

Original Research Article

Co-administration of Caffeine and Hydromethanolic Fraction of *Citrullus lanatus* Seeds

Extract improved Heamatological and Lipid Profile of Wistar Albino Rats

Abstract

Aim: This study was designed to evaluate the ameliorative effect of co-administration of effect 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 profile were determined using Randox diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder.

Results: The result showed that administration of CL and CF on WBC 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. While HB concentration in groups III and IV were significantly higher ($p < 0.05$) compared to the control group. The 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 concentration of rats in groups IV and V were significantly higher ($p < 0.05$) while HDL concentration in groups II and III were significantly lower ($p < 0.05$) compared to the group I. Also, LDL concentration in groups II and IV were significantly different ($p < 0.05$) while TG concentration of in groups II, III and IV were significantly different ($p < 0.05$) compared to the group I. 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.

Key Word: caffeine, *Citrullus lanatus*, Heamatological parameters, plasma lipid profile, Hydromethanolic

Introduction

The use of fruits has been highly recommended for healthy individuals and those who are ill or recuperating from an illness for ages. Mostly due to their effectiveness, less side effects and relatively low cost as compared to conventional drugs and therapies. Fruits used in the treatment of illnesses have been very successful, and sufficient use of its historic usage has been useful in drug discovery and development.

Watermelon (*C. lanatus* or CL) seeds are a source of protein, B vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others [1,2]. The fruits of CL help in Boosting Antioxidant Levels because it is exceptionally rich in carotenoids such as lycopene, lutein and β carotene [3]. A regular CL juice consumption result in significant increases in blood plasma concentrations of lycopene and β carotene [4]. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals [5,6]. The most important of these bioactive constituents (phytochemicals) of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. These phytochemicals are antibiotic principles of plants [5]. They are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques [7]. These phytochemicals have been reported to exhibit hemolytic and foaming activity, antifungal, anti-inflammatory, fungistatic, and molluscidal [5,6].

It is very important to note that all parts of the CL have something to offer. For example, the seeds are excellent source of protein. The good nutritional and functional properties of CL seed meal proteins suggest their potential use in food formulations and diet [8]. CL possesses numerous bioactivities from natural source which is of better advantage than conventional therapies. Moreover, CL-induced is also known to increase plasma antioxidant levels and may explain why epidemiological studies of the Chinese found greater watermelon intake to be associated with a lower risk of cancer [9].

Materials and Methods

Seeds Collection and Preparation

Watermelon seeds were collected from ripe watermelon. The seeds were allowed to dry under the sun. The dried seeds were grinded into fine powder using a laboratory grinding hand mill. The powder was weighed prior to extraction.

Preparation of Methanoic extract of CL seeds and CF

3398g of grinded watermelon seeds was extracted using hydro methanol (800 ml methanol & 200ml distilled water).1000ml hydro methanol was added to powdered watermelon seeds four consecutive times. The extract was kept in a bucket and left for exactly 24hours. After 24 hours the seeds were squeezed out of the mixture. The filtrate was filtered using whatman No.1 filter

paper placed on a clamp and stand. The filtrate was collected in beakers as a clear colored golden liquid and placed in a water bath at a temperature of 45°C for 7 days. After evaporation, the extract resembles a gelatinous substance and is diluted. The extract is kept in a refrigerator to prevent loss of potency. While 10g of caffeine was weighed and diluted in 1 liter of distilled water, a dose of 100mg/kg and 50mg/kg was used for the high dose and low dose administration respectively.

Experimental Animals

A total of twenty-five (25) albino wistar rats weighing between 160-300grams 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 12hour 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.

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

Group	Treatments	Durations	Number of Rats
Control	Normal feed + water	21days	5
50mg/kg CF	Normal feed + water + 50mg/kg CF	21days	5
100 mg/kg CF	Normal feed + water + 100mg/kg CF	21days	5
200mg/kg CL	Normal feed + water + 200mg/kg CL	21days	5
100mg/kg CF + 200mg/kg CL	Normal feed + water + 100mg/kg CF + 200mg/kg CL	21days	5

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.

88 The animals were grouped into five according to body weight with five rats in each group. The
89 extract was administered using average weight.

90 **Sample Collection**

91 At the end of twenty-one days (3 weeks), the rats were fasted for eight hours prior to sacrifice.
92 The animals were anaesthetized using chloroform in a chloroform chamber (following animal
93 care and guideline), and then sacrificed. Blood was collected via cardiac puncture with a 5ml
94 syringe; this process was repeated for each rat. The collected blood samples were taken to the
95 laboratory for analysis. A part of the blood was collected into clean dry labeled Ethylene
96 Diaminetetra-acetic acid bottles for the estimation of hematological parameters namely: white
97 blood cells (WBC), red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV),
98 Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC)
99 and hemoglobin (Hb) using an automated hematological machine (Cell-Dyn™ Abbot, US).
100 Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for
101 10 mins. The other part were separated, stored at -4°C used for evaluation of biochemical
102 parameters namely: Total cholesterol (TC), High density lipoprotein (HDL), Low density
103 lipoprotein (LDL), Triglyceride TG) using commercial kits obtained from Randox Laboratories,
104 UK.

105 **Determination of Hematological Parameters**

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

110 **Determination of Plasma Lipid Profile**

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

114 **Statistical Analysis**

115 The results obtained from this study were analyzed using statistical package for social science
116 (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 $\pm$ S.E.M. the graphical representations were designed in Microsoft Excel 2007 and SPSS.

Results

The result of the effect of oral administration of CL and CF on heamatological parameters are as shown in Tables 2. The WBC level 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 shows 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 shows no significant difference ($p > 0.05$) in comparison with the control group. Consequently, it's 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.

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

Group	WBC ($\times 10^9/L$)	RBC ($\times 10^{12}/L$)	HB (g/dL)	HCT (%)	MCV (f/l)	MCH (pg)	MCHC (g/dL)
I	7.35 $\pm$ 0.05	7.78 $\pm$.12	14.44 $\pm$ 0.45	42.65 $\pm$ 1.45	53.1 $\pm$ 1.41	19.2 $\pm$ 0.63	35.2 $\pm$ 1.21
II	6.12 $\pm$ 0.24	6.90 $\pm$ 0.25	13.7 $\pm$ 0.13	37.6 $\pm$ 2.45*	46.3 $\pm$ 1.89*	20.6 $\pm$ 0.55	39.7 $\pm$ 0.65*

111	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±2.35*
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 I; 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.

Results of the effect of administration of CL and CF on lipid profile in **Table 3** showed that, the TC concentration 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**.

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).

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].

There was a significant increase ($p<0.05$) in the white blood cell level of rats in Group II (16.66 ± 0.16) which received 200mg/kg CL seed extract & Group V (11.45 ± 0.23) which received 100mg/kg CF compared to the control group (7.35 ± 0.05). The increase in WBC level of rats in group II which received only CL seed extract could be attributed to the effect of the saponin which is a phytochemical constituent of CL and Saponins are reported to be suitable immunostimulators [14,15]. Since group II 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 neutrophilia (Ramanaviciene [16]). Therefore an increase in white blood cell in group V which received high dose CF could be attributed to the increasing effect CF has on white blood cells. The RBC level in group IV administered with 200mg/kg hydromethanolic fraction of CL seeds extract 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 V (17.60 ± 0.21) which received 100mg/kg in CF compared to control group (19.15 ± 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 II (42.83 ± 2.35) which received 200mg/kg CL seed extract and Group IV (39.70 ± 0.65) which received 50mg/kg CF. 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 4 and group 5 could be due to the presence of antioxidants in excess. CL contains antioxidants such as lycopene, flavonoids, 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 body [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 contradicting some research works, 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].

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.

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