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Effect of Different Dietary Fat Sources on Lipid Profile and Testosterone Level in Male Albino Rats

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Abstract

This study aimed to detect how different dietary fat sources (ghee, butter, and margarine) affected the lipid profile and testosterone level in male albino rats. For this purpose, four groups of 32 male albino rats were used. Group I was given a control diet (without the addition of any fat), Group II was given 20% ghee + 80% control diet, Group III was given 20% butter + 80% control diet, and Group IV was given 20% margarine + 80% control diet. After the end of the experiment, lipid profile and testosterone level were determined in serum. Also, testicular tissues were examined using hematoxylin-eosin staining. The results showed that the ghee group had a significant decrease in lipid profile and a significant increase in testosterone level compared with the control group. While in margarine group, there was a significant increase in lipid profile and a significant decrease in testosterone level as compared with control group. Butter group revealed non-significant increase in lipid profile while there was a significant decrease in testosterone level as compared with control group. Testes of rats received ghee showed normal architecture. On the other hand, butter group showed moderate vacuolation of the seminiferous tubules with a slight congestion of some blood vessels. Furthermore, margarine group had prominent vacuolation with great congestion of blood vessels. In conclusion, ghee is the best dietary fat followed by butter, while margarine is unhealthy fat source.

Keywords: Butter; Ghee; Margarine; Lipid profile; Testosterone

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1. Introduction

The link between food and health has been recognized for a long time, and today's essential thought of food is moving from one that focuses on preserving life to one that promotes and maintains health by avoiding chronic diseases **(Chinnadurai et al., 2013)**. Lipids are a class of molecules in our daily feed, including several subgroups. Dietary fat supplies essential nutrients, aids energy balance and controls blood lipid levels. These functions are necessary for good health, and they are often disrupted, leading to disorders like obesity, diabetes, cardiovascular disease, and cancer **(Gross and Silver, 2014; Krahmer *et al.*, 2013)**.

Although numerous synthetic drugs are available to lower cholesterol levels **(Eghdamian and Ghose, 1998)**, there are still various side effects. This has sparked a new wave of research to create new natural-components-based strategies to keep normal cholesterol levels with the least side effects **(Chinnadurai et al., 2013)**.

In Egypt, ghee is known as "samna baladi". It's usually made from buffalo and cow milk. Because of its pleasant taste and distinct aroma, most Egyptians prefer " samna baladi " **(Macrae et al., 1993)**. Ghee increases antioxidant status and antiatherogenic potency and improves lipid profiles **(Chaturvedi et al., 2016)**. In addition, it has beneficial effects on the reproductive system **(Hassanzadeh-Taheri et al., 2018).**

Butter is a dairy product made from cream that has been churned **(Dvořák et al., 2016)**. It has a fat level nearly comparable to ghee **(Gopalan, 1989)**, but cholesterol amounts in butter are higher than ghee (**Fındık and Andiç, 2017**). Also, **Karandikar et al. (2016)** found that ghee is slightly safer than butter when considering its lipid profile. On the other hand, **(Franczyk-Zarow *et al.* (2019)** found that butter increase HDL (good cholesterol) than other hydrogenated vegetable oils (margarine).

Margarine is used in the food sector. Trans fatty acids (TFAs) are produced during the hydrogenation process. They are recognized as a risk factor for coronary vascular diseases (CVD), insulin resistance, and the production of unfavorable serum lipoprotein profiles **(Sun et al., 2007)**. Furthermore, TFA consumption is linked to reduced total testosterone levels (**Arvizu et al., 2015)**.

Studies on the effect of dietary fat on human fertility have revealed that dietary lipids alter sex hormones and affect fertility in both men and women **(de Catalfo *et al.*, 2009; Mumford *et al.*, 2016; Segarra *et al.*, 2002).** In addition, hypercholesterolemia and high blood triglycerides levels have been related to a direct deleterious effect on testicular function **(Martínez-Martos et al., 2011)**. High-saturated-fat diets have been shown to affect testicular morphology by diminishing seminiferous epithelium size, height, and cell proliferation **(Campos-Silva et al., 2015)**

This study aimed to assess the effect of various dietary fat sources (ghee, butter and margarine) at a percentage of 20% of the diet on some physiological parameters including, lipid profile, testosterone level and histology of testicular tissues.

2. Materials and Methods

2.1. Experimental animals

In this experiment, thirty-two immature, thirty-five-day-old, apparently healthy male albino rats weighing 50 ± 10 g were used. Rats were obtained from a laboratory animal house in Tanta, Egypt, and housed in the Department of Physiology at the Faculty of Veterinary Medicine, Damanhur University. Rats were kept in plastic cages with sawdust bedding at room temperature and divided into four groups of eight rats. Before the experiment, rats were kept for ten days for acclimatization. Ration and water were added to rats *ad libitum*.

2.2. Dietary fat sources

Buffalo ghee was made by women in the villages of Behira Governorate using the traditional Egyptian method, which involves heating buffalo traditional butter at 1200°C until the fat melts and the moisture evaporates. To eliminate residues (morta), the product was strained. After that, the clear liquid was

transferred into clean, dry glass jars. The produced ghee has a minimum fat content of 99.5 % and a maximum moisture content of 0.1 % **(Abou-Donia, 2008)**.

Buffalo butter was made by women in the villages of Behira Governorate from the cream of buffalo milk by separating of cream from Buffalo milk manually, followed by mechanical churning to obtain the butter **(Macrae *et al.*, 1993)**. Margarine was purchased from a local market in Behira Governorate, Egypt.

2.3. Experimental design

A total of 32 rats were separated into four groups, each of eight rats. All animals were sacrificed after two months from the beginning of the experiment.

Group I, (control): rats received a control diet (without adding any fat) for 2 months.

Group II, (Ghee): rats received 20 % ghee + 80 % control diet for 2 months.

Group III, (Butter): rats received 20 % butter + 80 % control diet for 2 months.

Group IV, (Margarine): rats received 20 % margarine + 80 % control diet for 2 months.

2.4. Blood sampling

All rats were fasted overnight at the end of the experiment, and ether was used to anesthetize them. Blood samples were then drawn from the eye's medial canthus and placed in clean, dry Wasserman tubes. After allowing the blood to clot, it was centrifuged at 3000 rpm for 15 minutes, and the resulting serum was collected for further biochemical analysis.

2.5. Determination of serum lipid profiles

Serum triacylglycerol levels were measured according to **Fassati and Prencipe (1982)** by kit that was obtained from the Bio-diagnostic Company (Giza, Egypt).

Serum cholesterol levels were measured according to **Allain *et al.* (1974)** by a kit that was obtained from Bio-diagnostic Company (Egypt).

Serum high-density lipoprotein (HDL-c) levels were measured according to **Lopes-Virella *et al.* (1977)** by HDL kit that was provided by Bio-diagnostic Company (Egypt).

VLDL-c, LDL-c, and AI were calculated according to formula of **(Friedewald *et al.* (1972); Tremblay *et al.* (2004))**.

VLDL-c = TAG/5

LDL-c = Total Cholesterol - (HDL-c + VLDL-c).

Atherogenic index (AI)= LDL-c/ HDL-c

2.6. Enzyme-linked immunesorbent assay (ELISA) for determination of serum testosterone

Serum testosterone level was measured according to **Voller *et al.* (1978)** by ELISA kit that was obtained from Abcam Company (Waltham, MA, USA)

2.7. Tissue sampling for determination of lipid peroxidation

After blood sampling, rats were dissected, and the testicles were rapidly removed. One was preserved in liquid nitrogen until being frozen at -80 °C for biochemical examination. To eliminate red blood cells or clots, the frozen testicular samples were perfused with phosphate-buffered saline (0.1 M, pH 7.4) containing 0.16 mg/mL heparin. Afterward, 10% homogenate with cold potassium phosphate buffer (50 mM, pH 7.5) was prepared from each testicle using a potter-Elvehjem tissue homogenizer. The obtained homogenate was then centrifuged at 10,000 ×*g* for 15 min at 4°C, and the supernatant was taken off and kept frozen at -20°C **(Saleh *et al.*, 2017).** Lipid peroxidation was assayed in testicular aliquots by determining MDA level according to **Draper and Hadley (1990)** by using kits that were provided by Bio-diagnostic Company, Egypt.

2.8. Tissue sampling for histological examination

Testes were removed from animals after death and kept in 10% neutral buffered formalin after fixation. Samples from each testis were dehydrated in ascending grades of ethanol, starting from 50% to absolute ethanol. The clearance of the samples was then applied using xylene (three changes), and then paraffin impregnation was done in the hot oven using melted paraffin wax (three changes). Finally, paraffin blocks of the treated samples were prepared. The samples' blocks were cut into thin paraffin sections (3-7 µm thick), fixed on egg albumin-glycerin glycerin-coated glass slides, and dried in an electrical incubator for 30-60 minutes and then stained with Hematoxylin and Eosin (H and E). The previously mentioned histological staining methods were used according to **Bancroft and Gamble (2008)**. Examination of histological slides was done using light microscopy (*Labomed*, Labo, America, Inc.) supported by an ocular lens (X10, X20, and X40) and a digital camera for getting photos.

2.9. Statistical analysis

Data were analyzed by analysis of variance (One-way ANOVA) using the general linear model procedure of SPSS.

3. Results

3.1. Effect of ghee, butter, and margarine on lipid profile

Results in **Table 1** revealed that in the ghee group there was a significant decrease ($p < 0.05$) in cholesterol, TAG, LDL, and VLDL levels (except AI value showed a non-significant decrease) as compared with the control group. In contrast, the margarine group had a significant increase ($p < 0.05$) in TAG, LDL, VLDL, and AI levels (except cholesterol value showed a non-significant increase) as compared with the control group. The butter group revealed non-significant difference in levels of cholesterol, TAG, and VLDL as compared with the control group, meanwhile, it had higher levels of both LDL and AI value. On the other hand, the margarine group had a significant decrease in HDL-C value. Still, this decrement was non-significant in the butter group as compared with the control group while the ghee group showed a significant increase in HDL-C value compared with the control group.

3.2. Effect of ghee, butter, and margarine on lipid peroxidation

Results in **Table 2** revealed that there was a significant increase at ($p < 0.05$) in MDA level in the margarine group compared with the control group, but there was a significant decrease in the same value in the butter and ghee groups as compared with the control group.

3.3. Effect of ghee, butter, and margarine on serum testosterone level

Results in the Table 3 showed that there was a significant increase ($p < 0.05$) in testosterone level in the ghee group as compared with the control group, but there was a significant decrease ($p < 0.05$) in margarin, and butter groups as compared with the control group.

3.4. Histopathological assessment in rat's testicles tissue

The testis of control male rats showed regularly arranged undamaged pattern of seminiferous tubules with interstitial tissue, revealed a uniform shape with cellular components (**Figure 1A**). Sertoli cells showed long obvious processes which incubate the spermatogonia on intact basement membranes (**Figure 1A, 2A**). In ghee-treated rats, testis has a normal architecture with seminiferous tubules with epithelium, including Sertoli cells, similar to that of the control group, with more spermatids, were also found in the lumen (**Figure1B**). Sertoli cells have more obvious and clear processes than control group within normal epithelium height (**Figure 2B**). In contrast, butter group revealed loosely contacted seminiferous epithelium in some tubules and normal pattern in others. Moderate vacuolation in the interstitial tissue and degeneration of seminiferous tubules were also noticed (**Figure 1C**). In addition to slight congestion of some blood vessels was revealed (**Figure 2C**). Furthermore, Margarine treated group exposed a deterioration in the seminiferous tubules with the interstitial tissue; dissolute parts of some seminiferous epithelium with thin basement membrane and prominent vacuolation of fatty changes of the seminiferous tubules (**Figure 1D**). The seminiferous epithelium showed a significant decrease in its thickness and structural disorganization, great congestion of blood vessels was also noticed (**Figure 1D, 2D**).

Table 1. Effect of ghee, butter, and margarine on lipid profile after two months.

Group	Cholesterol (mg/dl)	TAG (mg/dl)	HDL-c (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI
Control	81.50 ± 0.65 [a]	99.80±0.86[b]	37.60 ± 1.86 [b]	22.55 ± 1.44 [b]	19.96 ± 0.17 [b]	0.40 ± 0.13 [c]
Ghee	73.00 ± 1.70 [b]	85.60 ± 1.03 [c]	41.80 ± 1.07 [a]	11.20 ± 1.40 [c]	17.12 ± 0.21 [c]	0.27 ± 0.04 [c]
Butter	82.33 ± 1.2 [a]	103.75 ± 1.80 [b]	33.60 ± 1.08 [b]	27.85 ± 1.94 [ab]	21.04 ± 0.4 [b]	0.78 ± 0.10 [b]
Margarine	86.33 ± 1.86 [a]	134.40 ± 4.06 [a]	29.00 ± 1.22 [c]	33.20 ± 2.04 [a]	26.88 ± 0.81 [a]	1.22 ± 0.10 [a]

All values are expressed as the mean ± SE.
Values with different superscript letters within the same column are significantly different ($p < 0.05$).

Table 2. Effect of ghee, butter, and margarine on lipid peroxidation

Groups	MDA (nmol/ g)
Control	7.15 ± 1.3 4[b]
Ghee	6.37 ± 0.26 [bc]
Butter	4.49 ± 0.25 [c]
Margarine	10.16 ± 0.22 [a]

All values are expressed as the mean ± SE. Values with different superscript letters within the same column are significantly different ($p < 0.05$).

Table 3. Effect of ghee, butter, and margarine on testosterone(ng/mL) value

Groups	Testosterone (ng/mL)
Control	3.1 ± 0.10 [b]
Ghee	5.5 ± 0.23 [a]
Butter	2.3 ± 0.11 [c]
Margarine	1.0 ± 0.08 [d]

All values are expressed as the mean ± SE.
Values with different superscript letters within the same column are significantly different ($p < 0.05$).

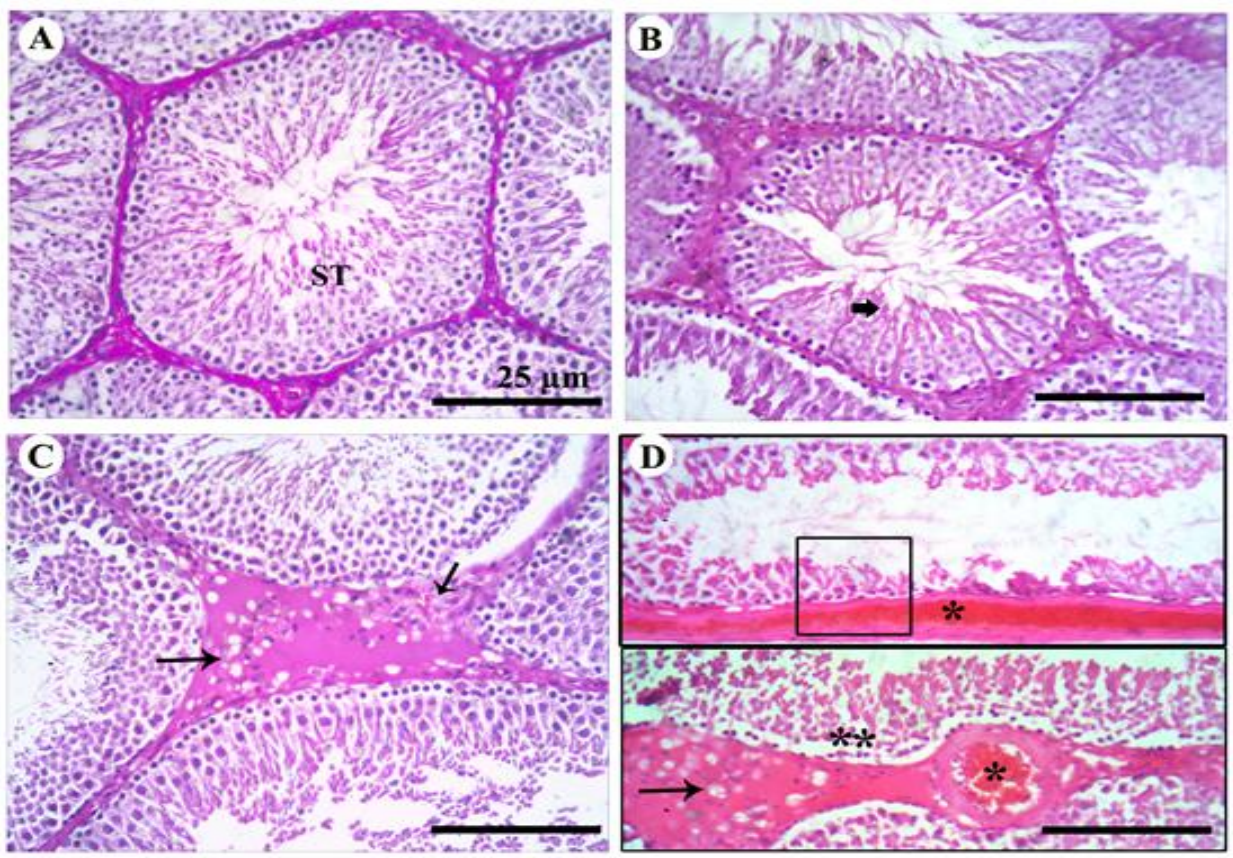

Figure 1. Histopathological examination of rat testes. A) Control group showing a normal histological structure of seminiferous tubule. B) Ghee group exhibiting normal histological structure of seminiferous tubule and epithelium also spermatids and spermatozoa (thick short arrow). C) Butter group showing moderate vacuolation of in interstitial tissue (long arrow) and slight congestion of blood vessels (short arrow). D) margarine treated group; seminiferous tubule displayed a significant decrease in the epithelium (box). Obvious blood vessels congestion (one asterisk) Dissolute parts of some seminiferous tubules (double asterisk). Vacuolation of interstitial tissue (long arrow). H&E stain. Scale bar= 25 µm.

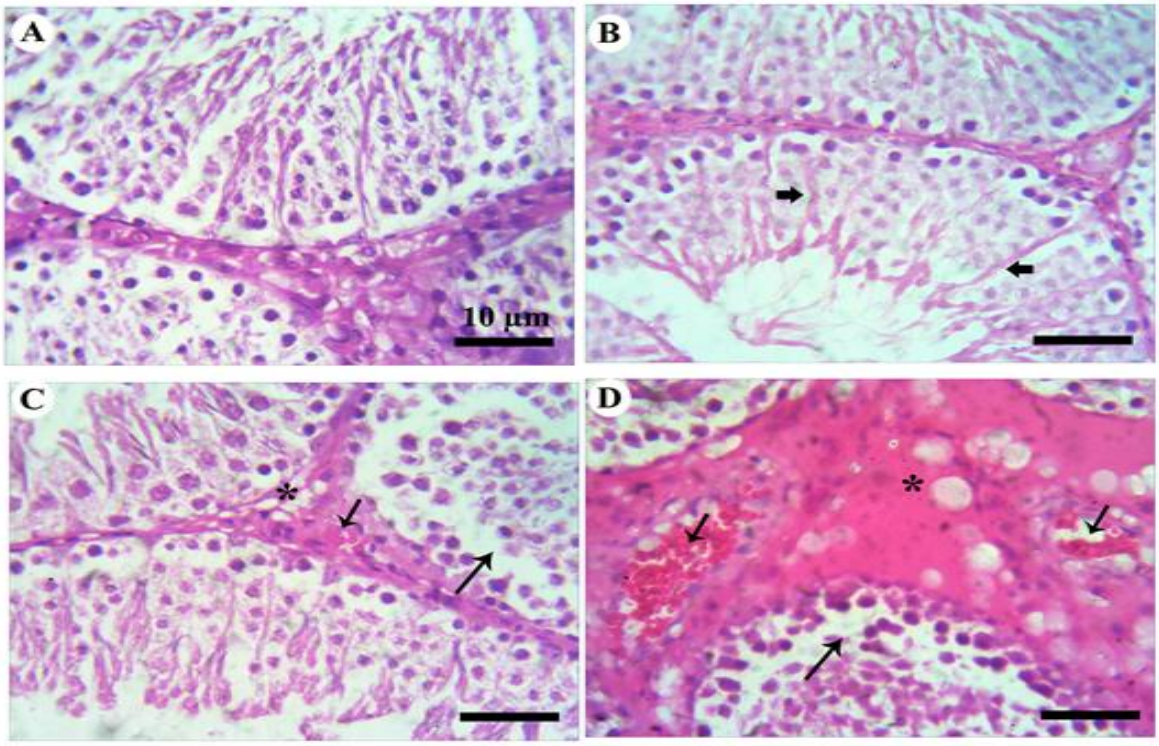

Figure 2. Histopathological examination of rat testes. A) Control group is displaying seminiferous tubules obvious processes of Sertoli cells and intact basement membrane and interstitial tissue structure. B) Ghee group revealing high magnification of Sertoli cells in the seminiferous tubules (Thick arrow**)**. C) Butter group high magnification of seminiferous tubules showing moderate vacuolation of fat (one asterisk), slight congestion of blood vessels (short arrow), dissolute parts of some seminiferous tubules (long arrow). D) margarine treated group, showing a high magnification of interstitial tissue displayed prominent vacuolation (one asterisk), congestion of blood vessels (short arrow), and dissolute parts of seminiferous tubules (long arrow). H&E stain. Scale bar= 10 µm.

4. Discussion

Lipids, such as fatty acids (FAs), are important components of biological membranes and signaling mediators **(Javaid *et al.*, 2019)**; however, lipids can become toxic and cause cell death if they are not properly managed **(Olzmann and Carvalho, 2019)**. The amount and type of dietary fats that person consumes directly affects their health and blood lipid profile **(Patel *et al.*, 2016)**.

Food sources of FAs include both plant and animal products. Plant sources as margarine **(Patel *et al.*, 2016)**, sunflower oil, cottonseed oil, Soybean oil, and canola oil **(Simopoulos, 2011)**. Also, animal sources of fatty acids are available in milk, dairy product (butter and ghee), meat and egg **(Kallio *et al.*, 2001)**.

According to the findings of this study, supplementation of rats with ghee for two months improved lipid profiles including (TAG, LDL-c, VLDL, AI, and cholesterol) meanwhile HDL-c increased as compared with the control group. This finding is in agreement with **(Kumar *et al.*, 2000),** who found that saturated fats in the diet, such as butter fat and ghee, had hypocholesterolemic effects by increasing both biliary constituent secretion and dietary cholesterol and bile acid excretion from the gastrointestinal tract **(Radhakrishnan *et al.*, 1993)**. Moreover, **Nour *et al.* (2018)** reported that Ghee raises HDL levels and lowers lipid peroxidation, which improves lipid profiles. Also, it increases antiatherogenic potency because of its high conjugated linoleic acid content that decreases serum LDL. In addition, short-chain saturated fatty acids are abundant in ghee which are easier to digest **(Vega *et al.*, 1995)** as they are digested directly into the bloodstream and delivered to the liver without being packed into lipoproteins. They are also easily digested since they do not require bile or pancreatic enzymes to break down. They have a low calorific value and give rapid energy due to their short carbon chain **(Karandikar *et al.*, 2016)**. On the other hand, **(Al-Othman (2000); Sharma *et al.* (2010)** stated that ghee significantly increased serum TAG and total cholesterol levels.

In this study, we found that ghee had reduced lipid peroxidation, which was proven by a significant decrease in MDA levels. Although ghee contains a high percentage of saturated fatty acids, it doesn't undergo lipid peroxidation, and therefore atherosclerosis is not induced by the consumption of fats containing saturated fatty acids **(Spiteller, 2005)**. Moreover, Sulfur-containing amino acids (cysteine), vitamins A, and E, carotenoids, enzyme systems, superoxide dismutase, catalase, and glutathione peroxidase are all antioxidants found in milk and dairy products**(Usta and Yılmaz-Ersan, 2013).** As a result, ghee is high in antioxidants such as Vitamin A, Vitamin E, and carotenoids, which may aid in preventing lipid peroxidation **(Sserunjogi *et al.*, 1998)**.

Testosterone is important for various physiological processes, including male secondary sexual development and fertility **(Tambo *et al.*, 2016)**. Leydig cells, found between the testis' seminiferous tubules, are in charge of testosterone synthesis and secretion. **(Neaves *et al.*, 1985)**. Testosterone level is essential for seminiferous tubules **(Wang *et al.*, 2019)**. Cholesterol is a necessary component of human nutrition. It is also a crucial raw material in the synthesis of steroid hormones at normal levels **(Lecerf and De Lorgeril, 2011)**.

In our study, there was a significant increase ($p < 0.05$) in testosterone levels in the ghee group as compared with the control group, this finding is in harmony with **(Hassanzadeh-Taheri *et al.*, 2018)**, who stated that this increase might be due to the high content of conjugated linoleic acid (CLA) in ghee. Also, **Barone *et al.* (2013) and Fındık and Andiç (2017)** stated that CLA supplementation enhances testosterone production in Leydig cells via an up-regulation of cytochrome P450 17A1 (CYP17A1) enzyme that is required for the production of androgenic steroids by converting 17α-hydroxypregnenolone to dehydroepiandrosterone (DHEA) in the endoplasmic reticulum of the Leydig cell.

Moreover, ghee is also an excellent source of lipophilic vitamins **(Upadhyay *et al.*, 2017)**, particularly vitamin A and E **(Antony *et al.*, 2018)**. Vitamin E is present in all cell membranes and works hard in this lipid environment to minimize free radicals and prevent significant lipid peroxidation caused by a free radical chain reaction **(Sharma, 2002)**. Moreover, it suppresses the apoptosis in the germinal epithelium and reduces oxidative stress in the testicular tissue **(Mumtaz *et al.*, 2020)**, which appeared in our histological findings.

Furthermore, our histological findings supported the elevated

testosterone level in the ghee-treated group, where they reported normal architecture of seminiferous tubules with seminiferous epithelium including Sertoli cells. More spermatids were also found in the lumen, and Sertoli cells have more obvious processes than the control group within normal epithelium height. Also, **Hassanzadeh-Taheri *et al.* (2018)** reported that colostrum is a full-fat milk product with fatty acid concentration similar to ghee. In diabetic rats, colostrum improves sex hormones and testicular morphology because it inhibits lipid peroxidation and protects the seminiferous epithelial layer in rats. **(Serki *et al.*, 2016)**. Also, the effect of ghee on testicular tissues may be attributed to its antioxidants such as CLA, vitamin E, and vitamin A. Vitamin A protects the testis from lipid peroxidation, promoting spermatogenesis and improving epididymis epithelial cell structural differentiation **(Fukuchi *et al.*, 2004)**.

On the contrary, adding margarine to the diet significantly increased serum lipid profile (TAG, LDLc, VLDL, AI, and cholesterol) and decreased HDLc compared to control group. This finding agrees with previous studies of **(Abd-Rabo *et al.*, 2020; Chardigny *et al.*, 2008; Hamza-Reguig *et al.*, 2017)** where they reported that significant biochemical problems had been identified with trans fatty acid intake, including undesirable serum lipoprotein profiles. Moreover, **(Longhi *et al.*, 2018; Ohlrogge, 1983)** stated that the physical characteristics of most TFAs are close to SFA, so TFA increases LDL as much as SFAs, but they lower HDL **(Mozaffarian *et al.*, 2004).** Moreover, **Han et al. (2002)** reported that, in persons with somewhat raised levels of LDL-c, the intake of hydrogenated fat with a high content of TFA is linked to an increase in inflammatory cytokines such as Tumor Necrosis Factor (TNF-) and Interleukin 1 (IL-6).

Furthermore, **Lee and Carr (2004)** stated that several mechanisms are proposed for the ability of margarine to increase LDL-c, such as decreasing the hepatic LDL-c receptors, aggregate esterification of cholesterol in lipoproteins containing apo B17, increasing the amount of esterified cholesterol transported in LDL-c due to chemical conformation of the fatty acid and increasing the activity of Acyl COA- cholesterol Acyl-transferase (ACAT). Therefore, dietary cholesterol content raises circulating cholesterol levels, and fatty acid-induced biosynthesis of cholesterol can increase serum cholesterol levels **(KATAN and BEYNEN, 1987).** In addition, Major SFAs present in margarine are myristic, palmitic, and stearic acids. Still, the most important one is palmitic acid **(Karabulut and Turan, 2006),** as it can stimulate cholesterol synthesis more than other dietary FAs **(Ohlsson, 2010)**. On the contrary, **Judd *et al.* (1998)**, **Brown *et al.* (2003)**, and **Morillas-Ruiz *et al.* (2014))** reported that margarine improved blood lipid profiles as it is rich in PUFA, which has a higher positive influence on lipoprotein metabolism.

In the current study, margarine induced lipid peroxidation, which was shown by a significant increase in MDA value in the margarine group as compared to control group, and this may be attributed to elevated dietary cholesterol that causes a significant decrease of testicular GSH, SOD with a significant increase of testicular MDA **(Matuszewska *et al.*, 2020)**. Moreover, **Nour *et al.* (2018)** reported that TFA induced lipid peroxidation.

Cholesterol is a necessary component of a balanced diet. Also, it is an essential raw material in synthesizing steroid hormones at normal levels **(Lecerf and De Lorgeril, 2011)**. However, accumulation of cholesterol in testicular Leydig cells leads to endoplasmic reticulum (ER) stress associated with decreased steroidogenic enzyme expression and testosterone synthesis **(Yu *et al.*, 2019)**. So, in this study, margarine-treated group had a significant decrease in testosterone level as compared with the control group. This finding agrees with **(Veaute *et al.*, 2007),** who stated that exposure to TFAs in male mice caused their accumulation in the testes, resulting in decreased serum testosterone and testicular atrophy. Moreover, **Okuyama *et al.* (2010)** found that testosterone levels decreased in rats that received hydrogenated vegetable oil compared with rats that received soybean oil. Also, gene expression of cytochrome P450 17A1(CYP17A1), steroidogenic acute regulatory protein (STAR), and hydroxysteroid 17-beta dehydrogenase (17β-HSD) was suppressed in rats received hydrogenated oil. Additionally, TFA consumption is linked to reduced total testosterone levels **(Arvizu *et al.*, 2015)**.

Excess cholesterol can be toxic **(Chen *et al.*, 2013)**. Free cholesterol deposition can raise the free cholesterol/phospholipid ratio in cellular membranes such as mitochondria and ER, which are important organelles in the manufacture of steroid hormones in Leydig cells like testosterone hormone **(Wang *et al.*, 2017)** and produce needle-shaped cholesterol crystals, causing integral membrane protein malfunction and cellular organelle disruption **(Tabas, 2002)**. Moreover, the marked increase in MDA in margarine group may be related to the overproduction of free radicals that mainly affect mitochondria, resulting in a decrease in testosterone synthesis **(Malek *et al.*, 2018)**

Both lipid profile and serum testosterone level in margarine group were supported by our histological analysis, where this group exposed a deterioration in the seminiferous tubules with the interstitial tissue, dissolute parts of some seminiferous epithelium with a thin basement membrane, and prominent vacuolization of fatty changes of the seminiferous tubules. The seminiferous epithelium showed a significant decrease in its thickness and structural disorganization, great congestion of blood vessels; this agrees with **(Ghosh and Mukherjee, 2018; Yu *et al.*, 2019)**, who reported that hypercholesteremia induced reproductive disorders. In addition, **Wu *et al.* (2020)** reported that rats that received oxidized fat oil that contained TFA showed disorganized seminiferous epithelium and atrophied Leydig cells. Testosterone binds to androgen receptors on Sertoli cells in seminiferous tubules **(Nieschlag *et al.*, 2012)**, so testicular degeneration is linked to lower testosterone levels in the blood **(Wu *et al.*, 2020)**.

When comparing the chemical composition of ghee and butter, we observed that, while they are nearly identical, butter contains more saturated fatty acids than ghee, and they also differ in the kind of fatty acid composition **(Johnson *et al.*, 2009; Joshi, 2014)**. Also, milk butter is higher in cholesterol, myristic (14:0), and palmitic fatty acids (16:0) **(Fındık and Andiç, 2017),** so LDL-c levels rise **(Katan *et al.*, 1994).** Also, ghee contain more PUFA than butter **(Karandikar *et al.*, 2016)**, so in this study, we found that the treatment with butter caused non-significant increases in cholesterol, TAG, VLDL but these increases were not as high as margarine group with non-significant decrease for HDL as compared with control group. These findings are in harmony with **(Wood *et al.*, 1993),** who stated that butter had increased total serum cholesterol levels. On the other hand, **Dorfman *et al.* (2005)** stated that hamsters that fed on butter showed high TAG levels compared to those fed on margarine because of the high saturated fatty acid content.

In butter treated group, there was a significant decrease in testosterone level as compared with the control group, that might be due to increased cholesterol level (as previously mentioned) that affects testosterone synthesis. The increase in lipid profile and the decrease in serum testosterone level in this group had induced histological alterations of testes including a slight decrease in the diameter of seminiferous tubules and loosely contacted seminiferous epithelium in some tubules. Normal pattern in others, moderate vacuolation in the interstitial tissue and degeneration of seminiferous tubules were also noticed. In addition, slight congestion of some blood vessels was revealed. Also, in our study, butter significantly decreased lipid peroxidation (MDA) as compared to control group, and this might be due to the presence of CLA in butter **(Franczyk-Zarow *et al.*, 2019)**, which lowered lipid peroxidation by improving oxidative stability in rats **(Jensen *et al.*, 2013)**.

5. Conclusion

From the above results, it is possible to conclude that ghee is the best dietary fat as it improved lipid profile, decreased lipid peroxidation, and increased testosterone level followed by butter. On other hand, margarine is unhealthy fat source.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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