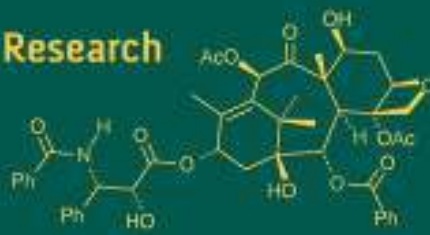
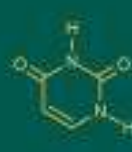


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Spectrophotometric evaluation of Slimming teas, Its effects on the hepatic and renal integrity in hyperlipidemia female rat model

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Abstract

Obesity and its related complications, including hyperlipidemia, pose significant health challenges globally. Amidst various weight management strategies, the consumption of slimming teas has gained popularity, yet concerns persist regarding their safety and efficacy. This study presents a comprehensive evaluation of the heavy metal content of two commonly consumed slim tea in Port Harcourt, Nigeria and further evaluated the effect of the consumption of the slim tea by Infusion on the hepatic and renal function of hyperlipidemia female rat models. The samples were treated using acid digestion method and concentrations of Copper, Lead, Cadmium, Nickel, Zinc and Chromium were assessed using the Atomic Absorption Spectroscopy. Forty-two female rats were divided into 7 groups for the evaluation of the effect of slim tea on the hepatic and renal function. At the end of 21 days, blood samples were collected. The results obtained revealed that Lead, Cadmium, Nickel, Zinc and Chromium were all above the WHO permissible range in both Tea 1 & 2, while Copper was within the permissible range. An increase in blood Urea and Creatinine levels in the rat models administered the teas when compared to the normal and positive control groups was observed. The hepatic markers; ALT, AST, ALP recorded a significant increase while the serum TB, CB and Albumin had a decrease in concentration for all tea samples when compared to the positive and normal control. The histological examination of the revealed lymphocytic infiltration of the portal tract and mild fatty infiltration of the hepatocyte for the groups administered 200 mg/kg tea 1 and 200mg/kg tea 2 respectively. The high ($p < 0.05$) levels of heavy metals in the tea samples raises long-term safety concerns of slimming tea consumption. The findings of this study highlights the need for evidence-based evaluations to inform regulatory decisions and promote consumer safety regarding slimming tea consumption.

Keywords: Administered, slimming, consumption, permissible

Introduction

The global burden of obesity and its associated metabolic disturbances, particularly hyperlipidemia, is on a concerning rise, posing significant challenges for healthcare systems worldwide (Ng *et al.*, 2014). In response to this growing public health concern, individuals are actively seeking effective weight management strategies (Yang *et al.*, 2010) [2]. Among the diverse approaches gaining popularity are dietary supplements, with slimming teas attracting considerable consumer interest (Ogbera *et al.*, 2020) [3]. These teas are often marketed with claims of promoting weight loss and improving overall health; however, significant scientific evidence regarding their safety and efficacy remains limited (Ogbera *et al.*, 2020) [3]. The availability of only a few studies on slimming teas raises critical questions about their potential benefits and risks (Ogbera *et al.*, 2020) [3]. While some studies suggest potential weight-loss effects (Hurtado-Barredo *et al.*, 2018) [4], concerns exist about possible damage to vital organs such as the liver and kidneys. Additionally, heavy metals or other contaminants in these teas can pose a serious health risk, particularly with long-term consumption (Yilmaz *et al.*, 2018) [6]. To address these uncertainties, the present study undertakes an evaluation of the heavy metal composition, hepatic and renal effects of selected slimming teas in high fat diet induced hyperlipidemia female rat models.

Additionally, the histopathological examinations of liver and kidney tissues were conducted to assess for any morphological changes indicative of potential damage.

Materials and Methods

Sample Collection

Two commonly consumed brands of Slimming tea ‘Healthy Hour Fat Burner (Tea 1) and Tummy and Fat Reducing Tea (Tea 2), were purchased from Mile one market in Port Harcourt, Nigeria. Each sample was analyzed to determine the amount Cd, Cu, Pb, Ni, Zn, Cr in the teas.

Apparatus

All glassware was soaked overnight in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid and rinsed with double distilled water and dried before using. A Perkin – Elmer 3030 and Shimadzu AA. 6650 atomic absorption spectrophotometers were used for the determination of heavy metals.

Reagents

Standard stock solutions of lead were prepared from Titrastol (1000 mg/l) and diluted to the corresponding metal solution. These reagents used were of analytical reagent grade (BDH, England). Also, a standard copper solution was prepared from CuSO₄, in distilled water and standardized. A 0.3% solution of NaBH₄ and 0.1% solution of NH₄VO₃ were prepared in 1% NaOH.

Sample preparation

Digestion Based on the method described by AOAC (Sour *et al.*, 2019) [8], 3 g of each tea particles sample were digested using 100 ml of concentrated HNO₃ for 10 min. The mixture was heated using electric heater until nearly dried. The mixture left to cooled at room temperature. The digested sample was mixed with 60 ml of a mixture (5:1 v/v) of concentrated HNO₃ and HClO₄. The mixture was heated on electric heater until the solution turned white and gave out the white fumes. The digest was transferred into 50 ml volumetric flask and the volume was adjusted to the mark using distilled water. Concentrations of heavy metals Cd, Cu, Pb, Ni, Zn, Cr were determined in the obtained clear solutions using atomic absorption spectrophotometer.

Validation of methods

Tea (digested) samples were spiked with various concentrations of heavy metals for the recovery repeatability tests and for verifying the analytical methodology. For each run, triplicate samples, spiked samples and blanks were carried through the digestion reaction.

Experimental animals

Forty-two adult female albino rats were obtained from the Animal House of Rivers State University, Port Harcourt and taken to the experimental laboratory. They were divided into seven groups of 6 albino rats each. The rats were given standard feed and water *ad libitum* and allowed to acclimatize for 7 days.

Substance of Study/Infusion

Two popular slimming teas; ‘Healthy Hour Fat Burner (Tea 1) and Tummy and Fat Reducing Tea (Tea 2), were sourced from mile one market, Port Harcourt, Nigeria. These teas are marketed for weight loss, diabetes control, and obesity

management, were prepared by Infusion. Tea infusion samples were prepared while taking into account the mode of consumption of the tea.

Two (2) grams of tea were infused in 50 ml of distilled water and for the same conditions incubated in 80°C. The mixture left to cool at room temperature for 10 min and then filtered to obtain the clear solution. The mixture was administered orally to rats at dosages of 200 mg/kg and 400 mg/kg. Both teas were stored in airtight glass containers in a dark, dry, and cool environment to maintain potency.

Experimental grouping/substance administration

The female albino rats, weighing between 250-300g, were divided into seven groups with six rats in each group:

- Group 1: Normal Control group (Feed + Water only)
- Group 2: High-Fat Diet (HFD) (40 mg/kg) without treatment
- Group 3: HFD (40 mg/kg) + Simvastatin (standard antihyperlipidemia drug)
- Group 4: HFD (40 mg/kg) + Slimming Tea 1 (200 mg/kg)
- Group 5: HFD (40 mg/kg) + Slimming Tea 1 (400 mg/kg)
- Group 6: HFD (40 mg/kg) + Slimming Tea 2 (200 mg/kg)
- Group 7: HFD (40 mg/kg) + Slimming Tea 2 (400 mg/kg)

Each day, the specified dosage of Slimming Tea 1 and Slimming Tea 2 was prepared by dissolving 2 grams of tea (equivalent to one tea bag) in 50 ml of distilled water and heating it to ensure proper dissolution. This preparation was constituted accordingly to obtained the desired concentrations.

The high fat diet contained 414.0 kcal/100 g with 43% as carbohydrate, 17% as protein, and 40% as fat (Table 1). The diet consists of a mixture of 68% normal rat chow pellet, 20% instant milk powder (peak milk), 6% corn oil (Mazola), and 6% ghee (popularly called Manshanu in Northern Nigeria). Meanwhile the normal rat chow diet contains 306.2 kcal/100 g with 48.8% as carbohydrate, 21% as protein, and 3% as fat. All ingredients of high fat diet were thoroughly mixed and baked in the oven at 65 °C overnight.

Table 1: Composition of high fat diet

High fat diet	
Nutrients	%/100 g
Carbohydrate	43
Protein	17
Fat	40
Ingredients	g/100 g
Powdered rat feed	68.0
Maize oil	6.0
Ghee	6.0
Milk powder	20.0
• Total energy	414.0
• (kcal/100 g)	

This diet was administered to the rats over a period of 15 days to induce hyperlipidemia, which was confirmed by measuring serum lipid levels. During the subsequent 21-day treatment phase, the rats received their respective treatments daily:

The administration was performed using an oral gavage tube to ensure accurate dosing, and precautions were taken to avoid esophageal injuries. Each dose was measured using a 2 ml syringe, adjusted according to the body weight of the rats to ensure precise administration. All animals had free access to feed and water throughout the study period.

Sample collection and procedure

On the last day (21st day from the onset of substance administration), the rats were fasted overnight, weighed, and euthanized by cervical dislocation. Blood samples were collected in plain sample bottles and centrifuged at 3000 rotations per minute for 10 minutes to separate the serum. The collected serum was then used for biochemical analysis. The liver samples were also collected and fixed in 10% formal saline for histological examination.

Sample analysis

Blood samples collected from the euthanized rats were stored in plain sample bottles and centrifuged at 3000 rpm for 3 minutes to separate the serum. The serum was used for biochemical assays, including Hepatic function markers (AST, ALT, ALP, bilirubin, total protein, and albumin) and Renal function markers (Urea, Creatinine, Na, K, Cl and HCO_3). These biochemical parameters were analyzed to assess the hepatic and renal function using the methods as described by Odinga *et al.*, (2016)^[1].

Data Analysis

Results obtained were analyzed using IBM SPSS v.25.0. Statistical evaluations of the differences between group mean values were conducted using one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc tests for multiple comparisons. The results were expressed as mean $\pm$ standard deviation (SD), and statistical significance was considered at $p \leq 0.05$.

Results

Heavy metal concentrations in Study slimming teas

The concentration of Cd, Cu, Pb, Ni, Zn, Cr were evaluated in the study tea; Tea 1 and Tea 2. From the result in Figure 1, the both teas were found to have concentrations of Pb, Cd, Ni, Zn, and Cr comparable with the values set by the WHO permissible range for heavy metals in tea. However, Cu had concentrations of 4.47 ± 0.22 and 5.32 ± 0.06 in Tea 1 and 2 respectively. These concentrations were within the WHO permissible range. Tea 2 was also observed to have higher concentrations of Cu, Pb, Ni and Cr than Tea 1. AL-Oud, (2003) in his study reported the presence of heavy metals in slim and heavy teas. He however stated that the amounts of heavy metals that one may take up through consumption of tea and herb beverages were found to match the acceptable daily intake that takes into account exposure from air, food and drinking water. Garba *et al.*, (2015)^[11] in their study, noted that heavy metal concentrations in teas could be attributed to geographical changes, seasonal changes and chemical characteristics of the growing regions.

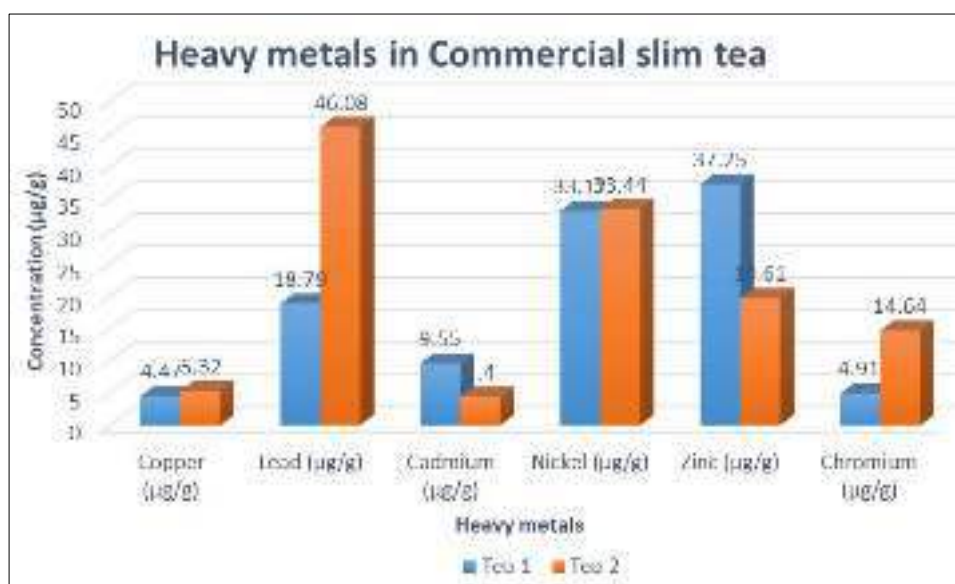


Fig 1: Concentration of Cadmium, Copper, lead, Nickel, Zinc & Chromium in slim teas of study.

Table 2: % Mean Body Weight Difference of Experimental Rats.

Group	Initial body weight (g)	Final body weight (g)	% Mean body weight Differences
Normal Control	129.50 $\pm$ 19.87	177.50 $\pm$ 23.32	37.10
Negative Control	175.83 $\pm$ 6.77	202.50 $\pm$ 10.52	15.17
Positive Control	176.83 $\pm$ 8.89	209.33 $\pm$ 23.44	18.38
200 mg/kg Tea 1	148.83 $\pm$ 7.14	178.50 $\pm$ 16.16	19.94
400 mg/kg Tea 1	140.67 $\pm$ 7.92	151.00 $\pm$ 8.34	7.34
200 mg/kg Tea 2	151.00 $\pm$ 5.76	175.67 $\pm$ 19.70	16.34
400 mg/kg Tea 2	159.12 $\pm$ 14.96	165.50 $\pm$ 16.37	4.01

Table 2 above shows the % mean body Weight difference of the rats. The percentage body Weight difference of the rats showed an increase in the Normal and Positive control

groups, but a drastic decrease in the % body Weight difference of 400mg/kgTea 1 and 400mg/kg Tea 2. This could be attributed to the effects of slimming tea high dose

(400 mg/kg) consumption by the experimental animals. Fan *et al.*, (2012) ^[12] in their study reported that herbal slim tea effectively reduced Obesity with its metabolic disorder hyperlipidemia. This could account for the reduction in body weight of the groups administered with the various concentrations of slim tea. However, tea 2 showed a more reduction when compared with tea 1.

Kidney function

From the results in Figure 2, it is observed that the concentration of serum urea increased after 7 and 14 days, however, a slight decrease was observed after 21 days of administration of the study Teas. Kwan *et al.*, (2006) ^[13] in their study observed that prolonged intake of slimming pills containing anthraquinones resulted in Acute renal failure. They also reported that prolonged use of slimming teas could lead to potential complications, including dehydration, electrolyte imbalance and strains on the kidney. This is evidenced in Figures 4,5,6 and 7 for the effects of slimming

teas on Sodium, Chloride, Potassium and bicarbonate respectively. Variations were observed in the serum concentrations of Na, Cl, K and HCO_3 in the experimental rat models. A relationship between tea consumption and incident acute kidney injury has also been reported by Liu *et al.*, (2023) ^[14]. The toxicity and problems associated with the kidney on acute consumption of slimming teas could be associated with the interactions of the heavy metals present in the tea at concentrations above the allowed daily intake (Asif, 2012) ^[15]. Heavy metals have also been observed to be a major threat with several health risk associated with it, including Renal effects (Jaishankar *et al.*, 2014) ^[16]. Figure 3 revealed the effect of slimming tea consumption on serum creatinine. An increase in the concentration of the serum creatinine was observed after 21 days of administration of both slimming teas of study. High serum creatinine level has been implied to be an indication of a range of underlying health conditions, including kidney infection and kidney failure (Fried, 2020; Richard *et al.*, 2006) ^[17, 18].

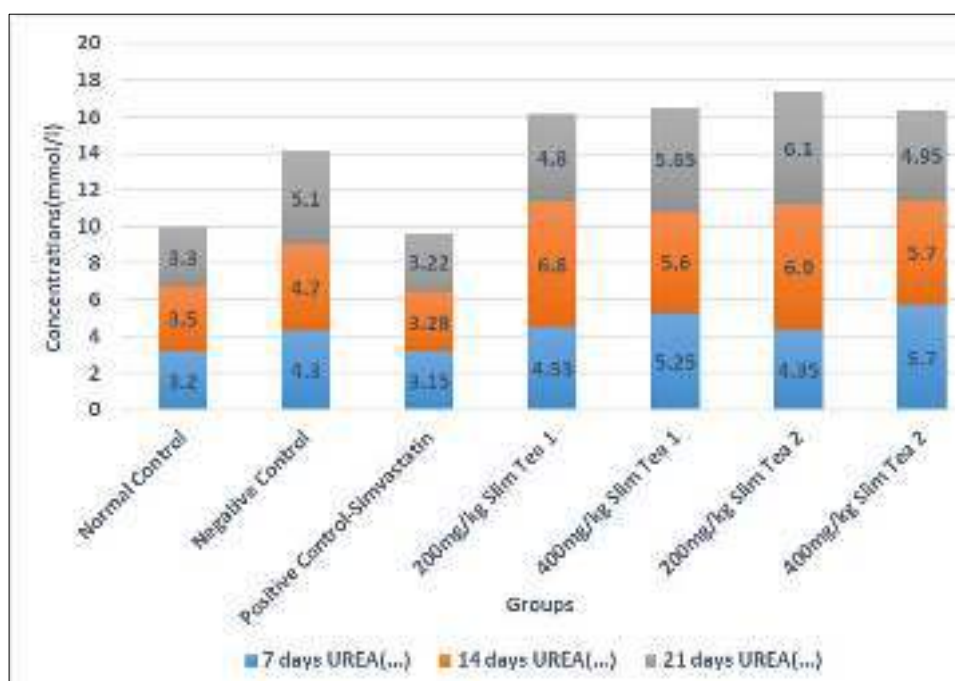


Fig 2: Concentrations of Serum Urea in Experimental animals after 7, 14 and 21 days

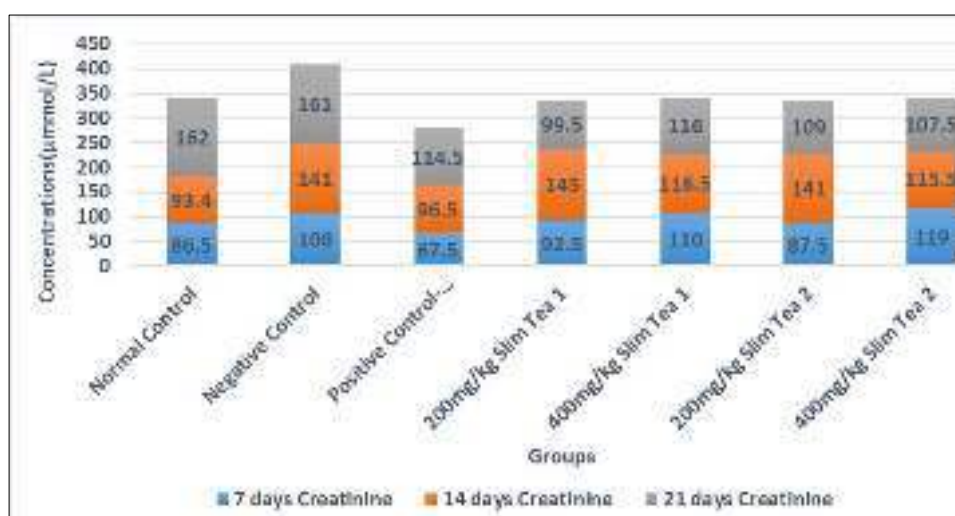


Fig 3: Concentrations of Serum Creatinine in Experimental animals after 7, 14 and 21 days

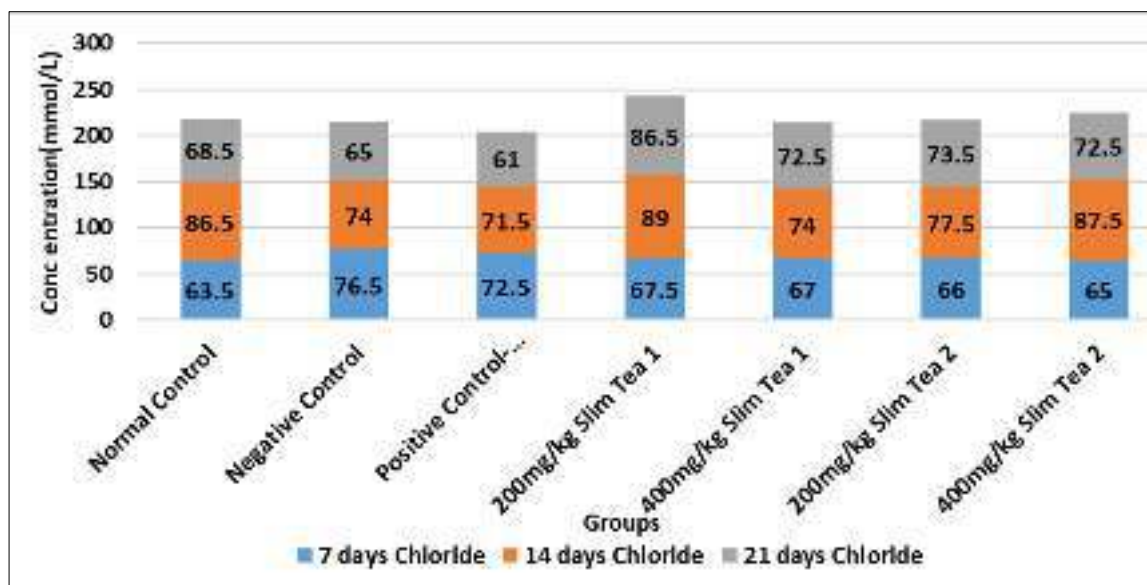


Fig 4: Concentrations of Serum Chloride in Experimental animals after 7, 14 and 21 days

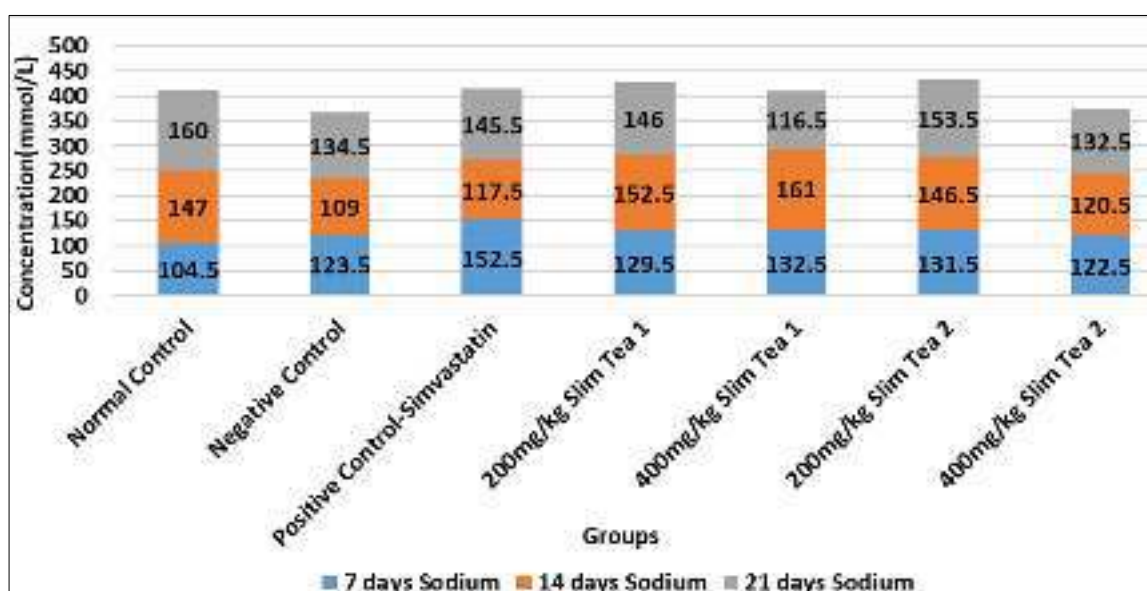


Fig 5: Concentrations of Serum Sodium in Experimental animals after 7, 14 and 21 days

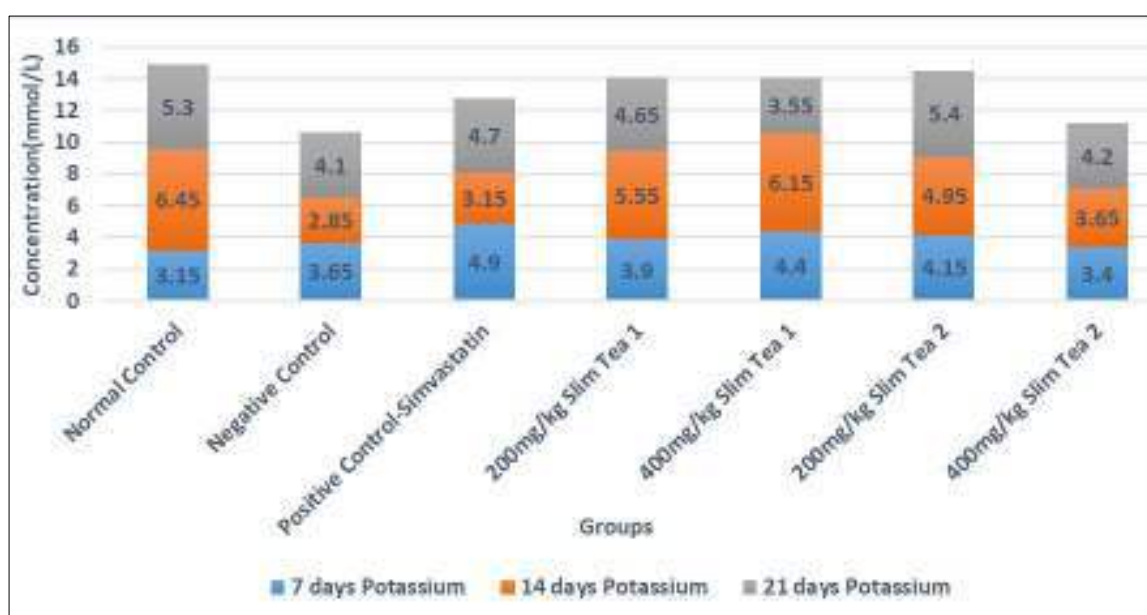


Fig 6: Concentrations of Serum Potassium in Experimental animals after 7, 14 and 21 days

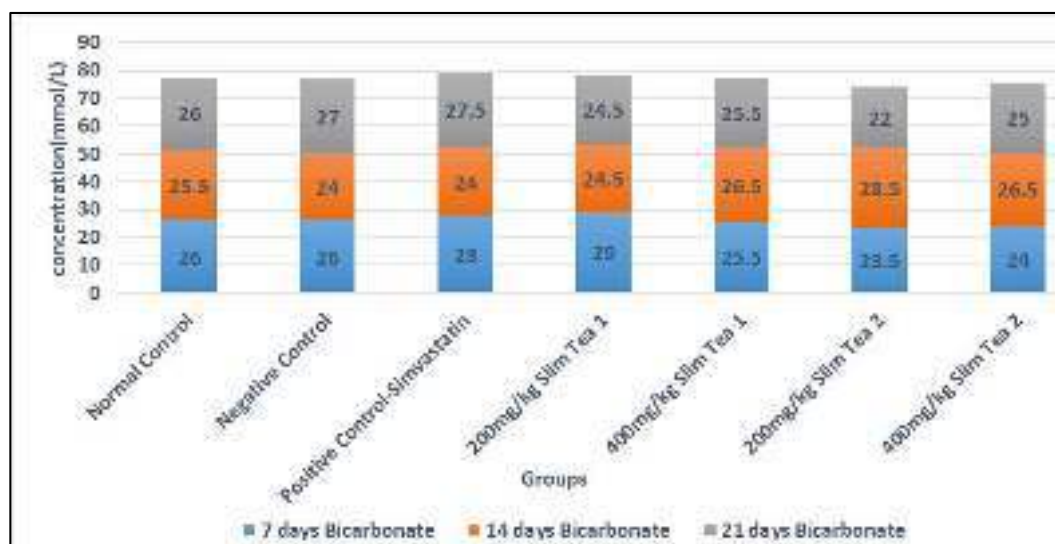


Fig 7: Concentrations of Serum Bicarbonate in Experimental animals after 7, 14 and 21 days

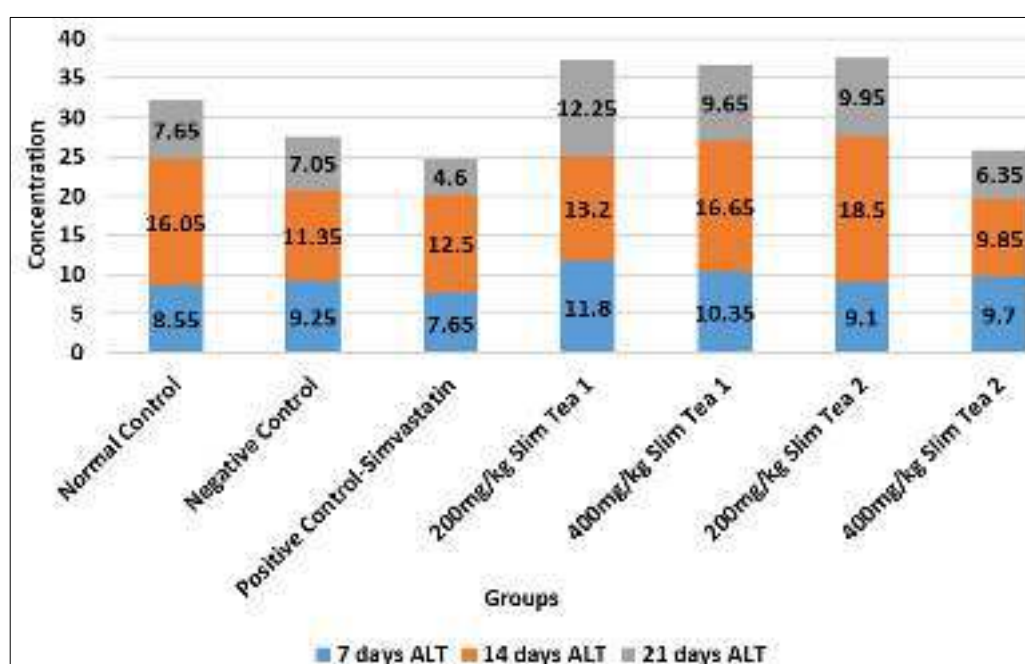


Fig 8: Concentrations of Serum ALT in Experimental animals after 7, 14 and 21 days

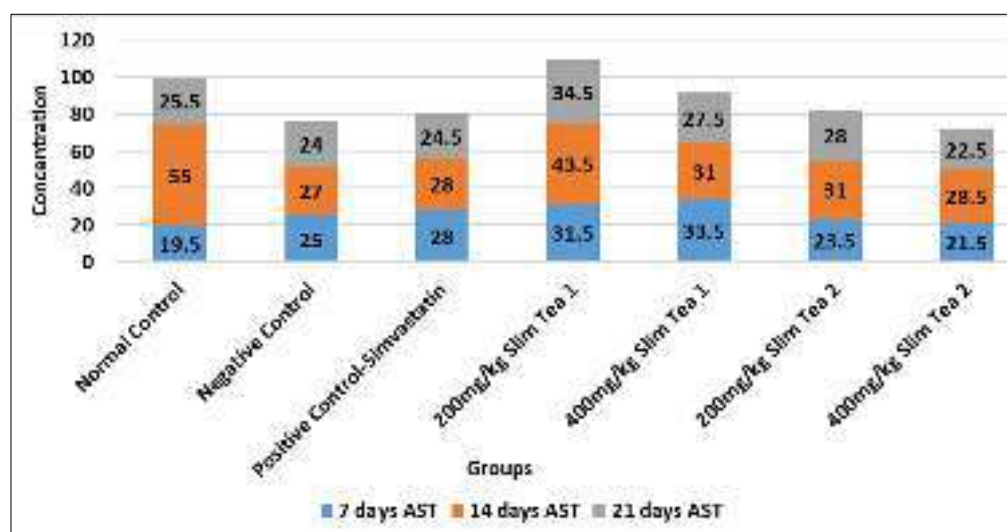


Fig 9: Concentrations of Serum AST in Experimental animals after 7, 14 and 21 days

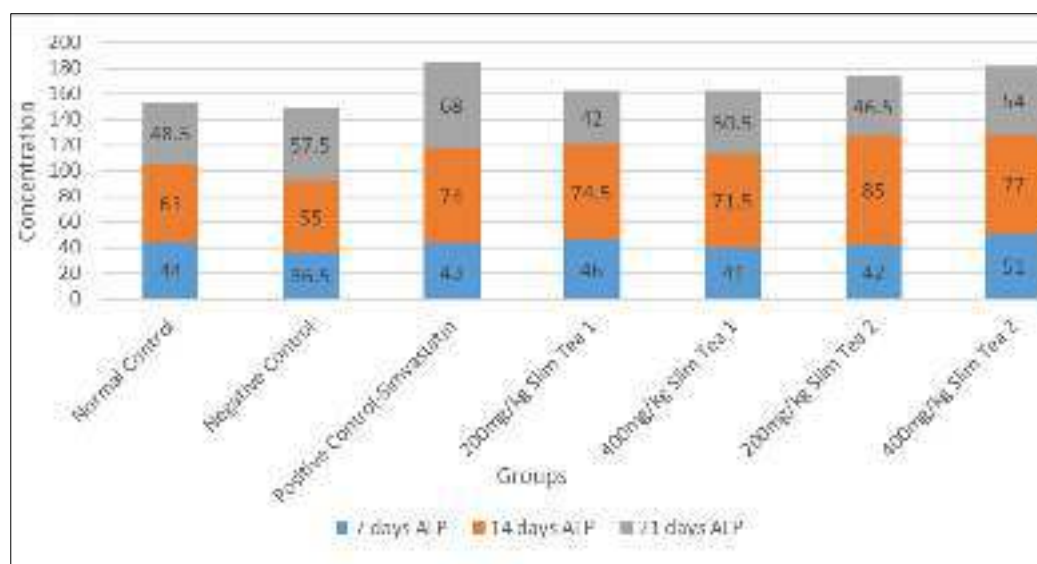


Fig 10: Concentrations of Serum ALP in Experimental animals after 7, 14 and 21 days

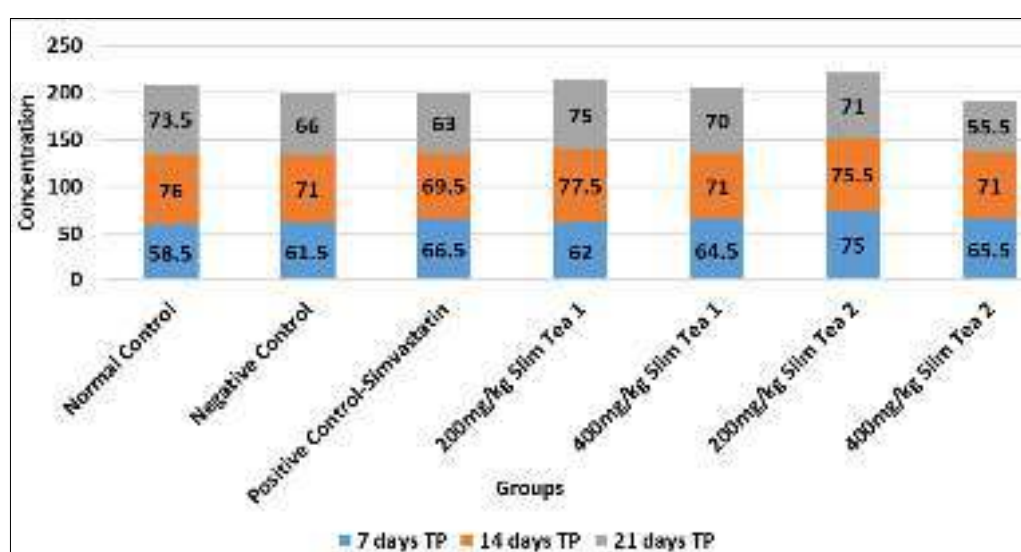


Fig 11: Concentrations of Serum Total Protein in Experimental animals after 7, 14 and 21 days

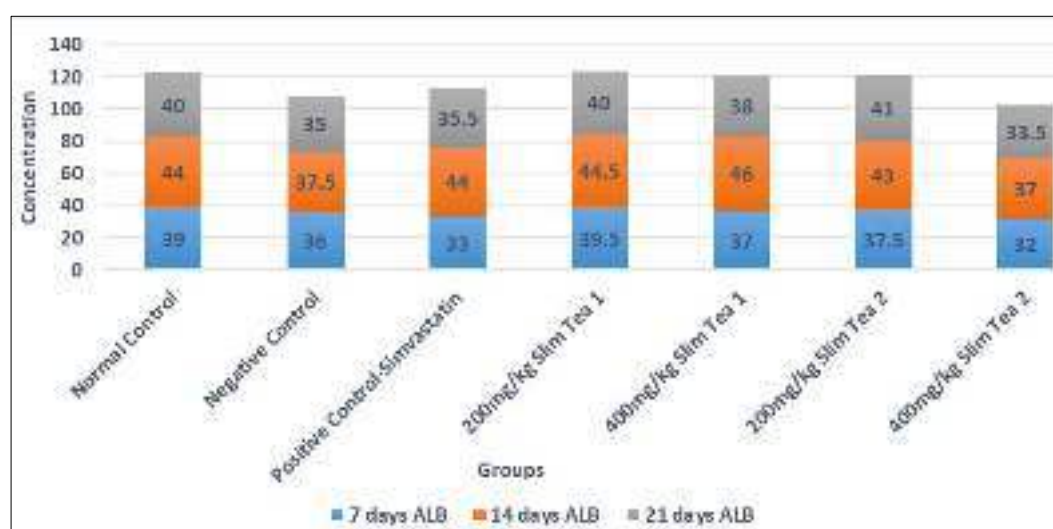


Fig 12: Concentrations of Serum Albumin in Experimental animals after 7, 14 and 21 days

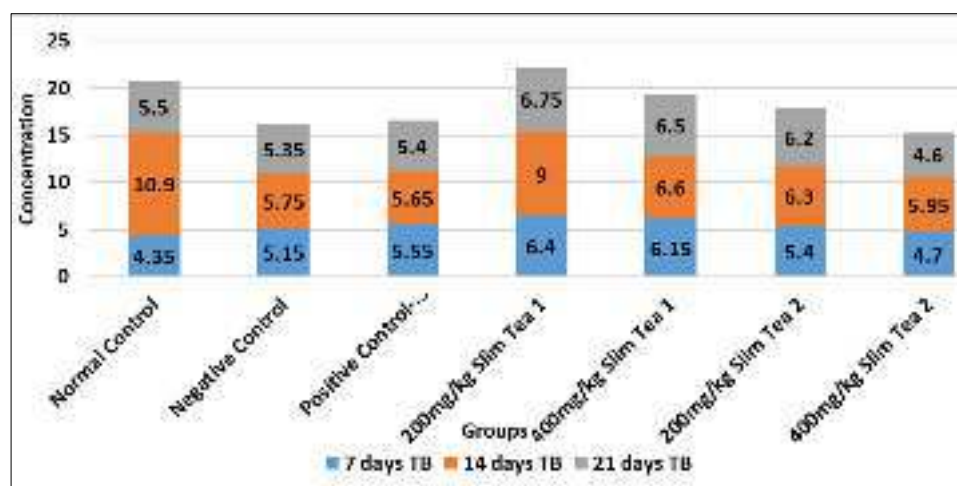


Fig 13: Concentrations of Serum Total Bilirubin in Experimental animals after 7, 14 and 21 days

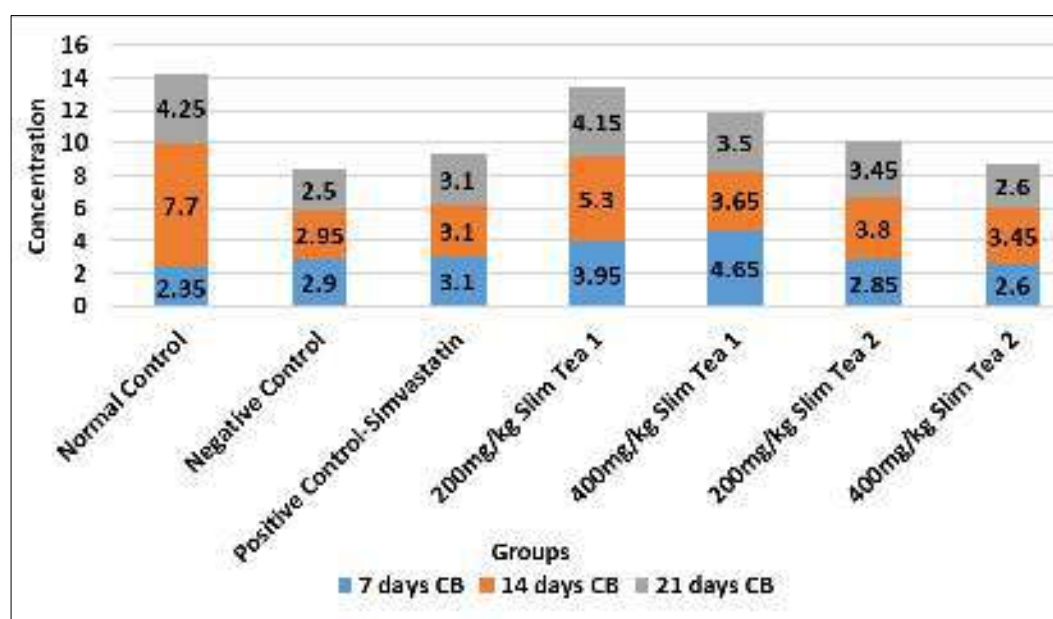


Fig 14: Concentrations of Serum Conjugated Bilirubin in Experimental animals after 7, 14 and 21 days

Zheng *et al.*, (2016)^[19] in their study, observed that prolong and frequent use of Slim tea could lead to severe acute hepatocellular liver injury, which may result in transplant. Several studies have added to the increasing number of reports of hepatotoxicity associated with dietary supplements containing uric acid, and highlights that herbal extracts from green tea or guggul tree may not be free of adverse effects (Yellapu *et al.*, 2011)^[20]. This is in consonance with the findings of this present study. Figures 8, 9, 10, 11, 12, 13, 14 which represents effects of slimming teas of study on ALT, AST, ALP, Total protein, Albumin, Total Bilirubin, Conjugated Bilirubin respectively revealed an increase in the serum ALT levels after 7, 14 days in all the experimental groups. An increase or high serum levels of ALT, AST, ALP has been implicative of damage or injury in the cells of the liver (Pei *et al.*, 2023; Odinga *et al.*, 2016; Galvin *et al.*, 2015; Wiwanitkit, 2001)^[24, 22, 9, 23]. Figure 8 revealed an elevation in the ALT level of the experimental rats administered slimming tea.

The histological examination of the cells of the liver revealed fatty infiltration of the hepatocytes as seen in figure 15.

The high/increased serum albumin level could be linked with the accompanying diarrhea associated with the intake

of slim teas, resulting to dehydration and loss of electrolytes. High Albumin levels may be a sign of dehydration, which may be caused by severe diarrhea or other condition (Ashraf, 2017)^[25].

Conclusion

This study suggests that the consumption of slimming teas, may significantly lead to weight loss, however, the variable impact on liver enzymes and the presence of heavy metals shows potential hepatotoxic risks and the need for stringent quality control. The elevated heavy metal content underscores potential adverse health effects. Thus, while slimming teas show promise for managing hyperlipidemia, their consumption must be approached with caution due to possible toxicological concerns.

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