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Research Article
Impact of Green Tea Extract on Reproductive Performance, Hematology, Lipid Metabolism and Histogenesis of Liver and Kidney of Rabbit Does

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Abstract

Background and Objective: Green tea has very strong antioxidant, antibacterial, antiviral, enzyme inhibitory, antiradiation and anticancerous properties. This study aimed to evaluate the efficacy of different levels of green tea extract on reproductive performance, hematology, lipid metabolism and histological structure of liver and kidney of rabbit does. **Methodology:** Total of 30 mature does and 10 fertile bucks of New Zealand White (NZW) rabbits were divided into 3 groups, 10 in each. The 1st group was control, while second and third groups were given, 1 mL distilled water containing 200 and 400 mg kg^{-1} b.wt. of green tea extract, respectively, orally for 30 days prior to natural mating. Doe weight at start and end of treatment, mating, kindling and weaning and kid weight at birth and weaning were recorded. Doe litter size and kid viability rate at birth and weaning were calculated. Hematological parameters and lipid profile were determined in blood plasma of does after weaning. Histological structure of liver and kidney of does was examined. **Results:** Doe body weight was higher in control and second than in third group at end of treatment, mating and kindling ($p<0.05$) and at weaning ($p\geq0.05$). Kindling rate, gestation period and litter size of does and body weight and viability rate of kids did not differ. Hemoglobin concentration, hematocrit, mean corpuscular volume and white blood cells count increased ($p<0.05$), while platelet count decreased ($p<0.05$) in third than in control and second groups. Plasma total lipids, total cholesterol, triglycerides and high density lipoprotein concentrations decreased ($p<0.05$), while low density lipoprotein concentration increased ($p<0.05$) in second and third than in control group. No pathological legions were noted in the histological structure of liver and kidney. **Conclusion:** Daily oral administration of rabbit does 30 days prior to mating with green tea extract at levels of 200 or 400 mg kg^{-1} is recommended to improve reproductive performance and lipid metabolism of rabbit does without adversely effects on hematological parameters and liver and kidney function.

Key words: Rabbit, green tea extract, reproductive performance, hematology, histology, liver, kidney

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rabbit husbandry has been gaining much attention in recent years due to their high prolificacy, short gestation length, high growth performance and high meat yield as compared to other live stocks[1]. Rabbits are used as a model in basic and applied reproductive biological studies for their high reproductive efficiency and economical purposes. Commercial rabbit breeder profitability has increased due to improving the genetic selection and reproductive management[2,3].

Reactive Oxygen Species (ROS) are important molecules (radical and non-radical) in biological systems, which are produced as by-products of normal cellular oxygen metabolism[4]. Reproductive tissues and cells remain stable when oxygen free radical production and the scavenging antioxidants remain in balance. At environmental stress, increasing production of ROS causes various diseases and can affect ovarian steroidogenesis, oocyte maturation, ovulation, implantation and formation of fluid filled cavity, blastocyst, luteolysis and luteal maintenance functions during pregnancy[5,6]. Antioxidants play a vital role in productive and reproductive performances[7], especially implantation, placentation, fetal growth and organ development[5]. In rabbits, dietary addition of antioxidants improved kindling rate, litter size at birth and weight of kids at birth and weaning[8,9], while decreased lipids and cholesterol contents and improved feed conversion in laying hens[10,11].

Herbal compounds such as Green Tea (GT), curcumin, indole-3-carbinol, resveratrol are currently undergoing clinical trials due to their chemo-preventive and antioxidant properties[1]. The GT became a very popular beverage in worldwide. Chemical composition of GT is similar to that of fresh tea leaves containing several compounds like, polyphenols, fluoride, vitamin K, caffeine, minerals (sodium, potassium, calcium) and trace elements (aluminum, chromium, selenium, manganese and iron)[12]. Majority of GT polyphenols (GTPs) components include 48-55% epigallocatechingallate (EGCG), followed by 9-12% epigallocatechin (EGC), 9-12% epicatechingallate (ECG) and 5-7% epicatechin[13]. The biological effects of GT seem to be mainly due to the high content of certain polyphenols. The GTPs are the major water-soluble components of GT infusions. The most prevalent among GTPs are a group of compounds called catechins which make up to 30% of the dry weight of GT leaves[14]. Several animal studies suggested that GTPs have very strong antioxidant capacity, antibacterial and antiviral agents, enzyme-inhibitory and antiradiation and anticancerous effects[12]. The EGCG is one of the most active and bioavailable component of GT catechins acting as an antioxidant and potent scavenger of free radicals and ROS in biological system[15], prevention of various diseases and also reproductive effects[16]. *In vivo* studies showed that EGCG in GTPs could inhibit key enzymes involved in lipid biosynthesis, lipid absorption, blood triglyceride and total cholesterol as well as stimulating energy expenditure, fat oxidation, High Density Lipoprotein (HDL) levels and fecal lipid excretion[12,17]. Adding GT leaves (1-5%) or GTPs (0.5-2.5 L/100 kg diets) to hen improved their productive performance[18].

There is no available information on the effect of oral Green Tea Extract (GTE) treatment on reproductive performance of rabbit does. Therefore, the goal of the present study was to investigate the efficacy of daily oral treatment of GTE, at levels of 200 and 400 mg kg^{-1} Live Body Weight (LBW) for 30 days as natural antioxidant on reproductive performance, hematological parameters, lipid metabolism and histological structure of liver and kidney of rabbit does.

MATERIALS AND METHODS

Animals: Total of 30 sexually mature New Zealand White (NZW) rabbit does (1-2 parity) as well as 10 fertile NZW rabbit bucks for natural mating were used in this investigation. Rabbit does were raised in rabbit farm of Sakha Station, Animal Production Research Institute (APRI), Agricultural Research Center, Egypt. Rabbit does were divided randomly into 3 groups, 10 in each according to LBW and parity. All animals (does with their kids and bucks) were housed in separate wire cages and fed *ad libitum* on a suitable commercial diet, while water was available all day time. All animals were kept under the same managerial system.

Experimental procedures

Green tea extract administration: Tablets containing GTE (Arab Company for Pharmaceuticals and Medicinal Plants, MEPACO-MEDIFOOD, Egypt) were used for treatment. Total of 10 or 20 tablets (each tablet contained 300 mg GT as a dry extract, 30% polyphenols) were dissolved in 15 mL distilled water for obtaining oral dose of 200 or 400 mg mL^{-1} of GTE, respectively.

Rabbit does in the 1st group were considered as a control group without treatment (G1), while does in the 2nd (G2) and 3rd (G3) groups were given 1 mL containing 200 or 400 mg kg^{-1} LBW of GTE, respectively, as daily oral doses for 30 days prior to natural mating.

Live Body Weight (LBW): The LBW of rabbit does was recorded at start of treatment (initial), end of treatment

(30 days of treatment and pre-mating), mating, kindling and weaning. Also, average kid weight at birth and weaning was recorded.

Reproductive measurements: At the end of treatment period, rabbit does in all groups were allowed to be naturally mated by fertile bucks and pregnancy was diagnosed by palpation on day 10-12 post-mating to detect the pregnancy. After the positive mating, the nest boxes were supplied with sawdust to provide a comfortable and warm nest for bunnies. Then, kindling rate and gestation period length were calculated. After 12 h of kindling, litters were checked and stillbirth was removed. Afterward, litters were examined each morning during suckling to remove the dead ones from the nest. Young rabbits were weaned at 35 days of age. Litter size at birth and weaning of does as well viability rate of kids were calculated. Weaning Rate (WR) was calculated as:

$$\text{Weaning Rate (WR)} = \frac{\text{No. of weaning kids}}{\text{No. of live kids}} \times 100$$

Blood sampling: Blood samples were collected from 6 does in each experimental group at the end of treatment period. Blood samples were taken from the ear vein into test tubes containing EDTA for determination of hematological parameters and lipid profile. Hematological parameters included hemoglobin concentration, hematocrit value, count of RBCs, WBCs and platelets, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and red cell distribution width (RDW). All hematological parameters were measured in whole blood using blood hematology analyzer (HB 7021).

Blood samples were centrifuged at 3000 rpm for 20 min to separate blood plasma using serological pipettes, then plasma was carefully decanted into labeled tubes and stored at -20°C for biochemical analysis of total lipids, total cholesterol, triglycerides, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). Commercial kids and spectro-photometer were used for determining all biochemical traits in blood plasma.

Histological study: At the end of the experimental period (at weaning), three rabbit does were sacrificed and small pieces of liver and kidney tissues were taken and fixed in 10% neutral buffered formalin solution for 24 h, dehydrated in ascending grades of ethyl alcohol, cleared and sections of 5-7 μm in thickness were cut, mounted on glass slides, deparaffinized and stained with hematoxyline and eosin as described previously by Al-Forkan *et al.*[19]. The sections were examined under a light microscope at magnifications of x100 and 400 for any morphological alterations in the histological structure.

Statistical analysis: Statistical analysis for all data was carried out using analysis of variance (ANOVA) using general linear model program of SAS[20]. Statistically significant difference among means were set at $p \leq 0.05$ level by using Duncan's Multiple Range Test (DMRT) procedure[21].

RESULTS AND DISCUSSION

Effect of GTE treatment on

Live body weight of rabbit does: The LBW of rabbit does was significantly ($p<0.05$) higher in G1 and G2 than in G3 at end of treatment, mating and kindling, while the differences in LBW of does at weaning among groups were not significant (Table 1).

The results indicated that increasing level of GTE administration only up to 400 mg kg^{-1} resulted in marked reduction in LBW of does. In agreement with the present results, dietary addition of GT leaves or its aqueous extract decreased final body weight of rabbits[22] and also decreased body weight gain[23] and body weight of rats[24] as compared to controls.

The mechanism of LBW decline as affected by GT treatment may be associated with an increase in expenditure of energy and oxidation of fat. Also, lipogenic enzyme fatty acid synthesis may be suppressed by GT treatment[25]. Also, hypo-lipidemic and antiobesity effects of GT or GTPs was reported by Lin and Lin-Shiau[26] in terms of suppression of molecular mechanisms of fatty acid synthase gene by EGCG and theaflavins in GTPs.

Table 1: Effect of GTE treatment on LBW of rabbit does

		GTE level	
Parameters (kg)	G1 (Control)	G2 (200 mg kg^{-1})	G3 (400 mg kg^{-1})
Initial live body weight	2.742±0.020	2.743±0.097	2.715±0.059
Average LBW at end of treatment	3.011±0.019[a]	3.003±0.038[a]	2.816±0.062[b]
Doe weight at mating	2.940±37.19[a]	2.946±91.79[a]	2.600±68.92[b]
Doe weight at kindling	3.050±35.36[a]	3.040±87.18[a]	2.760±53.39[b]
Doe weight at weaning	3.200±35.82	2.986±80.29	2.724±43.08

[a,b]Means within the same row having different superscripts are significantly different at $p \leq 0.05$, GTE: Green tea extract, LBW: Live body weight

Table 2: Effect of GTE treatment on reproductive performance of rabbit does and kid performance

		GTE level	
Parameters	G1 (Control)	G2 (200 mg kg^{-1})	G3 (400 mg kg^{-1})
Kindling rate (%)	100.00	100.00	100.00
Gestation length (days)	31.00±0.41	30.60±0.50	30.80±0.37
Total litter size at birth per doe	6.50±0.500	8.00±0.550	7.40±0.680
Live litter size at birth per doe	5.75±0.480	7.00±0.550	6.60±0.510
Viability rate at birth (%)	88.50±3.94	87.50±3.67	90.60±5.800
Litter size at weaning	5.00±0.410	6.40±0.600	6.20±0.580
Weaning rate (%)	87.30±4.40	91.00±3.92	94.30±5.71
Average kid weight at birth (g)	59.00± 1.4	59.60±1.44	59.40±1.50
Average kid weight at weaning (g)	407.50±17.50	415.00±11.83	418.80±14.8

Table 3: Effect of GTE treatment on some hematological parameters of rabbit does

		GTE level	
Parameters	G1 (Control)	G2 (200 mg kg^{-1})	G3 (400 mg kg^{-1})
Hemoglobin (g dL^{-1})	11.50±0.76^{b}	13.87±0.47^{a}	14.60±0.16^{a}
Hematocrit (%)	43.70±0.30^{b}	45.73±1.25^{b}	49.10±0.56^{a}
RBCs (×10^{6} mm^{-3})	6.13±0.59	6.72±0.11	7.35±0.11
WBCs (×10^{3} mm^{-3})	6.00±0.00^{b}	6.63±0.18^{b}	7.83±0.35^{a}
Platelets (×10^{3} mm^{-3})	328.0±1.00^{a}	198.00±0.00^{c}	302.00±2.00^{b}
MCV (μ^{3})	70.00±0.00^{b}	71.07±0.43^{ab}	71.53±0.58^{a}
MCH (pg)	20.67±0.44	20.20±0.64	20.73±0.64
MCHC (g dL^{-1})	29.17±0.17	29.73±0.38	30.13±0.94
RDW (%)	13.17±0.17	11.93±0.48	13.13±0.54

$^{a-c}$Means within the same row having different superscripts are significantly different at $p \leq 0.05$, RBCs: Red blood cells count, WBCs: White blood cells count, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width

Reproductive performance of rabbit does and kid performance: Reproductive performance including kindling rate, gestation period, litter size at birth (total and live) and weaning size as well as kid performance were insignificantly higher in G2 and G3 than in G1 (Table 2). Kindling rate of does was 100% in all groups, but there was an improved reproductive performance of does and growth performance of their kids in G2 and G3 treated with GTE at levels of 200 and 400 mg kg^{-1} LBW, being the best for those treated with 200 mg kg^{-1} LBW. In accordance with the present results, GTPs improved pregnancy rates which associated with the increase of antioxidant enzyme genes expression and decreased apoptotic index in bovine, blastocysts[27]. In addition, dietary cerium oxide as antibiotic at various concentrations significantly ($p<0.05$) improved the reproductive parameters in terms of embryo survival rate, litter size and weight as well as kid viability rate as compared controls[28]. Also, inclusion of extra vitamin E as antioxidant in the diet of does improved kid weaning rate[29,30]. Recently, growth of kids of rabbit does treated orally with Coenzyme Q10 or L-carnitine as antioxidants was significantly ($p<0.05$) higher than controls[31]. Generally, GTPs in GT have very strong antioxidant capacity, antibacterial and antiviral agents, enzyme-inhibitory and antiradiation and anticancerous effects[12].

Hematological parameters: Treatment of rabbit does with GTE at a level of 400 mg kg^{-1} LBW (G3) improved hematological parameters, but the effect was significant ($p<0.05$) only on hemoglobin level, hematocrit, MCV and WBCs as compared to control (G1). Reversely, G3 and G2 showed a significant ($p<0.05$) decrease in platelet count compared with G1. Meanwhile, RBCs, MCH, MCHC and RDW values showed no significant differences. Only hemoglobin concentration significantly ($p<0.05$) increased in G2 as compared to G1, but did not differ from that in G3 (Table 3).

Results of hematological parameters obtained in this study were within the normal range reported by several authors. In this respect, values of hemoglobin were inside the normal range (10.4-17.4 g dL^{-1}) for rabbits[32]. The obtained hematocrit values and RBCs count in this study are within a normal range of 30-50% and 5.46-7.94×10^{6} mm^{-3} as reported by Burns and De Lannoy Jr.[33] and Ahemen *et al.*[34], respectively.

The improvement in most hematological parameters after GTE treatment in this study might be related to the strong antioxidant effect of GTPs on hematopoietic cells. Hematopoietic cells appear to be particularly vulnerable in the presence of unchecked accumulation of ROS, because deficiencies in several ROS scavengers result in either anemia that is severe or even lethal in some cases and/or malignancies

Table 4: Effect of GTE treatment on lipid metabolites in blood plasma of rabbit does

		GTE level	
Parameters (mg dL^{-1})	G1 (Control)	G2 (200 mg kg^{-1})	G3 (400 mg kg^{-1})
Total lipids	360.00±5.77[a]	248.33±6.01[b]	170.00±11.55[c]
Total cholesterol	83.72±0.83[a]	79.43±0.47[b]	71.34±1.08[c]
Triglycerides	94.67±0.33[a]	85.00±1.73[b]	80.44±0.33[c]
High density lipoproteins	32.35±0.32[b]	35.30±0.30[a]	36.23±0.34[a]
Low density lipoprotein	32.43±0.64[a]	27.13±0.63[b]	19.02±1.35[c]

[a-c]Means within the same row having different superscripts are significantly different at $p \leq 0.05$

of hematopoietic tissues[35,36]. Also ROS have been implicated in the mechanism of damage of RBCs in diabetic patients[37]. As a result, hematological complications develop which consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets[38]. The normal hematocrit value is indicative of satisfactory nutritional condition of the rabbits[39], elevated RBCs count correlated with high quality food protein and the animal free disease[34] and RBCs index (MCH, MCV and MCHC) are essential morphological parameters of anemia[40]. Based on the present findings and previous results, rabbit does treated with GTE, in particular, at a level of 400 mg kg^{-1} LBW may improve health status.

Lipid metabolism: Treatment of rabbit does with GTE at both levels (G2 and G3) significantly ($p<0.05$) decreased lipid metabolites in terms of total lipids, total cholesterol, triglycerides and Low Density Lipoprotein (LDL) concentration in plasma of does as compared to control (G1). However, High Density Lipoprotein (HDL) concentration showed significantly ($p<0.05$) an opposite trend. It is of interest to note that increasing GTE treatment was more effective ($p<0.05$) on lipid metabolites at a level of 400 than 200 mg kg^{-1} LBW (Table 4).

In agreement with the present results, GT treatment significantly ($p<0.05$) decreased concentration of total lipids, cholesterol and triglycerides in growing rabbits[22] and rats[41]. Concentration of LDL significantly decreased ($p<0.05$), while HDL significantly ($p<0.05$) increased in growing rabbits treated with GT as compared to controls[22]. In laying hens, concentration of total lipids and total cholesterol significantly ($p<0.05$) decreased with dietary supplementation of GT leaves or aqueous GTE as compared to controls[18]. In growing Japanese quail, GTE addition to the diet significantly ($p<0.05$) decreased blood lipids fractions and increased HDL[42]. The moderate and high amounts of catechins reduced the postprandial triglycerides response in mildehypertriglyceridemic human subjects[43].

The effect of GTE on reducing blood lipids was explained by several authors. The mechanisms underlying the antiatherogenic effect of GTE may contribute to the strong antioxidant properties of its constituents (catechins) and their significant contribution to the total antioxidant capacity of blood plasma[44]. The catechins present in full GT bind to lipoproteins and possess antioxidant activities greater than α-tocopherol, effectively inhibiting lymphatic absorption of cholesterol and tocopherol from intestine in rats[45]. Crude catechin extract reduced cholesterol synthesis and increased LDL receptors which can both contribute to lowering plasma cholesterol concentrations[46]. Also, epigallocatechin in GT affect lipid metabolism by interfering with micellar solubilization of cholesterol in digestive tract, which in turn decrease cholesterol absorption[47]. Finally, Hasegawa *et al.*[48] observed that GTE inhibits lipogenesis in adipose tissues in rats.

Histological examination

Liver: Histological examination of liver in different experimental groups revealed that all groups showed normal architecture of hepatic lobule with intact central vein, portal area containing Glisson's capsule and hepatocytes arranged radially around the central hepatic vein of each lobule. This finding was observed in all groups, but some rabbit does in G2 treated with 200 mg kg^{-1} showed narrower central hepatic vein than in G1 and G3, while rabbit does in G3 showed more compacted hepatocytes than in G1 and G2 (Fig. 1).

Also, polyhedral hepatocytes having central, rounded, dark nuclei and vacuolated acidophilic cytoplasm were seen in radiated arrangement in all groups. The central hepatic vein was lined with flattened endothelial cells with wide lumen in G3 and lined with cuboidal cells with rounded nuclei, apical brush border and narrow lumen in G2 as compared to other groups. Moreover, blood sinusoids were more dilated with endothelial lining in G3 than in other groups. Also, few Von Kupffer cells were seen in between hepatocytes (Fig. 2).

The present examination indicated that histological structure of liver in treatment groups is similar or slightly improved as compared to control one. Such findings are agreement with that observed by Gad and Zaghloul[41] who found a significant improvement in liver of rat treated with GTE, in terms of hepatocytes with normal ultrastructural

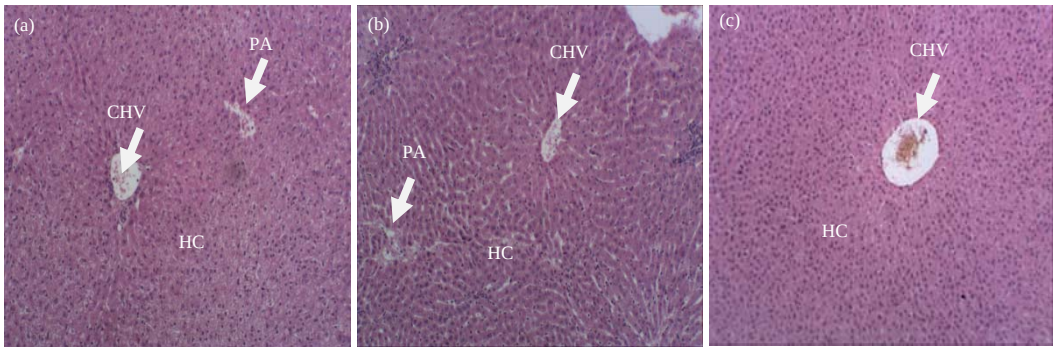

Fig. 1(a-c): Photomicrograph of the liver in (a) Control (G1), (b) 200 mg kg^{-1} (G2) and (c) 400 mg kg^{-1} (G3) rabbit does showing normal architecture of hepatic lobule with intact Central Hepatic Vein (CHV), Portal Area (PA) and arranged hepatocytes (HC) (H and E stain, x100)

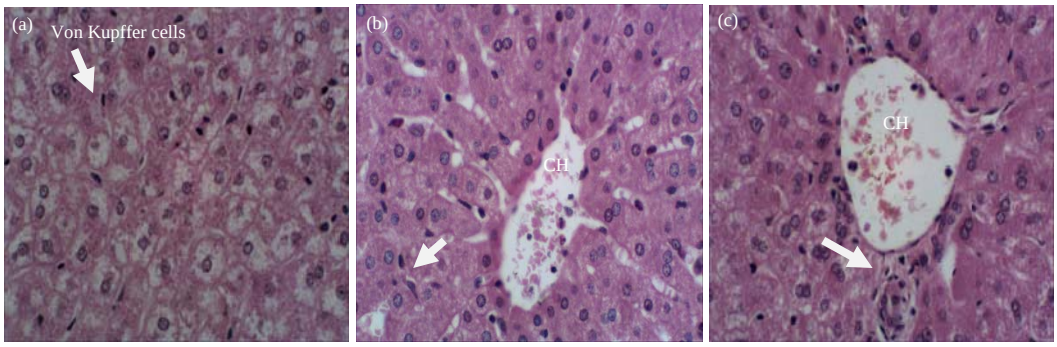

Fig. 2(a-c): A magnified section in the liver showing radiated arrangement around Central Hepatic Vein (CHV) of polyhedral hepatocytes having central, rounded, dark nuclei and vacuolated acidophilic cytoplasm. Also, few Von Kupffer cells (arrow) were seen in between hepatocytes (H and E stain, x400)

appearance. In this respect, some investigators suggested that GTE or EGCG treatment at high levels led to hepatotoxicity[49,50]. Also, others considered GT is safe in a wide range of doses, but hepatotoxicity was related to consumption of high dose of tea-based dietary supplements[51,52].

Using GTE at a level of 1.5% in rats led to hepatocyte vacuolar degeneration followed by destructed some hepatocytes, congestion of hepatic arterioles and blood sinusoids apparent intact well-developed active Kupffer cells and few fibrin deposits in the hepatic stroma, more glycogen deposition in the hepatocytes cytoplasm as compared to control[53]. In our study no pathological effects were observed in the histological structure of rabbit liver. The safe effect of using GTE at a level of 200 or 400 mg kg^{-1} in the present study may be attributed to low dose of GTE used, species differences in the experimental animals, body weight and type of feed or their interaction.

Kidney: Histological examination of kidney in different experimental groups revealed that all groups showed normal histological structure of the renal cortex with intact renal proximal tubules and renal corpuscles (Fig. 3).

The renal tubules are lined with cuboidal epithelial cells with rounded, central and dark nuclei, but the shape of renal tubules were elongated in G1 (control) and G2 (treated with 200 mg kg^{-1}) and was rounded in G3 treated with 400 mg kg^{-1}. Also, the lumen of the renal tubules was wider in G1 and G2 than in G3. In addition, renal corpuscles were intact in terms of normal Bowman's capsule, corpuscular space and glomerulosa in all groups (Fig. 4).

Similar observations were reported by Ortmann *et al.*[54] on kidneys of rats treated with GTE. Administration of GT catechins in diabetic animals drastically improved kidney function as a result of its anti-thrombogenicaction, which in turn controls the arachidonic acid cascade system[55]. The catechin in GT is clearly effective in reducing oxidative stress and inflammatory reactions in kidney tissue[56]. This may be interpreted as a result of the protective effects of GT in studied tissues and reduced the oxidative stress of alloxan that

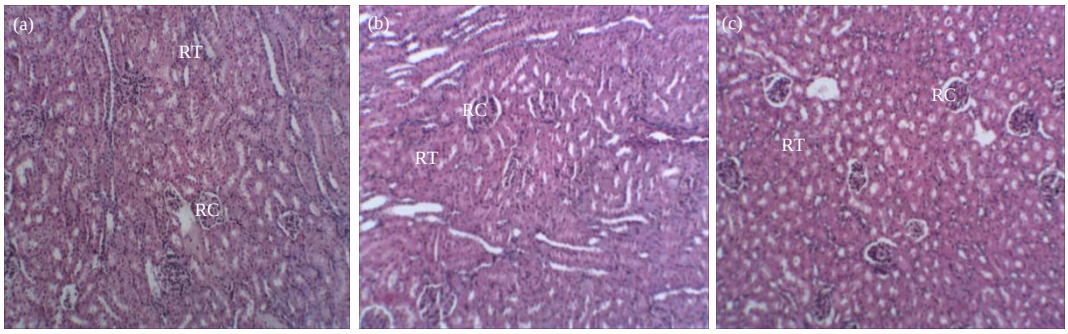

Fig. 3(a-c): Photomicrograph of the kidney of (a) Control (G1), (b) 200 mg kg^{-1} (G2) and (c) 400 mg kg^{-1} (G3) rabbit does showing normal histological structure of the renal cortex containing intact Renal Tubules (RT) and Renal Corpuscles (RC) (H and E stain, x100)

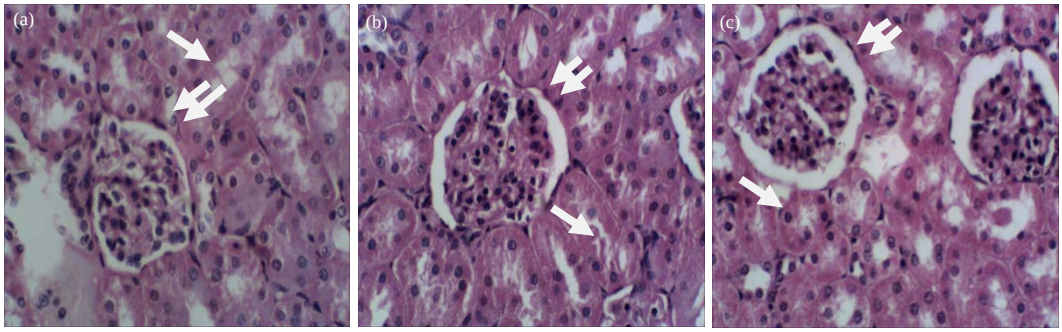

Fig. 4(a-c): A magnified section from kidney showing renal tubules (→) lined by cuboidal epithelial cells with rounded, central and dark nuclei. Also, intact renal corpuscles (⇉) with normal Bowman's capsule, corpuscular space and glomerulosa (H and E stain, x400)

causes the fatty degenerative and the aggregation of the inflammatory cells in these tissues.

The present findings on the histological structure of liver and kidney may indicate normal liver and kidney function of rabbit does treated with GTE. In this respect, GT catechins protect liver and kidney from lipid peroxidation injury[57]. Also, GTPs protects against alcohol induced liver, serum lipid peroxidation[58] and gentamicin induced oxidative stress in kidney[59].

CONCLUSION

Based on the foregoing results presented in this study, it was concluded that daily oral administration of rabbit does 30 days prior to insemination with GTE at levels of 200 or 400 mg kg^{-1} LBW is recommended to improve reproductive performance and lipid metabolism of rabbit does without adverse effects on hematological parameters and, liver and kidney functions.

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