilated follicles with thinning in granulosa cell lining (fig 3f), feeding the microcapsules of boswellia beside HFD showed obvious amelioration in the above mentioned lesions when compared with HFD group where the nomber of ovarian follicles markedly increased and the evidence of follicular cysts decreased (fig 3g). Immuno-staining of caspase-3 and iNOS in ovaries of HFD treated group revealed strong expression of both markers (figs 5c). Groups treated with HFD and boswellia microcapsules showed weak positive reaction of both markers in granulosa cells of ovarian follicles and stromal cells (fig. 5d).

Earlier studies reported that granulosa cells (GCs) provide oocyte with energy so that it influences the quality of oocytes and corpus luteum (CL) [**51**]. Hyperlipidemia and hyperglycemia and the associated oxidative stress [**52, 53**] results in endoplasmic reticulum (ER) stress and consequent DNA damage, excessive granulose cell apoptosis [**54-56**] and suppression of oocytes maturation [**57, 58**]. Also, insulin was reported to act as a co-gonadotropin with LH to enhance ovulation [**59**] so that, relative insulin deficiency, reduced thyroid iodine uptake, poor thyroid function, and low estrogen may result in oligo-ovulation/ anovulation, and multiple cysts formation in obese individuals [**60**].

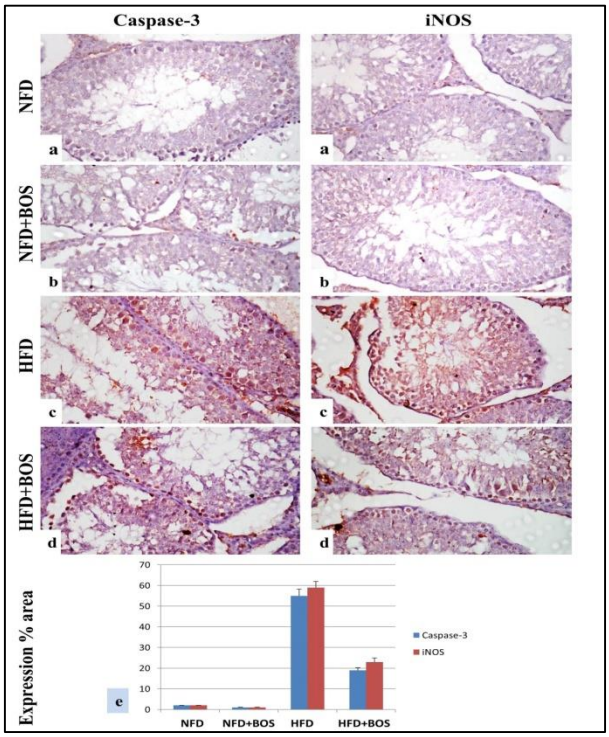

Figure 4. Immuno-staining of Caspase-3 and iNOS in male rats: (a) and (b) NFD and NFD+BOS treated groups showing no immune expression in testes for both markers. (c) HFD group showing strong expression of both markers in testes. (d) HFD+BOS treated groups showing weak immunoexpression for both markers. (e) Immunostaining

expression of caspase-3 and iNOS% area in testes (Data was expressed as mean ±SE) (Caspase-3 and iNOS X400).

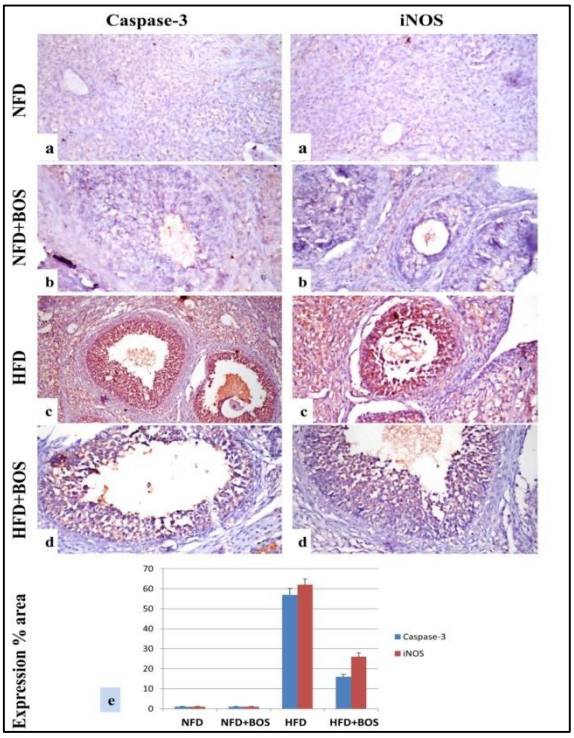

Figure 5. Immunostaining of Caspase-3 and iNOSin female rats: (a) and (b) NFD and NFD+BOS treated groups showing no immune expression in ovaries for both markers. (c) HFD group showing strong expression of both markers in ovaries. (d) HFD+BOS treated groups showing weak immunoexpression. (e) Immunostaining expression of caspase-3 and iNOS% area in ovaries (Data was expressed as mean ±SEM) (Caspase-3 and iNOS X400).

The present study showed that administration of BOS microcapsules beside HFD took all the finding back towards normal. This may be due to the high content of phenoilc compounds and antioxidant power of BOS [28]. This explanation was proved by the lower immune expression of Caspase 3 and INOS in ovarian tissue.

Conclusions

Obesity and hyperglycemia are considered as enemies to male and female fertility. Boswellia showed variety of pharmacological effects, particularly anti-hyperglycemia, antioxidant and anti-inflammatory effects Administration of Boswellia can correct the disrupted metabolic pathway. It showed positive effect on male and female fertility.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

This research was completely carried out in National Research Centre, Cairo, Egypt

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