

Hypoglycaemic and Hypolipidemic Effects of Black Brand of Lipton Tea (*Camellia sinensis*) on Normal Male Albino Rats

E.C. ANORUE¹, E.C. MBEGBU^{2*}, G.I. NGWU¹, K.N. IBEMENUGA³,
J.E. EYO¹

¹University of Nigeria, Department of Zoology and Environmental Biology, Nsukka, Enugu State, Nigeria; edmund.mbegbu@unn.edu.ng (*corresponding author)

²University of Nigeria, Department of Veterinary Physiology and Pharmacology, Nsukka, Enugu State, Nigeria

³Anambra State University, Department of Biological Sciences, Uli, Anambra State, Nigeria

Abstract

Recently, considerable attention has been focused on dietary supplements that possess hypoglycaemic and hypolipidemic properties in lieu of the synergistically synthesized drugs like statins and metiform which have been proven to have adverse effects. This present work was carried out to study the potential effects of Lipton tea aqueous extract (LTAE) on normal male albino rats. 36 six male Wistar rats weighing 115 - 150g were assigned into four main groups; each group was divided into three subgroups, consisting of three replicates namely R1, R2 and R3 making a total of nine rats in each of the main groups. Group 1 served as the control and received only water, Group 2 received 200 mg/kg of LTAE, Group 3 received 400mg/kg of the LTAE, while Group 4 received 800 mg/kg of LTAE. All treatment was given orally on daily basis for 28 consecutive days, but the parameters were assessed on weekly basis. The result revealed that, weight gain, fasting blood sugar and serum (total cholesterol, triglycerides, LDL-c and VLDL-c) significantly ($p < 0.05$) decrease in all the treated groups while HDL-c significantly ($p < 0.05$) increased when compared to the control group. The Lipton tea aqueous extract induced both a dose-dependent and a time-dependent effect in the parameters measured, apart from the total cholesterol which did not show a dose-dependent effect. This led to an overall decrease in the atherogenic index and coronary risk index, suggesting that LTAE possesses hypoglycaemic and hypolipidemic activities in normal male rats.

Keywords: atherogenic index; *Camellia sinensis*; coronary risk index; hypoglycaemia; rats

Introduction

Diabetes mellitus (DM) is the term used to refer to impairment in insulin metabolism (Aizaki *et al.*, 1999). Despite major investigations into understanding the pathophysiology and treatment of diabetes mellitus, it has remained a major health problem worldwide (Baynes and Thorpe, 1999). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Broadhurst *et al.*, 2000). The national diabetes information clearing house of the United States of America estimates that diabetes costs \$132 billion in the United States alone every year, and that one in three Americans born after year 2000 may develop diabetes in their lifetime (Broadhurst *et al.*, 2000).

There are several forms of diabetes mellitus but of all the forms, type 1 and type 2 are the major forms of diabetes mellitus and is the leading cause of abnormalities in the metabolism of carbohydrates and lipids in the body (Allain

et al., 1974; Eyo *et al.*, 2011). Lipid and carbohydrates are important metabolites in the body and abnormalities in their metabolism can lead to development and progression of many diseases and eventually mortality if not properly treated (Al Jamal, 2014). The abnormality in the regulation of these metabolites in the body leads to increase in their amount in the body, a condition referred to as hyperglycaemia and hyperlipidemia, leading to multifarious group of chronic disorders and morbidity in the body (Willett *et al.*, 2002; Abe *et al.*, 2005). Ahmed (2005) opined that several chronic diseases have been associated with an increased level of glucose and lipid in the body.

DM equally may be due to abnormalities in lipid metabolism, such as elevated circulating levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and usually accompanied by decreased circulating levels of high density lipoprotein cholesterol (HDL-c) (Murray *et al.*, 2014). These can lead to other complications such as obesity, heart attack, stroke,

hypertension and other related cardiovascular diseases (Cameron *et al.*, 2008).

Insulin is a key hormone in the regulation of carbohydrates, lipids and protein metabolism in the liver (Ahmad *et al.*, 1997). It is a hormone produced by the β -cells of islet of Langerhans in the pancreas, an organ located behind the stomach (Nelson *et al.*, 2008). Type 1 diabetes is also known as insulin deficiency, while type 2 is known as insulin resistance (King *et al.*, 1998). Based on the current understanding of pathophysiology of insulin resistance and insulin deficiency, multiple pharmacological and non-pharmacological interventions have been developed with the aim of improving both glycaemic and lipidemic control and prevention of diabetes complications.

Recently, the use of functional foods and their bioactive components have been considered as a new approach in the prevention and management of diabetes and its complications (Ozougwu *et al.*, 2008; Ozougwu and Eyo, 2010; 2011). Thus this has been an ever increasing area of research for scientist so as to find out diets that will help alleviate the skyrocketing effects of diabetes mellitus and other diseases in the society, especially in the face of the fact that efficacy and safety of known synthetic drugs are dwindling (Ezuruike and Prieto, 2014).

Camellia sinensis is a species of evergreen shrub whose leaves and leaf buds are used to produce tea (Li *et al.*, 2013). It is of flowering plants genus *Camellia* in the family Theaceae (Li *et al.*, 2013). Lipton tea (*Camellia sinensis*) is one of such dietary component of plant origin with folklore claim of lowering blood sugar and body fats among other health benefits. It has also been reported to possess anti-carcinogenic, anti-inflammatory, anti-mutagenic and anti-oxidative activities (Hasanein *et al.*, 2012). Generally the health benefits associated with black brand of Lipton tea (yellow label) are believed to be due to presence of high level of polyphenols as well as flavonoids (Hasanein *et al.*, 2012). These phytochemicals have the reputation of lowering the blood sugar and fatty acids (Dulloo *et al.*, 2000).

The present study therefore was designed to determine the hypoglycaemic and hypolipidaemic effects of Lipton tea, with respect to body weight, fasting blood sugar concentration, lipid profile, antherogenic index and coronary risk index of normal male albino rats.

Materials and Methods

Procurement of Lipton tea and extraction

Lipton yellow label tea, Unilever (*Camellia sinensis*) was purchased from Ogige market, Nsukka, Nigeria. Aqueous extract of the Lipton tea on daily basis was gotten by extracting 8 Lipton tea bags in 100 ml of hot distilled water. The aqueous extract was evaporated to dryness in a rotary evaporator (Stuart, model RE-300, UK). Concentration was determined thus:

$$\text{Extract concentration (mg/ml)} = (W_2 - W_1) / 100$$

Where: W1 (mg) = Weight of beaker and W2 (mg) = Weight of beaker + 100 ml of extract evaporated to dryness.

Animals

Thirty six (36) healthy male albino Wistar rats (*Rattus*

norvegicus) weighing 115 - 150 g were used for the study. They were purchased from the animal house of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were housed in clean and dry metal cages under 12 hours conditions of light, temperature (24 ± 2) and relative humidity ($55 \pm 5\%$). The animals were fed standard rat chow made up of 15% crude protein, and water *ad libitum*. Before sampling, the animals were fasted overnight but allowed free access to water. The ethical committee of the university approved the study protocol prior to commencement of the study. The study was carried out in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research (APA 2012).

Experimental design

The experimental setup was done using completely randomized block design. A total of thirty six rats were used for the study. The rats were randomly assigned into four (4) experimental groups (one control and three treatments). Each group was further divided into three replicates of 3 rats each. Group one served as control and received only distilled water, groups 2 - 4 received graded doses (200, 400 and 800 mg/kg respectively) of LTAE. All treatments were given on daily basis for 28 consecutive days. The body weight, lipid profile and the glucose level were monitored weekly for 4 weeks of experimentation.

Measurement of body weight

Body weights of the rats were measured weekly using electronic top loading balance (Mettler Toledo Series) to the nearest 0.01 g. The body weight gain and loose in grams was determined using the formulae; final weight - initial weight.

Blood collection and determination of fasting blood sugar

Blood samples for fasting blood sugar (mg/dl) were collected through the tail vein dripped directly into Accu Chek glucometer (Trinder, 1969). Blood samples for lipid profile were collected from the rats via the retrobulbar plexus at the median canthus of the eye (Parasuraman *et al.*, 2010), allowed to clot inside test tube and centrifuged at 10,000 rpm for 5 minutes; the supernatant (serum) was separated and poured into another test tube. The lipid profile was later measured using the serum.

Determination of total cholesterol concentration

The total cholesterol was determined after enzymatic hydrolysis and oxidation, according to the method described by Richmond (1973) using the Randox Kit for total cholesterol (Randox Laboratories Limited, London). One ml cholesterol reagent (R1) was put into two test tubes, labelled test and standard. Serum sample and standard cholesterol (10 μ l respectively) was added into the appropriate test tube, well mixed and incubated at room temperature for 10 minutes. Absorbencies were taken using a colorimeter, set at 500 nm.

The concentration of cholesterol (mmol/l) =
(Absorbance of standard \times Concentration of standard) / Absorbance of test samples

Determination of triglyceride (TG)

The triglyceride was determined after enzymatic hydrolysis with lipases according to the method described by Fossati and Prencipe (1982) is using the Randox Kit for triglyceride (Randox Laboratories limited, London). One ml triglyceride reagent (R1) was pipette into test tube, labelled test and standard. Serum sample and standard triglyceride (10 µl respectively) was added into the appropriate test tubes. It was mixed and incubated at room temperature for 10 minutes. Absorbances were taken using a calorimeter, set at 500 nm.

The concentration of triglyceride (mmol/l) = (Absorbance of standard × Concentration of standard)/Absorbance of test samples.

Determination of high density lipoprotein cholesterol (HDL-c)

The high density lipoprotein cholesterol was determined after using MgCl₂ and dextran sulfate to precipitate all low-density and very-low-density lipoproteins according to the method described by Finley et al. (1978) using the Randox Kit for high density lipoprotein cholesterol (Randox Laboratories Limited, London). 20 µl of serum was pipette into two test tube, labelled test and standard. Diluted precipitate (R1) (500 µl) was added into the appropriate test tubes. It was mixed and incubated at room temperature for 10 minutes. It was centrifuged for 10 minutes at 4000 rpm. The clear supernatant was put into other test tubes. Then 1ml cholesterol reagent (R1) was put into test tubes, labelled test and standard. Serum sample and standard cholesterol (10 µl respectively) was added into the appropriate test tube. It was mixed and incubated at room temperature for 10 minutes. Absorbencies were taken using a colorimeter, set at 500 nm.

The concentration of HDL-cholesterol (mmol/l) = (Absorbance of standard × Concentration of standard)/Absorbance of test samples

Determination of very low density lipoprotein-cholesterol (VLDL-c)

The very low density lipoprotein-cholesterol (VLDL-c) can be determined using the formula: VLDL-cholesterol (mmol/l) = Triglyceride ÷ 5 according to Friedewald et al. (1972).

Determination of low density lipoprotein-cholesterol (LDL-c)

The low density lipoprotein-cholesterol (LDL-c) was determined using the formula below:

LDL-cholesterol (mmol/l)) = Total cholesterol - (HDL-c + VLDL-c), according to Friedewald et al. (1972).

Determination of atherogenic index (AI)

Atherogenic index (AI) was calculated using the equation described by Adeneye and Olagunju (2009): AI = LDL-cholesterol/HDL-cholesterol.

Determination of coronary risk index (CRI)

Coronary Risk Index (CRI) was calculated using the equation described by Adeneye and Olagunju (2009): CRI = Total cholesterol/HDL-cholesterol.

Statistical analysis

Data obtained were analyzed using analysis of variance by employing the Statistical Package for the Social Sciences (SPSS version 18 for windows). Differences between means were separated using Duncan's Multiple Range Test (Duncan 1955). Statistical significance was set at p < 0.05. Results were reported as mean ± SEM.

Results

Concentration of the Lipton extract

The concentration of the extract was calculated as = 40 mg/ml.

Effect of Lipton tea aqueous extract on body weight gain (g) of normal albino rat

The result of the effect of LTAE on body weight gain showed that all treatments significantly (p < 0.05) precipitated dose-dependent decrease and time-dependent increase in body weight gain of the treated rats from week 1 to week 4 when compared to the control (Fig. 1).

Effect of Lipton tea aqueous extract on fasting blood sugar (mg/dl) of normal albino rats

The result of the effect of LTAE on fasting blood sugar level of normal albino rats showed that the extract significantly (p < 0.05) decreased the fasting blood sugar of the treated rats in both time dependent and dose dependent manner. The group treated with 800 mg/kg had the highest reduction in fasting blood sugar when compared to the 400 and 200 mg/kg doses except for week 2 and week 3. Equally, the median and low doses of the LTAE significantly (p < 0.05) reduced the fasting blood sugar when compared to the control. Generally, all the doses of the extract precipitated significant (p < 0.05) reduction in the fasting blood sugar (Fig. 2).

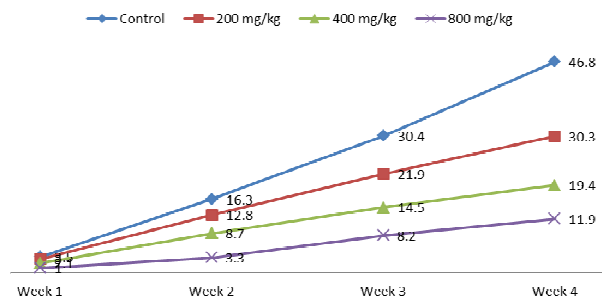


Fig. 1. Effect of Lipton tea aqueous extract on body weight gain of normal albino rat

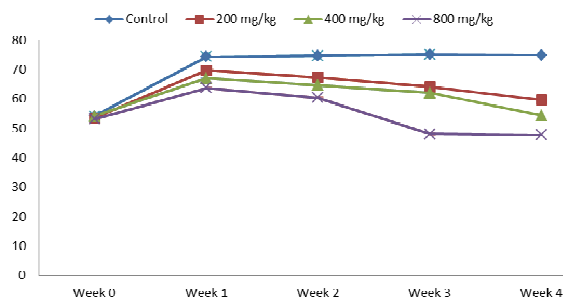


Fig. 2. Effect of Lipton tea aqueous extract on fasting blood sugar (mg/dl) of normal albino rats

Effect of Lipton tea aqueous extract on total cholesterol (mmol/l) of normal albino rats

The result of the effect of LTAE on total cholesterol level showed that the extract caused a time- dependent decrease in total cholesterol level as from week 2 of the experiment. However, the different doses of the Lipton tea aqueous extract used in this study significantly ($p < 0.05$) reduced the total cholesterol level from week 2 to week 4 when compared to the control. There is no significant ($p > 0.05$) difference among the treated doses except for the group treated with 800 mg/kg at week 3 where total cholesterol level was significantly ($p < 0.05$) lower than that of other treatment groups. The total cholesterol level of the control group increased significantly ($p < 0.05$) throughout the period of the experiment (Table 1).

Effect of Lipton tea aqueous extract on triglycerides (mmol/l) of normal albino rats

The result of LTAE on triglycerides showed that all the doses of the extract significantly ($p < 0.05$) decreased the serum triglycerides from week 3 of the experiment when compared to the control group. However, LTAE generally decreased ($p < 0.05$) the triglycerides in a time-dependent

manner from week 3 of the experiment (Table 2).

Effect of Lipton tea aqueous extract on HDL-C (mmol/l) of normal albino rats

The result of the effect of LTAE on high density lipoprotein cholesterol showed that the extract significantly ($p < 0.05$) increased the high density lipoprotein cholesterol of the treated rats in both time-dependent and dose-dependent manner when compared to the control. The extract at the highest dose (800 mg/kg) by week 4 gave the highest high density lipoprotein cholesterol value of 2.40 mmol/l (Table 3).

Effect of Lipton tea aqueous extract on LDL-C (mmol/l) of normal albino rats

The result of the effect of LTAE on low density lipoprotein cholesterol showed that the extract significantly ($p < 0.05$) reduced the low density lipoprotein cholesterol value of the treated rats in both time-dependent and dose-dependent manner when compared to the control. The LTAE at the highest dose (800 mg/kg) by week 4 gave the lowest low density lipoprotein cholesterol (0.76 mmol/l) (Table 4).

Table 1. Effect of Lipton tea aqueous extract on total cholesterol (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	4.2 ± 0.06 ^{a1}	4.8 ± 0.12 ^{b1}	4.9 ± 0.19 ^{bc1}	5.0 ± 0.17 ^{bc1}	5.2 ± 0.23 ^{c1}
200	4.1 ± 0.06 ^{a1}	4.7 ± 0.17 ^{b1}	4.6 ± 0.27 ^{b12}	4.2 ± 0.17 ^{ab2}	3.5 ± 0.17 ^{c2}
400	4.2 ± 0.12 ^{a1}	4.6 ± 0.12 ^{b1}	4.5 ± 0.15 ^{b2}	4.0 ± 0.23 ^{a2}	3.4 ± 0.17 ^{c2}
800	4.2 ± 0.06 ^{a1}	4.8 ± 0.60 ^{a1}	4.4 ± 0.18 ^{a2}	3.4 ± 0.19 ^{b3}	3.3 ± 0.15 ^{b2}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Table 2. Effect of Lipton tea aqueous extract on triglycerides of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	1.77 ± 0.03 ^{a1}	1.70 ± 0.12 ^{ab1}	1.60 ± 0.12 ^{b1}	1.70 ± 0.12 ^{ab1}	1.60 ± 0.12 ^{b1}
200	1.76 ± 0.03 ^{a1}	1.60 ± 0.12 ^{a1}	1.50 ± 0.06 ^{bc12}	1.40 ± 0.06 ^{c2}	1.30 ± 0.17 ^{c2}
400	1.63 ± 0.15 ^{a1}	1.70 ± 0.12 ^{a1}	1.40 ± 0.06 ^{b2}	1.20 ± 0.06 ^{c3}	0.90 ± 0.06 ^{d3}
800	1.70 ± 0.10 ^{a1}	1.60 ± 0.12 ^{a1}	1.30 ± 0.17 ^{b2}	1.00 ± 0.12 ^{c4}	0.73 ± 0.15 ^{c3}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Table 3. Effect of Lipton tea aqueous extract on HDL-C (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	0.80 ± 0.12 ^{a1}	1.30 ± 0.17 ^{b1}	1.20 ± 0.06 ^{b1}	1.00 ± 0.06 ^{c1}	0.80 ± 0.06 ^{a1}
200	0.93 ± 0.15 ^{a1}	1.40 ± 0.06 ^{b1}	1.53 ± 0.15 ^{bc2}	1.70 ± 0.12 ^{c2}	1.90 ± 0.06 ^{d2}
400	1.00 ± 0.17 ^{a1}	1.50 ± 0.12 ^{b12}	1.60 ± 0.17 ^{bc2}	1.80 ± 0.12 ^{c2}	2.10 ± 0.12 ^{d3}
800	1.00 ± 0.12 ^{a1}	1.60 ± 12 ^{b2}	1.70 ± 0.12 ^{b2}	2.10 ± 0.60 ^{c3}	2.40 ± 0.21 ^{d3}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Table 4. Effect of Lipton tea aqueous extract on LDL-C (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	3.06 ± 0.01 ^{a1}	2.90 ± 0.04 ^{b1}	2.88 ± 0.01 ^{b1}	2.90 ± 0.01 ^{b1}	2.88 ± 0.01 ^{b1}
200	3.04 ± 0.01 ^{a1}	2.83 ± 0.10 ^{b12}	2.70 ± 0.06 ^{b2}	2.22 ± 0.01 ^{c2}	1.34 ± 0.01 ^{d2}
400	3.06 ± 0.01 ^{a1}	2.76 ± 0.01 ^{b2}	2.62 ± 0.01 ^{c3}	1.96 ± 0.01 ^{d3}	1.12 ± 0.01 ^{e3}
800	3.05 ± 0.02 ^{a1}	2.58 ± 0.01 ^{b3}	2.44 ± 0.02 ^{c4}	1.60 ± 0.02 ^{d4}	0.76 ± 0.02 ^{e4}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Effect of Lipton tea aqueous extract on VLDL-C (mmol/l) of normal albino rats

The result of the effect of LTAE on very low density lipoprotein cholesterol showed that the extract significantly ($p < 0.05$) reduced the very low density lipoprotein cholesterol value of the treated rats in both time-dependent and dose-dependent manner from week 2 to week 4 of the experiment. As a result the highest dose of the extract by week 4 gave the lowest value of 0.14 mmol/l (Table 5).

Effect of Lipton tea aqueous extract on atherogenic index (mmol/l) of normal albino rats

The result of the effect of LTAE on atherogenic index showed that the extract significantly ($p < 0.05$) reduced the atherogenic index of the treated groups in both time-dependent and dose-dependent manner when compared to the control. The LTAE at the highest dose of 800 mg/kg by week 4 gave the lowest atherogenic index of 0.28 mmol/l (Table 6).

Effect of Lipton tea aqueous extract on coronary risk index (mmol/l) of normal albino rats

The result of the effect of LTAE on coronary risk index showed that the extract significantly ($p < 0.05$) reduced the coronary risk index of the treated rats in both time-dependent and dose-dependent manner when compared to the control.

The LTAE at the highest dose of 800 mg/kg by week 4 gave the lowest coronary risk index of 1.38 mmol/l (Table 7).

Discussion

In the present study, treatment with Lipton tea aqueous extract (LTAE) on normal albino rats produced significant decrease in weight gain of the treated groups with the group treated with the highest dose of LTAE having the highest decrease, followed by the group treated with the median dose and finally the group treated with the lowest dose having the lowest decrease.

All treated group with different doses of LTAE showed significant decrease in final weight gain as compared to the control group. This could be due to decrease in feed intake observed throughout the period of the experimentation. Wang *et al.* (2006) opined that the decrease in feed intake could be due to the presence of caffeine in LTAE which reduces the level of serum leptin and decreased their appetite for food which in turn had significant reductive effect on weight gain. It could also be due to the presence of catechins and caffeine which are effective promoters of thermogenesis which invariably impacts on the hypothalamus, and fat oxidation, resulting in reduction in body weight gain (Khan and Mukhtar, 2013; Yashin *et al.*, 2013).

Table 5. Effect of Lipton tea aqueous extract on VLDL-C (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	0.34 ± 0.01 ^{a1}	0.34 ± 0.01 ^{a1}	0.32 ± 0.02 ^{a1}	0.34 ± 0.01 ^{a1}	0.34 ± 0.01 ^{a1}
200	0.36 ± 0.02 ^{a1}	0.34 ± 0.01 ^{a1}	0.30 ± 0.01 ^{b1}	0.28 ± 0.01 ^{bc2}	0.26 ± 0.01 ^{c2}
400	0.34 ± 0.01 ^{a1}	0.32 ± 0.02 ^{a1}	0.28 ± 0.12 ^{b12}	0.24 ± 0.01 ^{c3}	0.18 ± 0.02 ^{d3}
800	0.32 ± 0.02 ^{a1}	0.32 ± 0.02 ^{a1}	0.26 ± 0.01 ^{b2}	0.20 ± 0.01 ^{c4}	0.14 ± 0.02 ^{d3}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Table 6. Effect of Lipton tea aqueous extract on atherogenic index (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	3.16 ± 0.01 ^{a1}	3.16 ± 0.01 ^{a1}	3.16 ± 0.02 ^{ab1}	3.17 ± 0.09 ^{ab1}	3.21 ± 0.03 ^{b1}
200	3.16 ± 0.01 ^{a1}	2.13 ± 0.02 ^{b2}	1.89 ± 0.01 ^{c2}	1.31 ± 0.01 ^{d2}	0.71 ± 0.01 ^{e2}
400	3.18 ± 0.01 ^{a1}	1.84 ± 0.01 ^{b3}	1.64 ± 0.02 ^{c3}	1.09 ± 0.02 ^{d3}	0.53 ± 0.02 ^{e3}
800	3.17 ± 0.01 ^{a1}	1.61 ± 0.01 ^{b4}	1.44 ± 0.01 ^{c4}	0.76 ± 0.01 ^{d4}	0.28 ± 0.01 ^{e4}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

Table 7. Effect of Lipton tea aqueous extract on coronary risk index (mmol/l) of normal albino rats

Dose (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
0.00 (Control)	3.69 ± 0.09 ^{a1}	3.69 ± 0.01 ^{a1}	3.69 ± 0.03 ^{ab1}	3.69 ± 0.01 ^{a1}	3.53 ± 0.09 ^{b1}
200	3.65 ± 0.03 ^{a1}	3.36 ± 0.01 ^{b2}	3.07 ± 0.02 ^{c2}	2.47 ± 0.01 ^{d2}	1.84 ± 0.02 ^{e2}
400	3.69 ± 0.08 ^{a1}	3.07 ± 0.01 ^{b2}	2.81 ± 0.01 ^{c3}	2.22 ± 0.01 ^{d3}	1.62 ± 0.01 ^{e3}
800	3.53 ± 0.09 ^{a1}	2.81 ± 0.01 ^{b4}	2.59 ± 0.03 ^{c4}	1.86 ± 0.02 ^{d4}	1.38 ± 0.02 ^{e4}

Mean values with different numbers as superscripts in a column are significant ($p \leq 0.05$). Mean values with different alphabets as superscripts in a row are significant ($p \leq 0.05$).

In human study, it has been shown that green tea helps to reduce obesity when consumed on daily basis. Anti-obesity effect of green tea has been associated with its content of caffeine and catechins particularly (-)-epigallocatechin-3-gallate (EGCG) (Al-Salafe *et al.*, 2014). Their findings showed a reduction in body weight and visceral fat after the period of experimentation. In another study, the consumption of two servings of an extra high-catechin green tea led to improvements in body composition and reduced abdominal fatness in moderately overweight Chinese subjects (Wang *et al.*, 2010). The result of the present study is in support of an earlier study that reported that the body weights of rats and their cholesterol, low-density lipoprotein cholesterol and plasma triglyceride were significantly reduced by feedings of green tea leaves to the rats (Lin and Lin-Shiau, 2006). Wickelgren (1998) also associated this to presence of caffeine in black tea which decrease food intake and increased thermogenesis and that the thermogenic effect induced the body weight reduction. Cao *et al.* (2004) further noted that the thermogenesis by caffeine was synergistically enhanced with catechins in rat adipose tissue. Dulloo *et al.* (2000) opined that caffeine ingestion elevated the metabolic rate and fat oxidation *in vivo* through lipolysis in fat cells and release of catecholamine. Bose *et al.* (2008) reported that mice fed diets supplemented with Lipton tea extract had significantly decreased food intake and subsequently decreased body weight and fat mass. Dulloo *et al.* (2000) added that serum leptin levels were also lowered in mice fed with diet supplemented with Lipton tea and decreased their appetite for food which in turn had significant reductive effect on their final weight gain as compared to the control. Ikeda *et al.* (1992) gave credit to this when he reported that a diet containing Lipton tea catechins at 0.2% showed a weight reductive action in mice at 27 weeks of feeding. On the other hand, Bailey and Day (1989) noted that Lipton tea reduced body weight gain in normal Thai subjects by increasing energy expenditure and fat oxidation. Wang *et al.* (2006) also suggested that catechin, polyphenols and caffeine which were present in Lipton tea may be effective promoters of thermogenesis and fat oxidation, resulting in reduction in body weight gain in Caucasians, Chinese and Japanese.

The result of this study further revealed that the LTAE caused significant decrease in fasting blood sugar for all the treated groups as compared to the control. This could be due to the presence of the phytochemicals such as flavonoids which have been suggested to directly improve the beta-cells of the pancreas (Mita *et al.*, 1985). It could also be due to the presence of caffeine, which has been suggested to cause reduction in food intake due to loss of appetite (Harbowy *et al.*, 1997). As a consequence, the liver could not metabolize any dietary glucose and this in turn led to decrease in fasting blood sugar. From the study of Anderson and Polansky (2002), fasting blood sugar reductive effect of Lipton tea may be due to their increase insulin stimulatory effect on the β -cells of the pancreas. Cameron *et al.* (2008) added that it might be due to inhibition of renal glucose reabsorption. Baynes and Thorpe (1999) purported that it is due to increase stimulation of

glucose uptake by peripheral tissues and inhibition of intestinal glucose absorption, while Ikeda (1992) connected it to the inhibition of endogenous glucose production. Furthermore, Lipton tea fasting blood sugar lowering effect can be attributed to the presence of polyphenolic compounds which have been suggested to have hypoglycaemic effect (Hasanein *et al.*, 2012). The hypoglycaemic effect of the polyphenols found in Lipton tea are mainly attributed to reduce intestinal absorption of dietary carbohydrate, modulation of the enzymes involved in glucose metabolism, modulation of intracellular signalling pathways and gene expression and increase glucose uptake in insulin-sensitive tissues (Mita *et al.*, 1985). One of the most well-known properties of these polyphenols found in Lipton tea on carbohydrate metabolism is inhibition of α -glucosidase and α -amylase, the key enzymes responsible for digestion of dietary carbohydrate to glucose (Khan and Mukhtar, 2007). Lipton tea polyphenols including the theaflavin, thearubigen, flavonoids and catechins could interfere with absorption of glucose from intestine through inhibition of sodium-dependent glucose transporters (SGLT), such as SGLT1 and SGLT2 (Leung *et al.*, 2001). Lipton tea polyphenols are able to regulate the key pathway of carbohydrate metabolism and hepatic glucose homeostasis. These polyphenols as contained in Lipton tea have been reported to attenuate hepatic glucose output through down-regulation and up-regulation of phosphoenolpyruvatecarboxykinase (PEPCK) (Li *et al.*, 2013). In an *in vitro* study, epigallocatechins gallate (EGCG), one of the most abundant catechins in Lipton tea could activate adenosine monophosphate-activated protein kinase (AMPK) as a required pathway for the inhibition of gluconeogenic enzymes expression (Murray *et al.*, 2014). AMPK, an important sensor of cellular energy status has a key role in metabolic control, it is the key cellular energy sensor that regulate glucose metabolism and is the main drug target of hypoglycaemic drugs such as metformin (Nelson *et al.*, 2008). Activation of AMPK leads to increased glucose uptake via up-regulation of glucose transporters, turning off anabolic pathways such as gluconeogenesis and increase catabolic pathways in cells (Nelson *et al.*, 2008).

The result of this research also revealed that the LTAE caused significant decrease in total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol, while there was an increase in high density lipoprotein cholesterol. This could also be attributed to the reduction in feed intake due to loss of appetite noticed within the period of experimentation. The reduction in feed intake might have reduced the amount of dietary source of lipids metabolized by the liver. And as a consequent effect it precipitated to the general decrease in the cholesterol level. The present study is in agreement with Atiku and Ubon (2011) that reported a decrease in total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol of rats, while there was an increase in high density lipoprotein cholesterol of the rats after the period of their experimentation. This can be attributed to the suppression of intestinal absorption of lipids by the Lipton tea polyphenols and increase in the

excretion of fats (Dulloo *et al.*, 2000). These polyphenols as contained in Lipton tea affect lipid metabolism by different pathways and prevent the appearance of atherosclerotic plaque which is a precursor to other cardiovascular diseases (Khan *et al.*, 2014). These polyphenols also has an inhibitory effect on acetyl-coA carboxylase which is essential for fatty acid biosynthesis *in vitro* (Prasad *et al.*, 2009). Several mechanisms have been proposed for the lipidemic lowering effect of the polyphenols as contained in Lipton tea. The two main potential mechanisms are decreased hepatic fatty acid synthase (FAS) and increased adenosine monophosphate-activated protein kinase (AMPK) activity (Maritim *et al.*, 2003). AMPK is also a key cellular energy sensor that regulate lipid metabolism and hence is the main drug target of lipid lowering drugs such as statins (Nelson *et al.*, 2008). Activation of AMPK leads to turning off anabolic pathways which include decreased fatty acid synthase for lipid synthesis and increased catabolic pathways in cells (Nelson *et al.*, 2008).

The result of this study showed a significant decrease in the atherogenic index and the coronary risk index of the treated group in both time-dependent and dose dependent manner when compared to the control. The decrease in atherogenic index can be attributed to decrease in low density lipoprotein cholesterol (bad cholesterol) and increase in high density lipoprotein cholesterol (good cholesterol) while the lowering of coronary risk index is due to decrease in total cholesterol and increase in high density lipoprotein cholesterol (Adeneye and Olagunju, 2009). The Atherogenic index is the tendency of developing atherosclerosis which is normally a precursor of cardiovascular diseases while coronary risk index is the tendency of developing heart related diseases like myocardial infarction, hypertension, high blood pressure etc. The finding of this study is in agreement with the results of Hasanein *et al.* (2012). Their finding showed a decrease in both atherogenic index and coronary risk index. He also opined that the decrease in atherogenic index can be attributed to decrease in low density lipoprotein cholesterol and increase in high density lipoprotein cholesterol while the lowering of coronary risk index is due to decrease in total cholesterol and increase in high density lipoprotein cholesterol.

Conclusions

Since the doses of Lipton tea extract used in this study significantly decreased the body weight gain of the normal male albino rats, reduced the fasting blood sugar, lowered the total cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, atherogenic index and coronary risk index, but increased the high density lipoprotein cholesterol, it is therefore concluded that Lipton tea extract possesses great potential for anti-obesity, hypoglycaemic and hypolipidemic properties. We recommend that further investigations should be made on this extract towards possible development of cheaper and safer alternatives to anti-diabetic chemotherapy.

References

- Abe K, Ijiri M, Suzuki T, Taguchi K, Koyama Y, Isemura M (2005). Green tea with high catechin content suppresses inflammatory cytokine expression in the galactosamine-injured rat liver. *Biomedical Research* 26(5):187-192.
- Adeneye AA, Olagunju JA (2009). Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn in Wistar rats. *Biology and Medicine* 1(1):1-10.
- Ahmad N, Feyes DK, Agarwal R, Mukhtar H, Nieminen AL (1997). Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *Journal of the National Cancer Institute* 89(24):1881-1886.
- Ahmed RG (2005). The physiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defense system. *Medical Journal of Islamic World Academy of Sciences* 15(1):31-42.
- Aizaki T, Osaka M, Hideyuki HA, Kurokawa S, Matsuyama K, ... Izumi T (1999). Hypokalemia with syncope caused by habitual drinking of oolong tea. *Internal Medicine* 38(3):252-256.
- Al Jamal A (2014). Effect of rosemary (*Rosmarinus officinalis*) on lipid profiles and blood glucose in human diabetic patients (type-2). *African Journal of Biochemistry Research* 8(8):147-150.
- Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry* 20(4):470-475.
- Al-Salafe R, Irshad M, Abdulghani HM (2014). Does green tea help to fight against obesity? An overview of the epidemiological reports. *Austin Journal of Clinical Medicine* 1(3):1011.
- Anderson RA, Polansky MM (2002). Tea enhances insulin activity. *Journal of Agricultural and Food Chemistry* 50(24):7182-7186.
- APA (2012). Guidelines for ethical conduct in the care and use of nonhuman animals in research. USA, Washington DC: American Psychological Association. Retrieved June 13, 2015.
- Atiku MK, Ubon GA (2011). Studies on the hypoglycaemic, hypocholesterolaemic and acute toxicity of aqueous *Gymnema sylvestre* leaf extract in rats. *Bio-Research* 9(1):738-742.
- Bailey CJ, Day C (1989). Traditional plant medicines as treatments for diabetes. *Diabetes Care* 12(8):553-564.
- Baynes JW, Thorpe SR (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48:1-9.
- Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS (2008). The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. *The Journal of Nutrition* 38(9):1677-1683.
- Broadhurst CL, Polansky MM, Anderson RA (2000). Insulin-like biological activity of culinary and medicinal plant aqueous extracts *in vitro*. *Journal of Agricultural and Food Chemistry* 48(3):849-852.
- Cameron AR, Anton S, Melville L, Houston NP, Dayal S, ... Rena G (2008). Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell* 7:69-77.
- Cao J, Luo SF, Liu JW, Li Y (2004). Safety evaluation on fluoride content in black tea. *Food Chemistry* 88(2):233-236.

- Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J (2000). Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *International Journal of Obesity* 24(2):252-258.
- Duncan DB (1955). Multiple range and multiple F tests. *Biometrics* 11(1):1-42.
- Eyo JE, Ozougwu JC, Echi PC (2011). Hypoglycaemic effects of *Allium cepa*, *Allium sativum* and *Zingiber officinale* aqueous extracts on alloxan-induced diabetic *Rattus norvegicus*. *Medical Journal of the Islamic World Academic Sciences* 19(3):121-126.
- Ezuruike UF, Prieto JM (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *Journal of Ethnopharmacology* 155(2):857-924.
- Finley PR, Schiffman RB, Williams RJ, Licht DA (1978). Cholesterol in high-density lipoprotein: use of Mg^{2+} /dextran sulfate in its enzymic measurement. *Clinical Chemistry* 24(6):931-933.
- Fossati P, Prencipe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry* 28(10):2077-2080.
- Friedwald WT, Leve RI, Fredrichson DS (1972). Estimation of concentration of low density lipoproteins separated by three different methods. *Clinical Chemistry* 18(6):499-502.
- Harbowy ME, Balentine DA, Davies AP, Cai Y (1997). Tea chemistry. *Critical Reviews in Plant Science* 16(5):415-480.
- Hasanein MA, Gawad HS, El-Megeid AA (2012). Effect of water extract prepared from green tea, black tea and cinnamon on obese rats suffering from diabetes. *World Applied Sciences Journal* 20(7):976-987.
- Ikeda I, Imasato Y, Sasaki E, Nakayama M, Nagao H, ... Sugano M (1992). Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism* 1127(2):141-146.
- Khan M, Ali M, Ali A, Mir SR (2014). Hypoglycemic and hypolipidemic activities of Arabic and Indian origin *Salvadora persica* root extract on diabetic rats with histopathology of their pancreas. *International Journal of Health Sciences* 8(1):45-56.
- Khan N, Mukhtar H (2007). Tea polyphenols for health promotion. *Life Sciences* 81(7):519-533.
- Khan N, Mukhtar H (2013). Tea and health: studies in humans. *Current Pharmaceutical Design* 19(34):6141-6147.
- King H, Aubert RE, Herman WH (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21(9):1414-1431.
- Leung LK, Su Y, Chen R, Zhang Z, Huang Y, Chen ZY (2001). Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *The Journal of Nutrition* 131(9):2248-2251.
- Li S, Lo CY, Pan MH, Lai CS, Ho CT (2013). Black tea: chemical analysis and stability. *Food and Function* 4(1):10-18.
- Lin JK, Lin-Shiau SY (2006). Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. *Molecular Nutrition and Food Research* 50(2):211-217.
- Maritim AC, Sanders A, Watkins JB (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology* 17(1):24-38.
- Mita M, Borland K, Price JM, Hall PF (1985). The influence of insulin and insulin-like growth factor-I on hexose transport by Sertoli cells. *Endocrinology* 116(3):987-992.
- Murray RK, Granner DK, Mayes PA, Rodwell VW (2014). *Harper's illustrated biochemistry*. USA: McGraw-Hill.
- Nelson DL, Lehninger AL, Cox MM (2008). *Lehninger principles of biochemistry*. Macmillan.
- Ozougwu JC, Eyo JE (2010). Studies on the anti-diabetic activity of *Allium sativum* (garlic) aqueous extracts on alloxan-induced diabetic albino rat. *Pharmacologyonline* 2010(2):1079-1088.
- Ozougwu JC, Eyo JE (2011). Evaluation of the activity of *Zingiber officinale* (ginger) aqueous extracts on alloxan-induced diabetic rats. *Pharmacologyonline* 2011(1):258-269.
- Ozougwu JC, Nwachi UE, Eyo JE (2008). Comparative hypolipidaemic effects of *Allium cepa*, *Allium sativum* and *Zingiber officinale* aqueous extracts on alloxan-induced diabetic *Rattus norvegicus*. *Bio-Research* 6(2):384-391.
- Parasuraman S, Raveendran R, Kesavan R (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics* 1(2):87-93.
- Prasad SK, Kulshreshtha A, Qureshi TN (2009). Antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats. *Pakistan Journal of Nutrition* 8(5):551-557.
- Richmond N (1973). Colorimetric determination of total cholesterol and high density lipoprotein cholesterol (HDL-c). *Clinical Chemistry* 19:1350-1356.
- Trinder P (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology* 22(2):158-161.
- Wang H, Wen Y, Du Y, Yan X, Guo H, Rycroft JA, ... Mela DJ (2010). Effects of catechin enriched green tea on body composition. *Obesity* 18(4):773-779.
- Wang S, Noh SK, Koo SI (2006). Epigallocatechin gallate and caffeine differentially inhibit the intestinal absorption of cholesterol and fat in ovariectomized rats. *Journal of Nutrition* 136(11):2791-2796.
- Wickelgren I (1998). Obesity: How big a problem? *Science* 280(5368):1364-1367.
- Willett W, Manson J, Liu S (2002). Glycemic index, glycemic load, and risk of type 2 diabetes. *American Journal of Clinical Nutrition* 76(1):274S-280S.
- Yashin A, Yashin Y, Nemzer B (2013). Beneficial effect of tea on human health. *American Journal of Biomedical Sciences* 5(4):226-241.