Beneficial Effects of Green Tea Extract on Liver and Kidney Functions, Ultrastructure, Lipid Profile and Hematological Parameters in Aged Male Rats

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Abstract: Aging is characterized by a progressive decline in function and a decrease in the body’s ability to maintain homeostasis. This study aimed to examine the effect of green tea extract (GTE) on some parameters of liver and kidney functions and ultrastructure, serum lipid profile and hematological parameters in aged rats. A total of 20 Albino male rats of 24 months old were divided into two groups of 10 rats each. Rats in the first group were administered 300 mg/kg bwt. GTE daily for 14 weeks and the second group was kept as a control. Control group aged rats exhibited a significant decrease in liver and kidney functions, increase in serum lipids profile and decrease in hematological parameters. Treatment of aged rats with GTE caused a significant increase in levels of total protein, albumin, globulin, albumin/globulin (A/G) ratio, blood hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and platelets counts and level of liver and kidney reduced glutathione (GSH). Also, a significant decrease in the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and liver and kidney levels of lipid peroxidation product, malondialdehyde (MDA) and a highly significant decrease in levels of total lipids, total cholesterol and triglycerides compared to control rats. Electron microscopic examination of the liver and kidney in GTE treated rats revealed clear improvement of the hepatocytes' mitochondria and the rough endoplasmic reticulum compared to control aged rats. Also the bile canaliculi were not dilated as they were in control aged rats. While treatment of kidney with GTE did not induce the same degree of improvement in the ultrastructure as seen in liver but the glomerular capillaries returned normal without dilatation and the filtration slit membrane became intact again but large amount of collagen fibers were still associated with it. There was excessive blood infiltration between the tubules. In conclusion, GTE treatment was able to reverse the impairment in liver and kidney functions and architecture and reduced the negative impact on hematological parameters and serum lipid profile accompanied with aging in rats so it could be recommended as an anti-aging phytochemical for other animals.

Key words: Green Tea Extract • Aging • Ultra Structure • Liver • Kidney • Function

INTRODUCTION

Aging is simply an intrinsic biological process from which there is no way out. It is characterized by the degeneration of essential functions in the late or post-reproductive phase of all multicellular organisms [1,2]. Aging is the outcome of a balance between damage and repair and is probably related to a multi-factorial process [3,4] that causes loss of function and the inability to adequately respond to external stress [5]. The free radical theory of aging is one of the most popular, single mechanistic theories of aging, which discloses increased generation of free radicals as the major cause of cellular damage [6]. Oxidative stress, a condition of cellular pro-oxidant-antioxidant disturbance in favor of the pro-oxidant state, also induces the production of reactive oxygen species ROS, leading to serious functional impairments [7].

Although a modest level of reactive oxygen species (ROS) generated under physiological conditions participate in cell signal transduction cascades to regulate cell growth and differentiation[8], in contrast, severe ROS cause oxidative damage of cellular DNA, protein and lipids, resulting in the initiation or development of various diseases such as neurodegenerative diseases, cancer and type 2 diabetes mellitus [9,10].
There has been a global trend toward the use of natural phytochemicals present in natural sources as antioxidants and functional foods [5].

Tea is rich in polyphenols contained in the leaves and stems of the tea plant. Green tea polyphenols are the secondary metabolites in tea plants and accounts for 30% to 36% weight of the water extractable materials in tea leaves. The main polyphenolic components in green tea are epigallocatechingallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechingallate (ECG) [11]. EGCG, the major and most active component of green tea catechins, acts as an antioxidant in the biological system [12] and is rapidly absorbed and distributed mainly into the mucous membranes of the small intestine and the liver; more interestingly, it can cross the blood brain barrier [13].

The polyphenols in green tea can neutralize free radicals and may reduce or even help to prevent some of the damage caused by reactive oxygen species (ROS) [14]. Long-term intake of green tea catechins may be important because cells are constantly exposed to oxidative stress. It has been reported that, in addition to directly quenching reactive oxygen species, tea polyphenols have the ability to participate in vitamin E recycling [15]. Green tea extracts are more stable than pure epigallocatechingallate, (EGCG) one of the major antioxidants in the biological system of green tea, because of the presence of other antioxidant constituents in the extract [13].

The aim of the present study was to examine the anti-aging and protective potential of green tea extract on liver and kidney functions and ultrastructure, some hematological parameters and serum lipids profile in and ultrastructure, some hematological parameters and serum lipids profile in aged male rats.

**MATERIALS AND METHODS**

**Experimental Animals:** A total of 20 Albino male rats of 24 months old and weighing 275 - 300 g were used in this investigation. The rats were raised in the faculty of veterinary medicine, Alexandria University, Egypt and divided into two groups of 10 rats each. Rats of each group were housed in separate wire cages and maintained on a standard laboratory diet (16.3% crude protein, 6.8% fat and 3.8% crude fiber) according to [16].

**Green Tea Extract Administration:** Rats in the first group received 300 mg/kg bwt. green tea extract (GTE) [Multi-treat Arab Co. for Pharmaceutical & Medicinal plants (MEPACO- MEDIFOOD) Enahas El Ramia- Sharkeya- Egypt, each tablet contains 300 mg green tea dry extract, (30% polyphenols)] in 1 ml distilled water/ rat by gavages daily for 14 weeks. The second group was considered as a control and received 1 ml distilled water / rat in the same administration regimen. In our present study we chose to use a moderate dose of green tea extract (GTE) to avoid adverse effects of GTE on many body organs, as there were evidence of deleterious effects of high doses of GTE including treatment-related mortality occurred in male and female mice receiving 1000 mg/kg bwt. treatment dose which was likely related to liver necrosis, while using doses not exceeding 500 mg/kg bwt. showed no adverse effects in males and females of both species sexes[17]. Humane care for rats was provided according to the guidelines of the National Institutes of Health (NIH) of animal Care and the local committee approved this study. All animals survived till the end of the experiment.

**Blood Collection:** After one day from the end of treatment, individual blood samples were collected via retro-orbital bleeding under light ether anesthesia. Two blood samples from each animal were collected one sample was collected on EDTA for determination of hematological parameters and the other was left to clot for one hour at 37°C and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was collected and stored at -20°C for biochemical analysis.

**Tissue Preparation:** Rats were sacrificed by cervical dislocation under light ether anesthesia, abdomens were dissected and liver and kidneys were excised rapidly, kidneys were trimmed of fatty tissue, tissues were washed in ice cold phosphate buffer saline (PBS) solution pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots, blotted with filter paper and weighed. Part of liver tissues and alternating left and right kidneys were then homogenized in 5 ml cold buffer containing. 50 mM potassium phosphate, 1mM EDTA, pH 7.5 per gram tissue using tissue homogenizer (MSE, Voltage 200-250, AMPSI, Cycles 50, England). The resulting homogenate was centrifuged at 4000 rpm at 4°C for 15 minutes. The supernatant was collected, stored at -80°C and then used for estimation of the levels of malondialdehyde (as an indicator for lipid peroxidation) and reduced glutathione as an indicator of antioxidant activity.

**Histological Examination**

**Transmission Electron Microscopy:** Pieces of 1 mm were cut from fresh livers and kidneys of each animal in the two animal groups then immediately fixed in 6 % solution of...
phosphate buffered gluteraldehyde, pH 7.4 at 4 °C, serially washed in cold (4°C) 0.1 M phosphate buffer, post fixed in 1% solution of osmium tetroxide, processed and then embedded in epoxidearaldite[18].

Semithin sections (1µm) were cut and stained with toluidine blue then ultra-thin sections (60-100 nm) were cut and stained with uranyl acetate followed by lead acetate [18]. The sections were examined and photographed with Joel transmission electron microscope working at 80 KVs.

**RESULTS**

Effect of green tea extract treatment on liver and kidney functions, serum lipid profile and liver and kidney lipid peroxidation (MDA) and GSH contents:

The findings of the present investigation reveals a marked improvement in liver and kidney functions after green tea extract treatment in aged rats. Total protein (mg/dl), albumin (mg/dl) and A/G ratio increased significantly (p<0.05) in green tea group (7.32±0.16; 3.75±0.15 and 1.13±0.10, respectively) compared to their control values (6.49±0.17; 3.14±0.15 and 0.93±0.07, respectively). Also green tea treatment caused a highly significant (p<0.001) decrease in AST, ALT and ALP activities (U/L) and BUN levels (mg/dl) (23.20±2.82; 28.45±2.53; 60.23±3.836 and 39.09±1.406, respectively) compared to the control values (56.00±7.62; 73.50±3.99; 87.90±2.76 and 56.66±1.43, respectively), in addition to a significant (p<0.05) decrease in serum creatinine (mg/dl) (1.73±0.14) compared to its level in control aged rats (2.28±0.33) [Table 1]. The most pronounced observation was a highly significant (p<0.001) decrease in serum levels of total lipids (mg/dl) (299.37±17.96) and triglycerides (mg/dl) (119.97±4.48) in green tea group compared to control values (496.05±17.96) and (172.8±4.35), respectively [Table 2] and a significant (p<0.05) decrease in total cholesterol (160.86±8.40 mg/dl) compared to its corresponding value in control aged rats (212.58±9.87 mg/dl) [Table 2], the obvious improvement in liver and kidney function parameters was accompanied by a significant (p< 0.05) decrease in liver and a highly significant (P<0.001) decrease in kidney lipid peroxidation product (LPO) malondialdehyde (MDA), (29.60±1.02 and 5.89±1.00nmol/g, respectively) as compared to control (39.13±2.08 and 17.79±1.15 nmol/g, respectively). While there was a significant (p<0.05) increase in liver and kidney GSH(3.73±0.46 and 1.51±0.18mmol/g ) compared to control ( 2.64±0.54 and 0.86±0.13mmol/g ), respectively [Table 3].

**Statistical Analysis:** Data were analyzed using independent t-test by the aid of SAS [28] software for control and treated groups.

**Estimation of Serum and Tissues Malondialdehyde and Reduced glutathione:** Levels of malondialdehyde and reduced glutathione were determined in liver and kidney homogenates. The level of malondialdehyde was measured based on the formation of thiobarbituric acid-reactive substances (TBARS) when malondialdehyde reacts with thiobarbituric acid. The absorbance of the resultant pink product was measured spectrophotometrically at 534 nm according to the method of [26]. The level of reduced glutathione was measured based on the reduction of 5,5” dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione. The absorbance of the resultant yellow product was measured spectrophotometrically at 405 nm according to the method of [27].

**Hematological Estimation:** Blood hemoglobin (Hb), hematocrit% (PCV%), red blood corpuscles (RBCs), white blood cells(WBCs) and platelets counts and mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were estimated automatically using a 17 parameter, 3 part leukocyte differential veterinary hematology analyzer with international standards, EN591:2001, EN 61326 (1997) (Boule medical for multispecies veterinary applications, Stockholm, Sweden).
Table 1: Effect of Green Tea Extract administration (300 mg/kg bwt. daily for 14 week) on liver and kidney functions in aged male rats (serum Total protein, Albumen, Globulin, A/G ratio, (AST), (ALT) ALP, BUN and creatinine)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Green tea extract group</th>
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<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>6.49±0.17</td>
<td>7.32±0.16*</td>
</tr>
<tr>
<td>Albumen (g/dl)</td>
<td>3.14±0.15</td>
<td>3.75±0.15*</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.35±0.13</td>
<td>3.57±0.22</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.93±0.07</td>
<td>1.13±0.10*</td>
</tr>
<tr>
<td>AST(U/l)</td>
<td>56.00±7.62</td>
<td>23.20±2.82**</td>
</tr>
<tr>
<td>ALT(U/l)</td>
<td>73.50±3.99</td>
<td>28.45±2.53**</td>
</tr>
<tr>
<td>ALP(IU/l)</td>
<td>87.90±2.76</td>
<td>60.23±3.836**</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>56.66±1.43</td>
<td>39.09±1.406**</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.28±0.33</td>
<td>1.73±0.14*</td>
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All values are means±SE, n=10
Values with * at the same raw are significantly different at (p<0.05)
Values with ** at the same raw are significantly different at (P<0.001)

Table 2: Effect of Green Tea Extract administration(300 mg/kg bwt. daily for 14 week) on liver and kidney malondioldhyde (MDA) and reduced glutathione (GSH) levels

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Green tea extract group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney MDA (nmol/g)</td>
<td>17.79±1.51</td>
<td>5.89±1.00**</td>
</tr>
<tr>
<td>Liver MDA (nmol/g)</td>
<td>39.13±2.08</td>
<td>29.60±1.02*</td>
</tr>
<tr>
<td>Liver GSH (mmol/g)</td>
<td>2.64±0.50</td>
<td>3.73±0.46*</td>
</tr>
<tr>
<td>Kidney GSH (mmol/g)</td>
<td>0.86±0.13</td>
<td>1.51±0.18*</td>
</tr>
</tbody>
</table>

All values are means±SE, n=10
Values with * at the same raw are significantly different at (p<0.05)
Values with ** at the same raw are significantly different at (P<0.001)

Table 3: Effect of Green Tea Extract administration (300 mg/kg bwt. daily for 14 week) on serum levels of total lipids, total cholesterol and triglycerides in aged rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Green tea extract group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids (mg/dl)</td>
<td>496.05±17.96</td>
<td>299.37±19.78**</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>212.58±9.87</td>
<td>160.86±4.40*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>172.8±4.35</td>
<td>119.97±4.48**</td>
</tr>
</tbody>
</table>

All values are means±SE, n=10
Values with * at the same raw are significantly different at (p<0.05)
Values with ** at the same raw are significantly different at (P<0.001)

Table 4: Effect of Green Tea Extract administration (300 mg/kg bwt. daily for 14 week) on some hematological parameters in aged rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Green tea Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (x10⁹/cmm)</td>
<td>7.16±0.30</td>
<td>8.60±0.31*</td>
</tr>
<tr>
<td>WBCs (x10⁹/cmm)</td>
<td>7.56±0.17</td>
<td>8.76±0.32*</td>
</tr>
<tr>
<td>PCV %</td>
<td>38.09±0.35</td>
<td>41.48±0.63*</td>
</tr>
<tr>
<td>Platelets (x10⁹/cmm)</td>
<td>1084.40±9.38</td>
<td>1150.90±29.52*</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td>13.16±0.18</td>
<td>14.07±0.14*</td>
</tr>
<tr>
<td>MCV µ</td>
<td>41.62±0.41</td>
<td>42.05±0.45</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>14.84±0.15</td>
<td>14.64±0.28</td>
</tr>
<tr>
<td>MCHC %</td>
<td>36.32±0.35</td>
<td>35.40±0.16</td>
</tr>
</tbody>
</table>

All values are means±SE, n=10
Values with * at the same raw are significantly different at (p<0.05)

Histological Results: Transmission electron microscopy of the liver in the control aged group revealed hepatocytes with clear nuclei (Fig.1) which were sometimes binucleated (Fig.2) although some nuclei were pyknotic. The chromatin was moderate to dense in electron density with clear nucleolus in some cells, nuclear envelops were clear showing the nuclear pores. Mitochondria were elongated with circular cross sections, but sometimes the crestae were not recognized. Large twisted mitochondria could be observed revealing crestae in different directions and those mitochondria were very electron dense (Fig. 3). The rough endoplasmic reticulum was obviously dilated in all sites inside the cytoplasm of hepatocytes (Fig.1). The cytoplasm was dispersed and contained cytoplasmic vacuoles with different sizes surrounded by a membrane. Complete loss or absence of cytoplasm could be seen in some cells. The cell membrane of some hepatocytes were liquefied and dissolved, losing the trilaminar appearance (Fig.4). Desmosomes were still

Fig. 1: Transmission electron micrograph of the liver of control group rats showing a hepatocytes with dark spherical nucleus (N), electron dense mitochondria (m) and dilated cistenae of rough endoplasmic reticulum (rER). X5000.
Fig. 2: Transmission electron micrograph of the liver of control group rats showing binucleated (N1 & N2) hepatocytes, mitochondria (m), cytoplasmic vacuoles (v) inside the dispersed cytoplasm. Note the bile canaliculus (arrow). X 3000.

Fig. 3: Transmission electron micrograph of the liver of control group rats showing large dark twisted mitochondria (m) with long crestae, dilated rough endoplasmic reticulum (rER) and cytoplasmic vacuoles (v). X 10000.

Fig. 4: Transmission electron micrograph of the liver of control group rats showing a hepatocytes nucleus (N) and electron dense mitochondria (m). Note the liquefied cell membrane (arrows). X 2500.

Fig. 5: Transmission electron micrograph of the liver of control group rats showing desmosomes (arrow heads) between hepatocytes and bile canaliculus (arrow) with a cut microvilli. Dispersed cytoplasm (c) still has mitochondria (m). X 5000.

No stored glycogen could be detected in the hepatocytes. Von Kupffer cells were found beside the blood vessels and usually coarse and thick collagen fibers were associated to them (Fig. 6).
Transmission electron microscopical examination of the control group kidneys revealed podocytes with many foot processes (pedicles) around the blood capillaries (Fig.11), some of these pedicles were cut or not firmly attached to the basal lamina of the blood capillaries. Some podocytes had pyknotic nuclei and vesicular cytoplasm. The filtration slit membrane was missed or not clear at some sites. The glomerular basement membranes were thick and had some small dense granules. Glomerular capillaries were widely dilated and contained cell debris (Fig.11). Erythrocytes and blood platelets were seen with large number inside the glomerular capillaries.
Fig. 9: Transmission electron micrograph of the liver of green tea treated rats showing intact cell membrane of hepatocytes with desmosomes (arrow head) between two neighboring hepatocytes and bile canaliculus (arrow). Nucleus of one hepatocytes (N), mitochondria (m) and rough endoplasmic reticulum (rER). X 4000.

The proximal convoluted tubules of the kidneys of control group aged rats showed cells with a dark cytoplasm. The lining epithelial cells were apart from each other and some of them contained pyknotic nuclei.

Fig. 10: Transmission electron micrograph of the liver of green tea treated rats showing Von Kupffer cell (V) beside a blood vessel containing red blood cells (RBCs). X 3000.

Fig. 11: Transmission electron micrograph of the glomerulus of kidney of control group rats showing a wide longitudinal glomerular capillary (Ca) and pedicles (arrows) of podocytes. X 3000.

Fig. 12: Transmission electron micrograph of the proximal convoluted tubule of kidney of control group rats showing microvilli (mv) in the lumen with no arrangement, pyknotic nucleus (N) and sloughed cell (sc) in the lumen of the tubule. X 2000.

The mitochondria were dark (electron dense), many lysosomes could be seen inside the cells and the luminal microvilli were not clear and lost their arrangement (Fig.12). The cells lied on a thick basal laminae with dense granules. The basal portion of the lining cells had many vesicles and abnormal shaped...
Fig. 13: Transmission electron micrograph of the distal convoluted tubule of kidney of control group rats showing three lining cells with their spherical to oval nuclei (N), basal cell membrane infoldings carrying elongated mitochondria (m), some cytoplasmic vacuoles (v) and somewhat thin basal lamina (arrows). X 2000.

Fig. 14: Transmission electron micrograph of the glomerulus of kidney of green tea treated group rats showing parts of pedicles of podocytes (arrows), part of podocyte nucleus (N), blood (RBCs) inside the capillaries and collagen fibers (Co). X 4000.

mitochondria with strange crestae and some lysosomes. Sloughed cells might be seen inside the lumen of the tubule (Fig.12).

Fig. 15: Transmission electron micrograph of the proximal convoluted tubule of kidney of green tea treated group rats showing oval nucleus (N) of the lining epithelial cell, elongated mitochondria (m) and the basal lamina (BL). X 4000.

Fig. 16: Transmission electron micrograph of the distal convoluted tubule of kidney of green tea treated group rats showing one lining epithelial cell treated with spherical nucleus (N) resting on a basal lamina (BL), mitochondria (m), lumen of the tubule (L) and the junction between the cell and its neighbor cell (arrow). X 2500.

The lining epithelium of the distal convoluted tubules looked more normal as the cell nuclei were normally spherical in a cuboidal cells, mitochondria were good and primary and secondary lysosomes could be
noticed (Fig.13). Basal infoldings of the cells with mitochondria were seen. There were some vacuoles in the basal portion of the cells. The basement membranes were thin and sometimes lost and thick collagen fibers could be seen in these lost areas (Fig.13).

Electron microscopy of the kidneys of green tea extract treated group rats showed some improvement. Pedicles of podocytes were intact (Fig.14) and no vesicular cytoplasm could be detected. Glomerular blood capillaries were not dilated as in the control (aged) group rats. The filtration slit membrane is intact. But collagen fibers were found with large amount (Fig.14). Revealing to the proximal convoluted tubules (Fig.15), the lumena were occluded and not clear, also microvilli still losing their arrangement. The distal convoluted tubules showed normal cuboidal epithelial lining with basal infoldings carrying mitochondria and clear junctions between cells (Fig.16). There were excessive blood infiltrations between the tubules.

**DISCUSSION**

The cellular distribution and bioavailability of key antioxidants have become altered with age. A shift in the Oxidant: antioxidant balance because of increased production of free radicals is observed during aging.[29,30].

In the present investigation administration of green tea extract caused a significant increase in the levels of serum total proteins, albumin, insignificant increase in globulin and A/G ratio, significant decrease in serum AST, ALT and ALP activities and BUN and creatinine as shown in table [1] compared to their values in control aged rats. The significant decrease in total protein and albumin with aging indicates compromised liver excretory function and impairment of the liver synthetic function,[5]. Moreover AST, ALT and ALP enzymes may be released into blood plasma and serum, levels of these enzymes may increase due to cellular damage in the liver. The increase of the activities of liver enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as mentioned by [31] and this is coincident with our ultrastructural results in the aged control group, where the hepatocytes lost their limiting membranes at many sites and there were a complete loss of cytoplasm with a loss of the trilaminar appearance of the cell membranes and loss of cell junctions in those sites.

BUN and serum creatinine levels rise is an indicator of renal failure, where they are not adequately excreted by the kidney[32,33], and this agreed with the ultrastructure of the control group kidneys where there were a loss of contact of podocytes pedicles with the glomerular basement membrane leading to inadequate blood filtration due to complete loss of the filtration slit membrane in some sites. Thick glomerular basement membrane with dense granules and presence of many cell debris in the dilated glomerular capillaries also indicates in adequate filtration.

In our present investigation the impairment in hepatic and renal function parameters in control aged rats may be contributed to one of the results of oxidative stress associated with aging. That reduced hepatic and renal functions. Ample evidence showed that peroxidative damage to lipid and protein occurs with the aging process and that the products of these reactions accumulate with age[34]. One of the findings in our present study which favor the role of oxidative damage in compromising the physiological functions of liver and kidney is that aging in rats caused a significant elevation in liver and kidney lipid peroxidation product malondialdehyde (MDA) and a significant reduction in liver and kidney reduced glutathione (GSH). Lipid peroxidation is widely accepted as a sign of oxidative stress,[35].

In human and animal body, ROS can be neutralized by antioxidant defense systems including antioxidant enzymes [36] and antioxidant compounds [37]. Many investigators reported that the reduction of GSH levels leads to elevation of lipid peroxidation (LPO)[38]. The results of the present study were coincident with the histological findings where, the liver ultrastructure of the aged rats exhibited hepatocytes with pyknotic nuclei, abnormal mitochondria, dilated cisternae of rough endoplasmic reticulum and a dispersed cytoplasm with many cytoplasmic vacuoles and sometimes complete loss of cytoplasm with a complete absence of stored glycogen and these results agreed with [39] where aging enhances apoptosis of hepatocytes under normal physiological conditions and with [40] where he found dilated and tortuous bile canaliculi in liver-specific β-catenin knockout mice along with decreased canalicular and sinusoidal microvilli. Knockout mice on cholic acid diet had higher hepatic and serum bile acid levels, bile ductular reaction, increased pericellular fibrosis and dilated, misshapen bile canaliculi. This occurred in the present study where, bile canaliculi were dilated in aged rats with swollen and sloughed microvilli.
During aging, mitochondria decay, rates of oxidant production increase and oxidative damage to important biomolecules increases and may in part be responsible for aging as well as age–associated degenerative diseases[29,30],which agree with the present findings where the mitochondria of aged hepatocytes were electron dense, with unrecognized cristae sometimes and large twisted mitochondria in other sites because the main site of oxygen and hydrogen peroxide production in eukaryotic cells is the electron transport chain in the mitochondria [41,42]. Also age is associated with a decrease in mitochondrial membrane protein (30%) and an increase in mitochondrial size and in mitochondrial-peroxisome generation (23%), the intracellular peroxide levels were also increased [43] who also reported a damage of the mitochondrial crests and other structure and increased in mitochondrial size in old aged rats compared to young rats.

In healthy livers, hepatocytes produce small amounts of reactive oxygen species (ROS) and Kupffer cells, the resident macrophages in the liver, release ROS in response to bacterial stimuli. Hepatic stellate cells contain a non-phagocytic form of NADPH oxidase. This form is constitutively active, producing relatively low levels of ROS under basal conditions. ROS is also involved in the pathophysiology of inflammatory liver diseases [44]. In our present study Kupffer cells were mainly accompanied by coarse collagen fibers in liver of aged rats indicating a degenerative changes. On the other hand, green tea intake caused a significant reduction in liver and kidney malondialdehyde (MDA) a finding consistent with[45] and a significant increase in liver and kidney reduced glutathione which agree with the findings of[46] in addition to the previously mentioned improvement in hepatic and renal function parameters compared to their values in control aged rats which may be contributed to the powerful antioxidant capacity[47,48] and anti-inflammatory effect of green tea polyphenols[49].

The histological examination of the liver of green tea extract treated aged rats revealed a significant improvement especially in liver where the mitochondria of the hepatocytes exhibited normal ultrastructural appearance, no dilation of the cisternae of rough endoplasmic reticulum and lower number of cytoplasmic vacuoles were present. The cell membranes were not liquefied and stored glycogen was found in nearly all hepatocytes examined. Bile canaliculi were not dilated and with normally arranged microvilli. There were no thick collagen associated with Von Kupffer cells. This indicates the improvement in liver ultrastructure caused by the green tea extract and so improving their functions.

While in kidneys treated with green tea extract the pedicles of podocytes were intact and also the filtration slit membrane. No vesicular cytoplasm of podocytes and these results coincident with the suggestion that age-dependent glomerulosclerosis is not merely a “degenerative but a reversible process locally confined to the glomerulus in volving recovery of podocytes from previous injury [50]. But nearly no improvement of the proximal convoluted tubules where the lumens were occluded and the microvilli still with lost arrangement.

Green tea catechins protect the brain, liver and kidney from lipid peroxidation injury[51]. Moreover Green tea polyphenoles protects against alcohol induced liver and serum lipid peroxidation[52] and gentamicin induced oxidative stress in kidney[53,54] in a rat model.

It has been reported that, in addition to directly quenching reactive oxygen species, tea polyphenols have the ability to participate in vitamin E recycling [15] and thus complementing some of the functions of glutathione(GSH). [46] have reported that green tea by scavenging the free radicals directly in rats may reduce the utilization of GSH and thereby exhibiting an increase in the GSH content in diabetic rats treated with green tea extract which offers a good explanation of the significant increase in the GSH levels in green tea treated aged rats compared to that of non-treated aged rats.

Serum levels of total lipids, cholesterol and triglycerides, table [3] showed a significant (p<0.001) decrease in green tea extract administered aged rats compared to their levels in controls which agree with the previous findings of[55] who reported that rats fed green tea catechins showed a decrease in cholesterol and triacyloglycerol levels in blood and their non-toxicity in relation to liver and kidneys.

Green tea or its catechins lower the blood levels of cholesterol (CH) in CH-fed male rats [56], mice [57] and hamsters [58] and retard the development or progression of atherosclerosis in apolipoprotein E–deficient mice [59] and hypercholesterolemic hamsters [58]. These studies, along with the epidemiologic finding of an inverse association between coronary heart disease(CHD) risk and green tea (GT) consumption in humans [60] strongly suggest that GT and its constituents may be used as an effective means of lowering blood CH levels and hence reducing the risk of CHD. Also, [61] reported that Moderate and high amounts of catechins
reduced the postprandial triglycerides response in 9 mildehypertriglyceridemic human subjects. The mechanisms underlying the antiatherogenic effect of GT, may contribute to the strong antioxidant properties of its constituents (catechins) and their significant contribution to the total antioxidant capacity of blood plasma [62], where Numerous studies have shown that catechins bind to lipoproteins and possess antioxidant activities greater than alpha-tocopherol (_TP), effectively inhibiting LDL oxidation and lipid peroxidation in vitro [63,64]. Also the decrease in total lipids, cholesterol and triglycerides might be related to a decrease in their lymphatic absorption or their synthesis in GTE treated rats.A previous study reported that green tea extract administration inhibited the lymphatic absorption of cholesterol and tocopherol from intestine in ovariectomized rat [65] and in another study [66] reported that crude catechin extract reduced cholesterol synthesis and increased the LDL receptor which can both contribute to lowering plasma cholesterol concentrations.

Green tea extract administration caused a significant (p<0.05) increase in RBCs, WBCs and platelets count, PCV% and Hb g%, meanwhile, MCV, MCH and MCHC% values showed no statistical difference from control aged group, the improvement in hematological parameters after green tea intake might be related to the strong antioxidant effect of green tea extract catechins 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 of hematopoietic tissues [67, 68]. One of the more notable age-related changes is hematopoietic and specifically immunological decline [69-72]. Decreased immune function is not compartmentalized; reduced immune cell function (and in some cases cell numbers) has been observed in both the myeloid and lymphoid lineages[69,72]. Furthermore, recent studies have indicated that these functional reductions result at least in part from aging-associated defects in hematopoietic stem cell (HSC) function, which are transferred to the their lineage-committed progeny[69,72]. The causes of the aging-associated decline in HSC and hematopoietic cell function are still open for debate [73]. It has been suggested that Red and white blood cells counts decrease in diabetics than in non-diabetic people[74]. Reactive oxygen species, have been implicated in the mechanism of damage of red blood cells in diabetic patients[75].As a result, haematological complications develop which consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets[76,77].

**CONCLUSION**

The present study elucidated the anti-aging and beneficial effects of green tea extract evident by improvement of hepatic, renal and hematological parameters and serum lipid profile as well as the ultrastructure of the liver and kidney cells. Interestingly the positive effects of green tea extract were more pronounced on the liver than on kidney ultrastructure. So, our present work recommends the usage of green tea extract to overcome the abnormal changes in body functions accompanied with aging in many animal species especially pet animals where most pets do not have a massive life span and readily suffer from aging problems.

**REFERENCES**


