Research Article

Influence of Phytochemicals on Haemato-Biochemical Parameters, Oxidative Status, Semen Characteristics and Histological Changes in Damascus Goat Bucks under Heat Stress Conditions

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Abstract | Garlic, ginger, and turmeric are all herbs with antioxidant and immunological properties; however, their combination may have a complementary effect on each another. That study was carried out to determine the efficiency of supplementation phytochemicals mixture on semen quality, blood metabolism, oxidative status and testicular structures. Three groups of fourteen Damascus bucks were formed. The first group (control, n=4) served as the control group. The second group (n=5) supplemented with 10 g/head/day of plant herb mixtures (PHM), along with turmeric (*Curcuma longa*), ginger (*Zingiber officinaleor*), and garlic (*Allium sativum*). The third group (n=5) supplemented with 5 g/head/day of commercial Quebracho tannins extract (QT). The findings showed that the PHM and QT groups had higher (P≤0.05) mean values of sperm concentration, mass motility, sperm motility, sperm viability, and cell membrane integrity. Plasma testosterone concentration and number of red blood cells were higher (P≤0.05) in both treated groups. However alanine aminotransferase, cholesterol, total antioxidant capacity and glutathione peroxidase levels were reduced (P≤0.05) in both treated groups. It can be noted that, supplementing of plant herbs mixtures had positive effects on some reproduction and immunological traits in goat males under heat stress conditions.

Keywords | Herbs additives, Quebracho tannins, Antioxidants, Sperm quality, Damascus goats.

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INTRODUCTION

Small ruminants comprising goats and other are critical to the economy, especially in the food security of desert communities due to their ability to produce meat, milk and fibers under desert harsh conditions (Konlan et al., 2012). Animals in arid regions are subject to a variety of stresses, including physical, nutritional, and environmental stress. Heat stress is currently the most concerning among all due to climate change (Sarangi, 2018). Reactive oxygen species (ROS) are produced on by heat stress (Garrido et al., 2004) and have a number of negative impacts on male livestock animals' reproductive organs and testicular functions. (Pintus and Ros-Santaella, 2021). These adverse stressors include inhibiting spermatogenesis (Mieusset et al., 1998), decreasing germ cells, sperm mitochondrial malfunction (Baumber et al., 2000), bad semen quality, abnormal sperm and DNA structure (Perez-Crespo et al., 2008), and oxidative degradation of polyunsaturated fatty acid-rich spermatozoa membrane lipids and proteins (Bromfield,

2014; O'Flaherty, 2015). Additionally, heat stress affects males' endocrine and biochemical states, increasing levels of thiobarbituric acid reactive substances (TBARs) and decreasing levels of glutathione peroxidase (GPx) in seminal plasma (Kowalowka et al., 2008; Marti et al., 2007) and reduction of LH hormone in bulls and testosterone level in boars (Murase et al., 2007). Subsequently, the fertilization capacity of sperm is reduced by an average (30% to 80%) (Guthrie and Welch, 2012). Higher semen quality is required for obtaining high fertility rates following artificial insemination or natural mating in order to produce livestock animals effectively. The use of synthetic antioxidants in animal diets has been outlawed by the European Commission (EC Regulation No. 1831/2003) due to evidence of harmful effects on both human and animal health. Therefore, attention was drawn to natural plant antioxidant sources for their effectiveness, abundance, and low cost (Ajao and Ola, 2021). Previous (*in-vitro* or *in-vivo*) studies on different species of animals confirmed that the use of plant herbs such as garlic, ginger, and curcumin (Elazab et al., 2022; Afele et al., 2020), either in the form of extracts, oils, powders, or in mixtures with other herbs, has a positive impact on the fertility and animal health (Vizzari et al., 2020; Maria et al., 2016).

Phytochemical analysis of garlic reveals that garlic contains varieties of enzymes (allinase, peroxidase and miracynase), essential amino acids , carbohydrates (sucrose and glucose), vitamins, minerals, flavonoid (Ankri and Mirelman, 1999; Focke et al., 1990), sulphides active compounds such as organosulfur (ajoene, allicin, dially disulphide, s-allyl cysteine and s methylcysteine sulfoxide) (Shin and Kim, 2004), allicin and allinin (Amagase et al., 2001), which makes it antimicrobial (Suleiman and Abdallah, 2014; Zouari Chekki et al., 2014), anti-inflammatory (Bozin et al., 2008), antioxidant (Asdaq, 2015). It outperforms synthetic antioxidants in terms of cytotoxicity (Sen et al., 2010), anti- tumor activity (Wallace et al., 2013), and immune resistance stimulation (Gafar et al., 2012). Additionally, garlic reduces the LDL level and cholesterol oxidation rate (Yeh and Liu, 2001), regenerates liver tissue (Tatara et al., 2005), reduces blood pressure, and prevents heart disease (Varshney and Budoff, 2016). The phytochemical analysis of ginger reveals a variety of active components, including ascorbic acid, beta-carotene (de Lima et al., 2018) and trace elements (Fe, Zn, Cu, Se, Ca, Mg, P, and K) according to (Sorokina and Steinbeck, 2020). In addition to, ginger is a natural antioxidant that inhibits lipid peroxidation, increases blood levels of glutathione, (Zhong and Zhou, 2013; Danwilai et al., 2017) and has androgenic properties on males (Kamtchouing et al., 2002). Ginger also has anthelmintic properties (Kiambom et al., 2020), anti-inflammatory (Jeena et al., 2013), and activates the immune system (Ali et al., 2008). Turmeric has a variety of bioactive properties, including hydroxymethoxyphen and heptadiene (Anand et al., 2007), which play a positive role in sperm quality (Soleimanzadeh and Saberivand, 2013), energy promotion and protection in the testicular tissue (Bucak et al., 2012), as well as reducing ROS such as superoxide and hydroxyl (Pulla Reddy and Lokesh, 1996), nitrogen dioxide (Unnikrishnan and Rao, 1992), and lipid peroxidase (Sreejayan and Rao, 1994). Tannins (QT) are a chemical defence mechanism used by plants to protect them from stresses both biotic and abiotic (Huang et al., 2018). QT has a variety of biological properties that are beneficial to both human and animal health, including immune stimulant, antioxidant properties (Ahmed et al., 2021), coenzyme Q10 and vitamins (Lukusa and Lehloenya, 2017). Several tannins sources have been applied in livestock species diets it had positive effects on productivity and reproductive performance (Jerónimo et al., 2016). The objective of the current study was to investigate the impact of combination of garlic, ginger and turmeric compared to quebracho tannins as feed additives on hemato-biochemical parameters, testis structure, semen quality and antioxidant status of Damascus bucks under semi-arid conditions.

MATERIALS AND METHODS

Animals and management

The current study was carried out Mariout Research Station (Latitude 31° 00' N; Longitude 29° 47' E), Desert Research Centre, Alexandria, Egypt.

Fourteen Damascus bucks' goats were used in this study. They were aged 12 to 14 months and weighed an average of 30 kg ±1.23 kg. Bucks were kept in a fenced closed yard for the course of the experiment. All animals received 2.5% of concentrate feed mixture and 1.5% of Egyptian clover hay (*Trifolium Alexandrinum*) of their live body weight to meet their requirements (NRC, 2007). The chemical composition of concentrates and hay are presented in Table (1). Proximate analysis was done according to the official methods (AOAC, 2005). Neutral detergent fiber (NDF) was determined according to (Van Soest and Robertson, 1985). Gross energy was calculated according to (Corbett et al., 1990), GE (MJ/KgDM) = 0.0176 OM (g/kg) + 0.0064 CP (g/kg) + 0.0214 EE (g/kg). Fresh water was available twice a day. All bucks were free of diseases or sexual disorders. The experiment was conducted in conformity with the standards for protecting experimental animals established by the European Union in 2010/63/EU. Additionally, the study was approved by the animal care committee Ref NO: (VUSC 003-1-22), Faculty of Veterinary Medicine, University of Sadat City, Egypt

Meteorological data

Air temperature (AT, °C), Relative humidity (RH, %), data

Table 1: The chemical composition (g/kg DM) of concentrate feed mixture and Egyptian clover hay

Items	DM	OM	EE	CP	NDF	GE*
Egyptian clover hay	876.9	842.4	18.1	175.0	584.5	16.33
Concentrates Concentrate feed mixture	909.8	911.4	29.2	190.5	291.5	17.88

DM (Dry matter), OM (Organic matter), CP (Crude protein), EE (Ether extract), NDF (Neutral detergent fiber) and GE (Gross energy – MJ/KgDM)

GE (MJ/KgDM) = 0.0176 OM (g/kg) + 0.0064 CP(g/kg) + 0.0214 EE(g/kg) according to [53].

were collected from a climatological station at the Ministry of Agriculture and Land Reclamation's Nubaria Research Station. The Temperature-Humidity Index (THI, %) was calculated according to the (Amundson et al., 2006) following formula: THI = (0.8 °C) + [(% relative humidity/100) (14.4 °C)] + 46.4.

Figure (1) presents the mean values of various parameters.

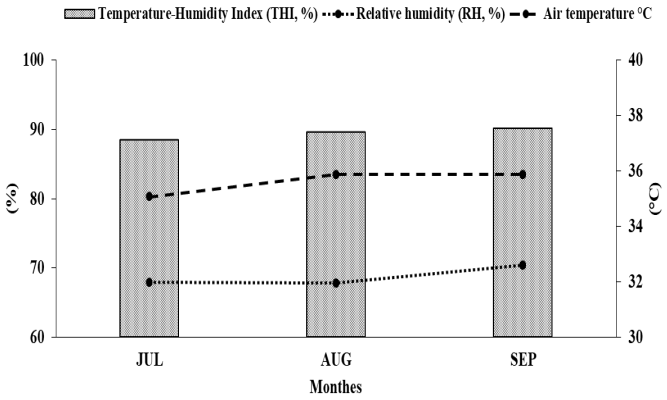

Figure 1: Changes in means of ambient temperatures, relative humidity temperature-humidity index (THI) throughout the experimental period in Damascus goats' bucks.

Experimental design

Fourteen Damascus bucks' goats were randomly divided into three groups. The first group (control, n=4) received the basal diet without any additive, while, the second group (n = 5) received the basal diet supplemented with plant herbs mixtures (PHM) (10 gm/head/day DM), which contain of garlic (*Allium sativum*) plus ginger (*Zingiber officinale*) plus turmeric (*Curcuma longa)* in proportions (1:1:1), respectively. The third group (n = 5) received the basal diet supplemented with (5 gm/head/day DM) of commercial Quebracho tannins extract (QT). The feeding trial lasted three months from September to December 2021.

Semen collection

Semen ejaculates were collected from animals using artificial vagina at monthly throughout experimental period (3 months).

Semen diluents and evaluation

Raw semen samples were transported immediately to the artificial insemination laboratory, Mariout research station, where ejaculate volume (mL) was measured using the graded collection tube. Additionally, each raw ejaculate's pH, mass motility, and sperm concentration were recorded. Thereafter, all good quality specimens (4-5) mass motility score according to (David et al., 2015) were further diluted (1:10) with an extender consisting of tris (3.8g), citric acid (2.2g), and glucose (0.6g) in 100 ml distilled water plus egg yolk (2.5%) according to Shamsuddin et al. (2000).

Semen assessment

Total sperm motility (%) was evaluated using a phase-contrast microscope (Leica) at 400 x magnification, and sperm vitality (%) was assessed using the differential staining technique. A mix of 10 µl of semen and 5 µl eosin-nigrosine stain was smeared on a warm stage and was examined at 1000 x magnification. Romanowski's triple-stain method was used to evaluate sperm abnormalities and acrosome integrity (DIFF-QUICK III, Vertex, Egypt); a phase-contrast microscope with a 1000x magnification was used to evaluate the stained smears. Sperm plasma membrane integrity (%) of at least 200 sperm at 400x magnification was determined by the hypo-osmotic swelling test (HOST) according to (Mosaferi et al., 2005).

Blood collection

Blood samples were collected monthly from all bucks throughout experimental period (3 months) in plasma vacutainer tubes 5 ml to determine biochemical and antioxidant enzymes activities. After centrifuging at 5,000 g for 10 minutes, plasma was collected and stored at -20 °C for later analysis. A whole blood samples were collected at the same times to determine hematological parameters.

Testosterone assay

Testosterone (TST, ng/mL) was analyzed using ELISA kit (Monobind, USA) according to (Lashansky et al., 1991).

Plasma biochemical and antioxidant enzymes activities analysis

The analysis of total protein (TP, g/dL), albumin (ALB, g/dL), glucose (GLC, mg/dL), cholesterol (CHO, mg/dL), triglycerides (TG, mg/dL), aspartate aminotransferase

(AST, IU/L), and alanine aminotransferase (ALT, IU/L) in plasma was performed colorimetrically using commercial kits (Spectrum Biotechnology, Egypt). Glutathione peroxidase (GPX, U/mL), malondialdehyde (MDA, nM/mL), and total antioxidant capacity (TCA, mM/L) were calorimetrically assessed using commercial kits (Biodiagnostic Research, Egypt).

Hematological parameters

A colorimetric kit was used to determine the concentration of hemoglobin (Hb, g/dL) according to (Diamond, Egypt). Thoms hemocytometer slide was used to count the total number of white blood cells (WBCs, $10^3/mm^3$), the number of red blood cells (RBCs, $10^6/mm^3$), and the packed cell volume percent (PCV, %) was estimated by the hematocrit tubes.

The equation used to calculate the mean corpuscular volume (MCV, m^3) was (MCV, m^3) = PCV (%) / RBCs ($10^6/mm^3$) × 10. The formula used to determine mean corpuscular Hb (MCH, pg) was MCH (pg) = Hb (g/dL) / RBCs ($10^6/mm^3$) ×10. The formula for calculating MCH concentration (MCHC, %) is MCHC, %=Hb (g/dL) / PCV (%) ×100.

Histological Examination

Three bucks from each group were slaughtered, and their testicles were collected and processed for histology examination at the end of the experiment and tissue specimens were rapidly fixed in 10% neutral buffered formalin solution for at least 24 hrs. The tissue was embedded and dehydrated in a variety of ethanol solutions. Xylene and paraffin wax were used for clearing and impregnation, respectively. Several paraffin sections of 3–5 micron thickness were obtained and stained with hematoxylin and eosin as described earlier (Bancroft and Gamble 2002) and examined by a light microscope at various magnifications for histological studies, as described by Amao et al. (2012).

Statistical analysis

Data of biochemical blood parameters, antioxidant activities, hormonal profiles, hematological parameters and semen characteristics throughout experimental period were statistically analyzed by one-way analysis of variance (ANOVA) to test the significance among means. The differences among means were detected by Duncan's post-hoc test. Significance was set at 5% and data were analyzed by IBMSPSS statistics program (IBM-SPSS, 2013). The statistical analyses were conducted according to following model:

$Y_{ij}=\mu+T_i+e_{ij}$.

Y_{ij} is the studied treat.

μ is the overall mean.

T_i is the effect of treatment (i= 1, 2, 3).

e_{ij} is the random error.

RESULTS

Changes in semen traits

The results showed that higher mean values of both mass motility (P=0.05) and sperm concentration (P=0.01) in PHM group (3.50±0.32 and 2268.33±104.33 $\times10^6$, respectively) followed by QT group (3.17±0.32 and 1708.00±104.33 $\times10^6$, in order), compared with control group (2.17±0.32 and 1830±104.33 $\times10^6$, respectively). Mean pH value was higher (P=0.04) in control group (6.68±0.07) compared with both treated groups PHM and QT (6.13, 6.30±0.07) respectively (Table 2). Increased mean values of sperm motility (P=0.02) and viability (P=0.01) in PHM specimens (91.66±2.15 and 92.66±1.36%) and QT (88.00±2.15%, and 85.00±1.36%), respectively compared to control specimens (80.00±2.15 and 76.33±1.36 %). Mean values of sperm cell membrane integrity percentage in both PHM and QT specimens were (P=0.01) significantly higher (87.00±2.29 and 80.33±2.29%, respectively) compared to control (68.46±2.29%). While the percentages of acrosomal integrity, normal sperm, primary, secondary sperm abnormalities, and semen volume were not affected (Table 2).

Testosterone hormone and blood biochemical constituents

The results showed that TST concentration was increased (P=0.01) in both PHM (10.00±0.46 ng/mL) and QT (7.76±0.46 ng/mL) bucks compared to those of control (4.93±0.52 ng/mL) Table. 2. However, levels of total TP, ALB, GLU, TG and AST were not affected. On the other hand, the mean level of ALT was declined (P =0.01) in the PHM group (71.48±3.76 IU) or QT group (82.58±3.76 IU) compared with control group (90.94±4.20 IU). Likewise, CHO concentrations followed the same trend, where both treated (QT and PHM) groups recorded lower (P=0.05) values (65.68±10.34 and 71.61±10.34 mg/dL) respectively compared to control (84.84±11.56 mg/dL) (Table 3).

Plasma antioxidant activities

Treatment with mixtures plant herbs additive (PHM) or Quebracho tannins (QT) decreased (P=0.01) the overall of GPX concentrations of (3.92±0.63 and 4.55±0.63 U/mL), respectively, compared to the control (7.08±0.71 U/mL). A similar trend was also observed in TAC concentration in both treated (PHM and QT) groups (0.83±0.01 and 0.89±0.01 mM/L), respectively, compared to control (0.93±0.02 mM/L). However, plasma MDA concentration was not affected (Table 3).

Hematological parameters

Overall means of WBC counts ($10^3/mm^3$), blood Hb

Table 2: Mean±SEM of physical sperm characteristics of Damascus bucks' semen fed a diet supplemented with mixtures plant herbs (PHM) or Quebracho tannins (QT)

Semen characteristics	Treatments groups			*P* value
	Control	QT	PHM	
Volume (mL)	1.00±0.16	1.4±0.16	1.6±0.16	0.09
pH	6.68±0.07^{A}	6.30±0.07^{B}	6.13±0.07^{B}	0.04
Sperm concentration (×10^{6}/mL)	1830.66±104.33^{B}	1708.00±104.33^{B}	2286.33±104.3^{A}	0.01
Mass motility score (5–0)	2.17±0.32^{B}	3.17±0.32^{AB}	3.50±0.32^{A}	0.05
Sperm motility (%)	80.00±2.15^{B}	88.00±2.15^{AB}	91.66±2.15^{A}	0.02
Live spermatozoa (%)	76.33±1.36^{C}	85.00±1.36^{B}	92.66±1.36^{A}	0.01
Normal spermatozoa (%)	88.33±1.65	90.66±1.65	95.00±1.65	0.07
Primary sperm abnormalities (%)	2.00±0.54	2.33±0.54	1.33±0.54	0.46
Secondary sperm abnormalities (%)	9.66±1.44	7.00±1.44	3.66±1.44	0.07
Intact acrosome (%)	86.00±2.26	84.00±2.26	92.00±2.26	0.09
HOST (%)	68.46±2.29^{B}	80.33±2.29^{A}	87.00±2.29^{A}	0.01

Table 3: Mean±SEM of plasma biochemical parameter of Damascus bucks' fed diets supplemented with mixtures plant herbs (PHM) or Quebracho tannins (QT)

Items	Treatments			*P* value
	Control	QT	PHM	
Blood biochemical parameters				
Total proteins (TP, g/dL)	7.06±0.42	6.77±0.37	7.58±0.37	0.32
Albumin (ALB, g/dL)	4.72±0.45	3.30±0.40	3.68±0.40	0.10
Glucose (GLC, mg/ dL)	55.45±7.05	68.78±6.31	55.61±6.31	0.28
Cholesterol (CHO, mg/ dL)	84.84±11.56^{A}	65.86±10.34^{B}	71.61±10.34^{B}	0.05
Triglycerides (TG, mg/ dL)	46.43±8.91	48.57±7.97	48.57±7.97	0.98
Alanine aminotransferase (ALT, IU/L)	90.94±4.20^{A}	82.85±3.76^{B}	71.48±3.76^{B}	0.01
Aspartate aminotransferase (AST, IU/L)	14.50±2.85	15.20±2.55	13.60±2.55	0.90
Antioxidant enzymes activities				
Total antioxidant capacity (TCA, mM/L)	0.93±0.02^{A}	0.89±0.01^{B}	0.83±0.01^{B}	0.05
Malondialdehyde (MDA, nM/mL)	10.39±1.50	6.77±1.34	5.77±1.34	0.10
Glutathione peroxidase (GPX, U/mL)	7.08±0.71^{A}	4.55±0.63^{B}	3.92±0.63^{B}	0.01
Hormonal assay				
Testosterone (TST, ng/mL)	4.93±0.52^{C}	7.76±0.46^{B}	10.00±0.46^{A}	0.01

concentration (g/dL), PCV %, MCHC %, MCH pg, and MCV μm^{3} were not affected by the supplementation. Overall mean of RBCs (10^{6}/mm^{3}) was increased (P=0.05) by PHM treatment (13.96±0.56 10^{6}/mm^{3}) compared to QT or control groups (10.27±0.56 and 9.49±0.63 10^{6}/mm^{3}) in order (Figure 2).

Histological Changes

The histological sections of the buck's testis of animals fed on mixtures of herbal plants (PHM) revealed the presence of intact seminiferous tubules and normal spermatogenesis (Figure 3, C), then the animals fed a diet supplemented with condensed tannins, which showed seminiferous tubules close to normal appearance with slight degeneration and edema Figure 3, B). While the histological examination of the control group showed damage of seminiferous tubules, vacuolation and necrosis of spermatogonic epithelial cells of seminiferous tubules, desquamated necrotic spermatogonic cells inside the lumen of the seminiferous, and thickening of interstitial tissue surrounding seminiferous tubules as shown in (Figure 3, A).

DISCUSSION

Regarding the effect of supplementation with mixtures of plant herbs (PHM) or quebracho tannins (QT) on the physical characteristics of buck's semen, the findings of the present study indicated that supplementing with (PHM)

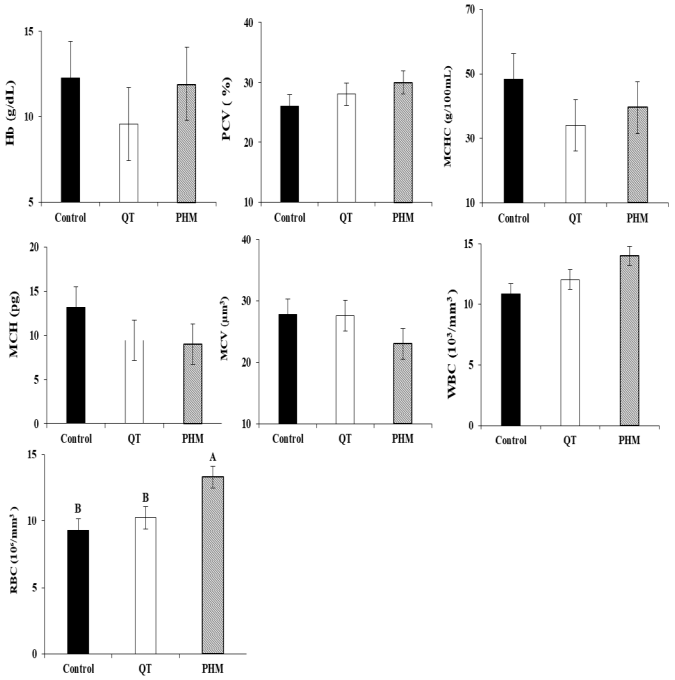

Figure 2: Effect of plant herbs mixture (PHM) OR Quebracho tannins (QT) supplemented diets in haematological parameters in Damascus bucks' goats (Mean ± SE).
A-B Letters among the group differ significantly (P ≤0.05).

or (QT) improved physical semen characteristics, including sperm concentration, mass motility, sperm motility, sperm viability, and cell membrane integrity percentage (Table 2).

Elkelawy et al. (2017) reported that semen parameters of rabbit bucks were enhanced after injection with a lower dose of garlic (3mg/kg) for 8 weeks. Ajao and Ola, (2021) concluded that supplementation with ginger or garlic (15g/kg feed) for 8 weeks caused an improvement of sperm motility (%). Similar results were reported with fresh ginger and garlic supplemented to rabbits' diets for 7 weeks (Adeyemi et al., 2020). Moreover, the addition of 0.025 g garlic to ram semen extender led to a slowdown in the deterioration of spermatozoa at 48 hrs of chilled preservation at 4°C (Jerez-Ebensperger et al., 2015). Tvrdá et al. (2018) revealed that the addition of (50µmol/L) curcumin (CUR) to the bull semen extenders led to improvements in motility, membrane integrity, and acrosomal integrity in frozen bull semen. All semen specimens treated with different levels of turmeric extract during cold preservation or post-thaw showed an increase in sperm motility, vitality, and sperm membrane integrity (HOST) (Elsheshtawy, 2020).

Supplementing rams' diets with encapsulated tannin or tannin extract (1.5, 3 g/h/d) for 16 weeks improved sperm concentration, sperm motility percentage and cell membrane integrity percentage (Wurlina et al., 2020). Tannins' beneficial effects on sperm quality are attributed to their antioxidant properties (Seifi, 2011; Bahmyari et al., 2020). Testes have a potent antioxidant defense system that includes the enzymes SOD and catalase to counteract the detrimental effects of ROS. In a biological system, the catalase enzyme protects SOD from H_2O_2 inactivation while SOD protects the catalase from superoxide anion inhibition. The elimination of superoxide and peroxide radicals produced in the tissues may therefore depend on maintaining the balance of this enzyme system (Sharma et al., 2012).

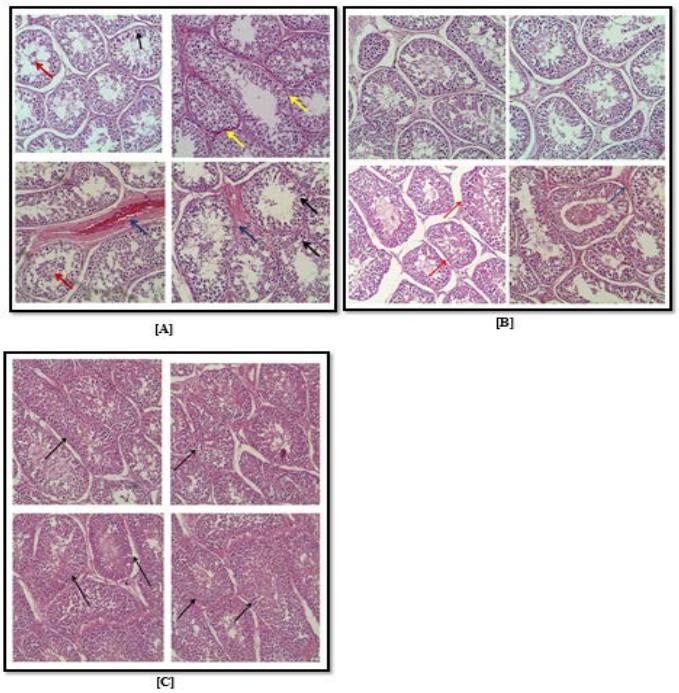

Figure 3: Photomicrograph of the testes of bucks Damascus goats fed mixtures plant herbs (PHM) or Quebracho tannins-supplemented diets (QT).
[A]: Histologic section of the testis of bucks in control group showing the damage of seminiferous tubules, desquamated epithelial cells in the lumen of seminiferous tubulles, haemorrhage and edema of interstitial tissue (blue arrow), vacuolation of spermatogenic epithelium of seminiferous tubules (black arrow), desquamated necrotic spermatogenic epithelium in seminifferous tubules (red arrow), thickening of interstitial tissue surrounding seminiferous tubules (yellow arrow) (H&E ×10).
[B]: Histologic section of the testis of bucks in group CTS testis, showing somewhat normal histology of testis (red arrow) with slight degeneration and edema (blue arrow) (H&E ×10).
[C]: Histologic section of the testis of bucks in group PHM testis, showing clearly defined, intact seminiferous tubules and normal spermatogenesis (black arrow) (H&E ×10).

Garlic, ginger, and curcumin include a wide range of

phytochemicals that can scavenge free radicals (Charles, 2013; Ghasemi et al., 2015), to protect DNA and other important molecules to spermatozoa from oxidation and damage (Nasimi et al., 2018), through several mechanisms such as improving cellular scavenging enzymes such as (CAT,GPX and SOD), inhibition of low density lipoproteins (LDL) oxidation, inhibition of lipid peroxidation, inhibition of homocystein ethiolactone formation, in addition to inhibition of nuclear factor activation (Ma et al., 2017). Additionally, antioxidants are compounds that inhibit ROS from damaging the spermatozoa's gene expression system, maintaining ATP synthesis in the mitochondria, which is necessary for sperm motility (Zhu et al., 2019; Barbagallo et al., 2020). Garlic, ginger and curcumin or condensed tannins supplementation may have an improvement effect by altering the shape of spermatogenic cells and leydig cells during the spermatogenesis process in the testicle (Figure 3. B & C).

Therefore, it appears from the results of current study, supplementation of (garlic, ginger and curcumin) combination or condensed tannins improved quality and quantity of sperm production sperm indicating good reproductive potential and fertility in either natural mating (Ekuma et al., 2021) or using advanced reproductive technology system (AI) and (IVF) in goats under Egyptian conditions (Abd El-Hamid, 2019).

Our results clarified that supplementation of mixtures of herbs (PHM) or condensed tannins (QT) to animal diets reduced CHO and ALT levels compared to control (Table 2). Similar findings were reported in different species in lambs (Sharifi and Chaji, 2019), sheep (Thamer et al., 2020), goats (Pirmohammadi et al., 2014), buffalo calves (Vamsi Duvvu et al., 2018), rabbits (Amaduruonye et al., 2020). Garlic includes the active ingredient (Allicin), which reduces fatty acid production and inhibit the production of cholesterol and triglycerides (Eidi et al., 2006). Kiambom et al. (2020) reported that levels of ALT and CHO were reduced by supplementing ginger to pigs' diets. Similarly, results were found in rabbits, (Shetaewi et al., 2017). Ginger has a hypolipidemic and hypocholesterolemic impact related to a reduction in dietary cholesterol absorption, an increase in bile acid production, and/or faecal losses (Goyal and Kadnur, 2006).

The same results were founded in the impact of curcuma longa or condensed tannins in different animals. In lambs (Al-Zabaie and Sultan, 2020), pigs (Djoumessi Tobou et al., 2020), rabbits (Ayoub et al., 2019) under heat stress. This could be because curcumin inhibited or reduced cholesterol absorption in the intestine, enhancing the activity of the enzyme cholesterol-7-hydroxylase and improving the liver's conversion of serum lipids into bile acids (Hussein et al., 2014). Also, the decrease in ALT activity obtained with rations herbs powder or condensed tannins were due to the properties of hepato-protective (Zhang et al., 2010).

The overall means of TAC and GPX activity were reduced in the treated groups (Table 2). Herbs or tannins have effects on antioxidant activity depending on their chemical composition (Vizzari et al., 2020), which contains flavonoids, fructans, alliin, allicin, gamma-glutamyl cysteine, and sulphur compounds, which are characterized by their powerful antioxidant activity It inhibits the activity of some enzymes, such as protein kinase C, which inhibits the production of ROS species by inhibiting lipid peroxide-producing reactions (Tan et al., 2018).

The present results showed that overall means of RBCs counts increased significantly in the MHP group compared to the QT or control groups (Figure 2).

The increase in RBC production was in agreement with the reports of (Alagawany et al., 2016; Onu and Aja, 2011). Active components of garlic, ginger, and turmeric, or tannins such as B1, B2, B6, B9, C, and E, have positive benefits for stimulating the thymus, spleen, and bone marrow for RBC production (Fazlolahzadeh et al., 2011; Samson et al., 2012). Additionally, it stimulates the kidney to produce and secrete erythropoietin and has a stimulatory effect on cytokines that interact with certain receptors on the surface of haematopoietic cells. (Attia and Ali, 1993; Samson et al., 2012).

In the current study, histological features of the testis showed an improved cytoarchitecture in the HMP group followed by the QT group compared with control. Our findings are similar to those of (El-kashef 2021; Ekuma et al., 2021), which confirmed the positive effect of mixtures plant herbs on the testicular structure of males in different animal species. This improvement may be due to the combined effect of the biologically active antioxidants in the PHM group that probably protected the testicular structure and sperm cells from depletion due to lipid peroxidation caused by ROS within the testes of animals exposed to heat stress conditions (El-Kholy et al., 2021; Shinkut et al., 2016). This result is supported by Table (2 and 3).

CONCLUSIONS

Our results indicated that both mixtures of plant herbs (PHM) and condensed tannins (QT) had a positive impact as an antioxidant on plasma biochemical parameters, immune and oxidative status, and improved physical sperm characteristics, enhanced testosterone production, and preserved the structure of the testicles. Further scientific

research is needed to investigate effects of these feed additives on the efficiency of *in-vitro* and *in-vivo* fertilization in goats and other domestic animals.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

NOVELTY STATEMENT

According to these results, the mixtures of plant herbs had a positive impact as an antioxidant on oxidative status, plasma biochemical parameters and immune functions, in addition to improving physical sperm characteristics, enhancing testosterone secretion and preserving testicular structures under heat stress conditions.

AUTHORS' CONTRIBUTIONS

Ibrahim S. Abd El-Hamid carried out blood biochemical and semen analysis in Artificial Insemination lab and DRC laboratories complex, performed the statistical analysis of the data and wrote this article. Alaa Emara Rabee and Moustafa, M.M.A. Ghandour designed the experiment and have carried out field execution to all experiment stages and collect blood samples and field data. Rasha Salah Mohammed histological examination of testis samples. Ahmed M Sallam collect the samples and revised the manuscript. All authors read and approved the final manuscript.

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