Bioactive Components and Antioxidant Properties of Aqueous Leaf Extract of Fresh and Processed *Camellia sinensis* in Alloxan-induced Albino Rats

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Original Research Article

ABSTRACT

The plant, *Camellia sinensis* (leaves) is popularly known for its usage as tea in many parts of the world, including Nigeria. The purpose of this study was to screen for the bioactive compositions and to determine the endogeneous antioxidant potentials of fresh and processed (tea) *C. sinensis* aqueous leaf extract. Phytochemicals, minerals and vitamins analysis were carried out following standard procedures. The extracts were further administered to alloxan-induced albino rats and its effects on serum Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH), acetyl cholinesterase (AchASE) activity and glucose concentrations were determined following standard procedures. The rats were randomly divided in to different groups thus; Group A: Normal control, Group B; Untreated toxic, Treatment Groups were further divided into four subgroups (1, 2, 3 and 4), Group C; Metformin Treated test (MTT), Group D; Fresh Leaves Treated Test, Group E; green tea, Group F; Black tea. Results showed the presence of presence of Saponin, Tannin, Flavonoid, Steroid, Terpenoid, Quinone, Alkaloid, cardiac glycosides, Proteins and phenol, vitamin C, Ca and Zn in all the extracts. Glucose concentrations significantly (p<0.05) increased upon induction with alloxan. MDA and AChE decreased while SOD, CAT and GSH increased significantly (p<0.05) when treated with

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the extracts. The aqueous extracts of *C. sinensis* was found to be rich in bioactive compounds and possess antioxidant properties, hence its continued usage could be encouraged.

Keywords: Camellia sinensis; antioxidants; intoxication; hyperglycemia; bioactive compounds.

1. INTRODUCTION

The plant, *C. sinensis* is described as an evergreen angiosperm dicotyledonous plant. Its leaves and leaf buds have been used for many years to produce flourishing tea. It is of the genus Camellia of flowering plants in the family Theaceae. It is native to mainland China, South and Southeast Asia [1].

It is commonly referred to as "tea plant", and its utilization as tea can be traced to the 16th century when it was taken to Europe from China as tea [2]. In Nigeria, the plant was brought in through Western Cameroun in 1952 by de Bouley [3]. The practice of growing the "tea plant" in Mambila started since 1975 with imported clones [4]. Today, one of the largest tea manufacturing industries in Nigeria is in Mambila Plateau, Gembu, Sarduna Local Government, Taraba State, Nigeria bearing the trade name "Highland Tea" [4]. The leaves of *C. sinensis* is being processed into different types and brands of consumable tea such as Green tea and Black tea in Mambilla Beverages Nigeria Limited Tea, for over 30 years [5].

Generally, tea is the most widely accepted and consumed liquid after water in the society nowadays [6]. Research has shown that tea derived from *C. sinensis* (Tea plant) contain compounds with therapeutic and pharmacological capabilities [7] after isolation, which is possibly while the tea is beneficial. These compounds have been shown to have antioxidant, anti-inflammatory, anti-bacterial, antiviral and anti-cancer properties against wide range of diseases [8] and disease-causing agents.

There has been wide speculation over the years that the use of fresh *C. sinensis* cultivated and processed (into forms known as green and black tea) in Mambilla Plateau, Taraba State, Nigeria have the ability to prevent and ameliorate many metabolic diseases that are predominantly orchestrated by oxidative stress such as diabetes, hypertension, obesity, and inflammations, hence the postulation that *C. sinensis* leaf extract may possess antioxidant properties.

The term "Phytochemicals" is used to refer compounds that are formed during plants normal metabolic activities and these chemicals are often referred to as "secondary metabolites" of which there are several classes which include alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins and terpenoids [9]. These can act as agents to prevent unwanted side effects of the main active substances or to help in the assimilation of the main substances. Phytochemicals are present in a variety of plants and plant parts utilized as important components of both human and animal diets.

One of the mechanisms in which phytochemicals adopt to fight against diseases is by free radical scavenging thereby acting as antioxidants. Free radicals could be referred to as any atoms which have one or more unpaired electron in the outermost shell [10]. These unpaired electrons are very unstable and can attack adjacent molecules such as lipids, proteins and carbohydrates and induce cellular damage [11]. Free radicals involving oxygen are termed reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide [12-15]. The oxidative damage caused by excess ROS may lead to development of many diseases such as heart diseases, congestive heart failure, hypertension, cerebrovascular accidents, and diabetic complications [16].

The term "antioxidant" refers to a molecule which can prevent the effects of oxidation in tissues and can protect the cells from getting damaged due to free radical effects [17], which normally results from oxidative stress. Plants are potential sources of natural bioactive compounds such as antioxidants and secondary metabolites or organic compounds that can defend the body system. When plants absorb sunlight, they can produce oxygen, necessary for respiration and secondary metabolites, which play vital role in cell protection from oxidative stress.

This study therefore sort to screen for the bioactive compositions and to determine the endogeneous antioxidant potentials of fresh and processed *C. sinensis* extract, hence the

outcome of the study may justify or undermine the use of the plant leaves as tea.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The fresh leaves of *C. sinensis* was harvested from Tea farm in Kakara village, Mambilla Plateau, Gembu, Sarduna Local Government of Taraba State, Nigeria and packaged in bags and labelled accordingly while the processed Green Tea and Black Tea samples were collected from Mambilla Beverages Nigeria Tea Limited; the production company. All the samples were stored at room temperature prior to the time of extraction.

2.1.2 Experimental animals

The animal models used in this study were adult albino rats of either sexes weighing between 70 - 110 g. They were obtained from the animal house of University of Nigeria Nsukka, Enugu state, Nigeria. The male rats were separated from the female rats and housed at the animal house in the Department of Biochemistry, Nasarawa State University, Keffi, Nigeria and acclimatized for seven days of twelve hours night and day, they were allowed free access to feed and clean water *ad libitum* during the acclimatization period.

2.1.3 Chemicals/reagents

All chemicals and reagents used in this study were of analytical grade and products of Sigma Aldrich, (U.S.A).

2.2 Methods

2.2.1 Aqueous extraction of fresh and processed *C. senensis* samples

Fresh leaves were extracted by simple maceration. 500g of the leaves were first ground in a ceramic mortar and then placed into glass conical flasks and macerated for 10 min in a water bath with 1L of deionized water at a temperature of 80 $^{\circ}$C. This extraction procedure was repeated until required volume of 4L was achieved. The mixture was filtered using Whatman No 1 filter paper; the filtrate was concentrated in a rotary evaporator at 48°C and later dried using a freeze dryer. The powder from this extraction were stored at room temperature prior to the experimental procedures.

2.2.2 Quantitative Phytochemical analysis

2.2.2.1 Determination of Saponins

Exactly 20 g of each sample was put into a conical flask and 100 cm^3 of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^0 C. The mixture was filtered and the residue re-extracted with another 200 mL 20 % aqueous ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separation funnel and 20 mL of di-ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated. 60ml of n- butanol was added, the combined n – butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath; after evaporation the samples was dried in the oven to a constant weight; the saponin content was calculated as a percentage.

2.2.2.2 Determination of total alkaloids

Exactly 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.2.2.3 Determination of tannins

The tannins was determined by Folin - Ciocalteu method. 0.1 ml of the sample solution was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) was prepared in the same

manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a Spectrum lab 752s UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

2.2.2.4 Determination of total phenols

Exactly 0.5 ml extract was mixed with 2.5 mL of Folin-ciocateu reagent. After 5 mins, 2 mL of sodium carbonate (75 g/ L) was added, after 120 min standing in dark, the optical density was measured at 760 nm against a blank. The total phenolic contents were calculated on the basis of the calibration curve of Gallic acid and expressed as Gallic acid equivalents (GAE), in milligrams per gram of the sample.

2.2.2.5 Determination of total flavonoids

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was prepared in the same manner as described earlier. The absorbance for test and standard solutions was determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

2.2.3 Mineral analysis

For mineral analysis in the *C. sinensis* samples, 0.5 mg Ca, (a macro-element) and 0.2 mg Zn (a micro-element) were measured in plant samples. Accurately weighed ash samples were boiled with 10 ml of 20% HCl in a beaker and then filtered into a 100 ml standard flask to determine the mineral content. The mineral content of the digested samples was determined by flame atomic absorption spectrophotometry using a Varian 220FS Spectra AA apparatus (Varian, USA) for calcium (Ca), zinc (Zn), was determined by spectro-photometric colorimetric method using UV spectrophotometer according to AOAC procedure 965.17 [18]. Mineral contents of plant samples were determined from calibration curves

of standards minerals. All samples were analyzed in triplicate.

2.2.31 Determination of Vitamin C content

The quantitative labeling of the content of ascorbic acid in the infusions was performed according to Tillmans et al.'s method. Titration is based on the reaction proceeding along with the change in the color of the solution of ascorbic acid under the influence of the solution of 2.6-dichloroindophenol. The samples were titrated until light-pink coloring was achieved, which lasted for 10 s. The result was presented in mg of ascorbic acid per 1 L of the infusion. Each labeling was performed three times.

2.2.4 Induction of hyperglycemia

Diabetes was induced by intravenous injection of in Alloxan (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of (150mg/kg) body weight. Rats with fasting blood glucose levels greater than 16.5 mmol/L were considered diabetic and then included in this study.

Albino rats of both sexes were randomly divided into groups A to F of five rats each and administered different treatments thus;

Group A: Normal control (NC), received distilled water only.

Group B: Untreated toxic: intoxicated with alloxan but not treated

Test Treatment Groups: These groups were further divided into four subgroups (1, 2, 3 and 4 respectively) of 5 rats each and administered 10mg, 20mg, 50mg 100mg per body weight of the treatment substances

Group C: This Test group was tagged Metformin Treated Test (MTT) Group: This was treated with different doses of standard drug (Metformin)10mg, 20mg, 50mg and 100mg per body weights after intoxication with alloxan.

Group D: This Test group was tagged Fresh Leaves Treated Test (FLTT) Group: This was treated with Fresh Leave Extract 10mg, 20mg, 50mg and 100mg per body weight after respectively after intoxication with alloxan.

Group E: This Test group was tagged Green Tea Treated Test (GTTT) Group: This was

treated with different dosage of Green Tea Extract 10mg, 20mg, 50mg and 100mg per body weight after respectively after induction with alloxan.

Group F: This Test group was tagged Black Tea Treated Test (BTTT) Group: This was treated with different dosage of Black Tea Extract 10mg, 20mg, 50mg and 100mg per body weight respectively after induction with alloxan. The administrations lasted for seven days. At the End of 7days of treatment final glucose reading was taken and the rats were anaesthetized with diethyl ether, the blood samples were collected via the retro orbital plexus after overnight fasting and it was used for separating the serum for analysis of malondialdehyde (MDA) and endogeneous antioxidant parameters.

2.2.5 Biochemical parameters

The biochemical parameters were estimated calorimetrically in separated plasma using commercially available diagnostic kits (Transasia Bio-Medicals Ltd., India). The estimations were performed as per the procedure mention in each kit protocol using biochemical analyzer (Erba Chem-7 Biochemistry Analyzer, Germany). Serum glucose level was determined by the glucose-oxidase method using ACUCHECK glucometer machine.

2.2.6 Assay for catalase activity

Catalase (EC 1.11.1.6) activity will be determined as described by Aebi [18]. A known volume (2.5 ml) of phosphate buffer + 2ml H_2O_2 + 0.5ml of sample were pipetted into a test tube. To 1ml portion of the reaction, 2 ml of Potassium dichromate and acetic acid were added. The absorbance was determined at 240 nm into 4 places at a minute interval. Catalase concentration was then calculated using the equation:

Catalase concentration
$$(\text{Unit/L}) = \frac{0.23 \times (\text{logAbs1}/\text{logAbs2})}{0.00693}$$

2.2.7 Determination of Glutathione-S-transferase (GST) activity

The GST (EC 2.5.1.18) activity will be determined by the method of Adedara et al. [19] using 1-chloro-2, 4- dinitrobenzene (CDNB) as substrate. The assay mixture consisted of 600 µL of solution A (20 µL of 0.25 M potassium phosphate buffer, pH 7.0 with of 2.5 mM EDTA,

and 510 µL of 0.1 M GSH at 25 °C), 60 µL of the sample (1:5 dilution) and 30µL of 25 mM CDNB. Increased absorbance will be measured at 340 nm for 2 min at 10 s interval using a spectrophotometer (Jenway). Data will be expressed in mmol/min/mg of protein utilizing the molar extinction coefficient (ε) of 9.6 mM1 cm1 of the coloured GS–DNB conjugate formed by GST.

2.2.8 Assay for Acetylcholinesterase (AChE) activity

The AChE (EC 3.1.1.7) activity was be determined by slightly modifying the method of Abolaji et al. [20]. The mixture consisted of 285 µL of distilled water, 180 µL of 100 mM PBS (pH 7.4), 60 µL of 10 mM DTNB, 15 µL of the sample, and 60 µL of 8 m Macetylthiocholine was added. The absorbance was monitored spectrophotometrically (UV-VIS spectrophotometer, Jenway 7315) at 412 nm for 2 min at intervals of 10 s. Data was calculated against blank and sample blank, and the activity expressed as µmol/min/mg protein.

2.2.9 Assay for Superoxide dismutase (SOD) activity

Determination of Superoxide Dismutase (SOD) activity The SOD activity was determined by the method of Iorjiim et al. [21]. Briefly, a volume of 200 µL of the sample will be diluted with 800 µL of distilled water (1:5). Then 200 µL of the diluted sample will be added to 2500 µL of 0.05 M carbonate buffer (pH 10.2) in the cuvette and allowed to equilibrate in a spectrophotometer. Then, 300 µL freshly prepared 0.3 M adrenaline will be added to start the reaction. The cuvette used as standard contained 2500 µL buffer, 300 µL adrenaline, and 200 µL of water. The absorbance will be monitored every 30 seconds for 150 seconds at 480 nm.

2.2.10 Determination of Malondialdehyde (MDA) concentration

Lipid peroxidation assay Lipid peroxidation, as an index of reactive oxygen species (ROS) generation, will be determined by measuring the level of Malondialdehyde using the method of Varshney and Kale as previously described Iorjiim et al. [21]. Briefly, 400 µL of the sample will be first mixed with 1600 µL of tris-KCl buffer followed by 500 µL of 30% TCA. 500 µl of 0.75% TBA will be added and placed in a water bath for 45 minutes at 800 C. Ice will be used to cool the mixture, then centrifuged at 3000 g for 5 minutes.

The clear supernatant obtained will be collected, and absorbance measured against distilled water as reference blank at 532 nm. Lipid peroxidation will be expressed as MDA formed/mg protein using a molar extinction coefficient of 1.56×10^5 $M^{-1} Cm^{-1}$

2.2.11 Statistical analysis

The data obtained were analyzed using one-way analysis of variance in IBM SPSS package, version 23.0 and the results were expressed as mean ± standard deviations. The level of significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical of Fresh Leaves Extract, Processed Green Tea and Black Tea Extracts from *Camellia sinensis*

The aqueous Extracts of Fresh leaves, Processed Green and Black Tea were analysed for various phytochemicals. The presence of Saponin, Tannin, Flavonoid, steroid, Terpenoid, Quinone, Alkaloid, cardiac glycosides, Proteins and phenol in extracts of Fresh leaves, Green and Black Tea. However, Coumarin was present but not Charcones in the Black Tea only and not in Fresh Leave and Green Tea Extracts where Charcones were present. Phlobatannin and Anthocyanins were all absent in the extracts of Fresh Leaves, Green Tea and Black Tea from *Camellia sinensis* as shown in Table 1.

3.2 Quantitative Analysis of Fresh Leaves Extract, Processed Green Tea and Black Tea Extracts from *Camellia sinensis*

The table, (Table 2) revealed that Total phenol of 62.909±2.273, 44.909±2.273 and 30.460±0.101 (mg GAE/g) were the highest contain in Fresh Leaves Extract, Processed Green Tea and Black Tea from *Camellia sinensis* respectively. This finding is in line with (Altemimi et al. 2017) who said the Phenols are the major component of *Camellia sinensis*. However, other compounds are found appreciable quantity are Tannin 9.360±0.011, 7.150±0.00 and 4.883±0.002(mg GAE/g). While Flavonoid in 0.891±0.020, 0.445±0.001 and 0.500±0.00 (mg QE/g) and %Alkaloid are 2.14±0.00, 1.48±0.01 and 0.09±0.01 respectively for Fresh Leaves Extract, Processed Green Tea and Black Tea from *Camellia sinensis* are the smallest in content.

This analysis also showed that contents decrease from fresh leaves sample to processed products.

3.3 Calcium, Zinc and Vitamin C Contents Fresh Leaves Extract, Processed Green and Black Tea from *Camellia sinensis*

Table 3 showed vitamin C content in Fresh Leaves Extract, Processed Green and Black Tea from *Camellia sinensis* to be 0.14±0.20, 0.04±0.00 and 0.05±0.00 (g/ml), Calcium was found to be 1856.45, 1031.60, and 14.00µg/g. Zinc was found to be 44.00, 33.30 and 14.00µg/g respectively.

3.4 Serum Glucose Level before and After Intoxication with Alloxan

The results of serum glucose levels before and after intoxication of the rats with alloxan showed a significant ($p < 0.05$) increase in glucose levels due to induction of hyperglycemia by alloxan

3.5 The Levels of MDA, Antioxidant Status and Acetyl Cholinesterase Activity in Alloxan Induced Albino Mice Induced

Table 4 is a presentation of the results of the effect of different dosage (concentrations) of metformin in Alloxan (150mg/kg) induced albino rats on MDA, SOD CAT, GSH and acetyl cholinesterase activity. MDA concentration and AchE activities increased significantly ($p < 0.05$) in the untreated group while SOD, CAT and GSH decreased significantly ($p < 0.05$) compared to the normal control. There is a raise in MDA concentration in untreated diabetes. In the Metformin treated groups, MDA and acetyl cholinesterase activities decreased significantly ($p < 0.05$) while SOD, CAT and GSH decreased significantly ($p < 0.05$) compared to the untreated groups.

3.6 Effects of Fresh Leaves Extract of *Camelia sinensis* on MDA, Antioxidant Status and Acetyl Cholinesterase Activity in Alloxan-Intoxicated Albino Rats

As shown in table 5, at 10mg/kg MDA decreased significantly ($p < 0.05$), CAT and GSH increased significantly ($p < 0.05$), at 20mg/kg, CAT increased significantly ($p < 0.05$), at 50mg/kg,

Table 1. Qualitative phytochemical of fresh leaves extract, processed green tea and black tea extracts from *Camellia senensis*

SN.	Phytochemicals	Fresh leave extract	Green tea extract	Black tea extract
1	Saponin	+	+	+
2	Tannin	+	+	+
3	Flavonoid	+	+	+
4	Steroid	+	+	+
5	Diterpenes	-	-	-
6	Terpenoid	+	+	+
7	Coumarin	-	-	+
8	Phlobatannin	-	-	-
9	Quinone.	+	+	+
10	Anthocyanins	-	-	-
11	Alkaloid	+	+	+
12	Cardiac Glycosides	+	+	+
13	Charcones	+	+	-
14	Phenols	+	+	+
15	Protein	+	+	+

Key: + =Presence/ Positive, - =Absence/ Negative

Table 2. Quantitative analysis of Fresh Leaves Extract, Processed Green Tea and Black Tea Extracts from *Camellia sinensis*

Phytochemicals	Fresh leaves extract	Green tea extract	Black tea extract
%Saponin	1.81±0.20	1.61±0.01	0.10±0.01
Tannin (mg GAE/g)	9.360±0.011	7.150±0.000	4.883±0.002
% Alkaloid	2.14±0.00	1.48±0.01	0.09±0.01
Flavonoid (mg QE/g)	0.891±0.020	0.445±0.001	0.500±0.000
Total Phenol (mg GAE/g)	62.909±2.273	44.909±2.273	30.460±0.101

Table 3. Calcium, Zinc and Vitamin C Contents Fresh Leaves Extract, Processed Green and Black Tea from *Camellia sinensis*

Components	Fresh leaves extract	Green tea extract	Black tea extract
Vitamin C (g/ml)	0.14±0.20	0.04±0.00	0.05±0.00
Calcium μg/g	1856.45	1031.60	943.13
Zinc μg/g	44.00	33.30	14.00

SOD and GSH increased significantly ($p<0.05$) while at 100mg/kg CAT and GSH increased significantly ($p<0.05$). AchNase decreased significantly ($p<0.05$) at 10, 20 and 100mg/kg of fresh leaves extract of *Camelia senensis* compared to the untreated groups.

3.7 Effects of Green tea Extract on MDA, Antioxidant Status and Acetyl Cholinesterase Activity in Alloxan-intoxicated Albino Rats

MDA was non-significantly ($p>0.05$) higher at all the administered doses of Green tea treatment. However, all the administered doses of green tea are significantly ($p<0.05$) increased in SOD, CAT and GSH. Increase in MDA revealed a well established hyperglycemic condition with increased oxidative stress which could have

triggered an increase in SOD, CAT, and GSH as mechanism of quenching and eliminating the production of free radical generation as a result of lipid peroxidation in hyperglycemia. Therefore, Green tea increases antioxidant activities in hyperglycemia albino rat induced with Alloxan which was not observed in untreated groups (Table 6).

Table 4. Serum glucose level before and after intoxication with alloxan

Before induction	After induction
86.00±7.96	403.40±51.77[*]
104.20±3.11	383.40±36.65[*]
101.40±7.40	389.40±25.99[*]
99.200±2.77	408.00±20.67[*]
101.00±3.39	388.40±17.64[*]

*Results are presented as Mean ± SD, mean values with * are considered statistically significant at $p<0.05$*

Table 5. Effect of Metformin on MDA, antioxidant status and acetyl cholinesterase activity in alloxan-intoxicated albino rats

Treatment group	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GSH (µmol/mg)	AchE (µmol/min/mg)
Normal control	4.256±0.46[a]	40.57±3.56[a]	12.53±0.235[a]	21.192±1.63[a]	1.69±0.119[a]
Untreated Toxic	8.928±0.34[b]	22.87±1.04[b]	7.07±0.526[b]	12.76±0.478[b]	3.53±0.328[b]
Met std 10mg/kg	4.77±0.91[a]	36.37±2.07[c]	10.54±0.327[c]	19.10±0.44[c]	1.96±0.066[c]
Met std 20mg/kg	5.25±0.17[c]	36.85±1.99[c]	12.32±0.285[d]	18.24±0.259[c]	2.08±0.069[c]
Met std 50mg/kg	5.20±0.18[c]	38.14±0.33[a]	12.578±0.30[d]	18.74±0.455[c]	2.138±0.093[c]
Met std 100mg/kg	5.83±0.105[c]	39.90±1.46[a]	12.268±0.267[d]	18.95±0.883[c]	2.256±0.217[c]

Results are presented in Mean ± SD, (N = 5), mean values with different letters as superscripts down the groups are considered significant at p < 0.05

Table 6. Effects of fresh leaves extract of *Camelia sinensis* on MDA, antioxidant status and acetyl cholinesterase activity in alloxan-intoxicated albino rats

Treatment group	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GSH (µmol/mg)	AchE (µmol/min/mg)
Normal control	4.26±0.46[a]	40.57±3.56[a]	12.53±0.235[a]	21.192±1.63[a]	1.69±0.11[a]
Untreated Toxic	8.92±0.345[b]	22.87±1.047[b]	7.07±0.526[b]	12.76±0.478[b]	3.53±0.32[b]
Fresh 10mg/kg	8.31±.28[c]	25.616±1.09[b]	7.77±0.24[c]	13.76±0.62[c]	2.47±0.09[c]
Fresh 20mg/kg	8.14±.38[b]	26.944±.57[b]	7.82±0.33[c]	14.22±0.48[d]	2.80±0.16[c]
Fresh 50mg/kg	9.19±.38[b]	27.23±2.52[c]	6.93±0.71[c]	13.798±0.23[c]	3.19±0.31[b]
Fresh 100mg/kg	9.15±.40[b]	25.95±1.62[b]	7.82±.15[c]	16.088±0.50[e]	0.26±0.12[d]

Results are presented in Mean ± SD, (N = 5), mean values with different letters as superscripts down the groups are considered significant at p < 0.05

Table 7. Effects of green tea extract on MDA, antioxidant status and acetyl cholinesterase activity in alloxan-intoxicated albino rats

Treatment group	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GSH (µmol/mg)	AchE (µmol/min/mg)
Normal control	4.26±0.46[a]	40.57±3.56[a]	12.53±0.24[a]	21.19±1.63[a]	1.69±0.11[a]
Untreated	8.92±0.345[b]	22.87±1.047[b]	7.07±0.526[b]	12.76±0.478[b]	3.53±0.32[b]
Green tea 10mg/kg	9.20±.36[b]	28.18±0.36[c]	8.34±.33[c]	14.47±.47[c]	2.87±0.10[c]
Green tea 20mg/kg	9.14±.26[b]	27.92±0.68[c]	8.07±.27[c]	13.57±.40[c]	2.85±0.18[c]
Green tea 50mg/kg	8.62±.23[b]	26.92±1.04[b]	7.51±.27[b]	15.91±1.00[d]	2.67±.50[c]
Green tea 100mg/kg	9.30±.48[b]	29.14±0.35[c]	7.64±.19[b]	15.24±.71[d]	3.17±.10[b]

Results are presented in Mean ± SD, (N = 5), mean values with different letters as superscripts down the groups are considered significant at p < 0.05

Table 8. Effects of black tea extract of *Camelia sInensis* on MDA, antioxidant status and acetyl cholinesterase activity in alloxan-intoxicated albino rats

Treatment group	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GSH (µmol/mg)	AchE (µmol/min/mg)
Normal control	4.26±0.46[a]	40.57±3.56[a]	12.53±0.24[a]	21.19±1.63[a]	1.69±0.11[a]
Untreated Toxic	8.92±0.345[b]	22.87±1.04[b]	7.07±0.52[b]	12.76±0.478[b]	3.53±0.32[b]
Black tea 10mg/kg	8.53±0.48[b]	27.14±0.87[c]	7.60±0.23[c]	14.57±.75[c]	2.71±0.44[c]
Black tea 20mg/kg	9.17±0.18[b]	30.224±2.37[d]	7.23±0.41[b]	19.708±2.37[d]	3.2±0.13[b]
Black tea 50mg/kg	8.70±0.60[b]	28.250±1.21[c]	7.78±0.45[b]	16.708±2.10[e]	3.15±0.30[b]
Black tea 100mg/kg	9.25±0.49[b]	27.41±1.53[c]	7.76±0.36[b]	16.51±.44[e]	3.33±0.27[b]

Results are presented in Mean ± SD, (N = 5), mean values with different letters as superscripts down the groups are considered at p < 0.05

3.8 Effects of Black Tea Extract of *C. sinensis* on MDA, Antioxidant Status and Acetyl Cholinesterase Activity in Alloxan-intoxicated Albino Rats

As shown in Table 7, MDA was non-significantly ($p > 0.05$) higher at all the administered doses of Black tea. All the administered doses of black tea significantly ($p < 0.05$) increased SOD, CAT and GSH. AchE decreased significantly ($p < 0.05$) at 10, 20 and 50 mg/kg as shown below. Effect of black tea on MDA, antioxidant status and AchE in albino rats induced with alloxan. Diabetes-induced oxidative stress in liver was manifested by significant increase in hepatic malondialdehyde (MDA).

4. DISCUSSION

In this study, the aqueous extracts of fresh leaves, processed (into green and black tea) of *C. sinensis* were analyzed for phytochemicals, minerals and vitamins composition. The presence of Saponin, Tannin, Flavonoid, steroid, Terpenoid, Quinone, Alkaloid, cardiac glycosides, Proteins and phenol in extracts of Fresh leaves, Green and Black Tea. However, Coumarin was present but not Charcones in the Black Tea only and not in Fresh Leave and Green Tea Extracts where Charcones were present. Phlobatannin and Anthocyanins were all absent in the extracts of Fresh Leaves, Green Tea and Black Tea from *C. sinensis* as shown in Table 1.

Previous research have shown that these phytochemicals have various health benefits such as antioxidant properties, anti-hyperglycemic activities, anti-lipideamic activities, anti-inflammatory properties, antibacterial properties, antiviral and antifungal properties against wide range of metabolic disorders including cancers. Also, in a bid to have an idea of the effect of the tea on nervous system, the activity of acetylcholinesterase was determined. A high activity of the enzyme is said to have negative effect on nerve function because of reduced amount of acetyl choline which is vital in nerve functions. Molecules that inhibit acetyl cholinesterase tend to favour nerve functions while molecules that activate it tend to negatively affect nerve function.

The table, (Table 2) revealed that Total phenol of 62.909 ± 2.273, 44.909 ± 2.273 and 30.460 ± 0.101 (mg GAE/g) were the highest contain in Fresh Leaves Extract, Processed Green Tea and Black Tea from *C. sinensis* respectively. This finding is in line with Altemini et al. [22] who said the Phenols are the major component of *Camellia sinensis*. However, other compounds are found appreciable quantity are Tannin 9.360 ± 0.011, 7.150 ± 0.00 and 4.883 ± 0.002(mg GAE/g). While Flavonoid in 0.891 ± 0.020, 0.445 ± 0.001 and 0.500 ± 0.00 (mg QE/g) and %Alkaloid are 2.14 ± 0.00, 1.48 ± 0.01 and 0.09 ± 0.01 respectively for Fresh Leaves Extract, Processed Green Tea and Black Tea from *C. sinensis* are the smallest in content. This analysis also showed that contents decrease from fresh leaves sample to processed products. This may be so due to the effect of processing as fermentation and oxidation occurs. Variation in these contents may have being the reason for slight changes in functional potency and effect on induced rats.

As displayed in Table 3, vitamin C content in Fresh Leaves Extract, Processed Green and Black Tea from *Camellia sinensis* to be 0.14 ± 0.20, 0.04 ± 0.00 and 0.05 ± 0.00 (g/ml), Calcium was found to be 1856.45, 1031.60, and 14.00µg/g. Zinc was found to be 44.00, 33.30 and 14.00µg/g respectively. Vitamin C is a water soluble vitamin and non-enzymatic antioxidants that play a great role in immune system defence mechanism. Calcium is a cofactor that serves as activator in enzymatic catalyzed reactions. Calcium is also needed in bone formation and other vital metabolic mechanisms. Zinc is a vital element in immune system defence mechanisms. The presence of these substances will have potential effects in chronic hyperglycemia and diabetes in stabilizing glucose levels [23]. Zinc supplementation have glycaemic control in diabetes [24, 25]. Similarly, Vitamin C supplementation have been associated to glycemic control by it potential healing power to repair damaged pancreas, reduction of oxidative [26] stress thereby curbing diabetes.

The elevated levels of glucose concentration (Table 4) following alloxan administration confirms the fact fact in many previous researches that alloxan can be a potent chemical agent for inducing hyperglycemia and eventually diabetes in animal models, thereby justifying its common use by many researchers to induce hyperglycemia and diabetes in rats for experimental purposes. Many researchers have previously used alloxan to induce hyperglycemia and diabetes in rat models and one of its

mechanisms of action involves the generation of free radicals which in turn mount an oxidative stress on tissues and organs.

As displayed in Table 5 which represents the results of the effect of different dosage (concentrations) of metformin in Alloxan (150mg/kg) intoxicated albino rats on MDA, SOD CAT, GSH and acetyl cholinesterase activity. Generally, the determination of the activities and concentrations of oxidative stress markers gives the researcher a great deal of insight into the health status of the test models and could suggest the possible mechanism of the observation. MDA concentration and AchNase activities increased significantly (p<0.05) in the untreated group while SOD, CAT and GSH decreased significantly (p<0.05) compared to the normal control. Previous research have shown elevated MDA concentration in untreated diabetes [27]. Malondialdehyde (MDA) is degradative product of peroxidation of polyunsaturated fatty acids (PUFA) in the cells membrane. Presence of higher MDA in the serum is an indication of induced lipid peroxidation and of oxidative stress of which has been reported as one of the underlying cause of diabetes mellitus [28]. The decrease in serum SOD, CAT and GSH is associated to untreated conditions of hyperglycemia or diabetes compared to normal control. Hyperglycemia can induce the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and which will cause oxidative damage to multiple organs leading significantly reduction in activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-reductase (GRD) [29].

In the Metformin treated groups, MDA and acetyl cholinesterase activities decreased significantly (p<0.05) while SOD, CAT and GSH decreased significantly (p<0.05) compared to the untreated groups as shown in Table 5. This findings was also similar to the [30] which revealed that metformin may prevent the increase in total antioxidant capacity in monotherapy or combined therapy with insulin due to metformin action controlling the ROS production of diabetic rats. This findings revealed that Metformin can significantly reverse high MDA, acetyl cholinesterase and low SOD, CAT and GSH activities in hyperglycemic treatment.

As shown in Table 6, which is a presentation of the results obtained when the intoxicated rats were treated with fresh leaf extract of *C. sinensis*,

at 10mg/kg MDA decreased significantly (p<0.05), CAT and GSH increased significantly (p<0.05), at 20mg/kg, CAT increased significantly (p<0.05), at 50mg/kg, SOD and GSH increased significantly (p<0.05) while at 100mg/kg CAT and GSH increased significantly (p<0.05). AchNase activity decreased significantly (p<0.05) at 10, 20 and 100mg/kg of fresh leaves extract of *C. sinensis* compared to the untreated groups.

This showed that increased concentration of fresh leaf extract of *C. sinensis* has various antioxidants effects on alloxan-induced diabetes in rats. Increased concentration of fresh leaf extract is capable of significantly increasing Catalase, SOD, GSH activities and reducing MDA and AchNase activities when compared with untreated groups. These results conforms to previous findings [31]. Therefore, the leave extract of *C. sinensis* may be used to ameliorate the effect of increased free radicals often observed in hyperglycemia hence providing a defensive reactive oxygen species mechanism against oxidative stress [32].

Table 7 represents the results of the effects of green tea extract on antioxidants and acetyl cholinesterase activity in rats. MDA was non-significantly (p>0.05) higher at all the administered doses of Green tea treatment. However, all the administered doses of green tea are significantly (p<0.05) increased in SOD, CAT and GSH. Increase in MDA revealed a well-established hyperglycemic condition with increased oxidative stress which could have triggered an increase in SOD, CAT, and GSH as mechanism of quenching and eliminating the production of free radical generation as a result of lipid peroxidation in hyperglycemia. AchNase decreased significantly (p<0.05) at 10, 20 and 50 mg/kg as shown in Table 8. Hence the green tea extract may be said to have posed a protective effect on central nervous system impairment due to the induced toxin "alloxan" thereby reversing the devastating effects of hyperglycemia which could have been as results of brain tissue (cognitive functions, such as memory) damage when causing increase in AchNase [33]. The suspected antioxidant effects of green tea was also a confirmation of previous studies.

As shown in Table 8, MDA was non-significantly (p>0.05) higher at all the administered doses of the black tea extract. All the administered doses of black tea significantly (p<0.05) increased SOD, CAT and GSH. AchNase decreased

significantly (p<0.05) at 10, 20 and 50 mg/kg as shown below. Effect of black tea on MDA, antioxidant status and AchNase in aloxan-induced hyperglycemia/diabetes in albino rats. Diabetes-induced oxidative stress in liver was manifested by significant increase in hepatic malondialdehyde (MDA), however, an increase the measured in antioxidant indices after administration of black tea extract is a potential indicator of its antioxidant effect.

5. CONCLUSION

In this study, the aqueous leaf extracts of *C. sinensis* were studied *in vitro* and *in vivo*. The outcome revealed that the aqueous extracts of fresh and processed leaves of *C. sinensis* are rich in phytochemicals, vitamin C, Zink and Calcium ions and possess remarkable potentials against lipid peroxidation due to reduced levels of MDA and improved neurotransmission due to reduced acetyl cholinesterase activities while also boosting the system's endogeneous antioxidant status due to increased activities of SOD, CAT and GSH concentrations.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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