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Co-administration of Caffeine and Hydromethanolic Fraction of *Citrullus lanatus* Seeds Extract Improved Heamatological and Lipid Profile of Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author GIO designed the study, wrote the protocol. Author KWN wrote the first draft of the manuscript and managed the literature searches. Authors JNO and OVAN performed the statistical analysis, managed the animals, collected all data and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to evaluate the ameliorative effect of co-administration of caffeine (CF) and hydromethanolic fraction of *Citrullus lanatus* (CL) seeds extract on heamatological and lipid profile of wistar albino rats.
Methods: Heamatological parameters were determined using a Synchron CX5 autoanalyzer according to the manufacturer's protocol while, plasma lipid profiles were determined using Randox diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder.

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Results: The result showed that administration of CL and CF on WBC and HB in group III and IV were significantly higher ($p<0.05$) when compared to the control group. Whereas RBC level in group IV was significantly higher ($p<0.05$) compared to the control group. The effect of CL and CF on the HCT, MCV and MCHC levels of rats in groups II and IV were significantly different ($p<0.05$) compared to the control group. TC concentrations of rats in groups IV and V were significantly higher ($p<0.05$). Also, LDL concentration in groups II and IV were significantly different ($p<0.05$) while TG concentrations of in groups II, III and IV were significantly different ($p<0.05$) compared to the control group. CF however caused a decrease in red blood cells and an increase in white blood cells.

Conclusion: It is therefore suggested that CF should be consumed only in moderate doses to ensure normal regulation of blood levels. The study also indicates the possible use of hydromethanoic extract of CL seed in the treatment of anemia. Furthermore, it showed that CF alone is not a risk factor for coronary heart disease, only when taken in conjunction with a diet high in cholesterol. CL when taken in moderate quantity is very good to the body due to its LDL lowering effects, although when taken too much it had a hypercholesterolemic effect.

Keywords: Caffeine; Citrullus lanatus; hematological parameters; plasma lipid profile; hydromethanolic.

1. INTRODUCTION

Caffeine is a trimethylxanthine whose primary biological effect is the antagonism of the A1 and A2A subtypes of the adenosine receptors [1]. Caffeine is found in many everyday products like coffee, tea, kola nuts, chocolate, soda beverages, drugs etc. Thus it is widely and immensely consumed. For example, an average American consumes 200 mg of caffeine daily [2]. In Nigeria and other third world countries, the consumption of caffeine has increased possibly as a result of culture assimilation. In humans, caffeine acts as a central nervous system stimulant, hence it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs [3]. It is metabolized in the liver into three primary metabolites, paraxanthine (84%), theobromine (12%) and theophylline (4%) [4].

The interest in researches pertaining to caffeine has been increasing in recent years, and this has resulted in a surge of publications dealing with a variety of pharmaco-physiological effects of caffeine. Caffeine has been shown to have various pharmacological and cellular responses in a wide spectrum of biological systems [5]. Onuegbu et al. [6] found significant increases in the mean total serum cholesterol concentration and LDL- cholesterol concentration were observed in healthy human subjects after regular administration of caffeine. No significant differences were obtained in the mean HDL cholesterol concentration and in the mean serum triglyceride levels.

C. lanatus of family Cucurbitaceae is commonly known as water melon. The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain [7]. It also reported having analgesic and anti-inflammatory of seeds [8], laxative activity of fruit [9], antioxidant of fruit and hepatoprotective [8]. This study investigated the effect of co-administration of caffeine and hydromethanolic fraction of CL seeds extract on hematological and lipid profile of Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty-five (25) albino wistar rats weighing between 160-300 grams were used for this study. These rats were purchased and housed in the experimental animal house of the department of medical physiology, Madonna University, Elele campus, Rivers state. The rats were kept under normal room condition and 12 hour light and dark cycles. The rats were randomized into control and experimental groups according to weight range and housed in sanitized wooden cages containing saw dust as bedding. The health status of animals was monitored and the rats were allowed to acclimatize for two weeks after which they were randomly grouped into five groups before

commencement of administration of extract and caffeine Table 1. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and approved by Departmental Animal Ethical Committee.

2.2 Preparation of Extract

A total of eighteen (18) fresh whole red watermelons were purchased from Elele local market, Rivers State, Nigeria. The seeds were separated, washed and dried for 5 days. The dried seeds were ground into powder with the aid of a Corona grinding machine. About 3.348 kg of the grounded seeds was measured out and poured into a neat white transparent bucket with a lid and then soaked in 4 liters of hydro-methanol (80% methanol and 20% distilled water). The mixture was properly stirred to make sure it was properly soaked and allowed to stand for a total period of 24 hours.

The soaked ground seeds was squeezed so as to separate the chaff from the liquid. The liquid obtained was filtered using Whitman's no. 1 filter paper placed in a funnel and held on a clamp stand. The brownish-yellow filtrate was collected in a beaker. This filtrate was then placed in a water bath set at 45ºC, covered with aluminum foil (to prevent reduction of its potency), and left to dry for 7 days (1week). This method of drying was in order to concentrate the extract. After a week the concentrated hydromethanolic extract of CL seeds was dissolved in 2 litters of water. A dose of 200 mg kg^{-1} was used for high dose and 100 mg kg^{-1} for low dose administration as shown on Table 1. Caffeine anhydrous ($CH_{10}N_4O_2$; 1,3,7-trimethylxanthine), obtained from SIGMA Chemical Co. (St. Louis, MO, USA) was dissolved in physiological saline and made available in doses of 100 mg/kg and 50 mg/kg, these doses was used for the high dose and low dose administration respectively.

2.3 Administration of CL and CF

The extract (CL) and CF were administered orally for twenty one days using gastric gavage and a 1ml syringe. Administration was done in the evenings before feeding the animals for the day. The animals were grouped into five according to body weight with five rats in each group. The extract was administered using average weight.

2.4 Sample Collection

At the end of twenty-one days (3 weeks), the rats were fasted for eight hours prior to sacrifice. The animals were anaesthetized using chloroform in a chloroform chamber (following animal care and guideline), and then sacrificed. Blood was collected via cardiac puncture with a 5 ml syringe; this process was repeated for each rat. The collected blood samples were taken to the laboratory for analysis. A part of the blood was collected into clean dry labeled Ethylene Diaminetetra-acetic (EDTA) bottles for the estimation of hematological parameters namely: white blood cells (WBC), red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and hemoglobin (Hb) using an automated hematological machine (Cell-DynTM Abbot, US). Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 mins. The other part were separated, stored at −4ºC used for evaluation of biochemical parameters namely: Total cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglyceride TG) using commercial kits obtained from Randox Laboratories, UK.

2.5 Determination of Hematological Parameters

Hematology profile, which covers white blood cell (WBC), red blood cell (RBC) count, hemoglobin level (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using a Synchron CX5 autoanalyzer according to the manufacturer's protocol.

2.6 Determination of Plasma Lipid Profile

The plasma total cholesterol, High density lipoprotein, Low density lipoprotein and triglycerides were determined using Randox diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder [10,11].

2.7 Statistical Analysis

The results obtained from this study were analyzed using statistical package for social science (SPSS version 16.0 for windows).

Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $p<0.05$. Post hoc multiple comparisons for differences between groups and within groups were established using Least Significant Difference (LSD). All results are presented as mean±S.E.M. the graphical representations were designed in Microsoft Excel 2010 and SPSS.

3. RESULTS

The result of the effect of oral administration of CL and CF on heamatological parameters are as shown on Tables 2. The WBC levels of rats in groups III and IV were significantly higher ($p<0.05$) compared to the control group. However, the groups II and V statistically show no significant difference ($p>0.05$) in comparison with the control group. Whereas, the RBC level of rats in group IV was significantly higher ($p<0.05$) compared to the control group. Although, there was no statistically significant difference ($p>0.05$) in the groups II, III and V when compared with the control group. Also, the HB concentration of rats in groups III and IV were significantly higher ($p<0.05$) compared to the control group. But, the groups II and V statistically show no significant difference ($p>0.05$) in comparison with the control group. Consequently, it was observed that a co-administration of CF and the CL seeds extract kept the level of hemoglobin at a normal level in consideration to the dose administered.

From the study, the effect of administration of CL and CF on the HCT, MCV and MCHC levels of rats in groups II and IV were significantly different ($p<0.05$) compared to the control group. Although, there was no statistically significant difference ($p>0.05$) in the groups III and V when compared with the control group, whereas, the MCH concentration of rats in group III was significantly different ($p<0.05$) compared to the control group. Notwithstanding, there was no statistically significant difference ($p>0.05$) in the groups I, II and III when compared with the control group.

Results of the effect of administration of CL and CF on lipid profile on Table 3 showed that, the TC concentrations of rats in groups IV and V were significantly higher ($p<0.05$) compared to the group I. However, the experimental groups II and III statistically were not significantly different ($p>0.05$) when compared with group I. Also, HDL concentration of rats in groups II and III were significantly lower ($p<0.05$) compared to the group I. However, the experimental groups IV and V statistically were not significantly different ($p>0.05$) when compared with the group I. Furthermore, LDL concentration of rats in groups II and IV were significantly different ($p<0.05$) compared to the group I. Although, there was no significant difference ($p>0.05$) in the groups III and V when compared with the group I, whereas, TG concentration of rats in groups II, III and IV were significantly different ($p<0.05$) compared to the group I. But, group V statistically showed no significant difference ($p>0.05$) when compared to the group I as shown in Table 3.

4. DISCUSSION

The use of herbal products is increasing, and over-the-counter herbal supplements are perceived by the public as “safe” and “harmless.” Although the majority of them are safe, some herbal medicines carry risks [12,13]. Hematological parameters are important health indices and are of diagnostic significance in routine clinical evaluation of the state of health [13]. CF significantly increases ($p<0.05$) hematological parameters and lipid profile; this is in agreement with what Onuegbu et al. [6] reported but CL alone exceedingly increases hematological parameters and lipid profile significantly ($p<0.05$) when compared with the administration of CF alone and co-administration of CF and CL except for MCV and LDL.

There was a significant increase ($p<0.05$) in the white blood cell level in Group IV (16.7±0.16) & Group III (11.5±0.23) compared to the control group (7.35±0.05). The increase in WBC level of group IV which received only CL seed extract could be attributed to the effect of the saponin

Table 1. The CL extract and caffeine administered orally for twenty one days

Group	Treatments	Durations	Number of rats
I	Normal feed + water	21 days	5
II	Normal feed + water + 50 mg/kg CF	21 days	5
III	Normal feed + water + 100 mg/kg CF	21 days	5
IV	Normal feed + water + 200 mg/kg CL	21 days	5
V	Normal feed + water + 100 mg/kg CF + 200 mg/kg CL	21 days	5

Table 2. Effect of CL extract and caffeine on heamatological parameters

Group	WBC ($X10^9$/L)	RBC ($X10^{12}$/L)	HB (g/dL)	HCT (%)	MCV (f/l)	MCH (pg)	MCHC (g/dL)
I	7.35±0.05	7.78±.12	14.44±0.45	42.65±1.45	53.1±1.41	19.2±0.63	35.2±1.21
II	6.12±0.24	6.90±0.25	13.7±0.13	37.6±2.45*	46.3±1. 89*	20.6±0.55	39.7±0.65*
III	11.5±0.23*	8.08±0.11	16.1±0.36*	44.7±1.42	49.0± 0.58	17.6±0.21*	36.1±1.21
IV	16.7±0.16*	9.47±0.27*	18.2±0.33*	48.9±1.18*	44.0±1.84*	20.3±0.77	42.8±2.3 5*
V	7.88±0.17	7.73±0.07	14.5±0.38	43.0±1.12	52.9±1.43	19.5±0.62	34.7±1.65

Data represented as Mean ± SEM; (*) p<0.05 significantly different in comparison with control group 1; n=4; WBC=White blood cell, RBC=Red blood cell, HB=Hemoglobin, HCT=Hematocrit, MCV= Mean Corpuscular Volume, MCH=Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration

Table 3. Effect of CL extract and caffeine on lipid profile

Group	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TG (mmol/L)
I	4.45±0.25	1.87±0.35	1.97±0.05	1.89±0.08
II	4.49±0.64	1.02±0.08*	2.05±0.19	1.48±0.38*
III	4.50±0.22	0.69±0.12*	2.18±0.27*	1.66±0.21*
IV	6.07±0.23*	1.98±0.35	1.48±0.13*	2.18±0.16*
V	6.34±0.51*	1.87±0.25	1.98±0.40	1.91±0.28

Data represented as Mean ± SEM; (*) p<0.05 significantly different in comparison with group I; n=4; Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglyceride (TG)

which is a phytochemical constituent of CL and Saponins are reported to be suitable immunostimulators [14,15]. Since group IV received only high dose of CL seed extract, saponin could immensely elevate white blood cell levels. CF changes the immune response inducing leucocytosis, lymphocytosis, and neutrophillia [16]. Therefore an increase in white blood cell in group III which received high dose CF could be attributed to the increasing effect CF has on white blood cells. The RBC level in group IV was markedly increased. Therefore an increase in red blood cells could consequently lead to an increase in hemoglobin concentration. Also, CF causes an increase in metabolic rate [16]. An increase in metabolic rate will require increased tissue oxygenation and consequently an increased demand for oxygen by the tissues. Therefore increased demand for oxygen will lead to increased concentration of hemoglobin. Hematocrit which is also called packed cell volume is the percentage (%) of red blood cells in blood. An increase in hematocrit level of group II could be a sign of polycythemia vera. Polycythemia vera is a proliferative disorder in which the bone marrow produces excessive number of red blood cells. This could lead to increase in blood volume and a consequent increase in hematocrit. Dehydration which is a consequence of the diuretic nature of CL could lead to increased red blood cells and ultimately cause an increase in hematocrit level. CF reduces iron absorption [17,18] which could result in thalassemia. Thalassemia is a disorder which results in the destruction of red blood cells leading to less formation of red blood cells. This could be a cause of the observed decrease in MCV levels of group IV.

There was a significant decrease ($p<0.05$) observed in the mean corpuscular hemoglobin in Group III (17.6±0.21) compared to control group (19.2±0.63). MCH is the hemoglobin amount per red blood cell. The decrease in iron absorption could cause a decrease in amount of HB in the RBC and consequent decrease in MCH as shown on Table 2. But there was marked increase in the MCHC in Group IV (42.8±2.35) and Group II (39.7±0.65). MCHC measures the average concentration of hemoglobin in the blood. And as such, the increase in MCHC would lead to an increase in the MCH.

The elevation of TC in group IV and group V could be due to the presence of antioxidants in excess. CL contains antioxidants such as lycopene, flavoniods, alkaloids, which binds to the free radicals in the body. In excess this strong reducing agent would bring about deficiencies in minerals such as iron, zinc and calcium, by preventing the absorption of these minerals from the gastro intestinal tract, therefore calcium's cholesterol lowering effects is lost bringing about an increase in total cholesterol in the 0body [19]. There was no significant increase in total cholesterol in groups given caffeine due

to the low antioxidant content of caffeine (an alkaloid). In this present study the levels of HDL in group II & III, decreased significantly, this decrease can be due to the fact that unfiltered caffeine contains a specific type of terpene called cafesol that might hijack the receptors in the intestine that regulate cholesterol, thereby, increasing the levels of LDL [20].

The level of LDL was significantly ($p<0.05$) elevated in groups II & III, this is an indication of cafesol as mentioned above. In group IV, there was a decrease in the levels of LDL this may be due to the fact that CL contains citrulline, an amino acid produced in the body from glutamate, citrulline is used in the body to make arginine which produces the nitric oxide vital in maintaining the vessels also L-Arginine and nitric oxide dilates vessels thereby aiding the kidney to function better also lowers the concentration of LDL [21]. The decrease in group II & III for TG was dose dependent, this decrease can be due to the fact that TGs and LDLs share the same carrier LDL-particles and if the levels of LDL increases most of these carriers would be occupied and therefore less available to TGs leading to the decrease in serum TGs [22]. Also in group IV the levels of TGs increased due to the decrease in LDL and also due to the presence of fructose present in watermelon, fructose is incorporated into triglycerides [23].

5. CONCLUSION

In this present study, it was observed that co-administration of CF and CL caused the blood parameters to remain relatively normal. CF however caused a decrease in red blood cells and an increase in white blood cells. It is therefore suggested that CF should be consumed only in moderate doses to ensure normal regulation of blood levels. Also from the result it was observed that CL seed extract causes an increase in white blood cell and red blood cell. These findings indicate the possible use of hydromethanoic extract of CL seed in the treatment of anemia. It also showed that CF alone is not a risk factor for coronary heart disease, only when taken in conjunction with a diet high in cholesterol, this is because the levels of HDL in the groups given CF was significantly decreased and their LDL levels increased significantly without a significant increase in total cholesterol. CL when taken in moderate quantity is very good to the body due to its LDL lowering effects, although when taken too much it had a hypercholesterolemic effect.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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