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Molecular and histopathological assessment of Arabic Coffee effect on fertility in Male Albino Rats.

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ABSTRACT

Coffee preparation and times of drinking in Arabic peninsula (AP) has ritual folk. Roasting and grinding levels of coffee beans, and additives vary between different parts in AP. the simplest form of Arabic-coffee adds cardamom and saffron to the roasted, ground and boiled coffee; however, the most complex form has more ingredients as ginger, cloves, and ajwain upon desire. Our study addressing the assessment of different forms of Arabic-coffee and their separate ingredients on testis-histology and mRNA expression level of some fertility genes in experimental animals. Fifty-four male albino-rats were divided into 9 groups (6 rats/group). One control group, 5 groups were separately treated with 5 usual Arabic-coffee ingredients, group was treated with coffee and other 2 groups were treated with 2 different Arabic coffee mix as folk recipes. Samples were administrated for 0, 15 and 30 days. The study resulted in non-significant down-regulation in mRNA expression of fertility genes (Testin, StAR and FSHR) However, no histological changes were recorded among the control group rats (CN) and the other treated groups, although there were statistically non-significant differences in between. On conclusion, the usual drinking of roasted Arabic-coffee may be non-effective on testis histology and molecular markers of fertility.

Keywords: Arabic coffee; testis; histopathology; fertility genes.

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INTRODUCTION

Coffee is one of the most widely consumed pharmacologically active beverages in the world and caffeine is consumed as psychoactive substance. Coffee is rich in phenolic compounds (as a plant adaptation to environmental conditions) with a strong antioxidant activity [1, 2]. Children and adolescents are the fastest growing population of caffeine users with an increase of 70% in the past 30 years [3]. They are well recognized as potentially protective factors against human chronic degenerative diseases, such as cancer and cardiovascular disease [4]. Regular drinking of coffee can reduce the oxidation of human Low-Density Lipoprotein (LDL) and the oxidation of LDL, decreasing the risk of atherosclerosis [5]. It also, reduces the risk of stroke, cerebral infarction and improves insulin sensitivity [6].

Coincident with this rise in caffeine use is the development of novel, caffeine-containing beverages called energy drinks. These drinks contain caffeine levels ranging from 50 mg (equivalent to a can of soda) to 500 mg (equivalent to five cups of coffee) and, often, very high levels of sugar [7].

Roasting is an essential step in coffee production for generating aroma, flavor, and color of the coffee beans. The mode of heat transfer and the applied temperature profile are the most critical processing parameters that affect the physical and chemical properties of roasted coffee beans [8]. The chemical reaction changes include Maillard reaction or nonenzymatic reaction, browning reaction and Strecker degradation of proteins, sugar, polysaccharides and other components. The degrees of roasting are controlled by roasting time and temperature and are necessary for the required chemical reactions without burning the beans and compromising the flavor of the beverage [9]. Coffee can be classified into light, medium or dark depending in roasting degrees [10]. However, over-roasted coffee could reduce antioxidant activity [11, 12]. Also, Parliament, 2000[1], found the major compositional changes occurring are the decrease of phenolic compounds and the formation of brown, water-soluble polymers called melanoidin, although the decrease in protein, amino acids, and other compounds is also described [13]. The oxidant/antioxidant imbalance in favor of oxidants contributes to the pathogenesis of different diabetic complications which are considered to result from enhanced reactive oxygen species generation via nicotinamide adenine dinucleotide phosphate-oxidase [14, 15, 16]. Previous reports stated that moderate daily consumption of coffee helped to reduce the risk of type 2 diabetes [17, 18] as there could be a strong inverse association between sex hormone-binding globulin (SHBG) levels and type 2 diabetes risk in men and women [19, 20]. Human studies show that caffeine enhances energy expenditure [21] and improves the clinical conditions of diabetic patients [22]. Another study by Greer et al., 2001[23] revealed that caffeine ingestion promotes glucose consumption with an increase in blood epinephrine while pre-exercise consumption promotes ventilation and enhances lipolysis [24]. Chlorogenic acid, another main constituent of coffee beans, has recently been reported to selectively inhibit hepatic glucose-6-phosphatase [25] which is a rate-limiting enzyme involved in gluconeogenesis. There is also evidence that the thermogenic effects of caffeine can increase energy expenditure [26, 27], and, perhaps, reduce weight gain over time [28]. Caffeine also appears to improve sports performance [29], including perceived exertion [30] and endurance [31]. Finally, there is some evidence of an inverse relationship between caffeine consumption and colorectal cancer [32, 33] and Parkinson's disease [34, 35], but the mechanisms for this apparent protection remain unknown [36]. Coffee polyphenol consumption improves postprandial hyperglycemia and vascular endothelial function, which is associated with increased Glucagon-like peptide 1 (GLP-1) secretion and decreased oxidative stress in healthy humans [37].

Arabic coffee (Coffee Arabica) is a name that refers to two types of coffee in some Arab countries: Turkish, and Saudi Coffee. The Saudi coffee or ''Al-Qahwa'' is made from coffee beans roasted very lightly or heavily between 165 -210°C and cardamom. Traditionally, it is roasted on the premises, ground, brewed and served in front of guests. This brewing method is common in Najd and Hijaz and sometimes prepared with other spices like saffron (to give it a golden color), cloves, and cinnamon. It is often served with dates [38]. Viani in 1993 [39], reported that coffee contains hundreds of biologically active compounds; phenolic polymers(8g/100g), polysaccharides (6g/100g), chlorogenic acids (4g/100g), minerals (3g/100g), organic acids (0.5g/100g), sugars(0.3g/100g) and lipids (0.2g/100g). These compounds; chlorogenic acid in coffee mediates the anti-diabetic effects of coffee.

In contrary to the previous reports, caffeine was found to promote lipolysis in rat adipocytes [40] over consumption than once per day led to a slight increase in blood pressure [41]. Similarly, coffee consumption may increase the risk of acute myocardial infarction [42] and stroke [43]. Chronic consumers of boiled coffee have been reported to elevate plasma cholesterol concentrations [44]. Intake of boiled coffee is associated with an increased risk of coronary heart disease [45]. Two constituents of ground coffee, cafestol, and kahweol, were found to be responsible for the cholesterolemic action of boiled coffee [46]. However, the roasting of coffee beans has been shown to reduce the content of chlorogenic acid in coffee [11]. Green coffee

beans are rich in chlorogenic acid and its related compounds that show hypotensive effect [47].

In previous reportes, the potential effects of coffee consumption on fertility, spontaneous abortion and prematurity were not clearly established but appeared to be quite limited. In rodents, caffeine induced malformations at doses never encountered in humans. However, the quantity of caffeine intake by human per day is divided to the limit that diminish the equivelent teratogenic effect in rodents. It was also reported adecrease in birth weight and hematologic changes corresponding to over intake of coeffeine during gestation. In animals, caffeine induces long-term consequences on sleep, locomotion, learning abilities, emotivity and anxiety, whereas, in children, the effects of early exposure to coffee and caffeine on behavior are not clearly established. On other hand, the maternal coffee or caffeine moderate (300 mg caffeine/day equivalent to 2-3 cups of coffee) consumption during gestation and/or lactation didn't have measurable consequences on the fetus of the newborn [48].

The contradictory reports and obscurity of molecular results encouraged us to design the current study. The study aims to assess the effects of KSA coffee on fertility of experimental animals at histological and molecular levels. Testis from female rats were used as the subject of the study. Some interesting genes with fertility impact such as testin, steroidogenic acute regulatory protein (StAR) and follicle stimulating hormone receptor (FSHR) .

Testin is a putative Sertoli cell secretory protein that can become tightly associated with the Sertoli cell surface (specialized inter-Sertoli or Sertoli-germ cell junctions) and is a sensitive marker to screen potential male contraceptives targeted at disrupting the Sertoli-germ cell junctions, inducing premature release of germ cells from the epithelium when specialized inter-Sertoli or Sertoli-germ cell junctions are formed .

Steroidogenic acute regulatory (StAR) protein is the protein responsible for regulation of steroid hormones biosynthesis. Steroid hormones are synthesized in steroidogenic cells of testis. StAR protein was reported to be an important factor in testosterone biosynthesis through transporting cholesterol from outer membrane to inner side of mitochondria [50]. StAR protein expression is regulated by LH-mediated activation of cAMP-dependent pathways leading to transcriptional activation in Leydig cells [51]. Successful spermatogenesis is dependent on somatic cells in the testis. Therefore, the mechanism contributing reduction in StAR protein expressions may be damage of Leydig cells, which associated with structure changes in testis [52].FSHR is a transmembrane G protein-coupled receptor that interacts with FSH. Its activation is necessary for the hormonal functioning of FSH. FSHRs in the ovary, testis, and uterus.

MATERIALS AND METHODS

Preparation of Arabic coffee brew:

Coffee seeds (Coffee Arabica) were bought from local markets at Taif city, KSA. Folk coffee was prepared as traditional Saudi method according to Mahmoud *et al*. [47] with some modifications. The coffee seeds were roasted for 10 min then milled into a powder. Coffee drink was prepared by boiling 30 g of coffee powder in one liter of water for 20 min. The rats' coffee dose was 8.6mg/100g of body weight, which equivalent to a human daily intake of Arabic coffee (5 small cups of coffee/person/day ≈150ml or 6g coffee /day). Additional herbs were added to boiling coffee in coffee-equivalent weight ratios (according to actual folk recipes), then further boiling for additional 5 min in Arabic coffee maker and cooling to room temperature (Arab Dalla Trading Est., KSA).

Animals:

Fifty-four male albino Sprague Dawley rats weighing 125 – 135 grams were used. The animals were housed in standard cages feed on defined basal diet and water ad libitum [53]. After a week of acclimatization rats were weighed and randomly divided into 9 equal groups (6 rats each) annotated as CN (control), AM (all mix), CF (coffee), CR (cardamom), GN (ginger), CL (cloves), AJ (ajwain), SA (saffron) and BM (basic mix).

Experimental Feeding Groups:

The control group (CN) was kept at the experiment conditions without any further treatments for 30 days. BM-group received a daily dose of standard Arabic coffee mix (8.6 mg coffee, 1.9mg cardamom powder and 0.31mg saffron/100g of body weight) prepared as previously mentioned. Each from other groups except

for AM received single oral daily doses of 8.6 mg/ 100 g/day coffee or single herb prepared as previously mentioned (1.9 mg/ 100 g/day cardamom, 1.4 mg/ 100 g/day ginger, 0.9 mg/ 100 g/day cloves, 2.5 mg/ 100 g/day ajwain, or 0.03 mg/ 100 g/day saffron). AM-group received mix contains all ingredients of herbs added as last mentioned concentrations to the boiled coffee.

At the end of treatment period, rats were fasted for 8 hr then anesthetized with diethyl ether and killed by exsanguinations. Right testis was taken after dissection and divided into two parts/each. One part was kept in neutral buffer formalin for histology and the other part was kept in RNA-Later for molecular assays.

Gene expression assay:

RNA extraction: Total RNA from about 40mg of right testis was extracted using Biozol Reagent and RNeasy animal Kit (Biolabs, USA) according to manufacturer manual. The prepared RNA integrity was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically using Genesys 10 UV (Thermo-Fisher Scientific, Waltham, MA, USA) at 260 nm for quantification and at 260/280 for impurities.

cDNA synthesis: RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) and Techne TC-3000x thermal Cycler (Bibby Scientific, England) were used in cDNA synthesis. Four micrograms of pure total RNA (with ratio of 1.7-1.9 at optical density 260/280nm) was incubated with 5µM of random hexamer primer in a total volume of 12 µl sterilized DEPC-water at 65 ◦C for 5 min for denaturation. Then, chilled on ice and mixed with 4 µl of 5X RT-buffer, 1 mM dNTPs (provided as 100mM), 200 U RevertAid Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV) and 20 U RiboLock RNase Inhibitor in total volume of 20 µl in sterilized DEPC water (all chemicals were provided in the kit). The mixture was then re-incubated in the thermal Cycler at 37 ◦C for 1 h, and then at 90 ◦C for 10 min to inactivate the enzyme then, held at 4 ◦C. cDNA was preserved at -20 ◦C until used [54 - 55].

Semi-quantitive PCR: PCR-amplification was performed using specific primers (synthesized by Macrogen Co., GAsa-dong, Geumcheon-gu., Korea) as summarized in table 1. Three microliter of cDNA and 1 µM of each primer were added to 12.5-µl 2xGoTaq PCR master mix (Promega Corporation, Madison, WI, USA). The volume was completed to 25 µl with DNase free water and samples were loaded into Techne TC-3000x thermal Cycler (Bibby Scientific, England). PCR conditions were 94 °C for 5 mins; 30 cycles of 1 min at 95°C, 1min at annealing temperature (table 1) and 1 min at 72 °C. The final extension step was 5 min at 72 °C, PCR products were separated on 1.5% agarose A (Bio Basic, Markham, ON, Canada) gel in 1.0 X- TAE (Tris-Acetate-EDTA) buffer (Sigma–Aldrich, St. Louis, MO, USA) at 100 volts for 30 min. The gel was stained with ethidium bromide (Sigma–Aldrich), visualized and photographed under UV light using Ingenius gel documentation system (Syngene Europe, Cambridge, UK). The amount of cDNA was determined after optimization of internal control gene amplicon [53]. AlfaEaseFC software, edition 4 (Alfa-Innotech cooperation, Kasendorf, Germany) was used in gel analysis and to determine integrated density values (IDV) of the studied genes (table 1) PCR product in relation to that of Beta-2-microglobulin (B2m).

Table 1: PCR primers, genes and reaction conditions.

Gene name-Accession No.		5'- Primer sequence-3'	Tm	Amplicon size (bp)
B2m (Y00441.1)	Fd	GTC TCA GTT CCA CCC ACC TC	60	199
	Rv	GAC GGT TTT GGG CTC CTT CA		
Testin (NM_173132.2)	Fd	AATGGGGCATGAAGGGCTAC	60	193
	Rv	TCACTACCCCTGTCCCCTTT		
Star(NM_031558)	Fd	CTCAACAACCAGGAAGGCTGG	56	405
	Rv	GCAGGTGGGACCGTGTTCAGC		
FSHR (NM_199237.1)	Fd	GTAACCTCGCCTTCGCTGAT	60	279
	Rv	TCCAGCCCAATACCATGACG		

Beta-2-microglobulin (B2m); steroidogenic acute regulatory protein (Star); follicle stimulating hormone receptor (FSHR).

Histopathological Examination:

Samples of, testes, were taken and fixed in 10% neutral buffered formalin for 24 hours. Paraffin sections 4-6 µm thick were prepared and stained with hematoxylin and eosin (H & E) for the examination by light microscopy. The stained sections of testis were examined microscopically using an eye-piece of X10 and an objective piece of X10, i.e. at a magnification of the X100. Serial fields were examined to get 12 readings per

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individual (slide) as regards the perimeters of seminiferous tubules, and another 12 readings for epithelial height, a mean value was calculated per slide then measurement of the mean value per each group (n=6)was done.

Data were obtained by using (Motic Image Analyser Plus) program. Using the menu bar of interactive measurements, measuring of the tubular perimeter was done by drawing a line along the circumference of the transversely and nearly round cut tubules. Also using the same menu, the tubular epithelial thickness or height was measured by drawing a straight line across the epithelium. Both tubular perimeter and thickness were measured in micrometers.

Data Analysis

Statistical analysis was carried out using SPSS program. In order to recognize the homogenous and non-homogenous data, Levene's test was applied for all samples under investigation. Consequently, statistical analysis using ANOVA and Kruskal-Wallis tests were performed. The estimated difference between samples' mean was considered as statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Gene expression:

This study records the effects of Arabic coffee mixes on the expression levels of genes, which have important roles in fertility e.g. testin, StAR and FSHR supported with histological studies.

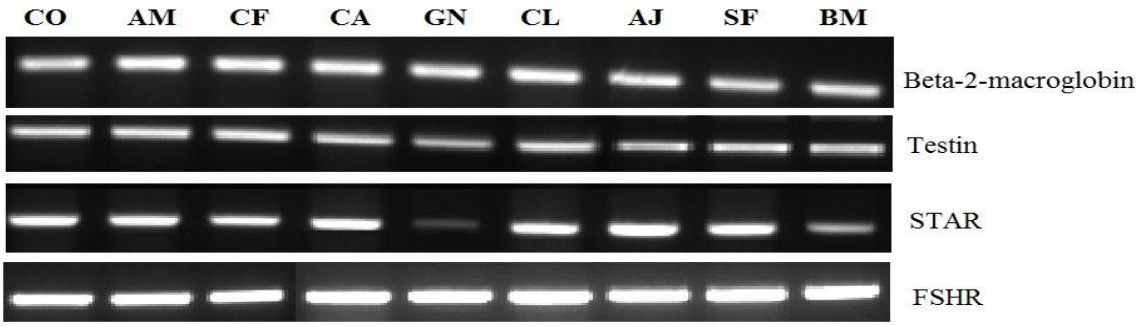

Figure 1. Electropherogram for PCR-products of Beta-2-microglobulin (B2m); steroidogenic acute regulatory protein (Star); follicle stimulating hormone receptor (FSHR) genes corresponding to control untreated group (CN); all mix group (AM); coffee group (CF); cardamom group (CR); ginger group (GN); cloves group (CL); ajwain group (AJ); saffron group (SF) and basic mix group (BM) of rats.

Table 2: The effects of Arabic coffee and separate Arabic coffee ingredients on gene expression.

Treatment Groups	FSHR	Testin	STAR
Control	0.690 ± 0.13	0.778 ± 0.31	0.925 ± 0.02^{a}
All Mix	0.508 ± 0.14	0.708 ± 0.30	$0.833 \pm 0.05^{a,b}$
Coffee	1.118 ± 0.67	0.925 ± 0.04	$0.763 \pm 0.11^{a,c}$
Cardamom	0.723 ± 0.38	0.890 ± 0.41	$0.813 \pm 0.07^{a,d}$
Ginger	0.773 ± 0.19^{a}	0.663 ± 0.20	$0.543 \pm 0.28^{a,e}$
Cloves	0.700 ± 0.09^{b}	0.748 ± 0.23	$0.800 \pm 0.08^{a,f}$
Ajwain	$0.443 \pm 0.11^{a,b}$	0.620 ± 0.22	$0.933 \pm 0.02^{b,c,d,e,f,g}$
Saffron	$0.555 \pm 0.03^{a,b}$	0.685 ± 0.27	$0.943 \pm 0.03^{b,c,d,e,f,h}$
Basic Mix	$0.578 \pm 0.03^{a,b}$	0.765 ± 0.12	$0.688 \pm 0.16^{a,g,h}$
LSD	0.196	0.1	0.086

Integrated density values (IDV) for genes are expressed as mean ± SD, n = 6. Data with the same letter at the same column have significant relation at $P \leq 0.5$. Follicle stimulating hormone receptor (FSHR), steroidogenic acute regulatory protein (StAR).

Testin-mRNA gene expression: Testin gene was non-significantly changed in response to all treatments. The mixed coffees decreased the expression of testin gene as the resulted IDVs were 0.71 ± 0.3 and 0.77 ± 0.12

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for AM and BM respectively as compared to the control 0.78 ± 0.31. The other groups (table 2 and Fig 1, 2) resulted in a decrease in expression of testin except for the CF and CR groups (0.93 ± 0.04 and 0.89 ± 0.41 respectively). The least significant intergroup difference was 0.1.

The non-significant decrease in mRNA expression of testin due to treatment with Arabic coffee mix could indicate to disruption of the cytoskeletal elements that causes premature release of germ cells into tubular lumen, making the seminiferous epithelium virtually devoid of elongate and round spermatids as well as spermatocytes and consequently negative affect on male fertility [56-57].

Stroidogenic acute regulatory (StAR)-mRNA expression: IDVs for Star-gene in samples show significant down-regulations in expression of StAR mRNA for all groups except for AJ (0.93 ± 0.02) and SF (0.94 ± 0.03) as compared to the control group (0.93 ± 0.02). However, the remaining treated groups have down-regulation patterns; AM (0.83 ± 0.05), CF (0.76 ± 0.11), CR (0.81 ± 0.07), GN (0.54 ± 0.28), CL (0.80 ± 0.08), and BM (0.69 ± 0.16). The minimum down-regulation was recorded after treatment with ginger. There were significant intergroup variations with a least significant difference of 0.09 (table 2 and Fig 1, 2).

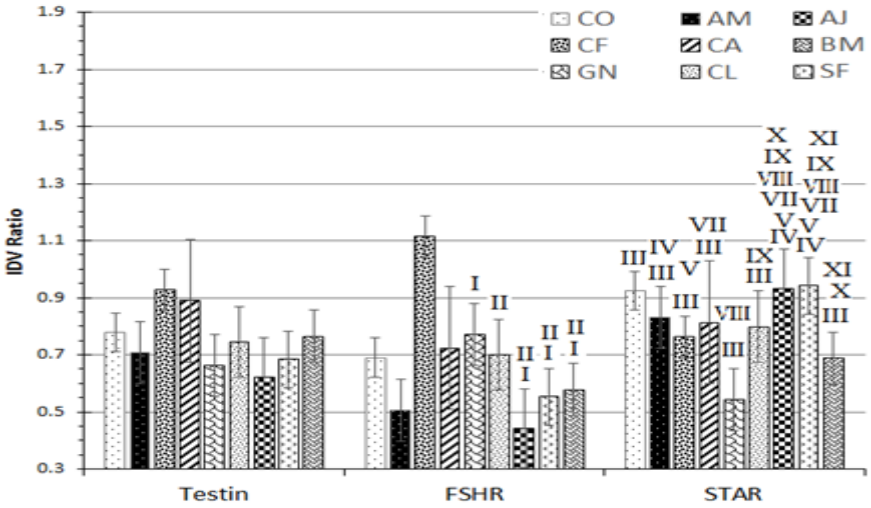

Figure 2. Histogram representing expression patterns for Beta-2-microglobulin (B2m); steroidogenic acute regulatory protein (Star); follicle stimulating hormone receptor (FSHR) genes corresponding to Control untreated group (CN); all mix group (AM); coffee group (CF); cardamom group (CR); ginger group (GN); cloves group (CL); ajwain group (AJ); saffron group (SF) and basic mix group (BM) of rats. Columns with same Latin number are significantly different (p < 0.5).

The current down regulation of StAR-mRNA in response to coffee treatment could affect Leydig cells that in turn could reduce fertility. It could be explained according to Stocco report StAR upregulatory and downregulatory signals affected steroid biosynthesis converge, and it might play a major biological function, in view of its high degree of conservation among all species tested, namely the mouse, rat, sheep, cow, rabbit and human [49].

Follicle stimulating hormone receptor (FSHR) expression: There were non-significant changes in FSHR gene expression due to all treatments as compared to the control group (0.69 ± 0.13). Down-regulation occurs in groups AM (0.51 ± 0.14), AJ (0.44 ± 0.11), SF (0.56 ± 0.03), and BM (0.58 ± 0.03). In the other hand, upregulations were recorded in the other groups; CF (1.12 ± 0.67), CR (0.72 ± 0.38), GN (0.77 ± 0.19), and CL (0.70 ± 0.09). There were significant intergroup variations as shown in table 2 and figures 1, 2. The least significant intergroup difference was 0.2.

Histological results:

The control group (CN) showed the usual features of seminiferous tubules with 4-6 cell layers thickness inside named, from outside to inside, spermatogonial cells, 1ry spermatocytes, 2ry spermatocytes, round spermatids. Multiple Sertoli cells rested on the tubular basement membrane. The tubular lumens were

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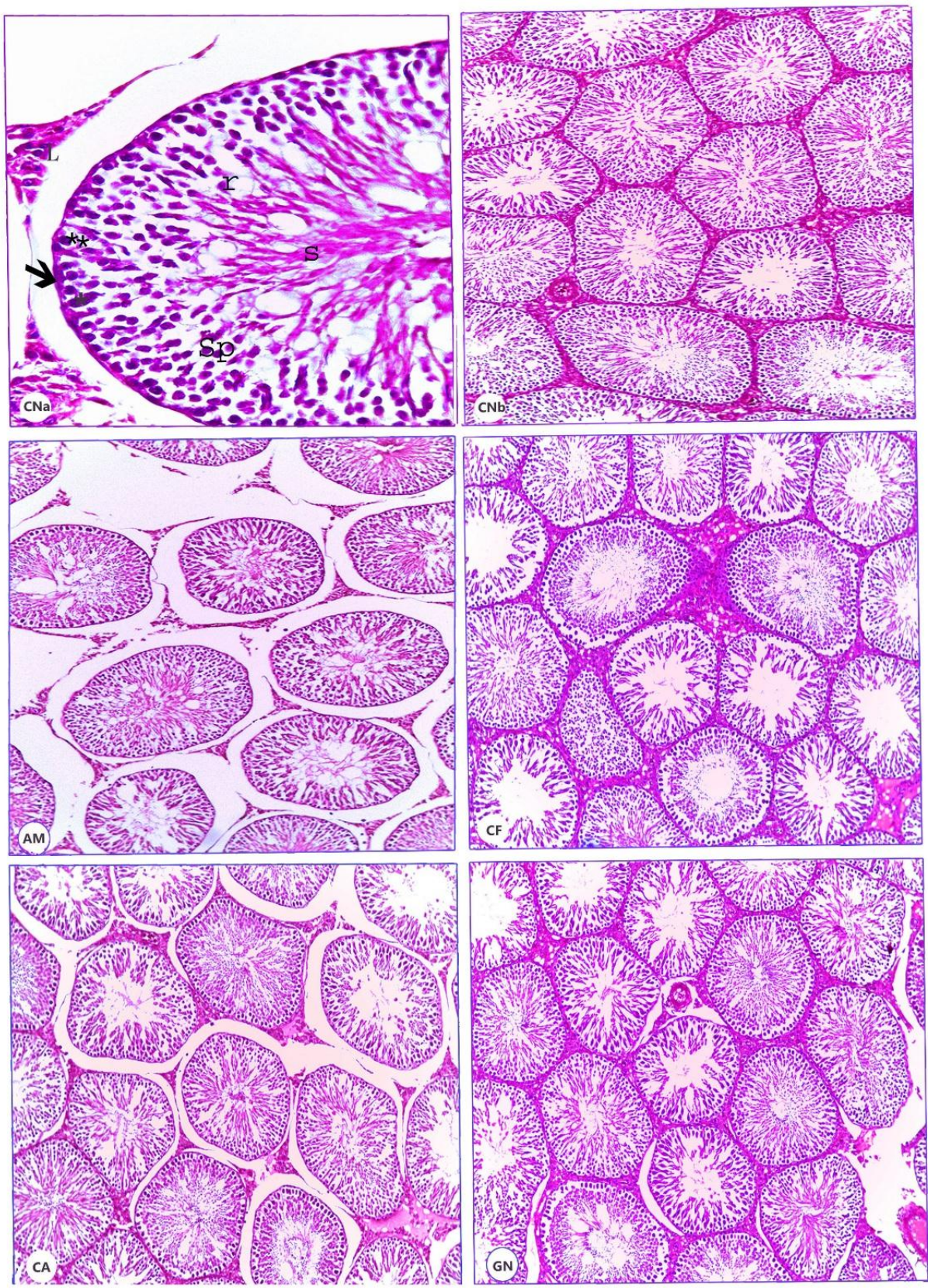

Figure 3.: Photomicrograph of a section of control group (CNa, CNb) and other 4 treated groups (all mix group (AM); coffee group (CF); cardamom group (CA); ginger group (GN)) is showing normal seminiferous tubule(s) with 4-6 cell layers thickness rested on basement membrane (black arrow), spermatogonial cells (*), 1ry and 2ry spermatocytes (Sp), round spermatids (r), Multiple Sertoli cells(**) rested on the tubular basement membrane. The tubular lumens were occupied by an abundant amount of formed sperms and there were numerous extra-tubular cells of Leydig (L). (H&E; X400, X100).

occupied by an abundant amount of formed sperms and there were numerous extra-tubular cells of Leydig (Fig.1; CNa, CNb).

Histologically, no changes were detected among the control group rats (CN) and the other treated groups (AM, CF, CA, GN, CL, AJ, SF, and BM), although there were non-significant differences among them obtained by the statistical image analysis results (Fig. 1, 2).

Table 3: The effects of Arabic coffee and separate Arabic coffee ingredients on rat testis parameters.

Treatments	Perimeter	Thickness	Thickness/Perimeter
Control	1016.0±88.0	94.4±7.0	9.3±0.3
AMG	1041.9±45.6	97.4±3.9	9.4±0.5
CFG	1039.2±48.6	93.6±8.6	9.0±0.6
CRG	1042.1±45.3	97.4±3.9	9.3±0.4
GNG	1039.2±48.6	97.5±3.7	9.4±0.4
CLG	1009.6±100.8	97.5±3.9	9.7±1.2
AJG	1059.7±38.0	97.4±3.9	9.2±0.3
SAG	1061.5±47.1	94.5±7.0	8.9±0.8
BMG	1045.2±42.4	93.6±8.6	9.0±0.7
LSD	37.8	3.8	0.4

Mean ± SE Rat body weights are expressed as mean ± SD. Relative tissue weights are expressed as mean ± SD, n = 6. Control untreated group (CO); all mix group (AM); coffee group (CF); cardamom group (CA); ginger group (GN); cloves group (CL); ajwain group (AJ); saffron group (SF) and basic mix group (BM). Data with the same letter at the same column have significant relation at P ≤ 0.5.

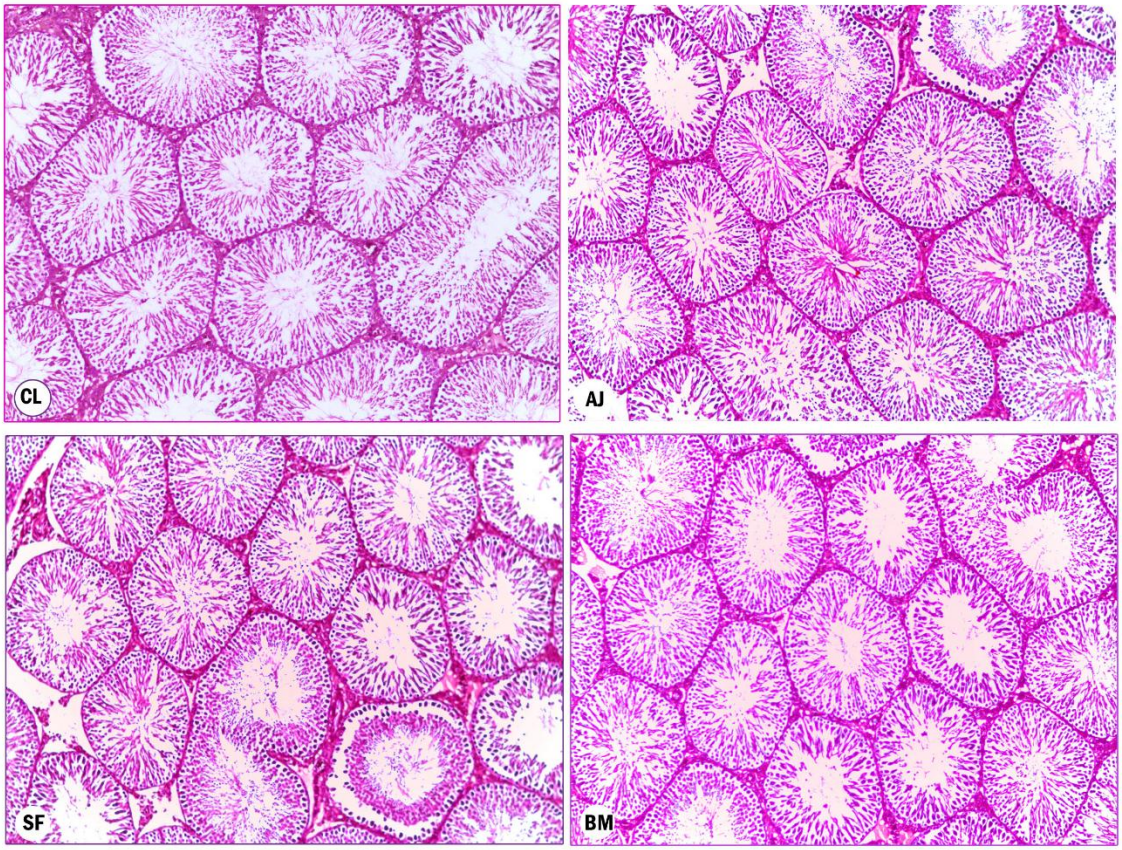

Figure 4. Photomicrograph of a section the other 4 treated groups (cloves group (CL); Ajwain group (AJ); Saffron group (SF) and basic mix group (BM)) is showing many normal seminiferous tubules with 4-6 cell layers thickness rested on basement membranes and interstitial cells of Leydig. (H&E; X100).

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The tubular perimeter measurements by image analysis showed that the mean control group (CN) was 1016.0 micrometers with SD of ?88.0, while that of AM, CF, CR, GN, CL, AJ, SA, and BM groups were (1041.9 ± 45.6, 1039.2 ± 48.6, 1042.1 ± 45.3, 1039.2 ± 48.6, 1009.6 ± 100.8, 1059.7 ± 38.0, 1061.5 ± 47.1,and 1045.2 ± 42.4) respectively.

Statistical analysis of the tubular perimeters presented high non-significant differences ($p \leq 0.5$) between control group (CN) and each of the other treated groups (AM, CF, CR, GN, AJ, SA) while CL group showed non-significant low difference in comparison to control group ($p \geq 0.05$) (Table1, Fig.3).

The tubular epithelial height or thickness measurements by image analysis method showed that the mean control group (CN) was 94.4±7.0, while that of AM, CF, CR, GN, CL, AJ, SA, and BM groups were (97.4 ± 3.9, 93.6 ± 8.6, 97.4 ± 3.9, 97.5 ± 3.7, 97.5 ± 3.9, 97.4±3.9, 94.5 ± 7.0, 93.6 ± 8.6) respectively.

Statistical analysis of the tubular thickness presented nearly no differences ($p \geq 0.05$)between control group (CN)and each of the other treated groups (CF,SA, and BM) while AM, CR, CL, and AJ group showed non-significant high difference in comparison to control group($p \geq 0.05$) (Table 1, Fig.4). The previous literature has shown contradictory results on caffeine intake as a lifestyle factor. Furthermore, some studies show adverse effects on sperm quality while others show positive results on sperm kinematic parameters [59]. A significant positive finding manifested as severe bilateral testicular atrophy with aspermatogenesis or oligospermatogenesis in 85-100% of the rats fed caffeine or theobromine. Cytogenetic analysis of testes from caffeine-or theophphylline-treated rats revealed a significantly reduced number of mitotic cells in the caffeine-treated group [60]. Caffeine has been described as a modulator of cellular metabolism. Hence, the authors hypothesized that it alters human Sertoli cells (hSCs) metabolism and oxidative profile, which are essential for spermatogenesis. Consequently, moderate consumption of caffeine appears to be safe to male reproductive health since it stimulates lactate production by SCs, which can promote germ cells survival. Nevertheless, caution should be taken by heavy consumers of energetic beverages and food supplemented with caffeine to avoid deleterious effects in hSCs functioning and thus, abnormal spermatogenesis [61]. A study indicated that caffeine consumption during gestation could reduce testicular parameters during postnatal development in male offspring Wistar rats [62]. It was reported that long term intake of caffeine induced suppression of spermatogenesis mainly through inhibition of FSH release and this effect was maintained even in the presence of normal or high levels of testosterone and LH [63]. The effect of caffeine on biological systems has been examined and the results differ according to the dose, tissue, duration of treatment. Acute treatment and in vitro administration of caffeine increases the motility of epididymal sperm in the rat [64]. In our experiment, Thickness/Perimeter ratios of seminiferous tubules were non-significantly increased in AM (9.4 ± 0.5), GN (9.4 ± 0.4), CL(9.7 ± 1.2) groups as compared to the control group(9.3 ± 0.3), while there were non-significant decreases in ratios of CF(9.0 ± 0.6), AJ(9.2 ± 0.3), SA(8.9 ± 0.8),and BM (9.0 ± 0.7) groups (Table1, Fig.5). The thickness/Perimeter ratios of both CR group (9.3±0.4) and CN group (9.3±0.3) were nearly equal.

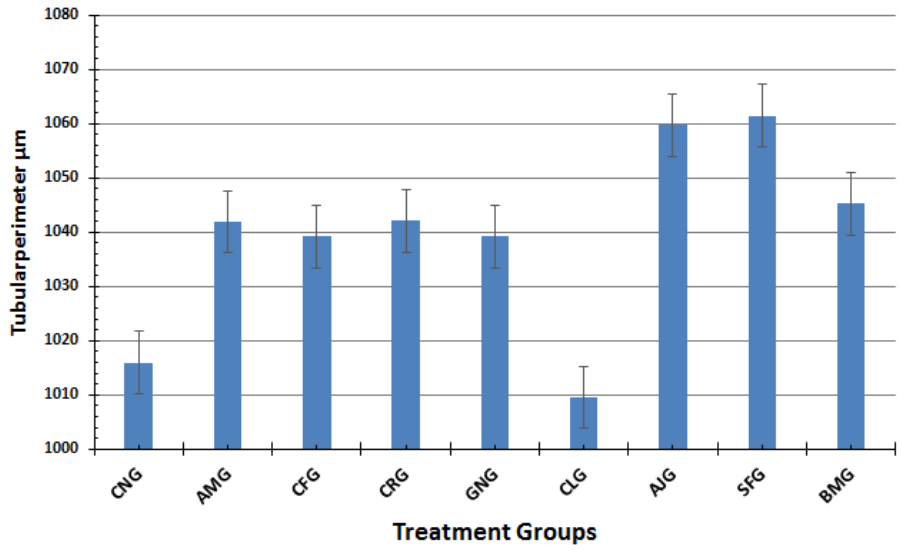

Figure 5. Histogram represents rat testicular tubule perimeter measurements after treatments with Arabic coffee ingredients and different Folk mixtures. Control untreated group (CO); all mix group (AM); coffee group (CF); cardamom group (CA); ginger group (GN); cloves group (CL); Ajwain group (AJ); Saffron group (SF) and basic mix group (BM). Columns with the same letter are significantly ($p \leq 0.5$) different.

CONCLUSION

From our current study, we can conclude that the method of Arabic coffee preparation can make positive changes in histological parameters of testis and down-regulate many genes of fertility impact at the mRNA level as well. However, the end product of gene expression (the protein) is important therefore further studies are needed to provide more insight into the effects of Arabic coffee on fertility genes at the molecular level.

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