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Anti-Atherogenic Effect of Green Tea

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ABSTRACT

Cardiovascular disease is one of the major causes of death worldwide. Diet is the most editable factor in the prevention of this disease. Green tea beverage (GTB) as a water extract was prepared and biologically evaluated for its anti-atherogenic potential in Wister rats. GTB prepared in the present study showed high antioxidant activity as obtained in radical scavenging activity (86%), Ferric reducing antioxidant power (2.566) and reducing power (1.687). GTB had high total phenolics (13.252 mg GAE/ml) and flavonoides (0.525mg QE/ml). In GTB-group (G3), administration of GTB led to improve serum lipid profile and oxidative stress parameters compared to the other two groups (atherogenic control group (G2) and negative control group (G1)). The histopathological investigation of the aorta, heart and liver assured these results which reflected the preventative impact role of GTB against atherosclerosis and cardiovascular diseases.

Keywords: Green tea; Antioxidant activity; Anti-atherogenic.

INTRODUCTION

Tea is consumed worldwide and is the second drink after water. Consequently, the health impacts of consuming tea have a significant leverage on public health. Tea has been consumed for more than 2000 years and is currently consumed in most parts of the world. Green tea (*Camellia sinensis*) contains many bioactive components, which play a role in preventing and lowering the hazard of many diseases, for example liver disease, obesity, cancers, vascular disease and diabetes (Higdon and Frei 2003; Li *et al.*, 2013; Wang *et al.*, 2014; Li *et al.*, 2014; Grosso *et al.*, 2017; Xu *et al.*, 2019). Green tea has antioxidant ability and a greater level of polyphenols compared to black tea, so green tea has positive effects on human health (Koo and Cho, 2004; Anuevo and Nomura, 2014), and considered as a source of dietary bioactive components such as (-)-flavan-3-ols including (-)-epigallocatechin gallate, epicatechin, (-)-epigallocatechin and (-)-epicatechin gallate or (-)-catechins (Budryn *et al.*, 2013; Zielinski *et al.*, 2015). The preparation of tea using hot water is better than cold water as it scavenging oxidative radicals (Lin *et al.*, 2010), may be due increased extraction of polyphenols. Bioactive components of green tea are known for their role in preventing vascular disease, through decrease of blood lipids, lower oxidative stress, mend ischemia , mitigate inflammation, preserve myocardial function and encourage endothelial function (Hodgson and Croft 2010; Xu *et al.*, 2016).

Many studies using animal models refer to catechins found in green tea demonstrated several benefits such as protection against degenerative diseases, from the oxidative stress (Pervin *et al.*, 2018), neurological damage, liver toxicity (Albokhadaim, 2016), and breast cancer (Yu *et al.*, 2019). Moreover, anti-proliferative activity on liver cells and hypolipidemia, anti-pain factors and immunity enhancement were also stated (Ganeshpurkara and Salujaa 2018).

Cardiovascular disease is a global epidemic that considered as one of the important leading causes of death worldwide and is responsible for 17.9 million deaths in 2016 (about 31% of all global deaths). In general, cardiovascular diseases could be resulted from several factors such as harmful use of alcohol, smoking, physical inactivity and fast food. Diet is the most editable factor in the prevention of cardiovascular disease. Up to a quarter of these diseases can be avoided through follow effective primary and secondary prevention strategies (Yusuf *et al.*, 2001 and Benjamin *et al.*, 2019).

Atherosclerosis is one of the main cardiovascular diseases that occur due to changing lifestyles and eating patterns. A diet rich in cholesterol leads to hypercholesterolaemic and the occurrence of atherosclerosis as a result of fat deposition in the artery wall, increased inflammatory cells and increased oxidative stress in many tissues. Oxidative stress is one of the factors linking high cholesterol to atherosclerosis. Increased low-density lipoprotein (LDL) cholesterol levels and low high-density lipoprotein (HDL) cholesterol levels are effective risk factors for atherosclerosis (Benjamin *et al.*, 2019).

The current study was intended to evaluate the anti-atherogenic impact of green tea beverage consumption on the histopathological changes and lipid profile in rats fed high fat diet. The antioxidant contents and activity of green tea beverage was also investigated.

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MATERIALS AND METHODS

Materials

The green tea (*Camellia sinensis*) was purchased from the local market in Changsha province, China. All chemicals were purchased from Sigma-Aldrich, Germany. The diagnostic biochemical kits were obtained from Bio Diagnostic Company, Al-Dokki, Giza, Egypt. Male albino rats of the Wistar strain were obtained from Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt).

The preparation of green tea

The green tea beverage (GTB) was prepared using the ideal conditions that ensure the highest antioxidant activity as demonstrated by Bakr *et al.,* (2018). One gram of green tea powder was weighted in beaker and then 100 ml hot water was added at 88.7 °C. Beaker content was leaved until take the room temperature and filtrated on Whatman filter paper. The GTB was stored at -70º C until further assay.

Total phenols content

Spectrophotometer using the modified Folin-Ciocalteau colorimetric method was used to estimate total phenols content (Eberhardt *et al.,* 2000). The total phenols content was expressed as a milligrams Gallic acid equivalent/ ml extract (mg GAE/ ml) by reference to the gallic acid standard calibration curve.

Total flavonoids content

Total flavonoids content in the beverage was determine spectrophotometrically by Aluminum chloride complex forming assay (Piyanete *et al.,* 2009). The total flavonoids content was expressed as a milligrams quercetin equivalent/ml extract (mg QE/ ml) by reference to the quercetin standard calibration curve.

DPPH˙ radical scavenging activity

Spectrophotometric method was used to test the Radical-scavenging activity of the prepared green tea beverage reported by (Brand-Williams *et al.,* 1995).

Ferric reducing antioxidant power

Ferric Reducing Antioxidant Power (FRAP) was estimated by the method of Benzie and Strain (1996).

Reducing power

The reducing power of prepared green tea beverage was estimated according to the method reported by Oyaizu, (1986).

Animals experiment and diets

GTB was biologically evaluated for its anti-atherogenic effect in male rats as model experimental animals. Twenty four adult male albino rats of the Wistar strain were housed in screen-bottomed aluminum cages in room maintained at 25 ± 1°C with alternating cycles of light and dark of 12h. For adaption, the rats were fed on the normal control diet for 7 days. Rats were randomly divided into three groups, each of eight rats, the first group was negative control (G1) fed on the normal control diet, was consists of corn starch (60%) casein (20%) corn oil (10%) cellulose (5%) salt mixture (3.5%) vitamin mixture (1%). The second was atherogenic control (G2) group fed on atherogenic diet, was consists of corn starch (59.2%) casein (20%) cellulose (5%) salt mixture (3.5%) vitamin mixture (1%) cholic acid (0.3%) cholesterol (1%) lard (10%), Salt and vitamin mixtures were presented in Table (1) according to AIN-93 guidelines (Reeves *et al.,* 1993). And the third was green tea group (G3) fed on atherogenic diet and given orally green tea in a dose of 1 mL/100 g body weight per day for successive 60 days. The calculation was based on a consumption of 275 mL/day for a 70 kg human as reported by Rouanet *et al.,* (2010). Blood samples also obtained from the retro-orbital plexus of the eyes from all animals of each group on 0, 30and 60 days according to the procedure of Schermer (1967). After the end of the experiment, the rats were slaughtered and organs were excised, specimen for aorta, heart and liver were obtained and preserved in formaldehyde (10%) for the histopathological examination. Serum was separated and the serum biochemical analyses were carried out.

Table 1. Compositions of the Salt mixture (g) and vitamins of basil diet.

Salt mixture		Vitamins mixture	
Salt	**Wight (g)**	**Vitamin**	**Wight**
$CaCO_3$	304.5	Vit. A	2000IU
KH_2PO_4	327.5	Vit. D	200IU
$CaHPO_4.2H_2O$	60.0	Methionine	0.5mg
$MgSO_4.7H_2O$	103.5	Inositol	10 mg
NaCl	170.0	Niacin	4 mg
$Fe(C_6H5O_7).6H_2O$	28.0	Ca-pa ntothenate	4 mg
KI	0.81	Riboflavin	0.8 mg
$MnSO_4$	5.12	Thiamine	0.5 mg
$ZnCl_2$	0.25	Pyridoxine	0.5 mg
$CuSO_4.5H_2O$	0.31	Folic acid	0.2 mg
		Cholic acid	0.2 g
		Biotin	0.4 mg
		Vit.B12	0.003 mg
		P-aminobenzoic acid	10 mg
		Glucose	1000 g

Biochemical analyses

Fully enzymatic determination of total triglycerides in serum was detected according to the method of Fossati and Prencipe (1982). Enzymatic determination of cholesterol was carried out according to the method of Roeschlau *et al.,* (1974). Low density lipoproteins cholesterol was estimated according to Wieland and Seidel, (1983). The high density lipoproteins-cholesterol (HDL-c) was evaluated according to the method of Lopez-Virella *et al.,* (1977). Cardiac Risk Ratio and atherogenic coefficient were calculated.

Cardiac Risk Ratio (CRR) = (total cholesterol/HDL-C).
Atherogenic coefficient (AC) = [(Total cholesterol - HDL-cholesterol)/HDL-C].

Determination of oxidative stress parameters:

Malondialdehyde (MDA) was estimated according to Ohkawa *et al.,* (1979). Reduced glutathione (GSH) was determined according to the method of Beutler *et al.,* (1963).

Histopathological examination:

Anatomy samples were taken from aorta, heart and liver of rats in different groups and fixed in a 10% formol solution for 24 hours. The samples were then washed with tap water and diluted alcohol was used in the following sequence (methyl, ethyl and absolute ethyl) for dehydration. Specimens were clarified in xylene and firmed in paraffin for 24 h at 56ºC. The paraffin wax blocks containing the tissue were sliced using microtome to 4 micron thickness. The tissue slices were deparaffinized and stained with hematoxylin and eosin and then examined by an electron optical microscope. (Banchroft *et al.,* 1996).

Statistical analysis

ANOVA analysis was achieved using the PROC ANOVA method of Statistical Analysis System (SAS, 2000). Duncan multiple ranges at 5 % significance were used as described by Duncan (1955) to compare between means. Means followed by different alphabetical letters significantly differed.

RESULTS AND DISCUSSION

Antioxidant components of green tea were shown in Table (2). The prepared GTB was characterized by its high content of antioxidants including total phenols (13.252 mg GAE/ml) and total flavonoids (0.525 mg QE/ml). The high content of antioxidants in GTB might be the cause of high antioxidant activity which was proved by high value of radical scavenging activity, ferric reducing antioxidant power and reducing power (Table 2).

Table 2. Antioxidant activity and contents of green tea.

Components	Concentration
Radical scavenging activity %	86%
Ferric reducing antioxidant power (OD_{593})	2.566
Reducing power (OD_{700})	1.687
Total Phenols (mg GAE / ml)	13.252
Total flavonoids (mg QE / ml)	0.525

Biological assay and histopathology

The GTB was biologically evaluated for its anti-atherogenic effect. Serum lipid profiles of the experimental animals were shown in Fig. (1). Gradually significant increases in serum triglycerides, total cholesterol, LDL-cholesterol, cardiac risk ratio and atherogenic coefficient at all time intervals during feeding period in AC group were observed. These values were 132 mg/dl, 139.56 mg/dl, 110.86 mg/dl, 4.27% and 3.27% respectively. On the other hand, significant declines of serum HDL-cholesterol 32.7 mg/dl was recorded at the end of feeding period compared to negative control (G1). These results are in a closed agreement with the results obtained by (Heber *et al.,* 2014; Yang *et al.,* 2015; Pan *et al.,* 2016 and Zhu *et al.,* 2017). The atherogenic effect in G2 was attributed to saturated fatty acids in high fat diet, while the elevated levels of LDL-C could be attributed to a reduction of LDL receptors by saturated fatty acids and cholesterol included in the diet. The oral administration with GTB (G3) for 60 days parallel to the feeding with high fat diet resulted in significant declines in serum triglycerides, total cholesterol and LDL- cholesterol, cardiac risk ratio and atherogenic coefficient, its values reached 88 mg/dl, 89.26 mg/dl, 25.1 mg/dl, 1.24% and 0.24% respectively. This was accompanied by significant elevation of serum HDL-cholesterol (72.16 mg/dl) at the end of feeding period compared to atherogenic control group (G2).

There was reduction in MAD value in GTB group from 5.45 nmol/ml in G2 to be 3.72 nmol/ml in GTB group (G3). Also, reduced glutathione (GSH) in GTB groups (G3) was 29.7 mg/dl, whereas it was 21.87 mg/dl in AC group (G2).

High-fat foods increase free radicals in the human body, which in turn increase the rate of lipid peroxides in the serum and liver. This may cause oxidative stress in the high fat group due to increased production of free radicals compared to the capacity of an antioxidant system (Yang *et al.,* 2010). The high content of antioxidant components; total phenols, flavonoids, , vitamin E and C as powerful natural antioxidants could be considered as the main reasons of increasing GSH and improve liver lipid profiles.

The physiological effects of GTB-containing flavonoids and other biologically active components, including antioxidant activity, indicate their role in preventing coronary artery disease including atherosclerosis by reducing LDL: HDL ratio, reducing oxidized LDL and making LDL less susceptible for oxidative stress. Flavonoids may work by making liver cells more effective for removing LDL cholesterol from the blood by increasing the density of LDL receptors in the liver and by binding to apolipoprotein B (Baum *et al.,* 1998). Trautwein *et al.,* (1997) reported that flavonoids make lower LDL-C and increased HDL-C that may accelerate the removal of cholesterol from peripheral tissues to the liver to catabolism and secretion. Moreover, elevated HDL-C levels may compete with LDL receptor sites for smooth arterial muscle cells and thus inhibit LDL absorption. The increase in HDL-C concentration can protect LDL from oxidation in vivo because lipids in HDL are preferentially oxidized before those in LDL (Bowry *et al.,* 1992 and Ge *et al.,* 2014). This could be explained by the ability of GTB to decreasing the cholesterol level due to inhibition of HMG-CoA reductase, which is likely a rate-limiting enzyme in cholesterol biosynthesis (Singh *et al.,* 2009). The consumption of green tea beverage was able to elevate both the LDL-receptor binding activity and relative amounts of LDL-receptor protein, and down regulates lipogenic enzymes in adipocyte cell lines and animal models. Treatment of hyperlipidemic rats with green tea showed significant reduction in CRR and AC as compared to hyperlipidemic group(G2) This finding was in harmony with those mentioned by Yang and Koo (2000). They demonstrated that, the inhibitory effect of green tea on endothelial cell induced LDL oxidation could be suggested a reason of delay atherogenesis and lower the risk of coronary heart disease.

Histological examination of liver, heart and aorta of rats were shown in Figs. (2, 3 and 4). Normal histological structure of the central veins, portal area and surrounding hepatocytes in the livers of rats in G1 was obtained (Figure 2.A). Also, non histopathological change in the myocardium (m) Figure (3. A). In the aorta, normal structures of tunica adventitia (a), media (m) and tunica intima (i) were illustrated in Figure (4. A). On the other hand, fatty changes in the hepatocytes with diffuse kupffer cells proliferation in between in liver of AC group (G2) Figure (2.B) and wide depression of myocardial bundles in heart, Figure (3.B) and vacuolization in tunic media in aorta , Figure (4.B),while GTB group(G3) found diffuse kupffer cells proliferation in between in liver, Figure(2.C) , normal intact histological structure for the myocardium, Figure (3.C) and mild swelling and protrusion of the cells in tunica intima, Figure(4.C).

The role of green tea for maintaining the biological organs of the experimental animals has been demonstrated due to its catechins content, particularly epigallocatechin-3-gallate(EGCG) (Ramesh *et al.,* 2012). This component has been known for its anti-atherogenic effect in Wistar rats fed on the atherosclerotic diet.

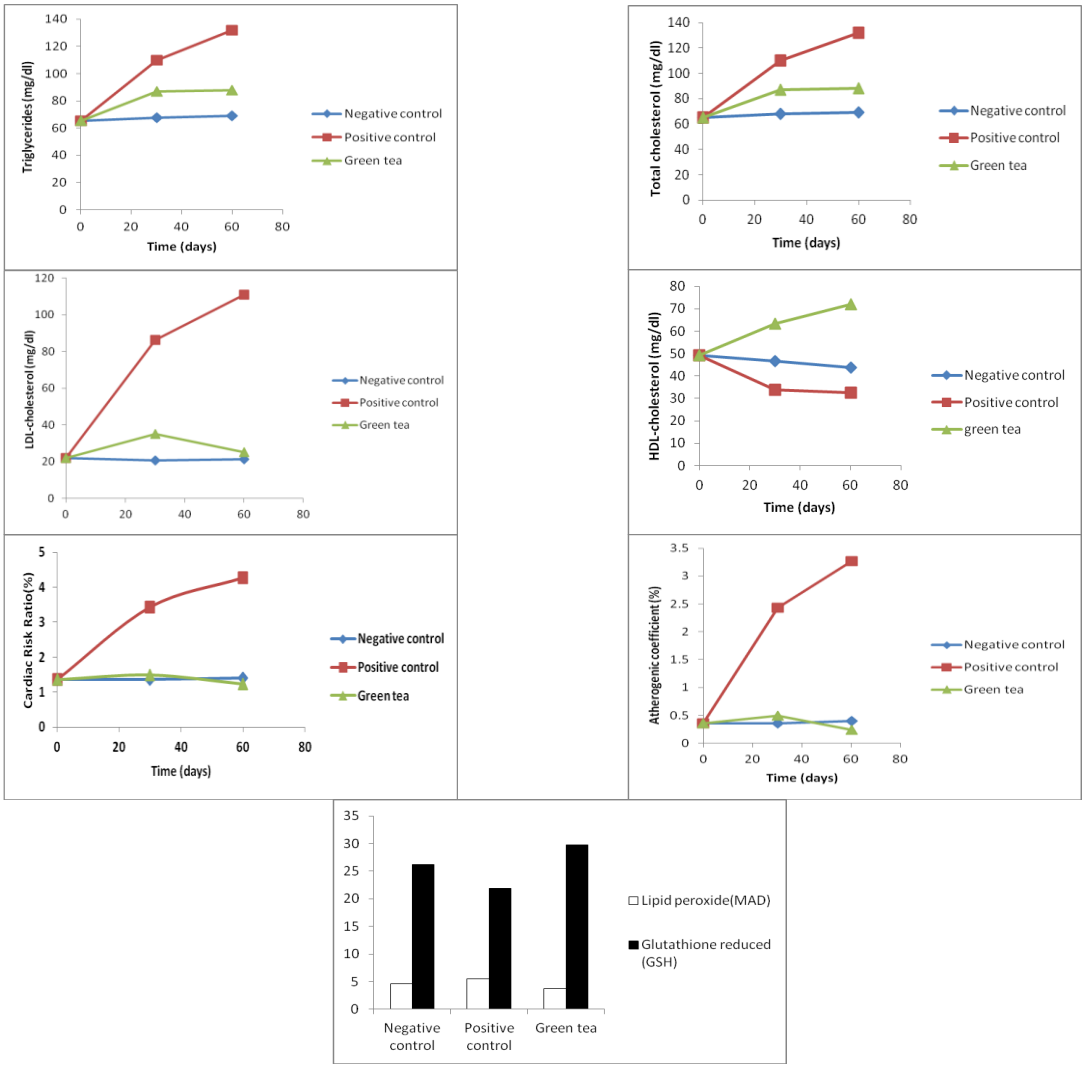

Fig. 1. Effect of oral administration of GTB on serum oxidative stress parameters and lipids profile in rats. (The data are presented as means ±SD from eight rats per group in 60days periods).

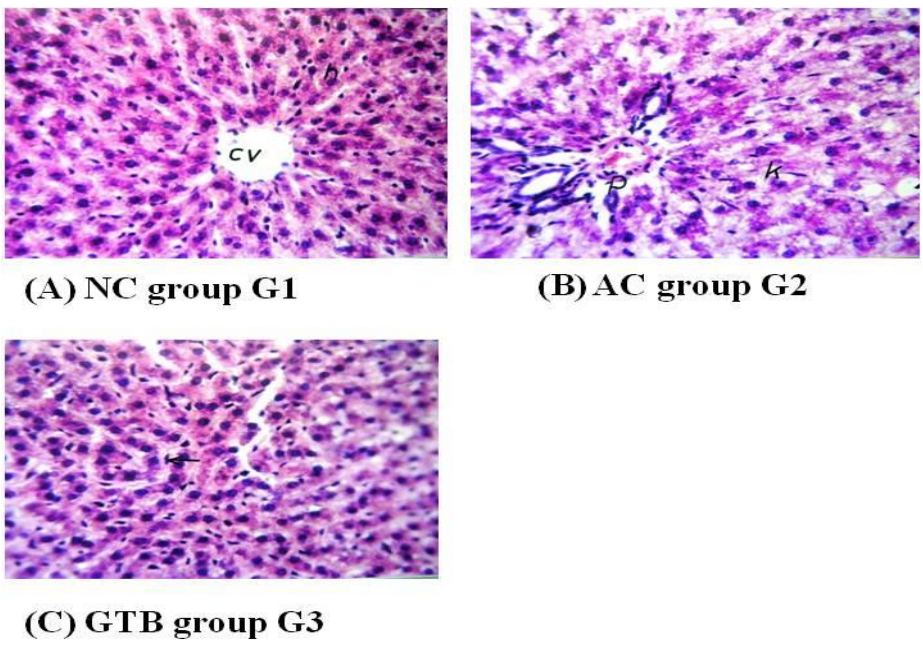

Fig. 2. Liver of negative control rats-G1 (A), atherogenic group- G2 (B)and GTB groups-G3 (C) (H & E, 80X).

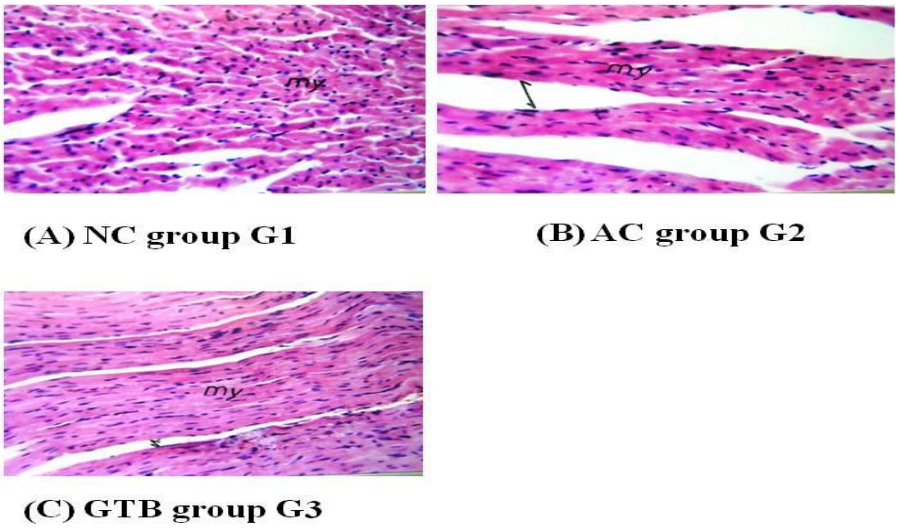

Fig. 3. Heart of negative control rats-G1 (A), atherogenic group-G2 (B), and in GTB group –G3 (C) (H & E, 64X).

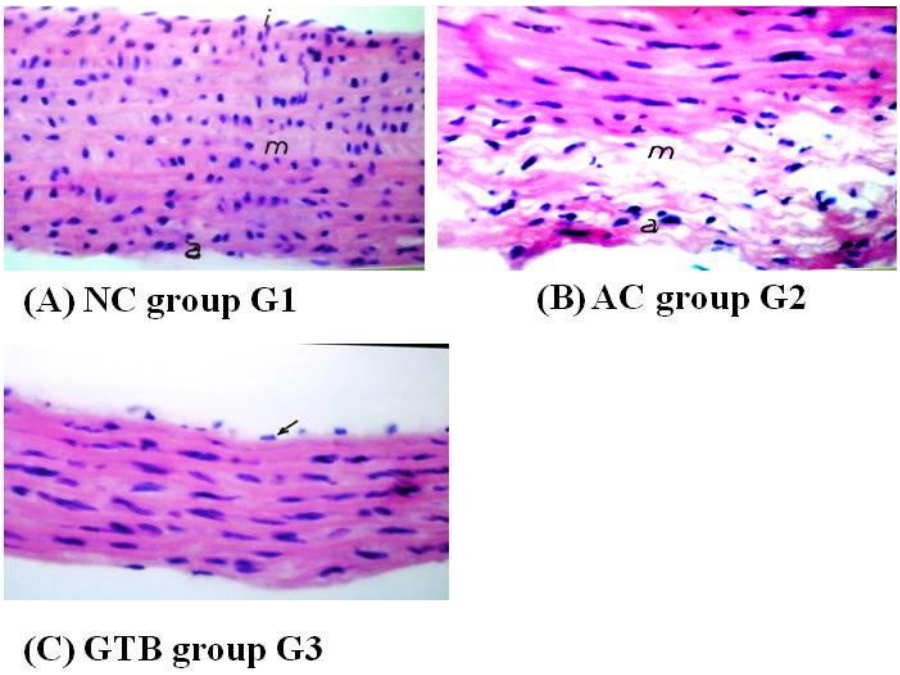

Fig. 4. Aorta of negative control rats-G1 (A), atherogenic group-G2 (B), and in GTB group –G3 (C) (H & E, 160X).

CONCLUSION

Green tea intake can alleviate or prevent atherosclerosis due to reducing the risk of atherosclerosis in the blood. Green tea contains many bioactive components which improve the metabolism of fats, antioxidant system and reduces the formation of atherosclerotic lesion. These beneficial potential of green tea may be caused by its ability to reduce oxidative stress and free radical quenching activity. Regular consumption of green tea in everyday life is considered a therapeutic agent for the prevention of atherosclerosis and cardiovascular disease.

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تأثير مشروب الشاى الأخضر المضاد لتصلب الشرايين

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تعتبر أمراض القلب والأوعية الدموية هي واحدة من الأسباب الرئيسية للوفاة في جميع أنحاء العالم. التحكم فى النظام الغذائي هو العامل الأكثر قابلية في الوقاية من هذة الأمراض. تم تحضير مشروب الشاي الأخضر (GTB) كمستخلص مائى وتقييمه بيولوجيًا لخصائصة المضادة لتصلب الشرايين لدى الجرذان. وأظهرمشروب الشاى الاخضرالذى تم تحضيرة في هذه الدراسة نشاط عالى لمضادات الأكسدة ودل على ذلك النتائج التى تم الحصول عليها في radical scavenging activity (86%), Ferric reducing antioxidant power (2.566) and reducing power (1.687) . كما أحتوى مشروب الشاى الاخضرعلى نسبة عالية من الفينولات الكلية (13.252 mg GAE/ml) وفلافونيدات (0.525mg QE/ml). في المجموعة التى تم معاملاتها بمشروب الشاى الاخضر (G3)، أدت المعاملة ب GTB إلى تحسين صورة الدهون في المصل ومعاملات الإجهاد التأكسدى مقارنة بالمجموعتين الأخريين (المجموعة الضابطه الأيجابية التى تغذت على حمية تصلب الشرايين (G2) ومجموعةالضابطة السلبية التى تغذت على الوجبة القياسية (G1)). وأكد فحص الأنسجة في الكبد والقلب والشريان الأورطي هذه النتائج التي تعكس دور التأثير الوقائى لمشروب الشاى الأخضر ضد تصلب الشرايين وأمراض القلب والأوعية الدموية.

الكلمات الدالة : الشاي الأخضر؛ النشاط المضاد للأكسدة؛ مضاد لتصلب الشرايين.