

## RESEARCH ARTICLE

## Effect of Tea Produced from *Citrus Sinensis* Rind and *Persea Americana* Seed on Obese Male Rats

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### Abstract

This study was designed to evaluate the effect of tea produced from *Citrus sinensis* rind and *Persea americana* seed on high-fat diet-induced obese male rats. Five groups of 6 male Wistar rats each were fed with a high-fat diet (HFD) for 2 months to induce obesity. These HFD-fed experimental groups were then treated for 30 days with either distilled water at 10 mg/kg (HFD control group), 400, 200, and 100 mg/kg of the tea (treatment groups), or the reference compound atorvastatin (10 mg/kg body weight). A sixth group, made up of rats (normal diet group) not subjected to an HFD, was used as a negative control. The body weight was recorded every week. The Lee index and lipid profile were assessed using animal body weight and biochemical methods, respectively. Key biomarkers for liver function (ALAT, ASAT) and kidney function (creatinine) were also assessed. HFD induced a significant increase ( $P<0.05$ ) in the body and liver weights of the animals in the experimental groups as compared to those in the normal diet group. The body weight reduced when obese rats received tea of CSR and PAS. The HFD control group showed significantly elevated levels of all parameters compared to the normal control. Both 100 mg/kg and 200 mg/kg effectively lowered ALAT and ASAT to levels comparable with the standard treatment and the normal control, indicating a strong hepatoprotective effect. Concentration of 200 mg/kg, in particular, demonstrated a superior lipid-lowering effect, significantly reducing triglycerides and total cholesterol. In contrast, 400 mg/kg, while effective in reducing ALAT, was associated with a significant increase in creatinine levels, suggesting potential renal toxicity. In conclusion, 200 mg/kg was identified as the most effective and safest dose, providing a strong therapeutic effect on both liver health and serum lipid profile without adverse effects on kidney function: CSR and PSA are interesting plant materials that could be exploited as weight-reducing agents.

**Keywords:** *Citrus Sinensis* Peel; *Persea Americana* Seed Tea; Obese Male Rats

## Introduction

Obesity results from complex interactions of genetic, behavioral, and environmental factors correlating with lifestyles, economic and social status. It can only occur when the energy value of food eaten exceeds energy expended. This is known as "a positive energy balance," a situation whereby excess intake of energy in the form of food will inevitably appear as deposits of fat. Regarding food consumption, a study has suggested the existence of a rural-urban trend towards an increase in lipid content of the diet, particularly in developing countries such as Cameroon. Obesity is associated with and contributes to a shortened life span, type 2 diabetes mellitus, cardiovascular disease, some cancers, kidney disease, obstructive sleep apnea, gout, osteoarthritis, and hepatobiliary disease, among others [1].

Obesity also induces dys-regulation of endocrine secretion of adipocytokines, or adipokines, characterized by chronic low-grade inflammation with increased oxidative stress. The latter damages cellular structures, leading to the development of obesity-related complications [2]. Different measures such as a change in diet, increased physical activity, and use of anti-obesity drugs, dietary supplements, and even surgery are proposed for management of obesity. However, some approved anti-obesity drugs have been withdrawn because of their side effects. Surgery is not only costly but can also lead to serious adverse outcomes [3]. It is therefore vital to look for new therapeutic options for better management of obesity.

Medicinal plants, thanks to their phytonutrients and their uses for millennia, may constitute good sources of anti-obesity drugs. Herbal remedies are gaining more interest across the globe due to their ready availability, low cost, and the common belief that they are natural and safer as compared to synthetic drugs. However, adequate scientific evidence on medicinal plants is needed for their optimal and rational usage. Hence, more detailed herbal studies are needed on suitable animal models in the search for novel anti-obesity agents. Previous reports showed the anti-obesity activity of some medicinal [4], such as *Citrus sinensis* rind extract, which is a good source of vitamin C and Mg, which can be developed as a nutraceutical for obesity treatment, and they suggested that further investigation is required to determine active compounds in the *Citrus sinensis* rind extract, which enhances the beneficial effects of vitamin C and Mg. However, further research is needed to find out the correct dosage regarding the use of *Citrus sinensis* rind extract as an alternative therapy for treating obesity [5].

Then, the aqueous extracts of *Persea americana* seeds exhibit antioxidant potential and can prevent radical-induced oxidative damage [6]. *Persea americana* seed displays in vitro antioxidant potential by stabilizing peroxy radicals and superoxide anions and ferric reducing power, inhibiting the  $\beta$ -carotene blanching and development of thiobarbituric acid reactive substances [7].

## Material and Methods

### Preparation of Tea Based on *Citrus Sinensis* and *Persea Americana* Proportions

*Citrus sinensis* rind and *Persea americana* seeds were purchased at Bamenda Main Market. Then they were washed separately with tap water and placed in a colander to drain.

The *Persea americana* seeds and *Citrus sinensis* rind were sliced thinly and dried in a dehydrator for 48 hours at 30 degrees Celsius. Then grind into powder and store in a glass container, in line with WHO recommendations for herbal medicine processing [8].

Three treatment groups were formulated: treatment 1 (75:25), treatment 2 (50:50), and treatment 3 (25:75) of *Citrus sinensis* rind and *Persea americana* seeds, respectively. Each mixture was homogenized in clean containers. Phytochemical screening (for flavonoids, phenols, tannins, and saponins) was performed. Treatment 2 exhibited the highest concentration of phytochemicals and was selected for further preparation. A total of 400 g of treatment 2 (200 g of *Citrus sinensis* peel + 200 g of *Persea*

americana seed) was added to 5 L of hot water (90°C). The mixture was infused for 6 hours, with stirring at 30-minute intervals to maximize extraction of bioactive compounds [9]. After infusion, the liquid was filtered through a sieve to remove residues, then through cheesecloth, and finally through Whatman Number 1 filter paper, yielding a clear herbal tea (supernatant). The filtrate was first stored in glass jars under refrigeration to maintain freshness. It was then concentrated to dry tea powder using an oven for 7 days. After drying, 24 g of tea powder was obtained and stored in an airtight container until use [9].

### **Nutritional and Phytochemical Analysis**

The moisture content, ash, and carbohydrate content of the tea were measured using the AOAC method. Crude fat was determined by Soxhlet extraction, and protein was determined using the biuret method. The phytochemicals, mainly total phenol, flavonoids, and tannins, were determined using the Folin-Ciocalteu colorimetric, aluminum chloride colorimetric, and Mital and Jah methods, respectively [10].

### **Effect of Citrus Sinensis and Persea Americana Seed Tea on Body Weight and Serum Lipid Profile**

#### **Diet Formulation and Animal Study**

Adult albino male Wistar rats were obtained from the animal house of the Department of Biochemistry (Faculty of Science, University of Bamenda, Cameroon). They were raised under standard conditions of temperature, humidity, and a natural light/-dark cycle. They had free access to food and drinking water during the experimental period [11]. The animals were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory as referenced by the approval and health control No. 001/17 CCS/MINEPIA/RD-NW/DD-ME/SSV.

The Lee index was defined a few decades ago to classify obesity in rats in a similar approach to the BMI used for human obesity. The Lee index is defined as the cube root of body weight (g) divided by the naso-anal length (mm). Lee index values above 310 g were considered an indicator of obesity in rats. Correlations were also found between the Lee index and body fat content.

Aside from the normal diet control group (fed on a normal diet composed of 70% corn flour, 10% palm oil, 10% soya bean flour, 8% fish powder, 1% bone powder, and 1% vitamin), the other rats (experimental groups) were given a high-fat diet (composed of 50% corn flour, 30% palm oil, 10% soya bean flour, 8% fish powder, 1% bone powder, and 1% vitamin) daily for 60 days. The obese status of the animals was confirmed with Lee's index  $\geq 300$ . Obese rats were partitioned into six groups of five animals each and orally treated for 30 days with either distilled water (10 ml/kg), 400, 200, or 100 mg/kg of the tea, or 10 mg/kg of atorvastatin. The choice of doses of the extract and atorvastatin was based on previous studies see above. The group of normal diet control rats was left untreated, and body weights of all animals were recorded every week [11].

#### **Biochemical Analysis and Atherogenic Index Determination**

At the end of the treatment, the animals were fasted overnight, then anesthetized using diazepam (10 mg/kg) and sacrificed. Capillary blood was collected, and serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at  $-20^{\circ}\text{C}$  until use for subsequent biochemical analysis. The liver and other organs were dissected out and weighed. Homogenates (20% w/v in phosphate buffer (pH 7.4, 50 mmol)) were prepared from the liver and used for the assessment of ALAT and ASAT. ALT, AST, creatinine, and lipid profile concentrations in samples were quantified using Chrono Lab kits according to the manufacturer's procedure. To this end, parameters such as triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL-C) concentrations were immediately determined using an automatic analyzer (COBAS-MIRA System of Roche Diagnostics, Indianapolis) [12].

Low-density lipoproteins (LD-CL), very low-density lipoproteins (VLDL-C), atherogenic index (AI), and coronary risk index

(CRI) were estimated using the following formulae.

$$\text{VLDL-C (mg/dl)} = \text{TG}/5$$

$$\text{LDL-C (mg/dl)} = \text{TC} - (\text{HDL-C} + \text{VLDL-C}) \quad \text{AL} = \text{LDL}/\text{HDL-C}$$

$$\text{CRI} = \text{TC}/\text{HDL-C}$$

### Statistical Analysis

The data obtained were processed using SPSS (Statistical Program for Social Science) version 22 and presented as the mean  $\pm$  standard deviation. Comparisons among the groups of data will be carried out using the one-way ANOVA followed by the Duncan's multiple range test. The statistical significance for the expression of the analysis will also be assessed by ANOVA, and the differences identified will be pinpointed by an unpaired Student's t-test. An associated probability (P value) of  $<5\%$  will be considered significant [13].

## Results

### Variation of Some Proximate Nutrients According to Treatments

Results from some proximate parameters did not show significant variations among treatments for some parameters, notably ash content, moisture content, and fat content. However, treatment 3 recorded the higher content of moisture ( $38.67 \pm 2.46$  mg/g) and fat ( $1.78 \pm 1.00$  mg/g), while treatment 2 recorded the higher value of ash content ( $8.43 \pm 1.45$  mg/g).

Significant differences were recorded for protein and carbohydrate content among treatments. For protein content, Treatment 3 recorded the highest content ( $1.24 \pm 0.14$  mg/g), followed by Treatment 2 ( $0.56 \pm 0.26$  mg/g) and lastly Treatment 1 ( $0.43 \pm 0.13$  mg/g). For carbohydrate content, treatment 2 ( $2.48 \pm 0.49$  mg/g) recorded the highest, followed by treatment 3 ( $1.84 \pm 0.21$  mg/g) then treatment 1 ( $1.41 \pm 0.94$  mg/g).

**Table 1:** Average means of some proximate nutrients according to treatments

Treatment	Ash Content	Moisture Content	Proteins (mg/g)	Fat Content (mg/g)	Carbohydrates (mg/g)
Treatment 1	$1.57 \pm 0.52^a$	$15.47 \pm 6.03^a$	$0.43 \pm 0.13^a$	$1.75 \pm 0.48^a$	$1.41 \pm 0.94^a$
Treatment 2	$8.43 \pm 1.45^a$	$21.07 \pm 1.85^a$	$0.56 \pm 0.26^b$	$1.74 \pm 0.08^a$	$2.48 \pm 0.49^b$
Treatment 3	$7.47 \pm 0.41^a$	$38.67 \pm 2.46^a$	$1.24 \pm 0.14^c$	$1.78 \pm 1.00^a$	$1.84 \pm 0.21^a$

Values represent mean  $\pm$  SD. Means with same letter is the same and are not significantly different (DMRT).

### Phytochemicals and Vitamin C

Results from some phytochemicals and vitamin C did not show significant variations among treatments for some parameters, remarkably vitamin C; however, treatment 2 ( $12 \pm 0.001$   $\mu\text{g/g}$ ) recorded the highest value, followed by treatment 3 ( $11.6 \pm 0.001$   $\mu\text{g/g}$ ) and treatment 1 ( $9.6 \pm 0.001$   $\mu\text{g/g}$ ).

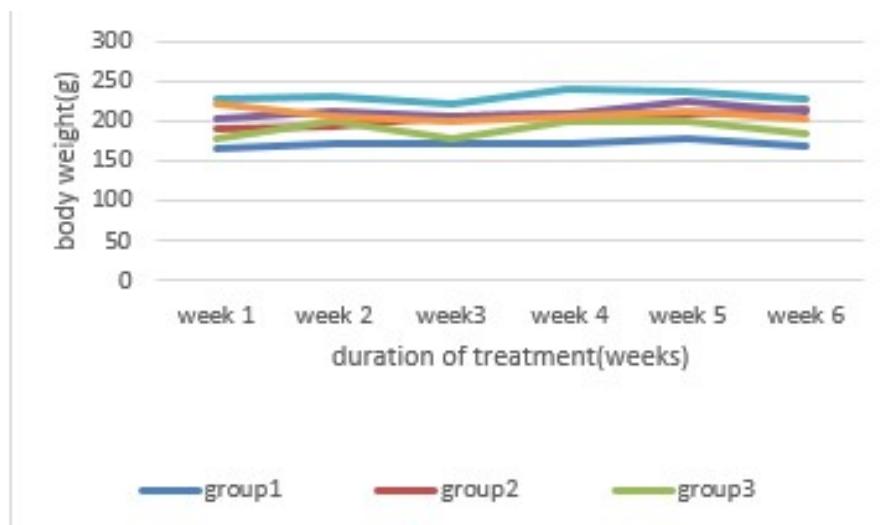
Statistical differences were recorded for phenols, flavonoids, and tannins. For phenols, the highest observation was in treatment 3 ( $0.38 \pm 0.01$  mg/g), followed by treatment 2 ( $0.37 \pm 0.04$  mg/g) and treatment 1 ( $0.32 \pm 0.01$  mg/g). For flavonoids, the highest observation was treatment 2 ( $0.28 \pm 0.01$  mg/g), followed by treatment 1 ( $0.25 \pm 0.001$  mg/g) then treatment 3 ( $0.07 \pm 0.01$  mg/g).

mg/g). For tannins, the highest observation was in treatment 1 ( $14.59 \pm 0.07$  mg/g), closely followed by treatment 2 ( $14.55 \pm 0.03$  mg/g), and lastly, treatment 1 ( $11.9 \pm 0.05$  mg/g).

**Table 2:** phytochemical and vitamin C analysis of citrus sinensis and persea americana seed tea produced

Code	Total Phenolic Content (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)	Vit C content (μg/g)
Treatment 1	$0.32 \pm 0.01^a$	$0.25 \pm 0.00^b$	$14.59 \pm 0.07^b$	$9.6 \pm 0.001$
Treatment 2	$0.37 \pm 0.02^b$	$0.28 \pm 0.01^c$	$14.55 \pm 0.03^b$	$12 \pm 0.001$
Treatment 3	$0.38 \pm 0.01^c$	$0.07 \pm 0.001^a$	$11.9 \pm 0.05^a$	$11.6 \pm 0.001$

Values represent mean  $\pm$  SD . Means with same letter is the same and are not significantly different (DMRT).



**Figure 1:** variation in the body weight of obese rats with different treatment

### Effect of CSR and PSA Tea on Lee Index

The table below shows the effect of the extract on male obesed rats. There was a significant decrease in the lee index in the different treatment groups compared to the control group except for group 3 that had a significant increase in body weight. This is in line with the previous research, which proved that the increase in body weight in all groups was directly proportional to the increase in body length in mice, so the researchers concluded that the determination of obesity in this study could not only focus on body weight variables, but needed to be compared with body length (Lee Index). In this study, we found that there was a decrease in Lee Index in the treatment group, which also align with previous studies showing

group 1: normal control, group 2:high fat diet control,group:3 atorvastatin(10mg/kg),group 4:CSR and PAS tea(400mg/kg) ,group5:CSR and PAS tea(200mg/kg), Group 6:CSR and PAS tea(100mg/kg)

**Table 3:** Effect of CSR and PSA tea on Lee index

Group	Lee index	
	before treatment	after treatment
Normal control	286.3	279.5
HFD control	322.2	340.5
Standard control	339	327.8

Dose 1	326.3	322.8
Dose 2	339.4	318.27
Dose 3	329.5	326.5

### Effects of Tea on Serum Lipid Profile

Serum lipid profile of rats after treatment with CSP and PAS or atorvastin is shown in table 4. The HFD significantly increased serum TC, TG, LDL-C, and VDL-C concentrations as well as AI and CRI while it reduced HDL-C levels. The treatment of rats with the tea and atorvastatin normalized these parameters as compared with the normal control animals.

**Table 4:** Serum lipid profile of different treatment groups

Groups	Triglyceride	Total cholesterol	High Density Lipo Protein	Low Density Lipo protein	Very Low Density Lipo protein	Atherogenic Index	Coronary Risk Index
Normal control	99.79±15.72 <sup>a</sup>	58.85±6.28 <sup>ab</sup>	41.50±4.0 <sup>a</sup>	38.79±9.76 <sup>a</sup>	19.15±3.15 <sup>a</sup>	0.94±0.18 <sup>a</sup>	2.43±0.23 <sup>a</sup>
HFD control	113.65±10.80 <sup>a</sup>	99.52±26.1 <sup>ab</sup>	30.59±8.05 <sup>a</sup>	56.33±13.6 <sup>a</sup>	22.73±2.16 <sup>a</sup>	1.17±0.78 <sup>a</sup>	3.47±0.98 <sup>a</sup>
Standard control	82.24±29 <sup>a</sup>	60.83±13.02 <sup>ab</sup>	36.38±7.53 <sup>a</sup>	29.41±2.32 <sup>a</sup>	13.45±5.80 <sup>a</sup>	0.85±0.66 <sup>a</sup>	2.30±0.83 <sup>a</sup>
dose 1	86.92±19.26 <sup>a</sup>	68.50±15.83 <sup>ab</sup>	37.50±5.96 <sup>a</sup>	32.02±12.94 <sup>a</sup>	17.38±3.85 <sup>a</sup>	0.87±0.36 <sup>a</sup>	2.34±0.44 <sup>a</sup>
dose 2	81.35±26.77 <sup>a</sup>	41.76±16.85 <sup>a</sup>	31.79±11.50 <sup>a</sup>	30.28±16.45 <sup>a</sup>	16.27±5.35 <sup>a</sup>	1.16±0.76 <sup>a</sup>	2.7±0.88 <sup>a</sup>
dose 3	93.92±24.78 <sup>a</sup>	67.26±16.79 <sup>b</sup>	33.01±15.12 <sup>a</sup>	42.12±27.70 <sup>a</sup>	18.78±4.96 <sup>a</sup>	1.64±1.28 <sup>a</sup>	3.30±1.16 <sup>a</sup>

Values represent the mean ± SD of 5 animals per group. Means with the same letter are the same and are not statistically significantly different (DMRT).

### Effect of Tea on Liver and Kidney Function

Results for the liver and kidney function showed significant variations for all the different functions; results for the HFD were generally greater compared to the other groups.

**Table 5:** ALAT, ASAT and creatinine of different treatment groups

Groups	ALAT U/L	ASAT U/L	CREATININE mg/l
Normal control	8.81±1.29 <sup>b</sup>	3.90±2.93 <sup>a</sup>	2.12±0.26 <sup>a</sup>
HFD control	12.18±1.70 <sup>c</sup>	7.1±2.61 <sup>b</sup>	2.88±0.33 <sup>a</sup>
Standard control	7.00±1.58 <sup>ab</sup>	2.98±1.45 <sup>a</sup>	2.74±0.45 <sup>a</sup>
Dose 1	5.70±2.53 <sup>a</sup>	2.98±0.93 <sup>a</sup>	2.25±0.91 <sup>ab</sup>
Dose 2	6.17±0.79 <sup>a</sup>	2.77±1.03 <sup>a</sup>	2.64±0.38 <sup>a</sup>
Dose 3	5.3±1.58 <sup>a</sup>	3.35±0.43 <sup>a</sup>	3.1±1.14 <sup>b</sup>

Values represent mean ± SD of 5 animals per group . Means with same letter is the same and are not significantly different.

## Discussion

This study investigated the impact of tea made from *Citrus sinensis* peel and *Persea americana* seed on obesity using body weight, Lee Index, and lipid profile in HFD obesity-induced male rats as markers.

The nutritional and phytochemical analysis of the formulated tea revealed notable variations influenced by the different proportions of CSP and PAS moisture contents obtained for samples. Treatment 1, treatment 2, and treatment 3 were consistently above the suggested moisture content of 10% for shelf [14] stability of food products. Treatment 3 showed the highest moisture content, implying that these tea blends, in their current form, would not be shelf-stable, as high moisture content facilitates biochemical reactions, promotes mold development, and increases microbial action in the samples. [15].

For total ash, treatment 2 showed the highest observation at treatment 2 followed by treatment 3 then treatment 1. The higher ash content in treatment 2 and treatment 3 may be an indication of a richer mineral content from their constituent materials. These 2 values are relatively higher than commonly reported ranges for *Citrus sinensis* peels typically 4-6% and *Persea americana* seeds typically 1-4% [16], while treatment 1 falls on the lower end. High ash content is vital for nutrition, as it indicates a good source of minerals that are involved in various physiological processes, including the digestion of basic biological complexes, compared to typical values for *Citrus sinensis* and *Persea americana*, suggesting enhanced mineral content complexes.

Protein content varied among the samples, with treatment 3 showing the highest content, followed by treatment 2 and treatment 1. These values are considerably lower than the typical protein content found in the dry matter of *Citrus sinensis* peel [16] or *Persea americana* seed treatment 1, seed, 3-6% [17]. This significant difference could be attributed to variations in the proportion of the main ingredients (*Citrus sinensis* rind and *Persea americana* seed) in their formulations or this formulations or inherent differences in analytical methods and sample processing; formulations processing: for example processing; aqueous extraction may not fully extract all.

The vitamin C content was significantly lower in treatment 1 compared to treatment 2 and treatment 3, and was lower than that of similar studies [16]. Vitamin C is a highly sensitive nutrient, particularly susceptible to degradation during processing and storage. However, its presence, even in smaller quantities, is very vital for human health.

Fat content was consistently low (1.75%), reflecting aqueous extraction's limited lipid solubility, yet sufficient for absorption of fat-soluble vitamins A, D, E, and K. The fat contents obtained were low across the samples: treatment 1, treatment 2, and treatment 3. These low values are typical for aqueous extracts of plant materials like *Citrus sinensis* rind and *Persea americana* seed, as lipids are generally not highly soluble in water. While *C. sinensis* rind and *Persea americana* seeds themselves contain some fat, the aqueous extraction process for tea significantly reduces this content.

Carbohydrate values were notably low with treatment 2, treatment 3, and treatment 1. These low values are significantly lower compared to values typically reported for *Citrus sinensis* peel or *Persea americana* seed, low with seed, which can be over 50% carbohydrates [17]. This implies that the tea blends, as prepared, would be poor sources of carbohydrates, which are primary energy sources. The seed, the discrepancy is likely due to the aqueous extraction method used for tea production, which may not efficiently extract complex carbohydrates like starch and fibers.

For total phenol content, treatment 3 showed the highest observation. Phenolic compounds are a diverse group of plant metabolites widely recognized for their potent antioxidant activities. *Citrus sinensis* rind and *Persea americana* seeds are both known to be rich sources of various phenolic compounds. Their presence contributes significantly to the overall therapeutic potential of these tea blends, as they play crucial roles in mitigating oxidative stress and inflammation, factors often implicated in various chronic diseases. The variation in phenolic content among the blends suggests that the blending ratios of *C. sinensis*

and *.americana* or specific constituent properties influence the final concentration.

For flavonoids, treatment 2 showed the highest, followed by treatment 1, and, treatment 3. Flavonoids are a key class of polyphenolic compounds known for their significant antioxidant potential, contributing to numerous vital biological actions and health benefits. *Citrus sinensis* rind, in particular, is a well-established source of various flavonoids. The higher concentration of flavonoids in treatment 2 and treatment 1 suggests that the specific blend ratio containing more *Citrus sinensis* rind might be particularly rich in these beneficial compounds. Flavonoids from plant sources have been shown to regulate blood lipids by improving oxidative stress capacity, inhibiting exogenous lipid absorption, inhibiting endogenous lipid synthesis, and promoting cholesterol and fatty acid decomposition [18].

Tannin content was high in treatment 1 and treatment 2. However, Tannins are known to form non-soluble compounds with proteins, potentially decreasing the breakdown and bioavailability of dietary proteins. Then, they also function as antioxidants, potentially reducing the risk of chronic diseases, and contribute to a food's characteristic flavor and color, making them important components in food formulations. The consistent levels across treatment 1 and treatment 2 suggest a stable contribution of these compounds from the constituent materials regardless of the specific blend ratio within the tested range.

Exposure of rats to HFD increased their body weights, the weights is a factor known as a hallmark of obesity, confirmed by the Lee index  $\geq 300$ . Body mass index is influenced by various factors such as age, gender, and demographics [19]. The increase in body weight is attributed to the rich caloric diet and fat accumulation in various parts of the body, leading to excessive growth of adipose tissue [20]. The body weight of the obese animals was significantly reduced by CSP and PAS tea at the dose of 200 mg/kg and dose of artovastatin (10 mg/kg). Similarly, studies were carried out by [16] and [21] revealed a decrease in body and organ weight in HFD-induced obese rats and mice after administration of *Citrus sinensis* peel extract then *Persea americana* seed oil, respectively. Cholesterol is an important structural component of the cell; it is the precursor for the synthesis of steroid hormones. Nonetheless, hyperlipidemia occurs when there is an abnormal increase in the total plasma concentrations of TC, TG, and LDL-C with a reduction of HDL-C level. Hyperlipidemia forms the basis of the development of coronary heart diseases, which, TG, by themselves, constitute a major health problem of great concern [22]. In this study, rats that received only HFD registered a significant increase in serum TC, TG, LDL-C, and VLDL-C levels, while HDL-C levels decreased. This may be due to an increase in both de novo-LDL-C, de novo-TG and cholesterol synthesis then de novo-synthesis and intestinal lipid uptake from the fat-enriched synthesis and-enriched diet [23]. A number of lipid parameters have been employed in predicting the risk-enriched the risk of coronary atherosclerosis and cardiovascular diseases. On a general note, recent data have shown that AI and CRI are more accurate predictors of cardiovascular risk than traditional lipid parameters [24]. Atherogenic dyslipidemia, characterized by a combination of increased TG, LDL-C, the LDL-C, AI levels and decreased HDL-C level, were observed in the HFD control animals. This confirms that HFD could predispose to atherosclerosis and consequently to cardiovascular diseases [22]. When the HFD-fed rats were administered the tea made from CSR and PAS tea, this resulted in a profound reduction in the atherogenic and coronary risk indices, in, thereby further supporting the hypolipidemic effect of the tea.

In the process of weight loss, most groups showed some fluctuations in body weight; this is due to the possibility that there will be an increase in body weight after a few days of treatment, called the phenomenon of weight fluctuation, and can occur for several reasons, including the fluctuation, the presence of fluid retention, hormonal changes, and the weight of undigested food that waste in the digestive system, as seen on the graph above; however, this weight fluctuation is normal and temporary [25].

At the start of the study (week 1), all groups had initial body weights generally clustering around 200-220 grams. Over the six-week treatment period, several groups exhibited a trend of body weight reduction or stabilization compared to their initial measurements. However, the normal control consistently showed a decrease in body weight, ending at a significantly lower weight than most other groups. Dose 2 and dose 3 demonstrated a clear reduction over the treatment. Group 5 showing the significant

weight loss, hence the most effective treatment group. Dose 2 (200 mg/kg) is the most effective dose; the treatment dose; this result ties with the work of [26].

In the sub-acute toxicity study, administration of avocado seed oil caused a significant increase in liver enzymes (ALT and AST) and renal function parameters (urea and creatinine). These results showed that avocado seed oil mildly impaired liver and kidney functions of the experimental rats [21]. Our results showed that administration of CSP and PAS tea increased AST and ALT, suggesting that CSP and PAS tea exhibit, mild toxicity following oral administration.

In this study, we found that there was a decrease in Lee Index and lipid profile in the treatment groups, which also aligns with previous studies showing that CSP and PAS were respectively effective in reducing Lee Index and lipid profile [27, 28]. The results from this study, particularly for dose 2, further support the hypothesis that tea blends derived from *Citrus sinensis* peel and *Persea americana* seed may contribute to the management of body weight and lipids profile. This study demonstrates the potential of CSP and PAS to be developed into a functional food for managing High-Fat-diet-induced obesity.

## Conclusion

The efficacy of this tea is attributed to its high content of phytochemicals such as flavonoids, phenols, tannins and vitamin C.

This study reinforces the beneficial effects of *Citrus sinensis* peel and *Persea americana* seed to ameliorated HFD diet-induced obesity. Improvement in obesity-related parameters: Notable improvements include reduced body weight, Lee index, and lipid profile.

This study demonstrates the potential of *Citrus sinensis* peel and *Persea americana* seed to be a functional tea for managing high-fat diet-induced obesity.

## Conflicts of Interest

No conflicts of interest

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