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Phytochemistry and effect of oral administration of (*Camellia sinesis*) Turkish and Ceylon tea on the development of metabolic syndrome in albino rats

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Abstract: Effect of oral administration of Turkish and Ceylon tea on the development of metabolic syndrome was investigated in 50 albino rats. The rats were divided into six (6) groups. The experimental groups (group 2, 3, 5 and 6) were administered orally 2.8mg/kg body weight of the teas under study for seven weeks. Groups 1 and 4 were not administered any tea and served as positive and negative control. Rats in group 4, 5 and 6 had their drinking water supplemented daily with 10%w/v fructose so as to induce metabolic syndrome. At the end of seven weeks, the rats were sacrificed and their fasting blood sugar (FBS), serum uric acid levels and serum lipid profile were determined. Significant difference at $P<0.05$ was observed between the mean FBS and serum lipid profile of the controls and experimental groups which is a pointer to hypoglycemic and hypocholesterolemic properties of the teas. Rats administered Turkish tea had lower mean serum uric acid levels than their controls. Qualitative phytochemical analysis revealed the presence of tannins, terpenoids, saponins, cardiac glycosides, resins and flavonoids in both teas. Alkaloids and chlorogenic acid were not detected in the teas; while anthraquinone was found in only Turkish tea. Vitamin C, tannins, saponins, cardiac glycosides and flavonoids were found in higher quantities in Turkish tea. This study has shown that Turkish and Ceylon tea contains medicinal important bioactive compounds which are of potential health benefit.

Keywords: Turkish tea, Ceylon tea, lipid profile, fasting blood sugar, serum uric acid.
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INTRODUCTION

Metabolic syndrome is a combination of medical disorders that when occurred together increase the risk of developing cardiovascular disease and type II diabetes[1]. Most patients happen to be older, obese and sedentary; and have a degree of insulin resistance. Recent report in Hispanics and China suggest that metabolic syndrome criteria are met by approximately 1 in 3 adults[2].

Dietary habits play a vital role in the risk of developing a variety of diseases; especially cardiovascular, cardio-renal disease and cancer. The use of dietary substances as a practical approach to reducing the risk of developing these diseases is receiving increasing attention. The utilization of tea and tea products is promising.

Tea is an aromatic beverage commonly prepared by pouring boiling hot water over cured leaves of the *Camellia sinensis* plant. Manufacturers process *Camellia sinensis* leaves in three different ways to produce the three major classes of teas known as green, black, and Oolong. When fermentation is completely arrested, the tea stays green. If fermentation time is long, the leaves darken and become black tea. Somewhere in-between these two extremes Oolong tea is produced[3].

Literature report[4] has shown that tea leaves contain several chemical components which are of potential health benefits. Among the compounds closely related to human health are flavonoids, amino acids, vitamin C, E and K, caffeine and other phytochemicals. Chinese researchers recently reported[5] green tea as a promising tool against metabolic syndrome. However the association between consumption of black tea and the incidence of metabolic syndrome is somehow controversial. Therefore this research was conducted to investigate the association between the intake of Turkish and Ceylon tea on the development of metabolic syndrome in albino rats through the observation of biochemical parameters.

Procurement of sample

The Turkish and Ceylon tea used in this research were obtained from Abuja and Kano respectively. They were stored at room temperature free from contact with moisture for the entire period of the study.

Preparation of aqueous tea extracts for phytochemical evaluation

Turkish tea (4g) and Ceylon tea (4g) were boiled in distilled water ($500cm^3$) for 10 minutes on a heating mantle. The mixtures were filtered and the resulting filtrates were used for phytochemical evaluation.

Preparation of aqueous Tea extracts for oral administration

Turkish tea (2g) and Ceylon tea (2g) were boiled in distilled water ($500cm^3$) for 10 minutes on a heating mantle. The volume and concentration administered were determined based on the average weight of the animals in to relation daily tea consumption of a 70kg man.

Experimental animals

Fifty albino rats (90-110g) were purchased from the Department of Biological Sciences, Bayero

University, kano and kept there; under laboratory condition and supplied food (vital feed; growers palletized feeds) and water for seven weeks (i.e. for the period of the research).

Experimental design

Albino rats (50) were divided into six groups. Group 1 and 4 comprise six (6) rats each, were not administered any of the teas under study and served as the positive and negative control respectively. The experimental groups (group 2, 3, 5 and 6) comprise ten (10) rats each. Rats in group 2 and 5were administered Ceylon tea orally, at concentration of (2.8mg/kg) body weight for seven weeks. Rats in group 3 and 6 were administered Turkish tea of same dose for same period. Rats in group 4, 5 and 6 were receiving tap water supplemented daily with 10% w/v fructose. At the end of seven weeks, the rats were sacrificed. Blood samples were collected in lithium heparin tubes and centrifuged at 3000 rpm for 10 minutes. Serum samples were separated for subsequent biochemical analysis.

Phytochemical analysis

Phytochemical evaluation (qualitative tests) for detection of phytocostituents from the aqueous tea extracts were carried out using standard procedures[6-10].

Biochemical analysis

The Serum Total- cholesterol, HDL-cholesterol,Serum Triglyceride and LDL-cholesterol, were determined respectively by standard analytical methods[11-14]. Fasting blood glucose level was estimated by glucose oxidase method in each case[15]. Serum uric acid was estimated by uricase method as describe[16].Colorimetric measurements (related to absorbance) were carried out using Ultrospec Plus 4054 UV/visible spectrophotometer (LKB Model Biochrom,Cambridge,England). All incubations were done using Heto water bath (type 11AT No496980A HetoHou ALS Gydevang 17-19DK-3450 Adered, Denmark).

Statistical analysis

Values obtained in table 3 were analyzed statistically using student's t-test; while values in table 4 and 5 were analyzed using ANOVA statistical analysis test. Instat statistical software was used for the analysis.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis revealed the presence of Cardiac glycosides, flavonoids, resins, saponins, tannins and terpenoid in the two brands of tea analyzed. Anthraquinone was detected in only sample A. However, alkaloids and chlorogenic acids were not present in both samples. Quantitative phytochemical evaluation showed that sample A had higher content of all the phytochemicals evaluated as well as Vitamin C.

Table 1: Qualitative phytochemical screening of Turkish and Ceylon tea

Phytochemicals	Sample A	Sample B
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Cardiac glycosides	+	-
Alkaloids	-	-
Anthraquinones	+	-
Chlorogenic acid	-	-
Resins	+	+

+=present; -=absent. Sample A=Turkish tea; Sample B=Ceylon tea.

Table 2: Quantitative phytochemical evaluation of Turkish and Ceylon tea

Phytochemicals	Sample A	Sample B
Tannins	0.63±0.01	0.53±0.01
Saponins	0.12±0.01	0.06±0.01
Flavonoids	0.06±0.01	0.05±0.01
Glycosides	0.59±0.01	0.35±0.01

Values are presented as mean ± standard deviation

Table 3: Fasting blood sugar, uric acid levels and serum lipid profile of rats administered aqueous extract of Turkish and Ceylon tea.

Parameters	Group A (N=25)	Group B (N=25)
FBS (mmol/L)	4.84^{a}±0.34	6.86^{a}±0.46
Uric Acid (mmol/L)	0.25^{b}±0.04	0.48^{b} ±0.02
TC (mmol/L)	3.7^{c}± 0.46	5.42^{c}±0.22
HDL-C (mmol/L)	1.15±0.08	1.08±0.09
LDL-C (mmol/L)	2.21^{d}±0.43	3.44^{d}±0.22
VLDL (mmol/L)	0.36^{f}±0.04	0.89^{f}±0.05
TG (mmol/L)	0.80^{g}±0.10	1.96^{g}±0.11

Results are presented as mean±standard deviation, figures in same column bearing same superscript are significant at P<0.05.
A =group of rats taking tap water; B= group of rats taking tap water supplemented with 10%w/v fructose.

Table 3 is a summary of all the biochemical parameters estimated in this research. It presents the mean fasting blood sugar, serum uric acid levels and serum lipid profiles of all the rats under study. Group A comprise the rats taking tap water and group B represents the rats taking tap water supplemented with 10%w/v fructose. The results show significant difference in all the biochemical parameters estimated except HDL-cholesterol. This confirms the fact from previous report that fructose is potent in inducing metabolic syndrome[16,17]. This is because fructose has the capacity of elevating blood sugar levels, uric acid levels, total cholesterol levels and LDL- cholesterol levels. All these aforementioned

biochemical parameters have been implicated in the incidence and development of metabolic syndrome.

Fructose ingested is metabolized principally in the liver. In this organ, fructose is converted to fructose-1-phosphate in a reaction catalyzed by fructokinase.Fructose-1-phosphate is fragmented to dihyroxyacetone phosphate and glyceraldehyde. This result in a bypass of a main regulatory step of glycolysis (phosphofructokinase catalyzed reaction).The implication is that while glucose can be negatively regulated, fructose can continuously enter the glycolytic pathway. This leads to continuous production of glucose, glycogen, lactate and pyruvate providing glycerol and acylglycerol molecules.

Table 4: Serum lipid profile of rats administered aqueous extract of Turkish and Ceylon tea.

Groups	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TG (mmol/L)
(Positive Control) N=5	4.61 ±0.13	1.21± 0.03	3.00± 0.15	0.40± 0.05	0.88± 0.11
Group 2 N=10	*3.51 ±0.06	1.12± 0.06	*2.04± 0.09	0.34± 0.04	0.79± 0.08
Group 3 N=10	*3.50 ±0.08	1.15± 0.18	*1.99± 0.18	0.35± 0.04	0.78± 0.11
Group 4 (Negative Control) N=5	5.83 ±0.14	1.08± 0.06	3.80± 0.15	0.94± 0.03	2.07± 0.07
Group 5 N=10	**5.32 ±0.03	1.07± 0.09	**3.37± 0.09	0.89± 0.05	1.95± 0.10
Group 6 N=10	**5.31 ±0.04	1.09± 0.10	**3.34± 0.13	0.88± 0.04	1.94± 0.10

Results are presented as mean± standard deviation, figures with single * differ significantly from positive control at P<0.05; while figures with double * differ significantly from negative control at P<0.05.N= number of animals.

Table 5: Fasting blood sugar and serum uric acid levels of rats administered aqueous extract of Turkish and Ceylon tea.

Groups	FBS (mmol/L)	Uric Acid (mmol/L)
Group 1 (Positive Control) N=5	5.41±0.29	0.27±0.03
Group 2 (N=10)	*4.76±.15	0.25±0.04
Group 3 (N=10)	*4.63±0.15	*0.24±0.04
Group 4 (Negative Control) N=10	7.70±0.28	0.50±0.01
Group 5 (N=10)	**6.66±0.13	0.47±0.02
Group 6 (N=10)	**6.64±0.13	**0.46±0.02

Results are mean±standard deviation, figures with single * differ significantly from positive control at P< 0.05; while figures with double * differ significantly from negative control at P<0.05.N= number of animals.

Fructose does not stimulate release of insulin upon ingestion but glucose does. Insulin regulated leptin will therefore have reduced concentration and a decreased effect on reducing appetite. This limited effect on appetite suppression of fructose coupled with the favored hepatic metabolism of fructose into lipid subsequently leads to weight gain, hyperinsulinemia and associated insulin resistance. Hormonal and physiological changes illustrate connections between energy intake, appetite control, weight gain and low circulating leptin, insulin and ghrelin in population consuming energy from this nutrient[18].

From table 4, the mean serum Total-cholesterol, LDL-cholesterol and triglyceride of the positive and negative control differ significantly at P<0.05. This implies that hepatic metabolism of fructose favors *De novo* lipogenesis. Liver metabolize dietary fructose to generate glycerol-3-phosphate which favors the esterification of unbound free fatty acid to form triglyceride. Fructose therefore leads to overproduction of triglyceride and inadequate clearance which is implicated in elevated T-cholesterol and LDL-cholesterol. The mean T-cholesterol (TC) and LDL-cholesterol (LDL-C) of rats in group 2 and 3 differ from the positive control; while that of rats in group 5 and 6 also differ from negative control at p<0.05. The reduced mean TC and LDL-C is indicative of the hypocholesterolemic effect of both teas administered [19].Reduced serum TC and LDL-C have been reported to be beneficial with regards to the development of cardiovascular disease[20].

Table 5 presents the mean fasting blood sugar (FBS) and serum uric acid levels. The mean FBS of the rats in group 2 and 3 differ from the positive control; while that of rats in group 5 and 6 differ from the negative control at P<0.05.The hypoglycemic effect exhibited by both teas confirms previous reports of hypoglycemic effect of tea[21,19] and .The mean serum uric acid levels of positive and negative control differ significantly at P<0.05. This difference shows that fructose raises uric acid levels and elevated uric acid predicts the development of hyperinsulinemia, hypertension, obesity and subsequently metabolic syndrome[18].The mechanism by which fructose raises uric acid levels can be partially accounted for as follows; ingested fructose enters the hepatocytes where it is phosphorylated by fructokinase to fructose-1-phosphate. During this reaction, ATP donates phosphate generating ADP which is catabolized to uric acid.

Fructose induced hyperuricemia predicts the development of hyperinsulinemia, hypertension, diabetes, stroke, cardio-renal diseases and subsequently metabolic syndrome[18].Several mechanisms have been put forward to explain how elevated uric acid levels account for the various components of metabolic syndrome. This includes; reduction or inhibition of bioavailability of nitric oxide, induction of endothelial dysfunction, increase hepatic production and decrease urinary excretion

due to vaso-renal constriction and endothelial dysfunction as well as activation of rennin-angiotensin system[22].

CONCLUSION

Dietary habits play a vital role in the risk of developing a variety of diseases; especially cardiovascular, cardio-renal disease and cancer. The use of dietary substances as a practical approach to reducing the risk of developing these diseases is receiving increasing attention. The utilization of tea and tea products is promising. Epidemiologic and laboratory studies suggest that tea consumption may have beneficial effects in reducing certain disorders which the present study also confirms. The reduction in serum T-Cholesterol, LDL-Cholesterol, fasting blood sugar and uric acid levels is a pointer to this claim. However, these observations and inferences are based on taking these teas without sucrose and milk.

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