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Haematological and Cortisol Dynamics in Albino Rats Stimulated By Intake of Methanolic Extract of *Cola acuminata*

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Abstract: The effects of four weeks administration of the methanolic seed extract of *Cola acuminata* on the haematology and serum cortisol profiles of adult male albino rats were investigated. Dose dependent significant effects of the extract were recorded in the white blood cell count and mean corpuscular volume ($p<0.05$). While the effects on the red blood cell count, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width-standard deviation were not significantly different ($p>0.05$). Significant increases were recorded in the serum cortisol concentration of the rats administered methanolic seed extract of *C. acuminata* ($p>0.05$) as compared to the control.

Key words: *Cola acuminata* · Haematology · Cortisol · Inflammatory Response · *Rattus norvegicus*

INTRODUCTION

Nutritional research in the present century is geared towards creating awareness among people about the health implications of their diets. Recently, healthy dietary ingredient exploration has diverted consumers towards natural resources [1]. Beverages (alcoholic and non-alcoholic) form a major component of our diet. Among the non-alcoholic are coffee and tea mostly taken by individuals avoiding much sugar intake associated with fruit juices, milk, yogurts, cocoa beverages and carbonated drinks. Man is endowed by nature with various plant species for use to improve the quality of life. Plants serve several purposes such as food, drug, raw material, etc. Kola nut is one of such plant species. It is believed to be indigenous to West Africa, Kola nut has over 40 different species, out of which four species (*Cola acuminata*, *C. vertialata*, *C. nitida* and *C. anomala*) are edible, thus widely cultivated [2]. Two out of these species, *C. acuminata* and *C. nitida* are common in Nigeria [3]. Locally, *C. acuminata* is chewed as a stimulant and used industrially in manufacture of dyes and beverages such as Coca cola, Pepsi cola and Afri Cola [3]. Kola nut is very indispensable in many social and religious gatherings and may also be used to quench hunger and taste [4]. According to Quarcoo [5], Nigeria accounted for almost 70% world kola nut production in the seventies. Approximately 90% of production was consumed locally, while l0% was exported [5].

Kola nut is greatly used by many professionals as a principal stimulant to keep awake and resist fatigue [6]. Principal chemical constituents of *C. acuminata* are caffeine, theobromine and theophylline which are xanthine derivatives [7]. High concentration of pure xanthine in the body may results in certain heart disorders [4]. Caffeine was consumed daily by estimated 85% of adults in the US either in coffee, tea or in soft drinks [8]. This high level of consumption persists despite more evidence being made available on the stimulatory effects of caffeine on the cardiovascular and neuroendocrine systems that could have serious negative health implications [9]. Laboratory studies have shown that a dose of caffeine equivalent to three cups of brewed coffee will raise resting blood pressure by 7-10 mmHg when given either to caffeine-naive individuals or habitual coffee drinkers after overnight abstinence [10].

Caffeine ingestion heightens the stress response by stimulating the release of stress-associated hormones

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such as cortisol, epinephrine and norepinephrine [11]. Continued presence of these hormones in the body not only has a damaging impact on some physiological systems, but can also accelerate the aging process. Short-term stress can be motivating, but long-term stress has a lot of harmful outcomes. Stress hormones can be elevated by stressful lifestyles, or the intake of caffeinated beverages. This can increase mental alertness as anxiety and feelings of tension increase, while fine motor control is hampered [12]. The immune system is suppressed, digestion and elimination of waste are impaired and the body repair mechanisms are inhibited, thereby accelerating the aging process [13-15]. Studies have shown that intake of kola nut and caffeine-containing beverages stimulate inflammatory response [16, 17], while some other studies suggested anti-inflammatory response [18, 19]. Cortisol or hydrocortisone is a steroid hormone or glucocorticoid, produced by the adrenal cortex [20]. Its functions include stimulating gluconeogenesis, suppression of the immune system and influencing fat, carbohydrate and protein metabolism [21]. Studies have shown a proportionate relationship between caffeine ingestion and blood cortisol levels [18, 22].

In Nigeria, kola nut is heavily produced in the west, traditionally and religiously celebrated in the east and heavily consumed in the north, thus kola nut is central to various socio-cultural and economic aspects of Nigerians. In light of the above, the present study investigated the effect of methanolic extracts of *C. acuminata* on the haematology and serum cortisol concentration of adult male albino rat. The findings of this study will provide baseline data needful for the management of *C. acuminata* stimulated inflammatory responses often associated with heavy kola nut consumption.

MATERIALS AND METHODS

Procurement and Preparation of Cola Acuminata Methanolic Extract (CAME): The seeds of *C. acuminata* were purchased from Ogige Market in Nsukka, Enugu State, Nigeria. Identity was authenticated at the Taxonomy Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where voucher specimen was kept (C. *acuminata* 2012/191). The seeds were taken to the Department of Zoology and Environmental Biology Physiology Laboratory where they were washed to remove impurities and then cut into smaller sizes. The seeds were air-dried under room temperature over a period of three weeks. The dried seeds were pulverized to get a fine powder used for the extraction. Fifty gram of the powdered *C. acuminata* was extracted with 500 ml of absolute methanol for 2 h in a Soxhlet apparatus, after which it was filtered using Whatman filter paper (Grade 1, 11 μm) and the filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK) at a temperature of 40°C and packed in air tight bottles pending use [23].

Experimental Animals: Adult male albino rats (*Rattus norvegicus*) were used for the present experiment. The rats were purchased from Genetics and Animal Breeding Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were allowed free access to feed (Chick Growers Mesh, Guinea Feed, Nigeria) and water *al libitum*. The animals were housed in stainless metallic cages at the Genetics and Animal Breeding Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Cages were cleaned every morning to ensure a healthy environment.

Experimental Design: The study was conducted using a four by three Latin square experimental design consisting of four treatment groups replicated thrice. A total of 60 adult male albino rats were used for the study. The animals were divided into four groups (1 - 4) of 15 animals per group. Each of the groups had three replicates of five rats each. Rats in groups 2, 3 and 4 received doses of 100 mg/kg, 150 mg/kg and 200 mg/kg of CAME by oral administration. Group 1 served as the control and received distilled water. At the end of every week, animals from all replicates were sampled for body weight (g) and blood (2 ml).

Blood Sample Collection: Blood samples were collected weekly from the orbital sinus of the rat. Samples were divided into two tubes; one containing EDTA for haematological analysis, while the second tube was allowed to clot and later centrifuged for serum collection [24].

Haematological Analysis: Haematological data such as white blood cells (WBCs) count, red blood cells (RBCs) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width-standard deviation (RDW-SD) were collected using an Autoanalyzer (Mindray BC 2800, China).

Cortisol Assay: Sera collected were used for cortisol

assay. Total serum cortisol concentration was determined using commercially available ELISA kit (RPC Diagnostic Systems, Russia).

Statistical Analysis: Data obtained were analyzed for their central tendencies using descriptive statistics and expressed as mean ± SD. One way Analysis of Variance (ANOVA) was used to determine significant difference between treatment means. Significant means were separated using Duncan's New Multiple Range Test (DNMRT). For all the analyses, the level of significance was set at $p<0.05$.

RESULTS

The effect of CAME on WBCs revealed that there were significant ($p<0.05$) increases in WBCs corresponding with increased dosage of CAME except for group 4 rats at weeks 3 and 4 when compared with the control (Table 1). Variation occurred at other weeks, but they were not significant ($p>0.05$) when compared with the control. The effects of CAME on the haemoglobin (Hb) concentration revealed a dose and duration dependent significant ($p<0.05$) increase in Hb levels except for weeks 1 and 4 when compared with the control (Table 1). The effect of the CAME on RBCs count was dose and duration dependent with significant reduction in RBC levels in weeks 1, 2 and 4 when compared with the control ($p<0.05$) (Table 1). CAME had a dose – duration dependent interaction on the haematocrit values of exposed rats with significant increased of haematocrit values in rats in groups 2 and 3 and a significant reduction in the same value for rats in groups 1 and 4 when compared with the control (Table 1). There was a significant decrease in the mean corpuscular volume (MCV) of rats in groups 1 and 3 when compared with the control, whereas there were significant increases in the MCV value of groups 2 rats and no significant difference in MCV of group 4 rats when compared with the baseline values ($p<0.05$) (Table 2). Despite changes seen in weeks 1, 2, 3, significant changes were not recorded in the mean corpuscular haemoglobin (MCH) values of rats at week 4 when compared to the control ($p < 0.05$) (Table 2). Fairly constant values were recorded in the MCHC rats throughout the duration of the experiment. A significant change was only recorded at week 3 where the value was significantly lower than that of the control rats at the end of the experiment when compared to the baseline ($p<0.05$) (Table 2). No significant MCHC values were recorded in the rats throughout the duration of the experiment, with a significant increase only recorded in week 3 when compare with the control ($p<0.05$) (Table 2). No significant ($p<0.05$) change was noticed in the RDW-SD of rats at the end of the experiment when compared to the baseline (week 0). But, at week 3, the RDW-SD of rats in group 4 had a significantly ($p<0.05$) lower value when compared with the control. Only rats in group 2 had a significant

Table 1: White and red blood cells and their associated indexes of rats stimulated by intake of methanolic extract of *Cola acuminata*

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
		White blood cell count ($\times10^9$/L)			
Group1 (Control) (0mg/kg)	11.00 ± 1.61^{ab1}	11.10 ± 2.65^{ab12}	7.63 ± 1.08^{a1}	8.73 ± 3.18^{a1}	13.90 ± 0.22^{b2}
Group 2 (100mg/kg)	10.67 ± 1.40^{a1}	11.47 ± 2.94^{a2}	10.40 ± 4.16^{a1}	9.53 ± 3.12^{a1}	9.40 ± 1.73^{a1}
Group 3 (150mg/kg)	9.70 ± 0.35^{a1}	14.40 ± 2.81^{b2}	7.60 ± 3.39^{a1}	8.93 ± 1.05^{a1}	10.67 ± 0.55^{a1}
Group 4 (200mg/kg)	8.90 ± 1.20^{ab1}	6.70 ± 1.18^{a1}	10.37 ± 3.60^{b1}	8.57 ± 0.67^{b2}	15.83 ± 0.40^{c3}
		Haemoglobin concentration (g/dL)			
Group1 (Control) (0mg/kg)	11.50 ± 2.97^{a1}	11.43 ± 1.80^{a2}	11.20 ± 1.00^{a1}	10.97 ± 1.82^{a1}	11.33 ± 0.74^{a12}
Group 2 (100mg/kg)	10.87 ± 0.59^{ab1}	11.37 ± 0.40^{ab2}	9.93 ± 2.80^{a1}	12.63 ± 0.55^{b1}	12.47 ± 0.58^{ab12}
Group 3 (150mg/kg)	11.93 ± 0.81^{ab1}	10.60 ± 1.23^{a12}	10.67 ± 1.89^{a1}	12.30 ± 0.56^{ab1}	13.07 ± 0.42^{b2}
Group 4 (200mg/kg)	11.77 ± 1.54^{b1}	8.40 ± 1.37^{a1}	11.43 ± 1.60^{b1}	12.47 ± 1.05^{b1}	11.10 ± 1.55^{b1}
		Red blood cell count ($\times10^{12}$/L)			
Group1 (Control) (0mg/kg)	6.97 ± 1.74^{a1}	6.64 ± 1.01^{a2}	6.94 ± 0.057^{a1}	6.26 ± 1.23^{a1}	6.53 ± 0.50^{a1}
Group 2 (100mg/kg)	6.79 ± 0.29^{a1}	7.06 ± 0.12^{a2}	6.02 ± 1.77^{a1}	7.60 ± 0.09^{a2}	7.10 ± 0.37^{a1}
Group 3 (150mg/kg)	7.41 ± 0.53^{a1}	6.28 ± 0.70^{a12}	6.09 ± 1.55^{a1}	7.26 ± 0.41^{a12}	7.31 ± 0.25^{a1}
Group 4 (200mg/kg)	7.29 ± 0.96^{bc1}	5.22 ± 0.73^{a1}	6.30 ± 0.94^{ab1}	8.00 ± 0.35^{c2}	6.27 ± 0.93^{b1}
		Haematocrit values (%)			
Group1 (Control) (0mg/kg)	37.93 ± 9.14^{a1}	37.57 ± 5.33^{a2}	37.60 ± 2.55^{a1}	35.47 ± 6.21^{a1}	36.97 ± 1.98^{a1}
Group 2 (100mg/kg)	33.27 ± 2.41^{a1}	36.83 ± 0.50^{a2}	33.43 ± 9.54^{a1}	42.07 ± 1.42^{a1}	41.60 ± 2.34^{a1}
Group 3 (150mg/kg)	38.80 ± 2.07^{ab1}	35.50 ± 3.73^{a2}	36.07 ± 5.28^{a1}	42.03 ± 0.70^{b12}	42.67 ± 1.03^{b1}
Group 4 (200mg/kg)	38.07 ± 4.83^{b1}	28.27 ± 3.71^{a1}	37.20 ± 3.71^{b1}	43.57 ± 1.55^{b2}	36.07 ± 5.90^{b1}

Mean value with different numbers as superscript in a column are significantly different (P < 0.05).

Mean value with different alphabets as superscript in a row are significantly different (P < 0.05).

Table 2: Mean corpuscular volume of rats stimulated by intake of methanolic extract of *Cola acuminata*

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
			Mean corpuscular volume (fL)		
Group1 (Control) (0mg/kg)	54.60±1.06^{a1}	57.70±1.30^{a3}	53.40±2.43^{a1}	56.93±2.40^{a1}	56.60±1.25^{a1}
Group 2 (100mg/kg)	52.03±1.78^{a1}	52.23±0.25^{a1}	55.67±1.74^{b1}	55.43±1.99^{b1}	58.63±0.67^{c1}
Group 3 (150mg/kg)	52.50±1.01^{a1}	56.70±0.52^{ab3}	60.50±7.54^{b12}	58.20±4.20^{ab1}	58.23±1.01^{ab1}
Group 4 (200mg/kg)	52.33±2.86^{a1}	54.30±0.50^{ab2}	66.03±3.17^{c2}	53.70±1.42^{ab1}	56.57±1.50^{b1}
			Mean corpuscular haemoglobin (pg)		
Group1 (Control) (0mg/kg)	16.43±0.29^{ab1}	17.17±0.72^{ab2}	16.10±0.95^{a1}	17.57±0.66^{b1}	17.07±0.23^{ab1}
Group 2 (100mg/kg)	15.97±0.25^{a1}	16.07±0.32^{a1}	16.50±0.030^{a1}	16.57±0.64^{a1}	17.50±0.23^{ab1}
Group 3 (150mg/kg)	16.07±0.25^{a1}	16.07±0.25^{a12}	17.70±1.73^{a1}	17.57±1.35^{a1}	17.40±0.17^{a1}
Group 4 (200mg/kg)	16.10±0.40^{a1}	16.03±0.40^{a1}	17.87±1.36^{b1}	16.83±0.75^{ab1}	17.00±0.44^{ab1}
			Mean corpuscular haemoglobin concentration (g/dL)		
Group1 (Control) (0mg/kg)	30.17±0.47^{a1}	30.37±0.75^{a1}	30.23±0.81^{a1}	30.90±0.60^{a2}	29.70±0.75^{a1}
Group 2 (100mg/kg)	30.77±1.08^{a1}	30.80±0.72^{a1}	29.70±0.62^{a1}	29.97±0.68^{a12}	29.93±0.35^{a1}
Group 3 (150mg/kg)	30.70±0.66^{a1}	29.77±0.60^{a1}	29.43±1.03^{a1}	29.50±0.70^{a12}	29.80±0.26^{a1}
Group 4 (200mg/kg)	30.85±0.93^{b1}	29.60±0.92^{ab1}	29.87±1.06^{ab1}	28.87±0.95^{a1}	29.77±0.35^{ab1}

Mean value with different numbers as superscript in a column are significantly different (P < 0.05).
Mean value with different alphabets as superscript in a row are significantly different (P < 0.05).

Table 3: Red cell distribution width-standard deviation (%) of rats stimulated by intake of methanolic extract of *Cola acuminata*

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Group1 (Control) (0mg/kg)	13.83±1.47^{a1}	17.20±0.75^{ab1}	17.80±2.19^{ab1}	18.70±4.60^{b2}	16.07±1.03^{ab1}
Group 2 (100mg/kg)	14.73±0.57^{a1}	15.13±1.10^{ab1}	16.13±0.42^{a1}	16.40±1.39^{ab12}	17.77±2.57^{b1}
Group 3 (150mg/kg)	13.83±0.31^{a1}	16.23±2.32^{ab1}	19.90±3.50^{b1}	18.27±0.70^{b2}	16.30±0.10^{ab1}
Group 4 (200mg/kg)	13.70±0.95^{a1}	14.80±1.04^{a1}	20.47±1.17^{b1}	13.03±0.71^{a1}	15.43±1.78^{a1}

Mean value with different numbers as superscript in a column are significantly different (P < 0.05).
Mean value with different alphabets as superscript in a row are significantly different (P < 0.05).

Table 4: Mean weekly serum cortisol concentration (nmol/L) of rats stimulated by intake of methanolic extract of *Cola acuminata*

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Group1 (Control) (0mg/kg)	1609.00±29.46^{b1}	1383.67±69.06^{a1}	1678.67±31.90^{b1}	1522.67±216.80^{ab1}	1459.47±88.22^{ab1}
Group 2 (100mg/kg)	1470.33±45.71^{a1}	1595.00±113.33^{ab2}	1759.67±104.04^{b1}	1770.00±22.92^{b12}	1548.00±223.5^{ab1}
Group 3 (150mg/kg)	1602.00±73.01^{a1}	1715.33±96.77^{a23}	1808±149.49^{ab1}	2013.33±194.08^{b2}	2649.67±65.23^{c2}
Group 4 (200mg/kg)	1512.33±167.89^{a1}	1839.33±118.93^{b3}	2115.673±60.00^{c2}	2339.33±3.06^{d3}	2713.33±178.89^{e2}

Mean value with different numbers as superscript in a column are significantly different (P < 0.05).
Mean value with different alphabets as superscript in a row are significantly different (P < 0.05).

change ($p<0.05$) in the RDW-SD at the end of the experiment when compared with the baseline (week 0). At week 3, the RDW- SD of rats in group 4 (13.03±0.71) had a significantly lower value when compared with the control value (18.70±4.60) (Table 3). There was significant ($p<0.05$) dose and duration dependent increase in serum cortisol concentration of rats in the CAME treatment groups across all the weeks when compared with the control group. The highest increase occurred in rats administered 200 mg/kg of CAME, from 1512.33±167.89 nmol/l in week 0 to 2713.33±178.89 nmol/l in week 4 (Table 4).

DISCUSSION

The present study recorded a significant leucocytosis at the end of the experiment in group 4 rats. This indicated a gradual stimulation of inflammatory or stress responses in the animals. The WBCs are at the centre of defense of the body against stressors and invading foreign antigens to which they employ several means such as formation of pseudopodia in order to get rid of whatever is foreign to the body [20]. The result of this work is in line with the findings in other studies of increases in WBC corresponding with increased dosage of CAME [17, 25, 26] and contradicts the report of Obidike *et al*. [27] that the extract of kola nut did not have a significant effect on WBCs count of rats. The Hb concentration of the treatments did not differ appreciably from the control at the end of the experiment, suggested that haem synthesis was not affected in rats given various doses of *C. acuminata*. Haemoglobin is the oxygen carrying pigment of the RBCs. It reversibly binds oxygen at the respiratory surfaces (Lungs and skin) and then delivers it at the tissues when the oxygen partial pressure is low [21]. So, its synthesis increases in prolonged hypoxia.

Abnormally high levels of RBCs, Hb and HCT could be as a result of dehydration, a congenital heart disease, kidney tumor and pulmonary fibrosis among other causes [20]. Low RBCs count, Hb and HCT can consider as indicators of anaemia. Certain types of anaemia result from vitamin and mineral deficiencies including iron, B12, B6 or folate. Anaemia can also occur as a result of bleeding from menstrual flow, or bleeding ulcers [20]. The red blood cell counts recorded for the control rats used in the experiment were low compared to the reports of Boukerche *et al*. [28] value of 8.45 x 10^6/mm^3 but agreed with that of Dougnon *et al*. [25] who recorded a value slightly higher than 7.1×10^6/mm^3 proposed by Lahouel *et al*. [29]. The result of this study suggested that erythropoiesis stimulated by the enzyme erythropoietin from the kidney was maintained fairly constant [20]. This also did not suggest any possible response to hypoxia in the tissues. The haematocrit of rats in the control and the CAME treatment groups did not differ at the fourth week. The control values were however, lower than the CAME treatment HCT values. No significant effects of *Datura stramonium* extract on HCT of rats have been reported [30]. The alterations noticed in the HCT value may be due to dehydration in the rats [31].

This is not surprising as caffeine and kola nut have diuretic properties. Adam *et al*. [26] reported similar findings but with significant leukocytosis in rats administered 75 mg/kg of both the methanolic and aqueous extracts respectively. Our study recorded non significant leukocytosis at doses (100,150 and 200 mg/kg) at the end of the experiment. The mean corpuscular volumes of rats in this study were similar to those of Bouzidi *et al*. [30]. The increase in the MCV values of the CAME treatment groups at the end of the experiment is indicative of macrocytosis which is associated with regenerative processes that may occur during tissue damage or heightened cellular activity [20]. The mean corpuscular haemoglobin (MCH) values in the rats in this study were similar to those of Bouzidi *et al*. [30]. The mean corpuscular haemoglobin values did not increase significantly at the end of the experiment. This was in line with the work of Bouzidi and colleagues [30]. The mean corpuscular haemoglobin concentration (MCHC) values of rat in the control group were slightly lower than those of Bouzidi and colleagues; the values decreased but were not significant. These declines though not significant, are indicative of some regenerative process taking place in the animals especially the liver [31]. The declines in MCHC values indicated hypochromasia [20]. High MCV and MCH values are usually due to macrocytic anaemia, which most of the time result from vitamin deficiencies. Higher than normal levels of MCV and MCH may also be caused by liver disease, hypothyroidism, myelofibrosis, reticulocytosis [20]. Low levels of MCV or MCH could result from lead poisoning, microcytic anaemia or haemoglobinopathy and the major cause of microcytic anaemia is iron deficiency [20]. An increase in the width of the red blood cells was not significant and so the flow of blood in vessels especially the smaller arterioles, capillaries and venules, may not be adversely affected as they will still maintain a diameter suitable for easy passage through the vessels [20]. The red blood cells distribution width-standard deviation values also increased, but the increase was not significant. Red cell distribution width (RDW) according to Arhan *et al*. [32] measures anisocytosis, which is the variability in the size of circulating erythrocytes. Red cell distribution width is usually increased as a result *Plasmodium falciparum* infection of the red cell, production of iron, foliate deficiency, haemolysis and after blood transfusion. Red cell distribution width has been proposed to be associated with several pathologies such as occult colon cancer, neoplastic metastases [33-35]. Red cell distribution width has been associated with inflammatory bowel disease as an additional inflammatory marker [36]. Other studies have also shown that RDW can be a potential marker for differentiating Crohn's disease and ulcerative colitis [37, 38]. Apart from the disturbances of micronutrient metabolism, inflammation may also affect RDW. The proinflammatory cytokines inhibit erythropoietin-induced erythrocyte maturation, which is reflected in part by an increase in RDW. There is one proposal that enzymes produced by leukocytes may lead to changes in red blood cell membrane (e.g. decrease in sialic acid), which results in variability in cell shapes. This may have contributed to elevation in RDW [39].

The present study showed a significant rise in serum cortisol concentration in the CAME treatment groups across all the weeks when compared with the control group. Though, no study has evaluated the effects of kola nut on serum cortisol concentration, several studies both in humans and animals have shown that increasing stressful state and inflammatory reactions cause an elevation of the serum cortical concentration. This is because they stimulate the hypothalamo-hypophysial-adrenal (HPA) axis resulting in the secretion of cortical from the adrenal cortex [20]. A study by Dallman *et al*. [22] showed that chronic elevation of blood cortisol level

caused by frequent stress or caffeine or coffee intake lead to increased fat build up in the abdominal region which further stimulates the release of more hormones. Elevation of stress-associated hormones brings about frequent sleep disruption and insomnia [40, 41]. Excessive levels of cortisol can lead to adrenal exhaustion or adrenopause. This is associated with a number of clinical conditions such as osteopathic, cognitive or mood impairment, progression of coronary heart diseases and immune deficiencies [42]. Studies over past decades consistently associated lack of social support, low socio-economic status, unhappy marriages and work stress with rising cases of cardiovascular diseases [43]. An abnormality in the regulation of the HPA axis, which helps control cortisol production and secretion, is emerging as a potential reason why elevated emotional stress is linked to cardiovascular diseases [43]. Chronic excessive cortisol release through constant psychological or physical stress, coffee or caffeine intake can negatively appetite the usually, tightly regulated hormonal balance. Even high blood pressure is associated with increased stress, coffee and caffeine consumption. The result of this study is in agreement with the work of Farag *et al*. [44] on the haemodynamic responses to mental stress due to caffeine consumption. Their results showed that caffeine ingestion before stress caused both men and women to have enhanced haemodynamic responses to the stressor associated with an increase in cardiac index and a drop in peripheral resistance index. They also found that caffeine enhanced the fight-or- flight response pattern to stress in men and women. The fight-or-flight response is triggered off by adrenaline [21].

CONCLUSION

From the present study, the methanolic extract of *Cola acuminata* had significant effects on the white blood cell count, mean corpuscular volume and serum cortisol concentration. We therefore recommend that the use of *Cola acuminata* as a stimulant especially during stressful states should be discouraged as it has the ability to potentiate the stress response adversely.

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