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Toxicological Assessment of Green Tea (*Camellia Sinensis*) and Chamomile (*Matricaria Chamomilla*)

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Research Article

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Abstract

The biochemical investigation and histological study of the liver sections established that supplementation with *Camellia sinensis, Matricaria chamomilla* and their composite blend (GT+C) teas infusion had no adverse effects on the liver histology and biomarkers (GGT, AST, ALT, ALP, T.PROT, LDH ALB, TBIL and glucose)which confirmed the safety in the consumption of the studied teas.

Keywords: Green tea; Chamomile; Histology; Biomarkers

Abbreviations: PT: Prothrombin Time; LFTs: Liver function tests; C: Control rats; DNMRT: Duncan's New Multiple Range Test.

Introduction

Tea is the most widely consumed drink in the world other than water. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemical's necessary for digestion [1]. Liver function tests (LFTs or LFs) are groups of blood tests that give information about the state of a patient's liver [2]. These tests include prothrombin time (PT/INR), albumin, bilirubin (direct and indirect), and others. Liver transaminases (AST and ALT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed on a patient's blood sample. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. 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. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications, such as anti-convulsants, to ensure the medications are not damaging the person's liver.

Numerous claims have been made for the health benefits of Green tea and Chamomile based on its chemical composition, *in vitro studies*, animal studies, and human epidemiological studies. Recent researches have reported the potentials of Green tea and Chamomile as antispasmodic, antinociceptive antioxidant, antimicrobial, antiviral, anticarcinogenic, anti allergic [3] and analgesic for diseases of mouth, pharynx or for relief of tension headache [4]. Thus the scientific validation of the safety of the teas for the maintanance of human health is expedient (Figures 1-3).

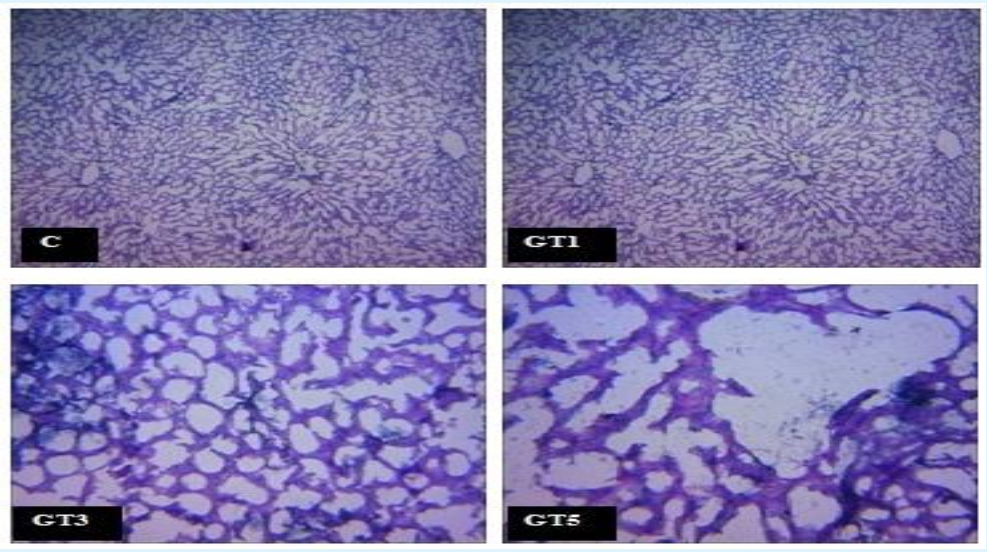

Figure 1: The histopathological picture of liver sections of normal Control rats (C), Green tea treated rats at doses 10, 30 and 50 mg/kg b.wt (GT1, GT3 and GT5). The design of hepatic structure showed evidence of normal histology (H&E; ×100).

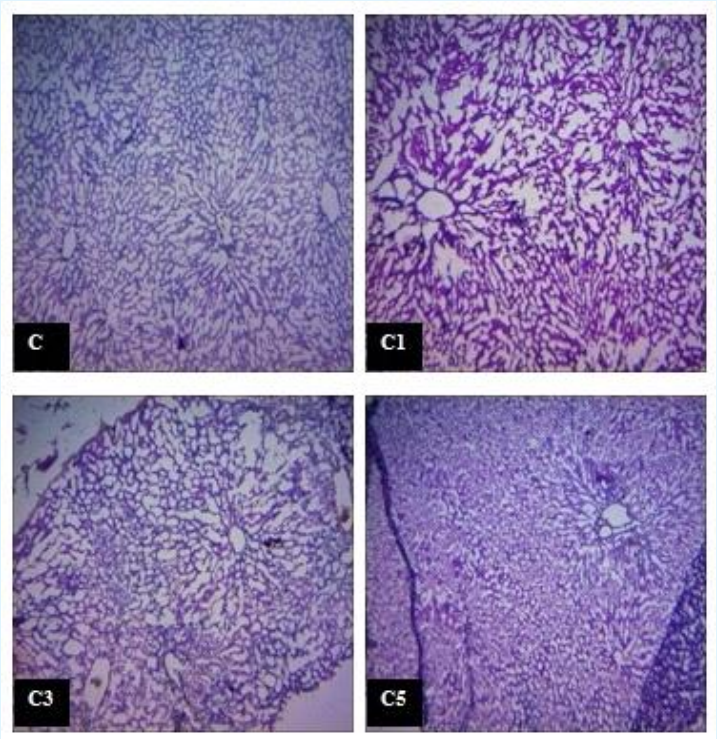

Figure 2: The histopathological picture of liver sections of normal Control rats (C), Chamomile tea treated rats at doses 10, 30 and 50 mg/kg b.wt (C1, C3 and C5). The design of hepatic structure showed evidence of normal histology (H&E; ×100).

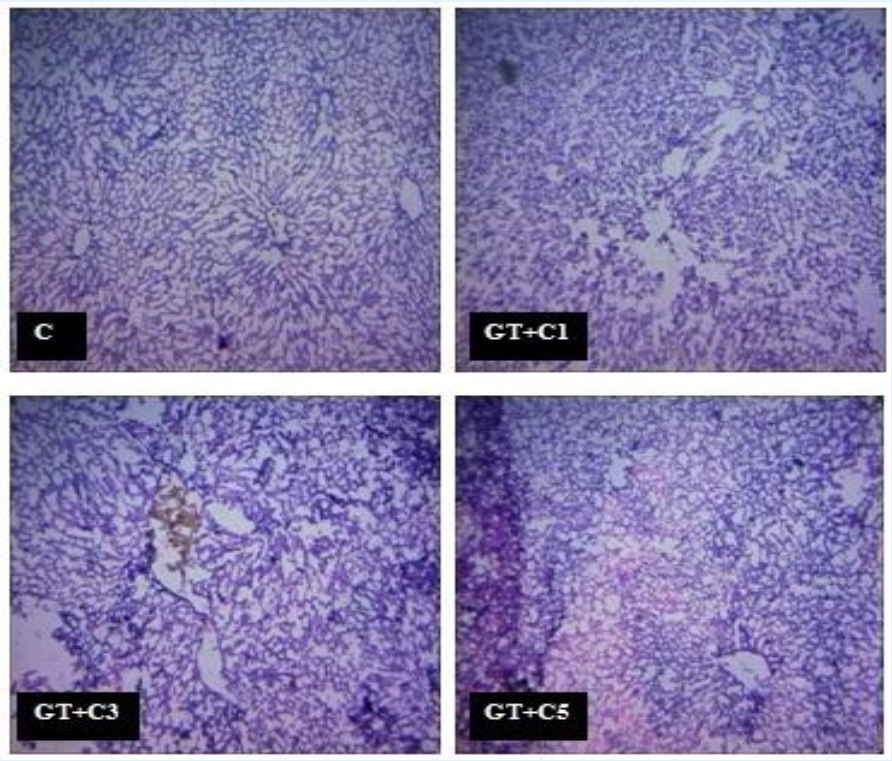

Figure 3: The histopathological picture of (C), mixture tea sample treated rats at doses 10, 30 and 50 mg/kg b.wt (GT+C1, GT+C3 and GT+C5). The design of hepatic structure showed evidence of normal histology (H&E; ×100).

Material and Methods

Chemicals

2-Deoxy-D-ribose (Cat No - #D5899), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Cat No -#11557), 2,2-Diphenyl-1-picrylhydrazyl (Cat No - #D9132) Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), ferrous sulphate, potassium dichromate ($K_2Cr_2O_7$), Ferric chloride ($FeCl_3$), Methanol, Folin-Ciocalteu's phenol reagent, sodium bicarbonate, aluminium chloride, potassium acetate, sodium phosphate dibasic, sodium phosphate monobasic, potassium ferricyanide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ammonia, sodium chloride, quercetin, tris salt, sodium nitroprusside, sulphanilamide, N-(1-Naphthyl ethyldiamine dihydrochloride (NED), orthophosphoric acid, xanthine, xanthine oxidase, sulphanilic acid, hydroxylamine, ammonium thiocyanate, potassium permanganate, hydrochloric acid, sulphuric acid, chloroform, tannic acid, sodium carbonate, aluminum chloride, quercetin, ascorbic acid, glacial acetic acid were obtained from Sigma chemical company, USA.

Sample Treatment and Preparation

Two different types of anti-stress teas, Peppermint and Licorice root were bought from Trado-medical Centre, Ibadan, Oyo state, Nigeria. The tea extracts were prepared using hot water infusion. 15g of each tea sample was infused in 1.2 Liters of hot water, the mixture was then filtered using No. 1 Whatman filter paper and the filtrate kept prior analysis.

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. The rats were allowed to acclimatize for a week before the experiment (Tables 1& 2).

SAMPLES	GGT (U/L)	ALP (U/L)	AST (U/L)	ALT(U/L)	LDH(U/L)
CONTROL	24.05 ± 0.58^{a}	56.22±4.14^{a}	36.00 ±1.67^{a}	25.70±1.10^{a}	231.43±66.03^{a}
GT1	26.73 ±4.05^{a}	73.12±1.38^{a}	44.83±4.5^{b}	31.90±0.10^{a}	351.11±53.65^{a}
GT3	23.69±13.32^{b}	63.46±9.66^{a}	37.00 ±0.83^{a}	26.90±2.50^{a}	259.05±63.97^{a}
GT5	21.63± 9.84a	50.70±2.76^{a}	35.17±2.33^{b}	25.70±0.90^{a}	213.18± 8.25^{b}
C1	28.69±4.05^{a}	64.50±5.52^{a,b}	41.00±1.50^{b}	29.40±0.60^{b}	373.65±45.40^{a}
C3	18.68±6.37^{a}	60.56±4.14^{b}	36.50± 2.00^{a}	24.80±2.20^{a}	229.37±10.32^{a}
C5	15.42±3.47^{a}	59.66 ± 4.14^{a}	32.67±0.67^{c}	21.10±1.50^{c}	218.42±37.14^{a}
GT+C1	25.58±9.26^{a}	66.22±1.38^{a}	42.00±2.33^{a}	37.4±0.80^{a}	367.94±41.27^{b}
GT+C3	21.53±29.53^{a}	53.42±4.14a	35.00±0.67^{b}	29.70±0.90^{b}	232.22 ±10.32^{a}

Table 1: Effects of teas extracts at different concentration on biochemical markers of the albino rats.

Values with different superscripts in the same column differ significantly (P<0.05). Values are expressed as mean ±SE. GT1 represents green tea infusion at 10mg/kg.BW/ml; GT3 represents green tea infusion at 30mg/kg.BW/ml; GT5 represents green tea infusion at 50mg/kg.BW/ml. N=5; C1 represents chamomile tea infusion at 10mg/kg.BW/ml; C3 represents L chamomile tea infusion at 30mg/kg.BW/ml; C5 represents chamomile tea extracts at 50mg/kg.BW/ml. N=5; GT+C1 represents green tea and chamomile infusion at 10mg/kg.BW/ml; GT+C3 represents green tea and chamomile infusion at 30mg/kg.BW/ml; GT+C5 represents green tea and chamomile infusion at 50mg/kg.BW/ml. N=5; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatise; AST: Aspartate transferase ; ALT: Alanine transferase; T. PROT: Total protein; ALB: albumin; BIL: Total Bilirubin; GLU: Glucose

SAMPLES	T. PROT(g/l)	ALB (g/dl)	BIL(mg/dl)	GLU(mg/dl)
CONTROL	68.41 ± 2.19^{a}	37.57±0.54^{a}	1.59 ±0.06^{a}	105.11±2.58^{a}
GT1	68.38 ± 2.56^{b}	31.74±0.19^{b}	1.09± 0.03^{a}	100.56±1.36^{b}
GT3	69.12 ± 0.93^{a}	41.65±2.79^{a}	0.62± 0.09^{a}	100.52±0.45^{b}
GT5	71.63± 2.28^{a,b}	46.05±0.20^{b}	0.43±0.03^{b}	92.34 ± 3.10^{a}
C1	68.65 ± 5.07^{a}	41.07±3.16^{a}	1.18 ±0.04^{b}	112.58±3.09^{a}
C3	72.61 ± 3.17^{a}	43.30±1.67^{a}	0.42 ±0.03^{a}	95.85±0.65^{b}
C5	73.24 ± 0.33^{a}	45.31±1.27^{a}	0.31 ±0.01^{a}	92.40±6.52^{b}
GT+C1	69.91±0.47^{a}	42.37 ±1.91^{a}	1.16 ±0.05^{a}	109.72 ±3.23^{a}
GT+C3	74.00±2.33^{a}	44.66 ±0.83^{a}	0.64 ±0.02^{a}	97.71 ±1.36^{a}
GT+C5	79.70±2.90^{a}	45.21 ±2.33^{a}	0.19 ±0.02^{a}	85.27 ±0.32^{a}

Table 2: Effects of teas extracts at different concentration on biochemical markers of the albino rats in different groups.

Values with different superscripts in the same column differ significantly (P<0.05).Values are expressed as mean ±SE. GT1 represents green tea extracts at 10mg/kg.BW/ml; GT3 represents green tea infusion at 30mg/kg.BW/ml; GT5 represents green tea infusion at 50mg/kg.BW/ml. N=5; C1 represents chamomile tea infusion at 10mg/kg.BW/ml; C3 represents L chamomile tea extracts at 30mg/kg.BW/ml; C5 represents chamomile tea infusion at 50mg/kg.BW/ml. N=5; GT+C1 represents green tea and chamomile tea infusion at 10mg/kg.BW/ml; GT+C3 represents green tea and chamomile infusion at 30mg/kg.BW/ml; GT+C5 represents green tea and chamomile infusion at 50mg/kg.BW/ml. N=5; T. PROT: Total protein; ALB: albumin; BIL: Total Bilirubin; GLU: Glucose

Mortality Study

There were 10 groups of 5 albino rats each; the experiment was carried out according to the methods of Li, et al. with slight modifications. Tap water, 10mg, 30mg and 50mg of the tea infusion was 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 hours for possible deaths of the rats.

Group 1: Control; group without treatment; normal diet and 0% of the tea samples
Group 2: aqueous extract of green tea; 10mg/kg b.wt
Group 3: aqueous extract of green tea; 30mg/kg b.wt
Group 4: aqueous extract of green tea; 50mg/kg b.wt
Group 5: aqueous extract of chamomile tea; 10mg/kg b.wt
Group 6: aqueous extract of chamomile tea; 30mg/kg b.wt
Group 7: aqueous extract of chamomile tea; 50mg/kg b.wt
Group 8: aqueous extract of green tea + chamomile tea; 10mg/kg b.wt
Group 9: aqueous extract of green tea +chamomile tea; 30mg/kg b.wt
Group 10: aqueous extract of green tea + chamomile tea; 50mg/kg b.wt

Dietary/Biochemical Study

None of the animals in the mortality study died. Therefore, administration of the infusions continued for a further 4 weeks before intoxication. At the end of four weeks, the rats were weighed and blood samples were collected through cardiac puncture under chloroform an aesthesia into EDTA bottles, centrifuged and the plasma was aspirated and analyzed for 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 by cervical dislocation and liver tissues were taken and immediately fixed in 10% formaldehyde for histological examination.

Statistical Analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and followed by one–way Anova Duncan's New Multiple Range Test (DNMRT) to compare the means that showed significant variation by using SPSS 17.0 and Graph Pad prism 6.0 for windows. The significance level was set at $p < 0.05$.

Results and Discussion

The liver has a wide range of functions that are important to life, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion [5]. It also plays a role in metabolism, regulation of glycogen storage, decomposition of red blood cells and hormone production [5]. The liver is the most important organ of the animal body and its functionality can be highly affected primarily by toxic or xenobiotics agents Brad berry, [6]. Assessment of liver function is vital and among the most sensitive and commonly used liver function markers are total protein content, bilirubin, albumin, glucose, gamma glutamyl transferase, aspartate transaminase, alkaline phosphatase, and alanine transaminase. These markers are predominantly found in the hepatocytes and to a lesser degree in the muscle cells. In conditions of damage to the hepatocytes, these markers may be spilled into the blood, raising their blood levels and indicating liver damage or disease. The result of the liver marker assessment revealed that the various concentrations of green tea, chamomile and the composite infusion show no significant damage to the hepatocytes as the values obtained from the biochemical investigation fell within the reference ranges: gamma glutamyl transferase, GGT (5-48U/L),albumin, ALB (35-50g/L), alkaline phosphatase, ALP(45-115U/L), glucose GLU(75-115mg/dL), total bilirubin BIL(0.1-1.2mg/dL), alkaline transferase, ALT (7-55U/L), and aspartate transferase AST (8-48U/L),LDH (122-320U/L) and T.Prot. (64-83g/L) respectively. This finding is in accordance with the work of Nyblom H, et al. [7] that having the liver enzyme markers fall within the reference values indicates by inference that there is no damage or impairment to the integrity of the liver.

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. As revealed by the histological studies, the studied tea infusions show no damaging effect on the hepatocytes. This finding is supported by Chacko SM, et al.; Shimizu M, et al. [8-11].

Conclusion

In line with the results obtained from this study, it can be concluded that at all tested concentrations of the teas extracts given to the rats had no adverse effects on liver biomarkers. Since the efficacy of a hepatoprotective drug could be judged through its capacity to restore or maintain the normal hepatic function that have been distorted by the hepatotoxic agent, the current study provides an evidence of the possible hepatoprotective effect of the studied teas against the hepatotoxic effect of ketamine.

Human investigations are recommended to be carried out to demonstrate whether similar results could be obtained.

Conflicts of Interest

None of the authors have any conflicts of interest to declare.

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