Journal of Food Measurement and Characterization (2019) 13:602–613
https://doi.org/10.1007/s11694-018-9973-3

ORIGINAL PAPER

Nutraceutical values of hot water infusions of moringa leaf (*Moringa oleifera*) and licorice root (*Glycyrrhiza glabra*) and their effects on liver biomarkers in Wistar rats

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Received: 26 September 2017 / Accepted: 7 November 2018 / Published online: 15 November 2018

Abstract
Globally, there is an increasing need to consume health promoting foods and beverages as alternatives to synthetic drugs. Moringa leaf (*Moringa oleifera*) and Licorice root (*Glycyrrhiza glabra*) have been widely harnessed for their innumerable bio-functions. Therefore, the present study sought to evaluate the mineral contents and antioxidant activities of hot water infusion made of Moringa leaf (M), Licorice root (LR) and their composite blends (M + LR), and to assess their effects on liver biomarkers in Wistar rats. The results of the mineral contents (Mg, Na, K, P, Zn, Fe, Mn) of M, LR and their composite blends (M + LR), showed high amounts of the evaluated minerals in M and M + LR, with the exception of Fe that was higher in the Licorice root. The calculated [phytate]/[Ca], [oxalate]/[Ca], [phytate]/[Zn] and [Ca][phytate]/[Zn] molar ratios of the tea infusions fell below the critical values, thereby revealing that Ca and Zn and by extension other minerals would be bio-available. The tea infusions have a measure of antioxidant action with M + LR ranking higher. The effects of the tea infusions on liver biomarkers and the histological examination of the liver tissue showed that the tested concentrations of M, L and M + LR had no damaging effects on the liver with the exception of L and M + LR at 50 mg/mL/kg, where degeneration of hepatocytes was observed. Overall, the results showed that the composite blends (M + LR), at a regulated dose, could be explored as functional food in the provision of nutritionally important minerals, and the management of stress-related diseases.

Keywords Antioxidant activities · Mineral bioavailability · Hepatoprotection · Herbal tea infusions

Introduction

Globally, tea is the most popular beverage after water, and historians have dated its consumption to thousands of years ago [1]. Tea and tea products originated from China and have gained the world's trust in the past 2000 years. Initially, it was consumed only by Chinese monks, but its use spread to other regions of the world, such as Great Britain, leading to its effective spread to other countries [2].

Tea and tea products have been proven to contain essential mineral elements like calcium, zinc, magnesium, manganese, sodium and potassium among others in significant amounts [1, 3], which have been proven to play important roles in tissue development, enzyme function, maintenance of cell walls, among others [4]. Tea contains a number of polyphenolic compounds, which are known to have a wide spectrum of biological activities, including the prevention of cancer, cardiovascular diseases, and anti-inflammatory, anti-arthritic, antibacterial, anti-angiogenic, anti-oxidative, antiviral, neuro-protective, and cholesterol-lowering effects of tea and isolated tea constituents are under investigation [2, 5, 6].

Antioxidants have become synonymous with good health because they contain classes of compounds that can prevent damages caused by the free radicals. Destroying free radicals may help fight ailments such as cancer, heart disease, stroke and other immune compromising diseases [7–9].

In this modern era, stress has become an integral part of human life [10]. Stress is considered to be any condition which results in the perturbation of the body's homeostasis [11]. Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, such as

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hypertension, diabetes, immuno-suppression, reproductive dysfunctions and behavioral disorders, which are due to the involvements of the central nervous system (CNS), endocrine system, and metabolic system [12]. If the level of stress is extreme, homeostasis may become altered and the survival of the organism may be threatened [11]. Some drugs having anti-stress properties induce a state of non-specific resistance against stressful conditions. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs [12].

Moringa (*Moringa oleifera*) and Licorice (*Glycyrrhiza glabra*) teas are nutraceuticals that have been widely used for their innumerable bio-functions. Previous reports have demonstrated that *M. oleifera* and *G. glabra* have been widely used in folk medicine mainly as anti-stress agent [13, 14], and in other conditions that are related to stress, such as anti-mutagenic, anti-diabetic, anti-bacterial, hypo-cholesterolemic, anti-inflammatory, anxiolytic, anti-ulcer, cytostatic, antimicrobial, flu vaccine, and in the treatment of cough, common cold, and gastrointestinal disorders [15].

Liver function tests are a group of blood tests that provide information about the state of the liver [16]. Elevated levels of some substances or enzymes in the blood may signify liver damage or diseased state [17]. 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 (GGT) and alkaline phosphatase (ALP)]. 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 [18]. 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 [19, 20].

The human body identifies all the drugs and nutraceuticals as xenobiotics and subjects them to various chemical processes to make them suitable for elimination [19]. 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 [21]. The central role played by the liver in the clearance and transformation of chemicals makes it susceptible to drug and natural products induced injury [22].

It is therefore expedient to assess the nutraceutical potentials of the selected teas, and 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 made from pure Licorice root and Moringa leaf were bought from Tradomedical Centre, Ibadan, Oyo state, Nigeria.

Methods

Sample treatments and preparation

The tea infusions (Licorice root, Moringa leaf and composite blends of both teas) were prepared using hot water infusion as follows: 15 g of each tea sample (Licorice root and Moringa leaf) was infused in 1.2 L of hot water and the tea infusions of the composite blends were prepared by mixing 7.5 g each of the studied tea samples, and subsequently infused in 1.2 L 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.

Mineral analysis

Five grams of each sample was dry-ashed in an electric furnace at 550 °C for 24 h. The resulting ash was cooled in a desiccator and weighed. The ash was dissolved with 2 mL 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 100 mL 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 [23].

Anti-nutrient assays

The phytate and oxalate contents of the studied tea were determined by the method described by Day and Underwood [24]. One gram of each tea sample was soaked in 100 mL of 2% HCl for 3 h, 6.25 mL was taken out of the filtrate and placed inside a conical flask and 1.25 mL of 0.3% of ammonium thiocyanate solution was added as indicator. Thereafter, 13.38 mL 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 mL until a brownish yellow coloration persisted for 5 min, and the phytate content was subsequently calculated. Oxalate content was determined by soaking 1 g of each of the samples in 75 mL of 1.5N H_2SO_4 for 1 h and then filtered. 5 mL of the filtrate was taken out and placed inside a conical flask, and then it was titrated hot at about 80–90 °C against 0.1M $KMnO_4$ until a pink color that persisted for 15 s was observed and the oxalate content was subsequently calculated.

The alkaloid content was determined according to the method described by Harbone [25] with slight modifications, by weighing 5 g of the sample into 250 mL beaker and 200 mL 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.

Antioxidant indices

A modified Folin–Ciocalteu method (Waterman and Mole [26]) and the modified method reported by Meda et al. [27], was used to measure the total phenol contents (TPC) and total flavonoid contents (TFC). Centrifuged tea infusions were reacted with Folin Ciocalteu phenol reagent and sodium carbonate (20%, w/v for 2 h) and the absorbance was read at 760 nm. 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% $AlCl_3$, 50 µL of 1 mol/L potassium acetate and 1.4 mL water, and incubated at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was measured at 415 nm. The TFC 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 [28], by reacting 1 mL tea infusions with 1 mL 200 mM sodium phosphate buffer (pH 6.6) and 1 mL 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 1 mL 10% trichloroacetic acid (TCA) was added. This mixture was centrifuged at 353×*g* for 10 min. Two milliliters (2 mL) of the supernatant was mixed with an equal volume of water and 0.4 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm. The ferric reducing antioxidant power was expressed as mg ascorbic acid equivalent per gram of the sample. Hydroxyl radical scavenging antioxidant activity of the tea infusions was determined by measuring the ability of the tea infusion to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose by reacting the tea infusion with a reaction mixture containing 120 µL of 20 mM deoxyribose, 400 µL 0.1M phosphate buffer pH 7.4, 40 µL 20 mM hydrogen peroxide and 40µL 500 mM $FeSO_4$, and the volume was made to 800 µL with distilled water. The reaction mixture was incubated at 37 °C for 30 min, and then stopped by the addition of 0.5 mL of 2.8% TCA. This step was followed by the addition of 0.4 mL of 0.6% TBA solution. The reaction tubes were subsequently incubated in boiling water for 20 min. The absorbance was then measured at 532 nm using the method of Halliwell and Gutteridge [29] and the percentage hydroxyl radical scavenging ability was subsequently calculated.

In vivo analyses

Animals

Adult male albino rats weighing 150–170 g 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 ten groups of five albino rats each; the experiment was carried out using the standard method. Tap water, 10 mg/mL/kg BW, 30 mg/mL/kg BW and 50 mg/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 24 h for possible deaths of the rats.

Treatment groups

Group 2	control; group without treatment; normal diet and 0% of the tea samples
Group 2	hot water infusion of Moringa tea; 10 mg/mL/kg BW
Group 3	hot water infusion of Moringa tea; 30 mg/mL/kg BW
Group 4	hot water infusion of Moringa tea; 50 mg/mL/kg BW
Group 5	hot water infusion of Licorice tea; 10 mg/mL/kg BW
Group 6	hot water infusion of Licorice tea; 30 mg/mL/kg BW
Group 7	hot water infusion of Licorice tea; 50 mg/mL/kg BW

Group 8 hot water infusion of Moringa + Licorice tea; 10 mg/mL/kg BW

Group 9 hot water infusion of Moringa + Licorice tea; 30 mg/mL/kg BW

Group 10 hot water infusion of Moringa + Licorice tea; 50 mg/mL/kg BW

Dietary/biochemical study

Since none of the animals in the mortality study died, further administration of the infusions continued for another 4 weeks. At the end of the 4 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 (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, gamma-glutamyltranspeptidase (GGT), albumin, glucose and lactate dehydrogenase (LDH). 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

Mineral analysis

Tea and tea products have been shown to contain essential mineral elements like calcium, zinc, magnesium, manganese, sodium and potassium [1, 3, 30]. The result of the mineral analysis of hot water infusions of Moringa leaf (*M. oleifera*) and Licorice root (*G. glabra*) is shown in Table 1. The result revealed the presence of some essential minerals (Mg, Na, K, P, Zn, Fe, Mn). The result showed that sodium was higher in M + LR (87.0 mg/g), with *M. oleifera* having sodium content of 86.5 mg/g, while the sodium content of Licorice root was significantly ($p < 0.05$) lower than the other infusions analysed (6.00 mg/g). Sodium is the principal cation in extracellular fluids, it regulates plasma volume and acid-base balance, and it is involved in the maintenance of osmotic pressure of the body fluids [31].

The result of the mineral analysis also revealed that potassium was the most abundant mineral in the studied tea infusions. Similarly, potassium was higher in M + LR (161.0 mg/g), followed by *M. oleifera* (158 mg/g), while Licorice root recorded the least potassium content (31.0 mg/g). 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 activity [32]. Potassium is also required during glycogenesis, and it helps in the transfer of phosphate from ATP to pyruvic acid. The potassium content of the studied tea infusions was found to be higher than the sodium content. The consumption of too much sodium and less amount of potassium contributes to a high prevalence of hypertension [32]. The sodium/potassium ratio in our body is of great concern as it prevents high blood pressure and the ratio should be less than one [33]. All the tea infusions were found to have sodium/potassium ratio less than one (Na:K for *M. oleifera*, Licorice root, and M + LR were 0.55, 0.19, and 0.54, respectively). Therefore, the consumption of these herb teas may be beneficial in the management and control of the high blood pressure.

Table 1 Mineral composition (mg/g) of *M. oleifera, G. glabra* and their composite blends

Sample	Ca	Mg	Na	K	P	Zn	Fe	Mn
M	149.94 ± 0.86^b	2.83 ± 0.08^a	86.5 ± 2.41^b	158 ± 9.90^b	1.06 ± 0.03^b	0.30 ± 0.01^b	2.42 ± 0.03^a	5.18 ± 0.03^b
LR	21.34 ± 0.45^a	2.77 ± 0.11^a	6.00 ± 0.53^a	31.0 ± 2.33^a	0.02 ± 0.00^a	0.15 ± 0.02^a	6.90 ± 0.28^c	0.32 ± 0.02^a
M + LR	151.31 ± 2.05^c	2.80 ± 0.08^a	87.0 ± 3.82^b	161.0 ± 3.82^c	1.05 ± 0.03^b	0.32 ± 0.03^b	5.80 ± 0.47^b	5.20 ± 0.14^b

Values represent mean ± SD of triplicate experiment. Values with different letters within a column are significantly different ($p < 0.05$) by Duncan test

M, *Moringa oleifera*; LR Licorice root; M + LR, Moringa leaf + Licorice

Calcium 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 [31, 33]. Calcium was also found to be higher in M + LR (151.31 mg/g), followed by Moringa (149.94 mg/g), while the least calcium content was recorded for Licorice root (21.34 mg/g). Phosphorus is located in every cell of the body and it is vitally concerned with many metabolic processes, including those involving the buffers in body fluids [34]. It also functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids [35]. Conversely, the phosphorus contents of *M. oleifera* and M + LR were not significantly ($p < 0.05$) different (1.06 mg/g; 1.06 mg/g), but these two values were significantly ($p < 0.05$) higher than that of Licorice root (0.02 mg/g).

The magnesium contents of the three tea infusions (Moringa leaf: 2.83 mg/g; Licorice root: 2.77 mg/g; M + LR: 2.80 mg/g) were not significantly different. Magnesium is involved in bone mineralization, protein synthesis, enzyme action, normal muscular contraction and nerve transmission [31]. Dietary deficiency of magnesium which is linked with ischemic heart disease [36] could be prevented by the regular consumption of any of the two tea varieties or their composite infusion as they are a good source of magnesium.

Conversely, the iron content of Licorice root (6.90 mg/g) was higher than that of Moringa (2.42 mg/g) and the composite blends (M + LR: 5.80 mg/g). Iron is a part of the heme of haemoglobin (Hb), myoglobin, and cytochromes [37]. Inadequate dietary intake and poor bioavailability of iron from food are the major etiological factors of anaemia [38]. Regular consumption of the two tea varieties would likely prevent the development of iron deficiency anaemia. Iron also functions as an essential component of enzymes involved in biological oxidation such as cytochromes c, c_1, a_1 and as an important constituent of succinate dehydrogenase [37, 39].

There was no significant difference ($p < 0.05$) in the zinc content of Moringa and M + LR. The zinc content of Moringa was 0.3 mg/g, while that of M + LR was 0.32 mg/g compared to that of Licorice root which had the least zinc content. The level of zinc of these studied herbal teas is higher than the value reported for some herbal teas; 0.21 mg/g was reported for lemon balm and 0.19 mg/g for dictamnus tea [40], indicating that the two tea varieties are better sources of zinc than the previously reported teas (lemon balm tea, dictamnus tea). Zinc is widely distributed in plant and animal tissues and occurs in all living cells. Zinc is essential for the functioning of over 300 enzymes, for example, LDH, alcohol dehydrogenase, glutamic acid dehydrogenase, ALP, carbonic anhydrase, carboxypeptidase, superoxide dismutase, DNA and RNA polymerase, and takes part in enormous number of biological processes such as protein synthesis, cellular differentiation and replication, immunity and sexual functions [41]. Zinc deficiency is associated with impaired gastrointestinal and immune function [42]. The manganese contents of *Moringa* (5.18 mg/g) and M + LR (5.20 mg/g) were significantly ($p < 0.05$) higher than that of Licorice root (0.32 mg/g). Manganese is essential for humans because it exhibits a wide range of biological functions such as a component of enzymatic and redox system [43].

Anti-nutrient composition

The result of the anti-nutritional factor of the studied tea infusions is shown in Table 2. The result revealed that the phytate contents of *M. oleifera* (1126.13 mg/100 g) and *G. glabra* (906.40 mg/100 g) were relatively low compared to the values reported by Ali et al. [44] for some other herbal teas such as Burdock root (2034 mg/100 g) and Cleavers (1918 mg/100 g). The low level of phytate in the studied tea infusions would make the tested tea products good for consumption, from a nutritional point of view. Phytate contents present in plants have been shown to depend on a variety and climate condition [45]. High levels of phytate are of nutritional significance as they might decrease bioavailability of minerals [46, 47]. 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 [48]. Phytate can also affect digestibility by binding with substrates or proteolytic enzymes [49].

The result also revealed that the oxalate content of *M. oleifera* was 39.02 mg/100 g, while that of *G. glabra* was 27.01 mg/100 g, which is less than the values reported for Burdock root (48.11 mg/100 g) and Cleavers (45.76 mg/100 g) herbal teas [44]. Although, the results revealed low oxalate contents in the studied tea infusions, an uncontrolled consumption of these herbal teas may deliver

Table 2 Anti-nutrient composition of *M. oleifera, G. glabra* and the composite blends

Sample	Phytate (mg/g)	Oxalate (mg/g)	Alkaloids (%)
Moringa	11.26 ± 0.48^{b}	0.39 ± 0.02^{b}	27.00 ± 0.67^{c}
Licorice	9.06 ± 0.82^{a}	0.27 ± 0.00^{a}	1.56 ± 0.19^{a}
Moringa + Licorice	11.81 ± 0.47^{b}	0.33 ± 0.05^{b}	8.00 ± 0.67^{b}

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 ± SD of triplicate experiment

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 [50, 51]. Oxalate bound to minerals such as calcium, magnesium, iron and zinc, makes the minerals unavailable for body use [52, 53].

The alkaloid contents of the two studied tea infusions *M. oleifera* (27%) and *G. glabra* (1.56%) are shown in Table 2. The alkaloid content of Licorice root (1.56%) was lower than that of *M. oleifera* (27%) and that of the composite blends (8.00%). 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 [46]. 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 [51]. 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 [54].

Mineral bioavailability

The result of the mineral bioavailability assessment in the studied tea infusions is shown in Table 3. It was observed from the results that [Phytate]/[Ca] molar ratios in all the tea infusions were below the critical level of 0.5 known to impair calcium bioavailability [55]. The estimated [Phytate]/[Ca] molar ratios of the tested tea samples ranged from 0.08 (*M. oleifera)* to 0.22 (Moringa leaf + Licorice root). Similarly, the calculated [Oxalate]/[Ca] molar ratios were below the critical value of 2.5 known to impair calcium bioavailability [56]. The calculated [Oxalate]/[Ca] ranged from 0.11 (*M. oleifera*) to 0.36 (*G. glabra*). This indicates a good calcium bioavailability by the two varieties of the studied tea infusions 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 [57, 58]. This index has been widely used and it is considered as a good estimate of zinc bioavailability [47]. The calculated molar ratios of phytate to zinc of both tea infusions and their composite mixtures were below the critical value of 15 as outlined by Fitzgerald et al. [53]. The values ranged from 3.08 (Moringa leaf + Licorice root) to 5.99 (Licorice). This showed that the concentration of phytate in *M. oleifera* and *G. glabra* will not affect the bioavailability of zinc. 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 [58, 59]. The calculated values for [Ca][Phytate]/[Zn] ratio for all the tea infusions were below the critical level of 200. The ratios ranged from 126.2 (Moringa leaf + Licorice root) to 169.4 (Licorice root). This implies that the concentration of calcium in the tea infusions will not affect the bioavailability of zinc in the presence of phytate. Since all the calculated indices of anti-nutrients to minerals in the tea samples are below their respective critical level, this shows that the two herbal teas are good bio-resources of calcium and zinc. In the same vein, the calculated indices are below the critical value for calcium and zinc; therefore, it can be inferred that iron and magnesium will probably be bioavailable in the studied tea infusions.

Table 3 Ca and Zn bioavailability of *M. oleifera, G. glabra* and their composite blends

Sample	[PHY]/[Ca]	[OXA]/[Ca]	[PHY]/[Zn]	[Ca][PHY]/[Zn]
M	0.08 ± 0.001^{a}	0.11 ± 0.03^{a}	3.72 ± 0.21^{a}	139.2 ± 5.77^{b}
LR	0.14 ± 0.02^{b}	0.36 ± 0.04^{c}	5.99 ± 0.14^{b}	169.4 ± 8.51^{c}
M + LR	0.22 ± 0.03^{c}	0.28 ± 0.04^{b}	3.08 ± 0.22^{a}	126.2 ± 7.23^{a}
Critical value	0.50	2.50	15.00	200.00

Values represent mean standard deviation of triplicate experiments

PHY, phytate; Ca, calcium; OXA, oxalate; Zn, Zinc, M, *Moringa oleifera*; LR Licorice root; M + LR, Moringa leaf + licorice root

Antioxidant indices

The result of the assessment of the antioxidant potential of the studied tea infusions is shown in Table 4. The result revealed that M + LR and *M. oleifera* have higher total TPC relative to Licorice root that displayed the least total phenolic content, with no significant difference ($p < 0.05$) in the TPC of Moringa and M + LR. The results are in agreement with the findings of earlier studies [60–62], where it was reported that the phenolic content and the free radical scavenging effect of *M. oleifera* are high enough compared with that of the tested reference antioxidants. Lower total phenol content in Licorice root compared with that of *M. oleifera* may possibly be due to the presence of a few phenolic compounds it possesses [63, 64]. Similarly, M + LR possessed the highest TFC, followed by *M. oleifera*, while Licorice root displayed the least TFC.

In the same vein, M + LR and Moringa leaf had higher reducing power compared with Licorice root which had the least reducing power, with no significant difference ($p < 0.05$) in the reducing power of Moringa leaf and M + LR

Table 4 Antioxidant indices of *M. oleifera, G. glabra* and their composite blends

Sample	TPC (mg TAE/g)	TFC (mg QE/g)	Reducing power (mg AAE/g)	% OH scavenging ability
M	97.08 ± 1.84^{b}	77.11 ± 2.54^{b}	32.79 ± 0.10^{b}	61.55 ± 1.28^{b}
LR	81.66 ± 3.22^{a}	70.34 ± 1.20^{a}	19.00 ± 0.55^{a}	52.05 ± 3.45^{a}
M + LR	97.87 ± 2.84^{b}	82.47 ± 3.52^{bc}	32.85 ± 1.00^{b}	60.90 ± 4.21^{b}

Values represent mean ± standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p < 0.05$)

M, *Moringa oleifera*; LR, licorice root; M + LR, moringa leaf + licorice root; TAE, tannic acid equivalent; QE, quercetin equivalent; AAE, ascorbic acid equivalent

Table 5 Effects of *M. oleifera, G. glabra* and their composite blends on liver markers

(a)

Samples	GGT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	LDH (U/L)
Control	5.05 ± 0.58^{a}	26.22 ± 4.14^{c}	36.00 ± 1.67^{b}	28.70 ± 1.10^{de}	231.43 ± 16.03^{e}
M1	7.35 ± 1.74^{b}	12.42 ± 1.95^{a}	44.83 ± 1.83^{d}	25.90 ± 4.50^{c}	168.26 ± 36.67^{ab}
M3	11.58 ± 6.95^{d}	20.70 ± 4.14^{b}	46.50 ± 1.17^{de}	40.90 ± 2.7^{g}	227.30 ± 26.67^{d}
M5	46.80 ± 2.90^{h}	75.60 ± 1.38^{j}	54.00 ± 2.00^{a}	49.60 ± 5.20^{h}	251.43 ± 6.67^{fg}
LR1	6.37 ± 1.74^{ab}	25.88 ± 2.76^{c}	42.50 ± 1.50^{cd}	18.90 ± 1.10^{b}	302.23 ± 24.92^{h}
LR3	7.16 ± 1.90^{b}	27.94 ± 1.38^{cd}	54.83 ± 0.83^{e}	19.30 ± 0.90^{b}	307.30 ± 45.40^{hi}
LR5	9.84 ± 4.05^{c}	31.74 ± 1.38^{e}	60.67 ± 2.67^{f}	27.20 ± 0.6^{d}	524.13 ± 33.02^{j}
M + LR1	20.27 ± 6.37^{e}	34.50 ± 4.14^{f}	41.50 ± 1.17^{c}	28.60 ± 0.80^{de}	182.54 ± 4.45^{b}
M + LR3	25.37 ± 0.41^{f}	42.78 ± 1.38^{h}	45.00 ± 0.67^{d}	29.40 ± 0.80^{de}	196.30 ± 6.81^{c}
M + LR5	41.69 ± 3.28^{g}	64.50 ± 1.38^{i}	107.33 ± 1.33^{g}	36.20 ± 6.40^{f}	240.86 ± 12.89^{f}

(b)

Samples	T.PROT (g/L)	ALB (g/dL)	BIL (mg/dL)	GLU (mg/dL)
Control	60.19 ± 0.42^{a}	37.57 ± 0.54^{ab}	0.59 ± 0.08^{b}	105.11 ± 2.58^{a}
M1	68.13 ± 0.47^{b}	44.58 ± 0.59^{d}	0.48 ± 0.02^{a}	75.56 ± 0.84^{a}
M3	73.72 ± 1.3^{cd}	44.78 ± 1.67^{d}	0.66 ± 0.03^{c}	76.95 ± 13.94^{a}
M5	76.29 ± 1.26^{d}	33.25 ± 0.39^{a}	0.73 ± 0.02^{cd}	83.40 ± 8.52^{b}
LR1	70.25 ± 0.42^{c}	35.77 ± 0.64^{ab}	0.81 ± 0.02^{d}	97.35 ± 6.97^{d}
LR3	71.11 ± 4.19^{c}	37.03 ± 1.37^{ab}	0.88 ± 0.02^{e}	102.89 ± 5.10^{ef}
LR5	80.47 ± 0.33^{e}	42.94 ± 2.96^{bc}	1.32 ± 0.22^{f}	112.60 ± 2.13^{g}
M + LR1	65.96 ± 0.23^{ab}	40.63 ± 0.27^{b}	1.18 ± 0.01^{f}	83.47 ± 1.58^{b}
M + LR3	69.49 ± 2.23^{b}	46.49 ± 1.08^{d}	1.27 ± 0.02^{fg}	$99.68 \pm 12.27^{de`}$
M + LR5	70.10 ± 0.70^{c}	49.69 ± 1.64^{de}	1.46 ± 0.02^{g}	109.65 ± 13.30^{f}

Values with different superscripts in the same column differ significantly ($p < 0.05$). Values are expressed as mean ± SE of triplicate experiments. M1, *M. oleifera* extracts at 10 mg/kg BW/mL; M3, *M. oleifera* extracts at 30 mg/kg BW/mL; M5, *M. oleifera* extracts at 50 mg/kg BW/mL; LR1, *G. glabra* extracts at 10 mg/kg BW/mL; LR3, *G. glabra* extracts at 30 mg/kg BW/mL; LR5, *G. glabra* extracts at 50 mg/kg BW/mL; M + LR1; *M. oleifera* + *G. glabra* 10 mg/kg BW/mL, M + LR3, *M. oleifera* + *G. glabra* 30 mg/kg BW/mL; M + LR5, *M. oleifera* + *G. glabra* 50 mg/kg BW/mL; N = 5; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; AST, aspartate transferase, ALT, alanine transferase; LDH, lactate dehydrogenase; T.PROT, total protein; ALB, albumin; BIL, total bilirubin; *GLU* glucose

(Table 4). The high reducing potential of Moringa leaf and the composite blends tea infusion is an indication that the two tested teas might contain reducing compounds, which can react with free radicals to stabilize and terminate radical chain reactions and reduce the oxidized intermediates of lipid peroxidation [65, 66]. *M. oleifera* and the composite blends of the tested teas (M + LR) had the best hydroxyl scavenging ability, with no significant difference ($p < 0.05$) between the hydroxyl scavenging abilities of *M. oleifera* and the composite blends, while Licorice root had the least

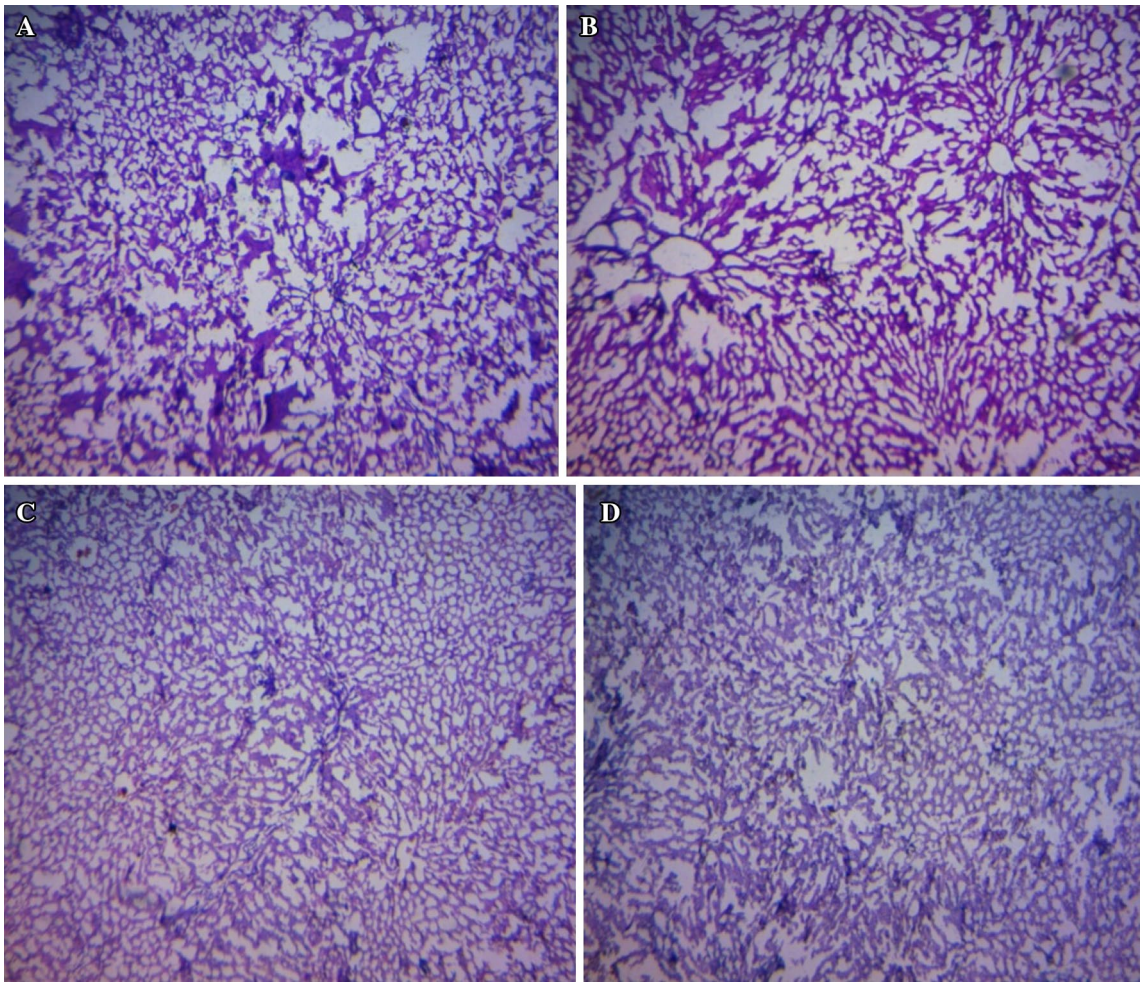

Fig. 1 Histopathological images of liver sections of albino rats. Magnification×400. **a** Control group not treated. **b** Pretreated with *M. oleifera* at dose 10 mg/kg b.wt. **c** Pretreated with *M. oleifera* at dose 30 mg/kg b.wt. and **d** Pretreated with *M. oleifera* at dose 50 mg/kg b.wt.

scavenging ability. The result is in accordance with the report of Anwar et al. [67], where it was reported that *M. oleifera* has a very strong hydroxyl radical scavenging activity which culminates in its overall ability to fight off radical-related diseases. The hydroxyl radical scavenging ability and the reducing power of the tested teas are in agreement with the previous report in which the higher antiradical action positively correlates with the phenolic content of phenolic extracts of plant food [68].

Liver function test and histopathological evaluation

The result of the rats' liver biomarkers after the administration of various concentrations of *M. oleifera, G. glabra* and the composite blends (*M. oleifera* + *G. glabra*), is as presented in Table 5a, b. The enzymatic activity of GGT, ALP, AST, ALT, and LDH was studied to evaluate liver malfunctions. The evaluated liver enzyme biomarkers elevated significantly in a dose dependent manner in all the test rats compared with the control, with few exceptions.

Analyses 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 [69, 70]. Enzymes like ALP, ALT, and AST have served as good liver biomarkers [71]. The observed dose showed a significant increase in the activities of liver enzymes as ALP, AST and ALT tend to suggest liver dysfunction in the experimental animals [72].

Usually an elevation in the liver enzymes may indicate 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. Similarly, total protein, albumin, total bilirubin

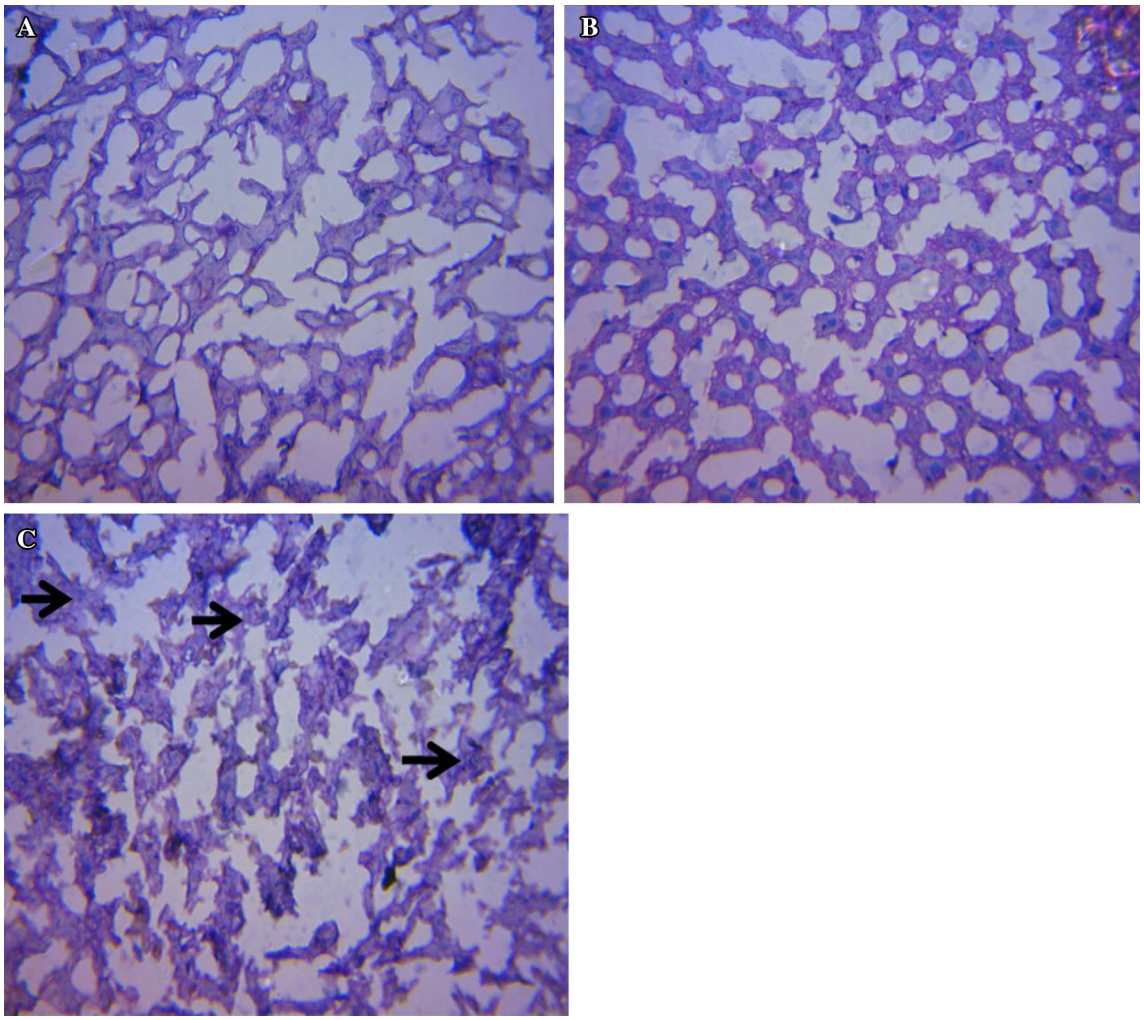

Fig. 2 Histopathological images of liver sections of albino rats. Magnification×400. **a** Pretreated with *G. glabra* at dose 10 mg/kg b.wt. **b** Pretreated with *G. glabra* at dose 30 mg/kg b.wt. and **c** Pretreated with *G. glabra* at dose 50 mg/kg b.wt.

concentration and glucose were significantly elevated in a dose dependent manner in all the test rats compared to the control, with few exceptions.

Histopathological study is recognized as a precise method for identifying and characterizing pathological changes associated with tissue lesions [73]. Histological examination of the rats' liver administered with various concentrations of *M. oleifera, G. glabra* and the composite blends (*M. oleifera* + *G. glabra*) 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 (Figures 1, 2, 3) revealed that the administration of the studied tea infusions showed no damaging effects on the liver, with the exception of Licorice root and the composite blends (M + LR) at a dose of 50 mg/kg b.wt. that caused mild to moderate degradation of hepatocytes.

Conclusion

The result of this investigation provides preliminary scientific information on the nutraceutical values (mineral composition, mineral bioavailability, antioxidant activities and hepatoprotective properties) of *M. oleifera* and *G. glabra* that are commonly used as folk medicine in the management of stress, anxiety, depression and other stress-related diseases. Therefore, the regular consumption of the studied tea and tea products, especially the composite blends at a regulated dose could be encouraged as a measure to keep free radical mediated diseases such as stress and stress-related diseases at bay.

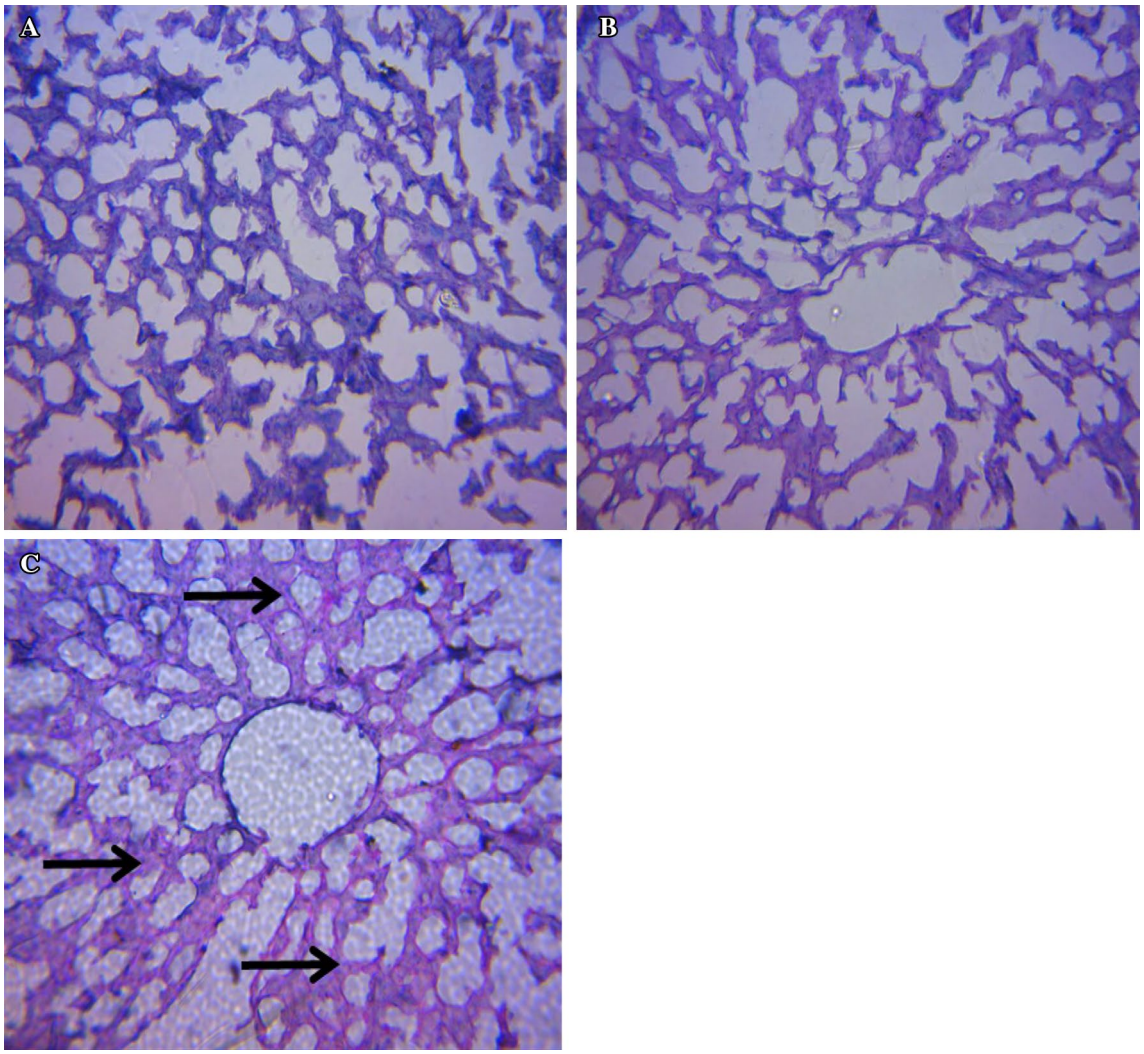

Fig. 3 Histopathological images of liver sections of albino rats. Magnification ×400. **a** Pretreated with *M. oleifera* and *G. glabra* (M+LR) at dose 10 mg/kg b.wt. **b** Pretreated with *M. oleifera* and *G. glabra* (M+LR) at dose 30 mg/kg b.wt. and **c** pretreated with *M. oleifera* and *G. glabra* (M+LR) at dose 50 mg/kg b.wt. Arrows pointing to regions having severe diffuse hepatic necrosis

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