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Original article

In vivo Effects of Black and Green Tea on Serum Lipid Profile and Cardiac Function in Hyperlipidemic Rats

*Olu Israel OYEWOLE, Omowumi Oyeronke ADEWALE and Juliana Bunmi ADETUNJI

Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria.

*Corresponding author: ioluoye@yahoo.com

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Abstract: Objective: The study was designed to investigate the *in vivo* effects of black and green tea consumption on serum lipid profile and cardiac function in hyperlipidemic rats. **Methods:** 24 male Wistar rats, average weight 125 g were sorted into four groups: A, B, C and D. Normal control group (A) were fed standard rat chow; the remaining three groups (B, C and D) received rat chow supplemented with 2% (w/w) cholesterol to induce hypercholesterolemia. Group C and D were administered 40 mg/ml of black and green tea respectively while group B (hyperlipidemic control) were not treated. The feeding and tea administration lasted 6 weeks. **Results:** Significant reduction in body weight and heart weight index was recorded in rats administered black and green tea compared with the untreated group. Black and green tea consumption also caused significant decrease in total cholesterol, LDL-cholesterol, triglycerides and coronary heart disease (CHD) risk ratio accompanied with elevated HDL-cholesterol compared to untreated group. Significant alterations in cardiac marker enzymes: creatine kinase (CK), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were also observed in serum and heart homogenates of untreated hyperlipidemic rats which were normalized in rats administered the teas. These results are synonymous with decreased risk of atherosclerosis and protective potential on cardiac function by green and black tea. **Conclusion:** We conclude in this study that regular intake of black and green tea might be useful in treatment of obesity and prevention of cardiovascular complications arising from hyperlipidemia.

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INTRODUCTION

Coronary heart disease remains the main cause of mortality and morbidity worldwide with 17.3 million deaths each year which might rise to 23.6 million by 2030 (American Heart Association, 2016) Elevated level of all lipids with exception to HDL is a risk factor in the pathogenesis of cardiovascular diseases including arteriosclerosis. An increase of 1% serum cholesterol is reported to have resulted in a 3% increase in coronary heart disease (V and Cox, 2000; Osmund, 2001). Equally a reduction in LDL-cholesterol by 2 mg/dl can result in 1% reduction in the risk of coronary heart disease. Deposit of cholesterol in blood vessels narrow the arterial channels and partly block the normal flow of blood through them (Haines, 2001) The decrease in blood flow and oxygen can result in stroke, partial paralysis, loss of speech and sometimes death (Olson, 1998, Khleifat et al., 2002) High levels of LDL cholesterol often called "bad cholesterol promote cardiovascular disease as opposed to HDL particles, which are referred to as "good cholesterol" or "healthy cholesterol". HDL particles are able to remove cholesterol from within the artery and transport them back to the liver for excretion or re-utilization. Those with higher levels of HDL cholesterol seem to have fewer problems with cardiovascular diseases, while those with low HDL cholesterol levels have increased rates of heart disease. Clinical studies have shown that elevated HDL-cholesterol as well as reduction in total cholesterol and LDL-cholesterol using diet or drugs decreases the incidence of coronary heart disease (Superko et al., 2002; Homady et al., 2002)

Low-fat diet is often prescribed for the management of arteriosclerosis as there are no specific treatments for the ailment. Some medicinal plants have been reported to lower blood cholesterol resulting in positive cardiovascular effects in experimental animals (Bhatnagar et al., 2008). Tea is one of the most widely consumed beverages in the world, next only to water and well ahead of coffee, beer, wine and carbonated soft drinks. Tea is produced by acceptable processes from the tender shoots of *Camellia sinensis*. It has an attractive aroma, good taste and health-promoting effects and has continued to be considered a medicine since the ancient times because of its polyphenol contents (Geleijnse et al., 1999). Several reports have indicated that polyphenols can reduce the risk of heart disease and cancer in humans (Hertog et al., 1995). Tea also has the potential of reducing the risk of atherosclerosis, cardiovascular diseases, myocardial infarction and decrease in serum lipid

concentration (Fraser et al., 2007; Gardner et al., 2007). The present study intends to assess the *in vivo* effects of black and green tea consumption on body weight, serum lipid profile and cardiac function in albino rat.

MATERIALS AND METHODS

Processed dried black and green tea (*Camellia sinensis*) leaves used are products of Greenfield Company, England. Total cholesterol, triglyceride, LDL, HDL, CK, GGT and LDH kits were obtained from Randox Chemical Hall, England.

Experimental Design and Animal Management

The experiment was conducted on 24 male Wistar rats weighing approximately 125 g. The animals were randomly assigned into four different groups: A, B, C and D. Normal control group (A) were fed standard rat chow; the remaining three groups (B, C and D) received rat chow supplemented with 2% (w/w) cholesterol to induce hypercholesterolemia. Group C and D were administered 40 mg/ml of black and green tea respectively while group B (hyperlipidemic control) were not treated. The feeding and tea administration lasted 6 weeks. The rats were housed in the Central Animal House, Osun State University, Osogbo, Nigeria. They were maintained in individual cages, under controlled temperature, humidity and illumination conditions with water and diet *ad libitum*.

Preparation of Serum

Rats were sacrificed at the end of experimental period by cutting through the jugular vein and blood sample collected into sterilized dry centrifuge tube. Blood was allowed to clot and then centrifuged at 3000 rpm for 20 minutes in a Uniscope SM902B Centrifuge (Surgifield Medicals, England). The clear supernatant (serum) was separated from the pellet and transferred into clean test tubes after which it was frozen until required for analysis.

Preparation of Heart Homogenate

The rats were quickly dissected and the heart removed. The heart was rinsed with 10% KCl, weighed and then homogenized in 4 volumes of 0.1 M Tris-KCl (pH 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 12,500 g for 15 minutes in a cold centrifuge (4 °C) to obtain the post mitochondrial fraction. The supernatant was collected and used for biochemical analyses.

Biochemical Assay

The enzymatic endpoint method (Zoppi and Fellini, 1976) was employed in the analysis of serum total cholesterol. Serum triglyceride was assayed using the GPO-PAP method (Trinder, 1969) while precipitant method of Wieland and Siedel (Wieland and Siedel, 1981) was used in the measurement of HDL-cholesterol. LDL-cholesterol was estimated using the procedure described by Friedewald *et al* (Friedewald *et al*. 1972). Coronary heart disease risk ratio (CHD risk ratio) was obtained by calculating the ratio of concentration of total cholesterol to HDL-cholesterol. Creatine kinase was estimated by the method of Hughes (Hughes, 1962) while LDH and GGT activities were measured by standard methods earlier described methods described (**Vanderlinde, 1985; Tiex et al., 1974). M**easurement of concentrations was done by the use of Camspec M106 UV spectrophotometer (Ohaus Corporation, Pine Brook, USA).

Statistical Analysis

Data were expressed as mean ±SD. The data were analyzed by analysis of variance (ANOVA) using SPSS version 22.0 computer software. Level of significance was determined by Duncan's multiple range tests. P values less than 0.05 were considered significant (Majali et al., 2015; Al-Asouf et al., 2017).

RESULTS

Results of body weight and heart weight index of rats are shown in Table 1. There was significant increase in body weight gain and heart weight index in hyperlipidemic untreated rats compared with the normal control. Treatment with green and black teas caused significant reduction in body weight gain with no significant change in heart weight index.

Table 1: Body weight and heart weight index of hyperlipidemic rats administered black and green tea

	Normal control	Hyperlipidemic (untreated)	Hyperlipidemic treated with green tea	Hyperlipidemic treated with black tea
Initial body weight (g)	125.24±4.36	124.34±5.11	126.32±3.69	124.14±4.21
Final body weight (g)	188.53±6.43	218.41±9.65**	166.58±6.23*	164.72±5.78*
Weight gain (g)	63.29±2.34	94.07±3.76**	40.26±3.22*	40.58±3.80*
Heart weight (g)	0.85±0.04	1.27±0.14**	0.78±0.06	0.79±0.05
Heart weight index (g/100g body weight)	0.45±0.02	0.58±0.05**	0.47±0.03	0.48±0.04

Values are mean of 6 rats ± SD. *Significantly lower than the control at p<0.05.
**Significantly higher than the control at p<0.05.

Table 2: Serum lipid concentration in hyperlipidemic rats administered black and green tea

	Normal control	Hyperlipidemic (untreated)	Hyperlipidemic treated with green tea	Hyperlipidemic treated with black tea
Total cholesterol(mg/dl)	158.21±5.33	188.70±6.86**	162.53±4.69	165.39±5.21
TAG (mg/dl)	107.62±4.69	131.82±3.50**	115.26±3.26	113.90±3.49
LDL (mg/dl)	85.44±3.66	113.27±4.11**	91.05±4.05	90.23±2.73
HDL(mg/dl)	63.44±3.12	44.66±2.90*	61.45±3.41	63.55±4.00
CHD Risk ratio	2.49±0.22	4.23±0.51**	2.24±0.23	2.22±0.24

Values are mean of 6 rats ± SD. *Significantly lower than the control at p<0.05.
**Significantly higher than the control at p<0.05.

Table 3: Activities of enzymes in the serum and heart homogenate of hyperlipidemic rats administered black and green tea

		Normal control	Hyperlipidemic (untreated)	Hyperlipidemic treated with green tea	Hyperlipidemic treated with black tea
Serum (IU/L)	CK	61.32±5.18	89.44±7.33**	65.57±5.73	63.29±4.58
	GGT	52.41±3.30	71.22±4.88**	57.12±3.13	56.32±3.65
	LDH	153.77±8.89	178.49±9.11**	157.45±8.67	160.33±7.21
Heart (IU/mg protein)	CK	26.56±3.87	16.44±2.20*	24.38±3.62	25.38±3.59
	GGT	32.77±3.30	20.33±2.43*	31.20±2.76	29.88±2.69
	LDH	94.21±5.55	68.57±4.24*	87.50±5.26	87.47±4.98

Values are mean of 6 rats ± SD. *Significantly lower than the control at p<0.05.
**Significantly higher than the control at p<0.05.

Table 2 show the lipid profile in the serum of control and test groups. There was significant increase in serum total cholesterol, triglycerides, LDL-cholesterol and CHD risk ratio in untreated hyperlipidemic rats. HDL cholesterol was also reduced in these rats compared to the normal control. These serum lipid abnormalities were normalized following administration of black and green teas.

Activities of enzymes in the serum and heart homogenate of control and test groups animals is shown in Table 3. There was significant elevation of enzymes (CK, GGT and LDH) in the serum with their concomitant decrease in the heart of untreated hyperlipidemic rats compared with normal control. Treatment with black and green teas normalized both the serum and cardiac enzymes levels in the rats.

DISCUSSION

The observed significant weight gain and heart weight index of untreated hyperlipidemic rats compared with normal control might result due to deposit of fat in the adipose and cardiac tissues respectively. It has been shown that cardiac enlargement, whether hypertrophic or dilated is an independent risk factor for sudden cardiac death, although the definition of what constitutes cardiac enlargement is not universally established (Neubauer, 2007). The increased heart weight was normalized following administration of green and black tea. The significant weight loss in rats treated with black and green teas indicate that the teas can be useful in the treatment of obesity and overweight.

The observed decrease in total cholesterol, LDL-cholesterol, triglycerides and CHD risk ratio accompanied with elevated HDL-cholesterol in the serum of rats to normal value following black and green tea consumption is indicative of their hypolipidemic property which is synonymous with decreased risk of atherosclerosis (Rifai, and Warnick, 2006). This imply that tea can be used to prevent cardiovascular complications arising from hyperlipidemia (Hodgson et al., 2000) This result might explain the traditional use of tea as a natural remedy against heart diseases. This property may be due to the flavonoid constituents of the teas (Ishikawa et al., 1997; Abboud et al., 2008; ALthunibat et al., 2010). Flavonoids are mostly hydrophilic with antioxidant and free radicals scavenging properties and do not bind LDL molecules (Shankar et al., 2007; Khleifat et al., 2019).

The significant reduction in serum LDL by black and green tea may be due to suppression of LDL oxidation (Luo et al., 1997; Shakhanbeh & Khleifat, 2004). The serum total cholesterol lowering effect of black and green tea may be attributed to their ability to increase the excretion of cholesterol. Certain drugs/herbs have been reported to cause enhanced excretion of acidic and neutral steroids. The significant reduction in serum level of serum triglyceride in rats administered teas may be due to a number of factors such as decreased availability of fatty acids for esterification, increased catabolism of LDL, activation of tissues lipases, inhibition of acetyl-CoA carboxylase and reduced production of triglycerides precursors such acetyl-CoA and glycerol phosphate (Coleman and Lee, 2004).

The observed elevation of serum CK, GGT and LDH in untreated rats with their concomitant reduction in heart homogenate could be due to leakage of the enzymes into the serum as a result of damage to heart integrity. Elevated serum activity

of these enzymes has been reported to be a strong risk factor of cardiovascular disease. The heart has a high content of these enzymes which leaks into the serum during myocardial infarction causing their elevation in the serum. These results indicate cardiovascular distress in the hyperlipidemic rats.
Creatine kinase is a muscle-specific enzyme used in routine diagnosis of myocardial infarction. Significant increase in serum CK was consistently observed in patients with myocardial infarction **(**Wallimann et al., 1992**).** LDH is an intracellular enzyme present in nearly all metabolizing cells with highest concentration in the heart, skeletal muscle and erythrocytes. Cellular damage to the heart increases serum LDH although this increase may be influenced by other body tissues other than the heart **(**Perry et al., 1997). Serum level of LDH1 isoenzyme is elevated within 24-48 hours and reaches a peak in 48-72 hours after episode of myocardial infarction. Elevated serum GGT has been reported to be a very strong indicator for early development of atherosclerosis. A correlation between GGT and cardiovascular mortality has been recorded indicating that the higher the elevation of GGT, the greater the risk of death (Jiang et al., 2013)

The observed hypolipidemic and cardiovascular protection properties of black and green teas in this study could be based on the antioxidant properties of the phytochemicals contained in the teas. Black and green teas are rich sources of antioxidants such as catechins, flavonoids, quercetin; flavones, tannins (Yashin et al., 2011). Catechins.is the most powerful antioxidant in black and green teas and is responsible for their color and taste. The known in vitro antioxidant properties of catechins and other polyphenolic compounds in tea have led to interest in the potential health benefits of tea consumption (Higdon and Frei, 2003)

Several epidemiologic studies have demonstrated inverse relationships between tea consumption and incidence of cardiovascular diseases (Riemersma et al., 2001) The antioxidant activity of tea polyphenols has been suggested as potential mechanisms for cancer prevention (Khleifat et al., 2007; Lambert and Elias, 2010). Apart from their potent antioxidant activity the thermogenesis (fat oxidation and energy expenditure) activities of tea has also been demonstrated (Liu et al., 2000).

CONCLUSION

Results obtained in this study suggest that green and black tea administration caused significant reduction in body weight and therefore could be useful in the treatment of obesity. The teas also have hypocholesterolemic property as well as modulatory effects on cardiac dysfunction caused by intake of high cholesterol diet in rats. These results suggest that black and green tea can be used as antiatherogenic agent for the management of atherosclerosis in man. The medicinal properties attributed to black and green teas could be based on the antioxidant properties of the phytochemicals contained in the teas.

Conflict of interest

The authors declare no conflict of interest.

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