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The Influence of *Camiella sinesis, Matricaria chamomilla* and their Blend Teas Infusion on Serum Lipids of Wistar Rats

Olanrewaju Roland Akinseye [1, a *], Aladenika Yetunde[2, b]

[1] Department of Biochemistry, Federal University of Technology, Akure, Nigeria,

[2] Gateway Polytechnic, Saapade Remo, Ogun State, Nigeria

[a] Akinseyeroland@gmail.com, [b] yetunde.aladenika@yahoo.com

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Abstract. Green tea (GT) and Chamomile tea (CT) are one of the most important beverages which are used worldwide. They have become a subject of interest because of the beneficial effects on human health in recent years. In this study which was to demonstrate the cholesterol reducing-effects of *Camiella sinesis*, *Matricaria chamomilla* and their blend teas on serum lipids of Wistar rats. The animals were divided into control group (CG) and nine treated groups: GT1, GT3, GT5 treated with green tea; CT1, CT3, CT5 treated with Chamomile tea and GT+CT1, GT+CT3, GT+CT5 treated with blend green tea and chamomile tea of 10 mg/kg.BW/ml, 30 mg/kg.BW/ml and 50 mg/kg.BW/ml concentrations respectively. The teas were administered orally once in a day for 30 days. GT3, GT5; CT3, CT5 and GT+CT3, GT+CT5 animals showed significant decrease in triglycerides and total cholesterol and slight increase in HDL-c levels. The levels of LDL-c decreased in the treated groups compared to control group. The results of this study say that the individual tea samples have a significant effect on lipid profile and the teas are capable of improving the cardiovascular health.

Introduction

Tea is one of the most popular beverages consumed worldwide. Teas, from the plants *Camiella sinesis* and *Matricaria chamomilla* are consumed in different parts of the world and in folk medicine its aqueous infusion are used.

Several epidemiological studies showed that the green tea may reduce chronic diseases and this effect is due to the presence of polyphenols which acts as antioxidants. Antioxidant potential is directly related to the combination of aromatic rings and hydroxyl groups causing the binding and neutralization of free radicals by the hydroxyl groups. Green tea is considered a dietary source of antioxidant nutrients: green tea is rich in polyphenols (catechins and gallic acid, particularly), but it also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as Cr, Mn, Se or Zn, and certain phytochemical compounds. Epidemiological studies suggest that green tea consumption is associated with a reduced cardiovascular disease risk, but the mechanisms for these observations have remained uncertain. Several studies have demonstrated that green tea may affect the cardiovascular function through mechanisms of action related to LDL-cholesterol oxidation. The oxidation of LDL cholesterol, associated with a risk for atherosclerosis and heart disease, is inhibited by green tea due to Epicatechin (EC) and Epigallocatechin gallate (EGCG) antioxidant activity. [1]

Chamomile has a widespread usage and holds a high reputation among herbal medicines during the history due to its anti-inflammatory, analgesic, antimicrobial, antispasmodic and sedative properties [2, 3]. The merit of the traditional use of *Matricaria chamomilla* has been supported by the isolation and identification of several biologically active chemicals including polyphenols (flavonoids) and essential oils extracted from chamomile flowers [2]. Chamomile is the herb that is used for antioxidant agent, pain management, antispasmodic, anti-inflammatory, anti-convulsant, anti-pyretic, sedation, and wound healing in traditional medicine [5]. Chamomile's cholesterol reducing potential has not been carefully documented. Our study aim is to determine the lipid-

lowering efficacy of green tea, chamomile tea and their blends in healthy male wistar rats with respect to control.

Material and Method

Animals

Healthy adult male albino rats of wistar strain weighing between 130 to 180 g were obtained from the animal house of the School of Agriculture, Federal University of Technology, Akure for the study. They were kept in rat cages at room temperature (27 ± 2°C) and humidity (55 ± 5%) and a 12 h cycle of light and dark. They were given free access to rat pellet and water *ad labitum*. The experiment was performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals. The rats were allowed to acclimatize for a week before the experiment.

Experimental design

There were 10 groups of 5 albino rats each, the experimental design were according to methods of [6] with slight modifications and it involved duration of 30 days.

1. Group 1: Control; group without treatment; normal diet and 0% of the tea samples
2. Group GT1: 0.4ml aqueous extract of Green Tea; 10mg/kg BW/ml
3. Group GT3: 0.4ml aqueous extract of Green Tea; 30mg/kg BW/ml
4. Group GT5: 0.4ml aqueous extract of Green tea; 50mg/kg BW/ml
5. Group CT1: 0.4ml aqueous extract of Chamomile Tea; 10mg/kg BW/ml
6. Group CT3: 0.4ml aqueous extract of Chamomile Tea; 30mg/kg BW/ml
7. Group CT5: 0.4ml aqueous extract of Chamomile Tea; 50mg/kg BW/ml
8. Group GT+CT1: 0.4ml aqueous extract of Green + Chamomile Tea; 10mg/kg BW/ml
9. Group GT+CT3: 0.4ml aqueous extract of Green + Chamomile Tea; 30mg/kg BW/ml
10. Group GT+CT5: 0.4ml aqueous extract of Green + Chamomile Tea; 50mg/kg BW/ml

Extract preparation and administration

Green and Chamomile teas were bought from the Tradomedical Centre, Ibadan, Oyo state, Nigeria. The tea extracts were prepared using hot water infusion. The mixture was filtered using No 1 Whatman filter paper and the filtrate kept prior analysis. The rats were weighed daily and the calculated volumes of the extracts in milliliters (mg/Kg. BW/ml) were administered orally for 30 days.

Phytochemical investigation of extract

The different chemical constituents present in aqueous extracts were subjected to the tests by Kokate [12] and Trease & Evans [11].

Total Flavonoid content

The total flavonoid content of the extracts was determined using a slightly modified method reported by Chung *et al* [15]. Briefly, 0.5mL of enzyme digested sample was mixed with 0.5mL methanol, 50µl of 10% $AlCl_3$, 50µl of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard by making use of a seven point standard curve (0, 20, 40, 60, 80,100 µg/mL). The total flavonoids content of samples was determined in triplicates and the results were expressed as mg quercetin equivalent per gram of the sample.

Total Phenolic content

The total phenolic content of the samples extract was determined by the Folin-Ciocalteu assay as described by Chanda *et al* [14]. 500µl of Folin reagent was added and mixed with a solution

containing 100µL of the extract and 2mL of distilled water. 1.5mL of 7.5% sodium carbonate was then added to the solution and the volume was made up to 10mL with distilled water. The mixture was left to stand for 2 h after addition of the sodium carbonate. The absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the results were expressed as mg tannic acid equivalents per gram of the sample.

Biochemical analysis

At the end of the experiment, blood was collected from each rat by cardiac puncture method. Blood samples were centrifuged (at 2000 g for 10 min); serum was obtained for the measurement of cholesterol, triglycerides, HDL by spectrophotometer using a commercial kit package (Randox Laboratories Limited). We used standard commercial kits for analysis as recommended by the manufacturer of these kits. LDL and VLDL-cholesterol were calculated following the method by Johnson et al [4], while the atherogenic index was calculated by using the method described by Muruganandan et al [7].

LDL = TC – (HDL + Triglyceride/5)

VLDL = TC-HDL-LDL

Atherogenic index = (TC-HDL)/HDL

Statistical analysis

Results are expressed as mean±SEM (standard error mean) and subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test and values with $p<0.05$ were considered to be statistically different.

Results and Discussion

Tables 1, 2 and 3 show the effects of the green tea, chamomile tea and the blend of the two teas extracts on the serum lipid parameters of wistar rats. The results showed that the different levels of green tea, chamomile tea and their blend extracts have no significant effects on serum HDL ($p > 0.05$). The serum contents of cholesterol, triglycerides, and LDL were significantly lower in the rats in groups GT3, GT5; CT3, CT5 and GT+CT3, GT+CT5 than in the control group ($p < 0.05$) with few exceptions in triglycerides and total cholesterol values of GT3, GT5 and LDL values for GT3, CT3, GT+CT3 which showed slight increase in mg/dl compare to control. The green tea, chamomile tea and their blend extracts, at high levels generally, showed increased effects on the serum lipid parameters (GT3, GT5; CT3, CT5 and GT+CT3, GT+CT5 Vs GT1; CT1 and GT1+CT1).

Table 1: Phytochemical screening of the two tea extracts and their blends

PHYTOCHEMICAL TESTS	GT	CT	GT+C
ALKALOIDS	-	-	-
SAPONINS	-	-	-
TANNINS	+	+	++
PHLOBATAMINS	-	-	-
ANTHRAQUINONES	-	+	+
STEROIDS	+	+	++
TERPENOIDS	+	-	+
FLAVONOIDS	-	+	-
CARDIAC GLYCOSIDE	+	-	+

Key: + = Present, - = Absent

Table 2: Effects of green tea extract on serum lipid parameters (mg/dl) of the rats in different groups

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	156.93±1.53[a]	78.49±1.72[a]	40.85±0.42	11.76±0.56[a]	31.39±0.31[a]	1.16±0.009[a]
GT1	160.69±3.45[a]	158.71±0.64[a]	40.42±0.42	27.88±0.15[a]	32.14±0.30[a]	1.66±0.003[a]
GT3	137.24±2.07[b]	132.26±2.58[b]	41.06±0.85	19.93±0.35[b]	27.45±0.41[b]	1.30±0.008[b]
GT5	104.83±1.38[c]	113.55±0.91[c]	42.12±0.85	13.54±0.17[c]	20.97±0.28[c]	0.95±0.008[c]

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE

Table 3: Effects of chamomile extract on serum lipid parameters (mg/dl) of the rats in different groups

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	156.93 ± 1.53 [a]	78.49 ± 1.72 [a]	40.85±0.42	11.76±0.56[a]	31.39±0.31[a]	1.16±0.009 [a]
C1	198.62 ± 2.76 [a]	77.42 ± 0.65 [a]	39.37±1.06	19.91±0.96[a]	39.72±0.55[a]	1.62±0.05 [a]
C3	151.72 ± 2.75 [b]	64.52 ± 0.65 [b]	40.00±0.84	14.44±0.54[b]	30.34±0.55[b]	1.18±0.002 [b]
C5	101.38 ± 6.21 [c]	26.13 ± 3.55 [c]	41.48±1.06	12.14±0.69[c]	20.28±1.24[c]	0.89±0.009 [c]

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE

Table 4: Effects of the blend of green tea and chamomile tea extracts on serum lipid parameters (mg/dl) of the rats in different groups

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	156.93 ±1.53 [a]	78.49 ± 1.72 [a]	40.85±0.42	11.76±0.56[a]	31.39± 0.31[a]	1.16±0.009 [a]
GT+CT1	204.14 ±1.38 [a]	88.71 ± 0.97 [a]	38.10±0.21	15.59±1.03[a]	40.83± 0.28 [a]	1.67±0.01 [a]
GT+CT3	120.00± 2.76 [b]	76.77 ± 1.29 [b]	39.15±0.63	13.29±0.22 [b]	24.00± 0.55 [b]	1.07±0.009 [b]
GT+CT5	74.48 ± 2.76 [c]	17.10 ± 3.55 [c]	40.00±0.84	9.95±0.61 [c]	14.90± 0.55 [c]	0.78±0.01 [c]

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE

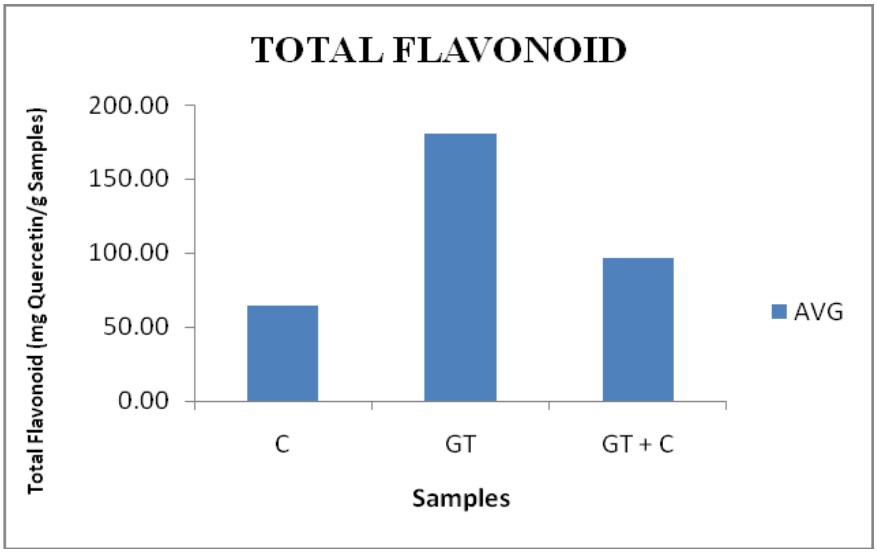

Figure 1: Total Flavonoid content of Green tea, Chamomile tea and their blend

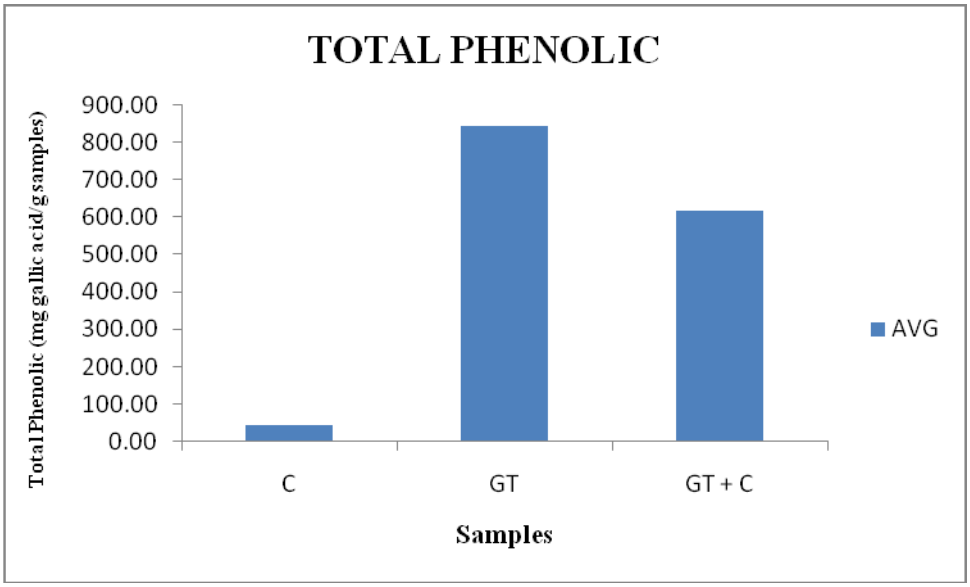

Figure 2: Total Phenolic content of Green tea, Chamomile tea and their blend

Discussion

In this study, green tea, chamomile tea and their blend extracts did not have a significant impact on the serum content of HDL. The absence of concurrence among these studies may be a result of the levels at which the extract was administered. In addition, other variables, such as differences in background of the selected animals, the types of animals or genera and age may have affected the efficacy of extract usage, and therefore it was difficult to directly assess different studies that used extracts.

It was suggested that flavanoids act in the aqueous phase perhaps on the surface of lipoproteins particles. These flavanoids plays an important role on lipid profile. Some studies shown that catechin in green tea normalized the concentration of plasma cholesterol without affecting the HDL cholesterol which is correlated with our study. With supplementation of green tea, chamomile tea and their blend for 30 days, cholesterol in our study decreased which is correlated with the study of Hsu et al [8] who states that intake of green tea decreases the cholesterol level.

In the present study the triglycerides in the individuals shown a significant decrease ($p < 0.05$) when compared with control value which is correlated with the study of Raederstorff et al [9], explained that green tea exerts the hypolipidemic action, the effect which was also observed in this study in chamomile tea and the blended one. The extracts from green tea, chamomile tea and the

blended tea normalizes the plasma triglycerides, cholesterol concentration and possibly, these teas extracts might decrease intestinal absorption of lipids. In our study, LDL significantly decreased ($p < 0.05$) after supplemented with green tea, chamomile tea and the blended sample. This is correlated with the study of Nikolaos Alexopoulos et al [10] who stated that consumption of green tea reduced the total and low density lipoprotein cholesterol.

This study provided information, which supported the conclusion, that green tea, chamomile tea and their blends have hypocholesterolemic properties. These results suggested that these teas may be incorporated into a targeted dietary program as part of public health policy to improve cardiovascular health. Flavonoid and phenolics rich foods and extracts like green tea, chamomile tea and their blend can reverses the endothelial dysfunction in patients with coronary artery disease. The mechanism in this disease is that these teas polyphenols inhibits the activity of angiotensin converting enzyme, increases nitric oxide production by endothelial cells and improved the bioactivity of endothelium derived nitric oxide. It seems to be there is an interrelation between antioxidant and endothelial effects of the teas.

Since green tea, chamomile tea and their blends' beneficial health effects were being increasingly proved, it could be advisable to encourage the consumption of these teas regularly which is alternative to other drinks. This study also confirmed that it is possible to combine equal quantity of two teas which have beneficial health potentials with the blended tea exhibiting the two potentials.

Conclusions

In our study consumption of green tea, chamomile tea and their blends produced a significant reduction in lipid profile with slight or no effects on HDL and ensuring antioxidant potential.

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