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Evaluation of two tea beverages (*camellia sinesis* and *matricaria chamomilla*) as functional foods and their effects on liver biomarkers in wistar rats

Abstract

The present study seeks to evaluate Green tea and Chamomile tea as functional foods, and also to assess their effects on liver biomarkers. HPLC-DAD quali-quantitative analysis of Green tea (GT) and Chamomile tea (C) revealed the presence of some phenolic compounds in both teas, with GT, having the higher total phenolic content. The results of antioxidant indices of the teas revealed that both teas demonstrate good antioxidant action with GT ranking higher. The mineral analysis of the teas showed varied levels of the evaluated minerals and the calculated [phytate]/[Ca], [oxalate]/[Ca], [phytate]/[Zn], [Ca][phytate]/[Zn] molar ratios of the teas fell below the critical values, thereby revealing that Ca and Zn and other minerals would be bio-available. The effects of the teas on the liver biomarkers and the histological examinations showed no damaging effects on the liver. This by implication is that the teas, most especially GT could be explored as functional foods.

Keywords: antioxidant activities, phenolic compounds, *camellia sinesis, matricaria chamomilla*, mineral bioavailability, liver biomarkers

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Practical application

The beverage industry can produce and package Green tea (*Camellia Sinesis*) and Chamomile tea (*Matricaria Chamomilla*), and their composite blends (*Camellia Sinesis + Matricaria Chamomilla),* which can be harnessed at regulated dose as functional foods in the management and prevention of stress related diseases.

Introduction

Tea is the most widely consumed drink in the world other than water.[1,2] It is well known that the tea plant originated from the southwest of China. As early as 4000–5000 years ago, the Chinese people had become aware that tea could promote health and prevent some human diseases. Tea has been proven to be reliable sources of minerals and phenolic compounds.[3,4] It has also been reported that teas and tea products possess good antioxidant activities, which are active against free radical related diseases.[5,6] Tea products have been used for the management of various diseases such as cancer, cardiovascular diseases, diabetes, neurodegenerative diseases, anxiety, insomnia, depression, and viral infection.[7–12] The term green tea refers to the product manufactured from fresh *Camellia sinensis* leaves in which significant oxidation of the major leaf polyphenols known as catechins is prevented.[13] Green tea have a very strong antioxidant activities and contains a variety of enzymes, amino acids, carbohydrates, lipids, sterols, related compounds, dietary minerals, phenolic compounds and caffeine.[14] Phenolic compounds found in teas tea includes but are not limited to epigallocatechin, epicatechin, epicatechin gallate and epigallocatechin gallate, flavanols such as kaempferol, quercetin, and myricitin are also found in green tea.[14]

Chamomile (*Matricaria chamomilla)* is one of the oldest, most widely used and well documented medicinal plants in the world and has been recommended for a variety of healing applications.[15] Chamomile is a native of the old World and is a member of the daisy family (*Asteraceae* or *Compositae*), that are commonly used to make herb infusions to serve various medicinal purposes.[14,16] Popular uses of chamomile preparations include treating hay fever, fungal growth, inflammation, muscle spasm, menstrual disorders, insomnia, ulcers, gastrointestinal disorder, and haemorrhoids.[17,18] Liver function tests are a group of blood tests that provide information about the state of the liver.[19] Elevated levels of some substances or enzymes in the blood may signify liver damage or diseased state.[20] Some tests are associated with functionality (*e.g. albumin*), some with cellular integrity (*e.g. transaminases*) and some with conditions linked to the biliary tract (*gamma-glutamyltransferase* and *alkaline phosphatase*). These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment.[21] However, the consumption of food and phytochemicals, either directly or in the form of nutraceuticals can either enhance, maintain or impair the integrity of the liver in carrying out its biological functions.[22,23]

The human body identifies all the drugs and nutraceuticals as xenobiotics and subjects them to various chemical processes to make them suitable for elimination.[22] This involves chemical transformations to reduce fat solubility and to change biological activity. Although almost all tissues in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in the liver is the principal "metabolic clearing house" for both endogenous and exogenous substances.[24] The central role played by the liver in the clearance and transformation of chemicals makes it susceptible to drug and natural products induced injury.[25] It is therefore expedient to assess the potentials of the selected teas as functional foods, and also evaluate their effects on the functionality and integrity of the liver upon their usage as functional food.

Materials and Methods

Sample collection

Two different types of anti-stress teas, Green tea (*Camellia sinensis*) and Chamomile (*Matricaria chamomilla L.*) were bought from the Tradomedical Centre, Ibadan, Oyo state, Nigeria.

Methods

Sample treatments and preparation

The tea infusions (Green tea, Chamomile tea and composite blends of both teas) were prepared using hot water infusion as follows: 15g of each tea sample (Green tea and Chamomile tea) was infused in 1.2litres of hot water and the tea infusions of the composite blends were prepared by mixing 7.5g each of the studied tea samples, and subsequently infused in 1.2L of hot water. The prepared tea infusions were filtered using No. 4 filter paper. The filtrates of the three infusions were stored in amber bottles and kept in the refrigerator at the Department of Biochemistry, Federal University of Technology, Akure, Ondo, State, Nigeria.

HPLC-DAD analysis of *Camellia sinensis* tea and *Matricaria chamomilla tea*

Camellia sinensis tea at a concentration of 12 mg/mL was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C_{18} column (4.6 mm x 250 mm x 5µm particle size). The mobile phase was water with 1% acetic acid (v/v) (solvent A) and HPLC grade acetonitrile (solvent B) at a flow rate of 0.6mL/min and injection volume 40µL. The composition gradient was: 5% solvent B reaching 15% at 10min; 30% solvent B at 25min, 65% solvent B at 40min and 98% solvent B at 45min, followed by 60min at isocratic elution until 65min. At 70min the gradient reached the initial conditions again, following the method described by Boligon *et al*.,[26] with slight modifications. The sample and mobile phase were filtered through 0.45µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the acetonitrile: water (1:1,v/v) at a concentration range of 0.025 – 0.400mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254nm for gallic acid; 280nm for catechin and epicatechin; 325nm for caffeine and chlorogenic acid, and 366nm for quercetin, rutin and kaempferol. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600nm).

Chamomile tea at a concentration of 10mg/mL was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C_{18} column (4.6 mm x 250 mm x 5 µm particle size). The mobile phase was water with 1% formic acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 mL/min and injection volume 40µl. The composition gradient was: 2% solvent B reaching 15% at 10min; 30% solvent B at 25min, 65% solvent B at 40min and 98% solvent B at 45min, followed by 50min at isocratic elution until 55min. At 60min the gradient reached the initial conditions again, following the method described by Boligon et al.[26] with slight modifications. The sample and mobile phase were filtered through 0.45µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the methanol: water (1:1, v/v) at a concentration range of 0.030 – 0.500mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 280 nm for catechin; 327nm for chlorogenic, *p*-coumaric acid and caffeic acids, and 366 for quercetin, rutin, luteolin, apigenin and kaempferol. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). The limit of detection (LOD) and limit of quantification (LOQ) for both samples were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve. All chromatography operations were carried out at ambient temperature and in triplicate.

Antioxidant indices

A modified Folin-Ciocalteu method[27] and the modified method reported by Meda et al.,[28] were used to measure the total phenol (TPC) and total flavonoid contents. Centrifuged tea infusions were reacted with Folin Ciocalteu phenol reagent and sodium carbonate (20%, w/v for 2h) and the absorbance was read at 760nm. Tannic acid was used as a standard and the TPC expressed as mg of Tannic Acid Equivalents (TAE) per g. Similarly, the centrifuged tea infusions were reacted with 0.5 mL methanol, 50 µL of 10% $AlCl_3$, 50µL of 1mol L^{-1} potassium acetate and 1.4mL water, and incubated at room temperature for 30min. Thereafter, the absorbance of each reaction mixture was measured at 415nm. The total flavonoid content was calculated using quercetin as standard and a seven point standard curve (0-100 µg/ml). The ferric reducing properties of the tea infusions were determined using the method of Oyaizu[29] by reacting 1ml tea infusions with 1mL 200mM sodium phosphate buffer (pH 6.6) and 1mL 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min and then 1mL 10% trichloroacetic acid (TCA) was added. This mixture was centrifuged at 353 x g for 10min. Two milliliters (2mL) of the supernatant was mixed with an equal volume of water and 0.4mL of 0.1% ferric chloride, and the absorbance was measured at 700nm. The ferric reducing antioxidant power was expressed as mg ascorbic acid equivalent/g of the sample. Radical scavenging antioxidant activity of the tea infusions were determined using the 1, 1 diphenyl-2-picrylhdrazyl (DPPH) radical scavenging assay according to the method of Brand-Williams et al.[30] Trolox was used as a standard for DPPH anti-radical assay, and the values reported as µmol trolox equivalent anti-oxidant capacity per gram (µmol TE/g).

Mineral analysis

Five grams (5g) of each sample was dry-ashed in an electric furnace at 550°C for 24h. The resulting ash was cooled in a desiccator and weighed. The ash was dissolved with 2ml of concentrated HCl and a few drops of concentrated HNO_3 were added. The solution was placed in boiling water bath and evaporated almost to dryness. The content was then transferred to 100ml volumetric flask and diluted with deionized water. Appropriate dilution was made for each element before analysis. The mineral analyses carried out on the sample were calcium, magnesium, potassium, sodium, phosphorus, manganese, lead, zinc, cobalt and iron contents, and were quantified using Buck Atomic Absorption Spectrophotometer model 210A, as described in the official method of AOAC.[31]

Anti-nutrient assays

The phytate and oxalate contents of the studied tea were determined by the method described by Day & Underwood.[32] One gram of each tea sample was soaked in 100ml of 2% HCl for 3h, 6.25ml was taken out of the filtrate and placed inside a conical flask and 1.25ml of 0.3% of ammonium thiocyanate solution was added as indicator. Thereafter, 13.38ml of distilled water was added to give the proper acidity and it was titrated against iron (III) chloride solution that contained about 0.00195 g of iron per milliliter until a brownish yellow coloration persisted for 5min, and the phytate content was subsequently calculated. Oxalate content was determined by soaking

1g of each of the samples in 75ml of 1.5N H_2SO_4 for 1hour and then filtered. Five milliliters of the filtrate was taken out and placed inside a conical flask, and then it was titrated hot at about 80-90^{0}C against 0.1M $KMnO_4$ until a pink color that persisted for 15seconds was observed and the oxalate content was subsequently calculated. The alkaloid content was determined according to the method described by Harbone,[33] with slight modifications, by weighing 5g of the sample into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min. The obtained mixture was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added dropwise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with diluted ammonium hydroxide, and then filtered. The residue was the alkaloid which was dried and weighed.

In Vivo analyses

Animals

Adult male albino rats weighing 150-170g were used according to the standard guidelines of the Care and Use of Experimental Animal Resources. This was also approved by the Ethical Committee of the Federal University of Technology, Akure, Nigeria on the use of animals (Approval/Ethic Number: FUTA/BCH/FPT/005). The rats were allowed to acclimatize for a week before the experiment.

Mortality study

There were 10 groups of 5 albino rats each; the experiment was carried out using the standard method. Tap water, 10 mg/ml/kg BW, 30mg/ml/kg BW and 50mg/ml/kg BW of the tea infusion were given to the rats in the groups respectively. The animals given tap water served as controls. The tea was administered orally and all the rats were placed under observation for 24h for possible deaths of the rats.

Group 1: Control; group without treatment; normal diet and 0% of the tea samples

Group 2: hot water infusion of green tea; 10mg/ ml/kg BW

Group 3: hot water infusion of green tea; 30mg/ ml/kg BW

Group 4: hot water infusion of green tea; 50mg/ ml/kg BW

Group 5: hot water infusion of chamomile tea; 10mg/ ml/kg BW

Group 6: hot water infusion of chamomile tea; 30mg/ ml/kg BW

Group 7: hot water infusion of chamomile tea; 50mg/ ml/kg BW

Group 8: hot water infusion of green tea + chamomile tea; 10mg/ ml/kg BW

Group 9: hot water infusion of green tea +chamomile tea; 30mg/ ml/kg BW

Group 10: hot water infusion of green tea + chamomile tea; 50mg/ ml/kg BW

Dietary/biochemical study

Since none of the animals in the mortality study died, further administration of the infusions continued for another four weeks. At the end of the four weeks, the rats were weighed, and blood samples were collected through cardiac puncture under chlorohydrate anaesthesia into EDTA bottles, centrifuged and the plasma was aspirated to analyze the effect of the tea samples on liver markers: alanine transaminase, aspartate transaminase, alkaline phosphatase, total protein, gamma-glutamyl transpeptidase, albumin, glucose and lactate dehydrogenase. The animals were subsequently sacrificed and the liver tissues were taken and immediately fixed in 10% formaldehyde for histological examination.

Statistical analysis

All the analyses were conducted in triplicates. Results were computed using Microsoft Excel, 2010 software (2010 Microsoft Corporation, Redmond, WA, USA) and followed by analysis of variance (ANOVA) Duncan's multiple range test to compare the means that showed a significant variation by using SPSS 11.09 for Windows (IBM SPSS, Inc., Armonk, NY, USA). The significance level was set at $P<0.05$.

Results and Discussion

HPLC-DAD analysis of *Camellia sinensis tea and Matricaria chamomilla tea*

The adaptability of medicinal plants in human health has been documented for thousands of years,[35,36] while the utilization of medicinal plants for both traditional and non-traditional forms of medicine is dated back to at least 5000 years.[35,37] Phenolic compounds are commonly found in plant kingdom, which represent one of the major groups of compounds acting as primary antioxidants or free radical terminators, with several health promoting benefits.[38–40] The HPLC-DAD quali-quantitative analysis of phenolic compounds of green tea (*Camellia sinensis*) and chamomile tea (*Matricaria chamomilla L.*) is as presented in Figure 1a and Figure 1b. Qualitatively, the result revealed the presence of quercetin, rutin, chlorogenic, kaempferol, catechin in both green tea (*Camellia sinensis*) and chamomile tea (*Matricaria chamomilla L.*), with gallic acid, epicatechin and caffeine only present in green tea, while luteolin, caffeic acid, *p*-coumaric acid and apigenin are only present in chamomile tea. The quantitative estimates (mg/g) of the identified phenolic compounds revealed a higher total phenolic compounds in green tea (34.85), compared with chamomile tea. The result further showed that the most abundant phenolic compound in green tea is caffeic acid (10.63), and in chamomile tea, *p*-Coumaric acid (4.86 mg/g) ranked higher, while the least abundant phenolic compound in green tea is gallic acid (1.19) and in chamomile tea, kaempferol (0.29) ranked the least.

Previous work revealed that most of the polyphenols in green tea are flavanols, commonly known as catechins.[41] The primary catechins in green tea are (-) epicatechin (EC), (-) epicatechin-3-gallate (ECG), (-) epigallocatechin (EGC), and (-) epigallocatechin-3-gallate (EGCG). In addition, caffeine, theobromine, theophylline, and phenolic acids, such as gallic acid, have also been reported to be present as minor constituents of green tea.[41,42] The regular consumption of green tea is related to benefits in some diseases as atherosclerosis and cancer.[41] Green tea has shown remarkable anti-inflammatory and cancer chemopreventive effects in many animal tumor bioassays, cell culture system and epidemiologic studies.[42] Epigallocatechin-3-gallate (EGCG), the major catechin component in green tea, selectively prevents cytokine-induced VCAM-1 expression and reduces monocyte adhesion to endothelial cells independently of NF-k-B activation. Since VCAM-1 is one of the key molecules involved in the early atherogenic process, this emphasizes a novel mechanism by which tea catechins may exert anti-atherogenic effects.[41,43]

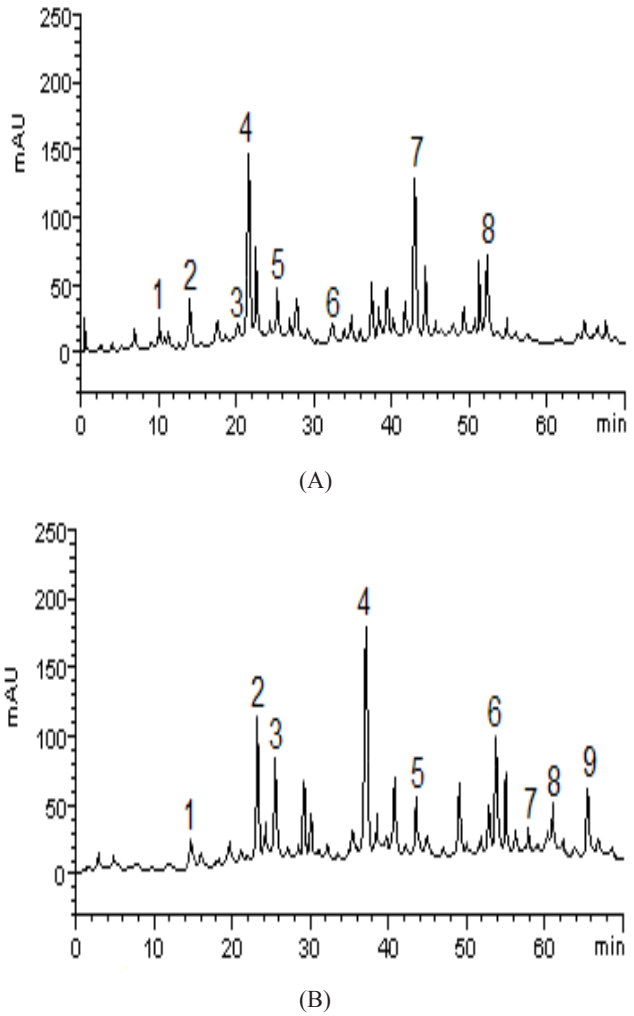

Figure 1 (a-b) Representative high performance liquid chromatography profile of (a) *Camellia sinensis* (GT) tea: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeine (peak 4), epicatechin (peak 5), rutin (peak 6), quercetin (peak 7) and kaempferol (peak 8); (b) *Matricaria chamomilla* (C): catechin (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), *p*-coumaric acid (peak 4), rutin (peak 5), quercetin (peak 6), kaempferol (peak7), luteolin (peak 8) and apigenin (peak 9)

The phenolic profile of chamomile using ultra performance liquid chromatography (UPLC) coupled with photodiode array (PDA) detector as previously reported identified some phenolic compounds especially flavonoids such as apigenin 7-glucoside, apigenin, quercetin, luteolin, kaempferol, rutin and isorhamnetin.[44] *Matricaria chamomilla* L. (Asteraceae) is often used as a medicinal plant, due to its anti-inflammatory, analgesic, sedative, antimicrobial, anti-allergic, anti-hyperglycemia and anti-spasmodic effects. It is also used in a number of alimental, cosmetic, pharmaceutical industries. Previous research supports this use and shows these properties are partly due to its phenolic compounds.[45,46] The spasmolytic and antiphlogistic activities are mainly attributed to the presence of apigenin, apigenin-7-O-glucoside and its acetylated derivatives.[47] The flavones are also known to play an important role in the overall anti-inflammatory activity, chemopreventive activity against UV-radiation and/or anti-cancer properties against a number of tumor types and sedative effect of chamomile extracts.[48–50]

Antioxidant Indices of *Camellia sinensis tea and Matricaria chamomilla tea*

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed their antioxidant activities and their ability to control free radical mediated diseases.[51,52] The result of antioxidant indices (Figure 2a-d) showed that green tea have the highest total phenolic content (844.97mg/g), followed by the tea infusion prepared from the composite blends (green tea + chamomile: 619.43 mg/g), while chamomile tea has the least total phenolic content (43.57mg/g). Various studies have reported high total polyphenol content in green tea.[53–,55] Flavonoids are the most numerous of the phenolics and are found throughout the plant kingdom.[3] They are present in high concentrations in the epidermis of leaves and fruits and have important and varied roles as secondary metabolites. Similarly, the results revealed that green tea have the highest amount of total flavonoid content (180.39mg/g); followed by the composite blend (96.46mg/g), while the least content was recorded for chamomile (64.38mg/g). The total flavonoid content in the studied tea samples is in agreement with in the previous report.[56] The results obtained for ferric reducing antioxidant power (FRAP) and DPPH radical scavenging ability was similar to the result obtained for the (TPC and TFC). The observed antioxidant activity elicited by the evaluated tea infusions could be attributed to the constituent phenolic and could therefore be harnessed as functional food in the prevention and management of free radical mediated diseases. The ferric reducing antioxidant power, radical scavenging ability and the reducing power of the tested teas are in agreement with the previous report in which the higher antioxidant and antiradical action positively correlates with the phenolic content of phenolic extracts of plant food.[57] The high antioxidant activities in green tea compared to chamomile tea could be ascribed to a higher phenolic content as revealed in the quantitative estimation of the two tested teas (Table 1).

Mineral analysis of *Camellia sinensis tea and Matricaria chamomilla tea*

It has been reported that teas and tea products contain essential mineral elements like calcium, zinc, magnesium, manganese, sodium and potassium.[58–60] The result of the mineral analysis (mg/g) of hot water infusions of Green tea (GT), Chamomile tea (C) and the composite tea blends (GT+C) *is* shown in Table 2. The result showed varied levels of minerals **(**Ca, Mg, Na, K, P, Zn, Fe, Mn) in the evaluated tea infusions, with calcium and zinc ranking high in GT (Ca: 143.64±0.00; Zn: 11.89±0.00); magnesium, iron, manganese and phosphorus ranking high in C (Mg: 2.98±0.20; Fe: 5.50±0.60; Mn: 1.10±0.10; P: 1.89±0.10), while sodium and potassium ranked high in GT+C (Na: 65.14±2.34; K: 157.35±11.56). The results further revealed that the evaluated tea infusions are rich sources of potassium and calcium. Potassium is the principal cation in intracellular fluid and functions in acid-base balance, in the regulation of osmotic pressure, muscle contraction and Na^+/K^+ ATPase.[61] Potassium is also required during glycogenesis, and it helps in the transfer of phosphate from ATP to pyruvic acid. The consumption of too much sodium and less amount of potassium contributes to a high prevalence of hypertension.[61]

The potassium content of the studied tea infusions was found to be higher than the sodium content. The sodium/potassium ratio (Na: K) in our body is of great concern as it prevents high blood pressure and the ratio should be less than one.[62] The three tested tea infusions

were found to have Na: K less than one (GT: 0.33; C: 0.35 and GT+C: 0.41, respectively). Therefore, the consumption of these herb teas may be beneficial in the management and control of the high blood pressure. Calcium on the other hand functions as a constituent of bones and teeth, the regulation of the nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin. Calcium also activates a large number of enzymes such as adenosine triphosphatase (ATPase), succinate dehydrogenase, and lipase.[62,63] Calcium was also found to be higher in GT+C (77.52±11.15), followed by Chamomile (74.14±8.00), while the least calcium content was recorded for Green tea (63.74±0.00).

Table 1 Phenolic composition of *Camellia sinesis, Matricaria chamomilla*

Componds	Camellia sinensis (mg/g)	Matricaria chamomilla (mg/g)	LOD µg/mL	LOQ µg/mL
Gallic acid	1.28±0.03a	-	0.025	0.083
Catechin	3.17±0.01b	0.59±0.01a	0.024	0.079
Epicatechin	3.14±0.01b	-	-	-
Chlorogenic acid	1.19±0.02a	2.97±0.04c	0.008	0.043
Caffeic acid	10.63±0.04e	2.13±0.02b	0.008	0.052
Apigenin	-	1.18±0.02b	0.011	0.036
Rutin	1.25±0.01a	1.15±0.01b	0.013	0.082
Quercetin	9.06±0.03d	2.19±0.02c	0.009	0.089
Kaempferol	5.13±0.02c	0.29±0.02 a	0.009	0.085
p-Coumaric acid	-	4.86±0.03d	0.017	0.056
Luteolin	-	0.62±0.02a	0.015	0.049
Total Phenolics	34.85	15.98		

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters at each column differ by Turkey test at p<0.05. Abbreviation: LOD; Limit of detection, LOQ; Limit of qualification

Table 2 Mineral composition (mg/g) of Camellia sinesis, Matricaria chamomilla and their composite blends

Sample	Ca	Mg	Na	K	P	Zn	Fe	Mn
GT	143.64±0.00	2.73±0.1	51.50±5.00	154.00±0.00	0.46±0.06	11.89±0.00	3.05±0.67	0.61±0.04
C	107.14±8.00	2.98±0.20	53.50±5.00	152.01±0.00	1.89±0.10	7.01±0.10	5.50±0.60	1.10±0.10
GT+C	127.52±9.15	2.97±0.54	65.14±2.34	157.35±11.56	1.51±0.76	10.80±0.21	4.87±0.55	0.59±0.04

Values represent mean ± standard deviation of triplicate experiment. Values with different letters within a column are significantly different (*P*< 0.05) by Duncan Test. GT; *Camellia sinesis, C; Matricaria chamomilla,* GT+C; *Camellia sinesis + Matricaria chamomilla*

Anti-nutrient assays of *Camellia sinensis tea and Matricaria chamomilla tea*

The result of the some selected anti-nutrient content (phytate, oxalate, alkaloids) in the evaluated tea samples is as presented in Table 3. The result showed that Green tea have the highest phytate content (1117.60mg/100g), followed by the composite blends (1019.70mg/100g), while the least value was recorded for Chamomile tea (804.27mg/100g). The phytate content of Green tea, Chamomile and the composite blend tea infusion were lower compared with the values reported by Ali *et al*.[64] for some other herbal teas such as Burdock root (2034mg/100g) and Cleavers (1918mg/100g). High concentrations of phytate are of nutritional significance as they might decrease bioavailability of minerals.[65] Phytate acts as a strong chelator forming protein and mineral-phytate complexes, thereby, decreasing protein and mineral bioavailability. It has been reported that high phytate contents have decalcifying effects, resulting in nutritional disorders such as rickets and osteomalacia in children and adults, respectively.[66] Phytate can also affect digestibility by binding with substrates or proteolytic enzymes.[67]

The oxalate content of the evaluated tea samples are as follows: Green tea (93.05mg/100g), composite blend tea infusion (60.03mg/100mg) and the least value was recorded for Chamomile tea (41.01mg/100g). Oxalate is regarded as undesirable constituents of the diets, reducing assimilation of calcium. The result of the oxalate content of the tested teas, with the exception Chamomile tea were higher than the values reported for Burdock root (48.11mg/100g) and Cleavers (45.76mg/100g) herbal teas.[64] An uncontrolled consumption of herbal teas with high oxalate content may deliver toxic levels of the anti-nutrient into the body with attendant health problems of oxalate toxicosis, which may ultimately result in hypocalcaemia, kidney stone and reduced bioavailability of the minerals to the body.[68] Oxalate bound to minerals such as calcium, magnesium, iron and zinc, makes the minerals unavailable for body use.[69,70]

Table 3 Anti-nutrient composition of Camellia Sinesis, Matricaria Chamomilla and their composite blends

Sample	Phytate (mg/g)	Oxalate (mg/g)	Alkaloids (%)
GT	1117.60±21.32	93.05±7.40	19.33±0.67
C	804.27±17.51	41.01 ± 5.20	16.56± 0.51
GT+C	1019.70± 7.22	60.03±5.20	1.00±0.33

Values are given as mean ± SD of independent experiment performed in triplicate. Values with different letters at each column are significantly different (P< 0.05) by Duncan Test. Values represent mean ± standard deviation of triplicate experiment. GT; *Camellia sinesis, C; Matricaria chamomilla,* GT+C; *Camellia sinesis + Matricaria chamomilla*

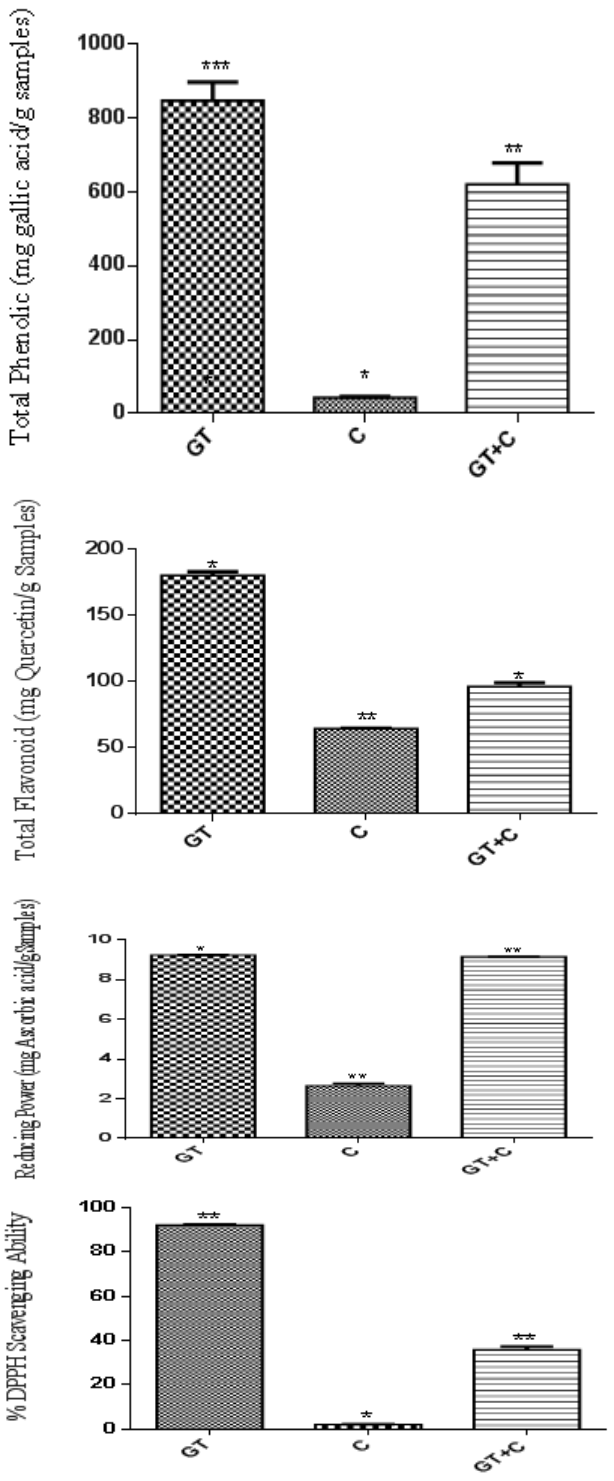

Figure 2 a) Total phenolic content (mg tannic acid equivalent/g of sample), b). Total flavonoid content (mg quercetin equivalent/g of sample), c). Reducing power (mg ascorbic acid equivalent/g of sample), and (d). % $DPPH^{\cdot}$ radical scavenging activity of tea infusions of *Camellia sinesis* (GT), *Matricaria chamomilla* (C) and their composite blend (GT+C). Values are given as mean ± SE of the independent experiments performed in triplicate. Bars with different asteriks are significantly different (*P<0.05*) by Duncan Test. GT; *Camellia Sinesis, C; Matricaria Chamomilla,* GT+C;. *Camellia sinesis* **+** *Matricaria chamomilla*

The alkaloid content of the studied tea samples revealed that Green tea have the highest value (19.33%), followed by Chamomile tea (16.56%) and the least value was recorded for the composite blend tea infusion (1.00%). A wide range of biological activities of alkaloids have been reported: emetic, anti-cholinergic, antitumor, diuretic, sym-pathomimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antitussigen, antimicrobial and anti-inflammatory.[71] However, only a moderate level of alkaloid in food is an indication that the food is safe and free from the attendant cytotoxic effect of alkaloids.[72] Alkaloids are considered to be anti-nutrients because of their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission. The levels are normally low and without adverse effects on food safety and culinary quality. However, the consumption of unusually high contents of alkaloids in food has occasionally been associated with acute poisoning, including gastrointestinal and neurological disturbances.[73]

Ca and Zn bioavailability of *Camellia sinesis, Matricaria chamomilla and their composite blends*

The result of Ca and Zn bioavalabity in the studied tea infusions is as presented in Table 4. The result revealed that [Phytate]/[Ca] molar ratios of the three tested tea infusions were slightly below the critical value of 0.5, which is known to impair calcium bioavailability.[74] The estimated [Phytate]/[Ca] molar ratios of the tested tea samples ranges from 0.46 (Chamomile tea*)* to 0.49 (Green tea + Chamomile tea). In the same vein, the calculated [Oxalate]/[Ca] molar ratios were below the critical value of 2.5 known to impair calcium bioavailability,[75] with values ranging from 0.17 (Chamomile tea) to 0.29 (Green tea). Although, the results of [Phytate]/[Ca] molar ratios do not provide a clearer information about the bio-availability of calcium in the studied tea infusion when compared with the result of the [Oxalate]/[Ca] molar ratios which clearly showed that calcium would be bio-available in the two varieties of the studied tea infusions and the composite blend tea infusion in the presence of anti-nutrients like phytate and oxalate. Zinc bioavailability is usually predicted by phytate to zinc molar ratio of the food and the amount of calcium in the food.[76,77] This index is considered as a good estimate of zinc bioavailability and it is widely used in evaluating the bioavailability of zinc.[78]

Table 4 Ca and Zn bioavailability of Camellia sinesis, Matricaria chamomilla and their composite blends

Sample	[PHY]/[Ca]	[OXA]/[Ca]	[PHY]/[Zn]	[Ca][PHY]/[Zn]
GT	0.47±0.013	0.29±0.014	9.31±0.12	33.44±0.87
C	0.46±0.022	0.17±0.013	11.37±0.13	30.45±0.93
GT+C	0.49±0.016	0.21±0.011	9.36±0.21	29.83±0.76
Critical value*	0.50	2.50	15.00	200.00

Abbreviations: PHY; Phytate, Ca; Calcium, OXA; Oxalate, Zn; Zinc, GT; Camellia Sinesis, C; Matricaria Chamomilla, GT+C; Camellia sinesis + Matricaria chamomilla. Values represent mean standard deviation of triplicate experiments.

The calculated molar ratios of phytate to zinc of both tea infusions and their composite blend tea infusion were below the critical value of 15 as outlined by Fitzgerald *et* al.[70] The values ranged from 9.31(Green tea) to 11.37 (Chamomile). This showed that the concentration of phytate in the three tested tea infusion will not affect the bioavailability

of zinc. Furthermore, the calculated [Ca][Phytate]/[Zn] molar ratio is considered a better index for predicting zinc bioavailability compared to [Phytate]/[Zinc] ratio because the inhibitory effect of phytate on zinc absorption increases as the amount of dietary calcium increases and this is caused by the synergistic interaction between calcium, phytate and zinc.[79,80] Similarly, the calculated values for [Ca][Phytate]/[Zn] ratio of the three tested tea infusion were below the critical level of 200, ranging 29.83 (Green tea + Chamomile) to 33.44 (Green tea). This implies that the concentration of calcium in the tea infusions will not affect the bioavailability of zinc in the presence of phytate. Therefore, calcium and zinc would be bio-available in all the tested tea infusions, since all the calculated indices of anti-nutrients to calcium and zinc are below their respective critical values; therefore, it can be inferred that iron and magnesium will probably be bio-available in the studied tea infusions.

Effects of *Camellia sinesis, Matricaria chamomilla and their composite blends on liver markers*

The result of the rats' liver biomarkers after the administration of various concentrations of the three tested tea infusions (Green tea infusion, Chamomile tea infusion and the composite blend tea infusion), is as presented in Table 5 (a, b). The enzymatic activities of GGT (Gamma-glutamyltransferase), ALP (Alkaline phosphatase), AST (Aspartate transferase), ALT (Alanine transferase), and LDH (Lactate dehydrogenase) was evaluated to assess the liver function. The evaluated liver enzyme biomarkers decreases in a dose dependent manner after the administration of the three tested teas in all the test rats compared. Evaluation of liver enzyme biomarkers and other biochemical profiles of blood are widely used as indicators to access the functional status of the animal health and the internal environment of the organism.[81,82] Estimation of Alkaline phosphatise (ALP), Aspartate transferase (AST), and Alanine transferase (ALT), usually served as good liver biomarkers.[83] The elevation in the liver enzyme biomarkers beyond the threshold values indicates liver inflammation or damage to the cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes into the blood stream which can result in elevated liver enzymes on blood tests. The observed dose dependent decrease in the activities of liver enzymes as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin and glucose, with exception of total protein and albumin after the administration of the tea infusions tend to suggest the hepato-protective properties of the tested tea infusions. The results further revealed that all the evaluated liver biomarkers fall within the reference values. This by implication is that there is no damage or impairment to the integrity of the liver.[84]

Table 5a Effects of Camellia sinesis, Matricaria chamomilla and their composite blends on liver markers

SAMPLES	GGT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	LDH (U/L)
Control	24.05±0.58	56.22±4.14	36.00±1.67	25.70±1.10	231.43±33.03
GT1	26.73±4.05	73.12±1.38	44.83±4.5	31.90±0.10	351.11±23.65
GT3	23.69±3.32	63.46±9.66	37.00±0.83	26.90±2.50	259.05±31.97
GT5	21.63±9.84	50.70±2.76	35.17±2.33	25.70±0.90	213.18±8.25
C1	28.69±4.05	64.50±5.52	41.00±1.50	29.40±0.60	373.65±25.40
C3	18.68±6.37	60.56±4.14	36.50±2.00	24.80±2.20	229.37±10.32
C5	15.42±3.47	59.66±4.14	32.67±0.67	21.10±1.50	218.42±27.14
GT+C1	25.58±9.26	66.22±1.38	42.00±2.33	37.40±0.80	367.94±21.27
GT+C3	21.53±2.53	53.42±4.14	35.00±0.67	29.70±0.90	232.22±10.32
GT+C5	17.64± 3.57	48.17±2.18	31.54±1.37	24.67±3.21	215.56±3.43
Normal value 5-48U/L		45-115U/L	8-48U/L	7-55U/L	122--320U/L

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE of triplicate experiments. GT1 represents *Camellia sinesis* extracts at 10mg/kg.BW/ml; GT3 represents *Camellia sinesis* extracts at 30mg/kg.BW/ml; GT5 represents *Camellia sinesis* extracts at 50mg/kg.BW/ml; C1 represents *Matricaria chamomilla* extracts at 10 mg/kg.BW/ml; C3 represents *Matricaria chamomilla* extracts at 30mg/kg.BW/ml; C5 represents *Matricaria chamomilla* extracts at 50 mg/kg.BW/ml; GT+C1 represents *Camellia* sinesis + Matricaria *chamomilla* 10 mg/kg.BW/ml; GT+C3 represents *Camellia* sinesis + Matricaria *chamomilla* 30mg/kg.BW/ml; GT+C5 represents *Camellia* sinesis + Matricaria *chamomilla* 50mg/kg.BW/ml; N=5; GGT; Gamma-glutamyltransferase, ALP; Alkaline phosphatise, AST; Aspartate transferase, ALT; Alanine transferase, LDH; Lactate dehydrogenase

Histology, which is the microscopic study of tissues, including their anatomy, interaction with body systems and the way they are affected by diseases plays a vital role in the diagnosis of disease due to its ability to reveal changes in tissue arrangement and organization. The liver is the target tissue in this context because it functions as the site for the metabolism of various substances and xenobiotics and damage to the hepatocytes may be deleterious. Histo-pathological examination is identified as a better method for evaluating and characterizing pathological changes associated with tissue lesions.[85] Histological examination of the rats' liver administered with various concentrations of Green tea, Chamomile tea and the composite blends (Green tea + Chamomile tea) was carried out to ascertain the effects of the tea infusions on the functionality and integrity of the liver. The result of the histological investigation (Figure 1-3) revealed that the administration of various concentrations (10, 30, and 50mg/kg b.w) of the studied tea infusions showed no damaging effects on the liver.

Table 5b Effect of Camellia Sinesis, Matricaria Chamomilla and their composite blends on liver markers

SAMPLES	T.PROT (g/l)	ALB (g/dl)	BIL (mg/dl)	GLU (mg/dl)
Control	68.41±2.19	37.57±0.54	1.39±0.06	105.11±2.58
GT1	68.38±2.56	31.74±0.19	1.09±0.03	100.56±1.36
GT3	69.12±0.93	41.65±2.79	0.62±0.09	100.52±0.45
GT5	71.63±2.28	46.05±0.20	0.43±0.03	92.34±3.10
C1	68.65±5.07	41.07±3.16	1.18±0.04	112.58±3.09
C3	72.61±3.17	43.30±1.67	0.42±0.03	95.85±0.65
C5	73.24±0.33	45.31±1.27	0.31±0.01	92.40±6.52
GT+C1	69.91±0.47	42.37±1.91	1.16±0.05	109.72±3.23
GT+C3	74.00±2.33	44.66±0.83	0.64±0.02	97.71±1.36
GT+C5	79.70±2.90	45.21±2.33	0.19±0.02	85.27±0.32
Normal value 64-83g/L		35-50g/L	0.1-.2mg/dL	75-115mg/dL

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE of triplicate experiments. GT1 represents *Camellia sinesis* extracts at 10 mg/kg.BW/ml; GT3 represents *Camellia sinesis* extracts at 30 mg/kg.BW/ml; GT5 represents *Camellia sinesis* extracts at 50 mg/kg.BW/ml; C1 represents *Matricaria chamomilla* extracts at 10 mg/kg.BW/ml; C3 represents *Matricaria chamomilla* extracts at 30 mg/kg.BW/ml; C5 represents *Matricaria chamomilla* extracts at 50mg/kg.BW/ml; GT+C1 represents *Camellia* sinesis + Matricaria *chamomilla* 10mg/kg.BW/ml; GT+C3 represents *Camellia* sinesis + Matricaria *chamomilla* 30 mg/kg.BW/ml; GT+C5 represents *Camellia* sinesis + Matricaria *chamomilla* 50mg/kg.BW/ml; N=5; T. PROT: Total protein; ALB; albumin, BIL; Total Bilirubin, GLU; Glucose

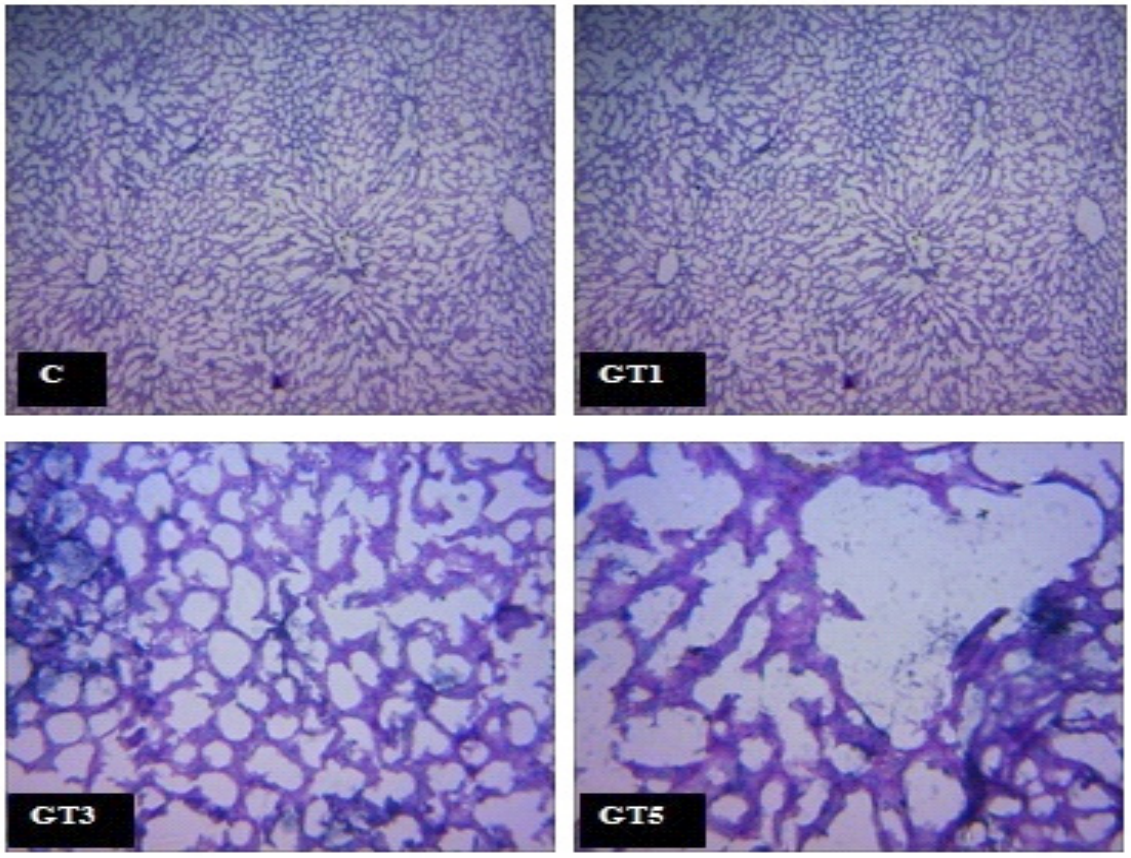

Figure 3a The histopathological picture of liver sections of normal Control rats (C), Green tea treated rats at doses 10, 30 and 50 mg/kg b.wt (GT1, GT3 and GT5). The design of hepatic structure showed evidence of normal histology. (H&E; ×100) H and E means Hematoxylin and eosin **stain** or haematoxylin and eosin **stain**.

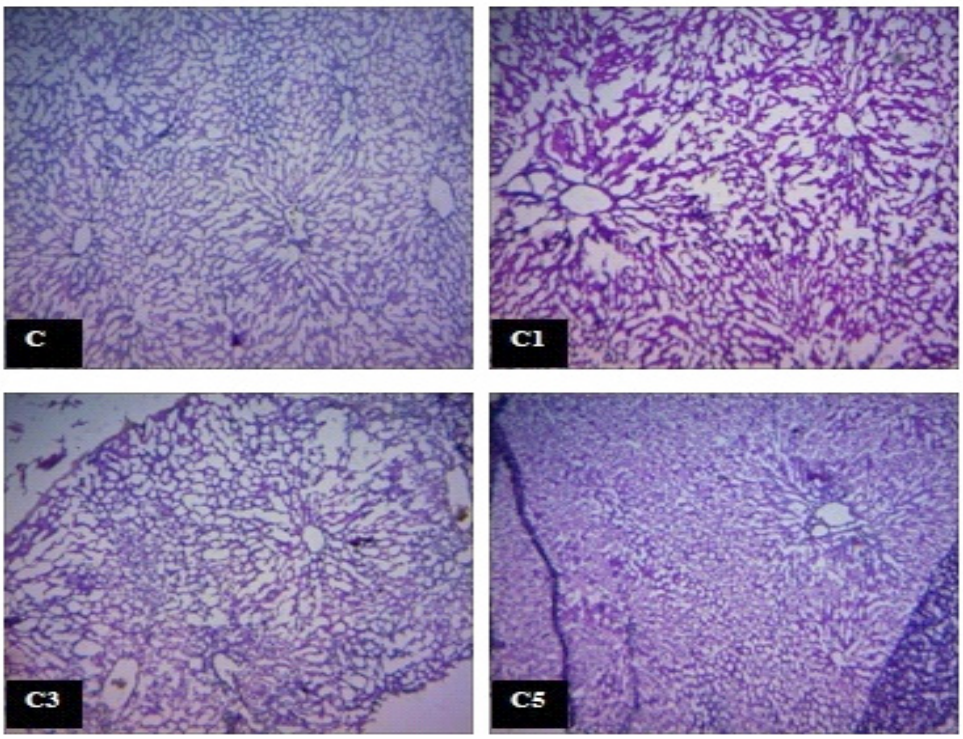

Figure 3b The histopathological picture of liver sections of normal Control rats (C), Chamomile tea treated rats at doses 10, 30 and 50 mg/kg b.wt (C1, C3 and C5). The design of hepatic structure showed evidence of normal histology. (H&E; ×100) H and E means Hematoxylin and eosin **stain** or haematoxylin and eosin **stain**.

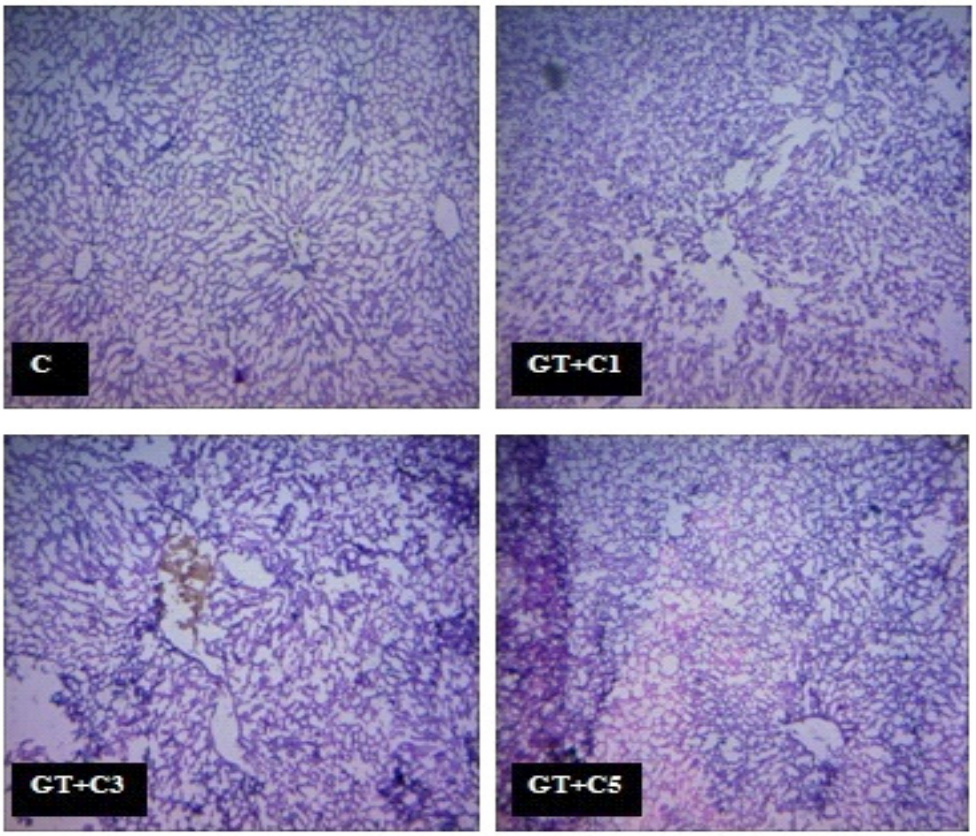

Figure 3c The histopathological picture of liver sections of normal Control rats (C), mixture tea sample treated rats at doses 10, 30 and 50 mg/kg b.wt (GT+C1, GT+C3 and GT+C5). The design of hepatic structure showed evidence of normal histology. (H&E; ×100) H and E means Hematoxylin and eosin **stain** or haematoxylin and eosin **stain**.

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Conclusion

The result of the present investigation provides a scientific insight to the potential use of the hot water infusions of Green tea (*Camellia Sinesis*) and Chamomile tea (*Matricaria Chamomilla*), commonly used as folk remedy, as functional foods and also provides information on their effects on liver biomarkers in Wistar rats. Therefore, the regular consumption of the studied tea infusions at a regulated dose could be used in the management and prevention of stress related diseases.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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