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Effect of *Vernonia amygdalina* supplemented diet on selected tissues function in diet-induced obese rats

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The present study investigated the impact of *Vernonia amygdalina* (VA) supplemented diet on blood parameters, and liver and kidney functions in diet-induced obese rats. VA supplemented diet (5 and 15%) was fed for 4 weeks and compared to orlistat (5.14 mg/kg b.w., p.o.), an anti-obesity drug. Full blood counts and some biochemical indices were measured at the end of the study. Platelet count which decreased in obese rats (56.17%, *P* < 0.05), was increased by orlistat (40.70%), 5% (51.34%) and 15% (97.63%, *P* < 0.05) VA diets, respectively. Also, 15% VA diet only, lowered leukocyte counts by 31.75% (*P* < 0.05), indicative of anti-inflammation. Obesity-induced hepatotoxicity indicated by elevated serum alanine amino transferase (32.58%, *P* < 0.05), aspartate amino transferase (19.69%) activities and lowered AST:ALT ratio (14.62%) were respectively ameliorated. However, both treatments failed to up regulate the hitherto decreased total protein level (29.84%, *P* < 0.05), but modulated the altered albumin levels. Depressed sodium level in obese rats (58.24%, *P* < 0.05), was upregulated (*P* < 0.05). Orlistat and 15% VA diet only, respectively increased chloride (*P* < 0.05) and calcium (38.15%, *P* < 0.05 and 22.00%). The depressed urea level (58.48%, *P* < 0.05) was partially resolved, but null effect on increased creatinine (*P* < 0.05). VA diet may exert anti-inflammatory, hepatopretective and nephrotoprotective actions in obesity.

Key words: *Vernonia amygdalina* Del., diet-induced obesity, liver function, kidney function, haematological indices.

INTRODUCTION

The World Health Organization Global Health Observatory (2013), indicated that about 2.8 million people die worldwide each year as a result of being overweight or obese; and the worldwide prevalence of obesity has more than doubled between 1980 and 2008. According to the report, as at 2008, an estimated 205 million men (10%) and 297 million women (14%) in the world, over the age of 20 were obese (body mass index (BMI) ≥ 30 kg/m^2) - a total of more than half a billion adults worldwide. On the contrary, pharmacotherapeutic effort at mangement of obesity seem not to commensurate with the prevalence rate. For instance, only two drugs are currently approved and available for the long-term treatment of obesity: orlistat and sibutramine (Ioannides-Demos et al., 2011). The latter drug, sibutramine was incidentally withdrawn from distribution in the European Union countries in January, 2010, due to a reported 16% increase in risk of cardiovascular events in the sibutramine treated patients (Hsu et al., 2010), leaving just one approved anti-obesity drug,

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orlistat, on the market. Treatment with orlistat, a typical gastric and pancreatic lipase inhibitor, is known to be associated with troublesome gastro-intestinal tract side effects including diarrhoea, bloating, flatulence, abdominal cramps, and dyspepsia besides expressed concerns of some severe liver disease (Ioannides-Demos et al., 2011). Other side effects include fatty and oily stools, fecal urgency, and oily spotting, fecal incontinence, malabsorption of fat-soluble vitamins - vitamins A, D, E and K (Hsu et al., 2010). These may not be acceptable to some patients on long-term treatment. Therefore, there is a dire need for a broad spectrum approach to the search for obesity therapy given its prevailing incidences. Incidentally, empirical evidence and preliminary pharmacological studies with anti-obesity natural products have shown significant improvement in body weight control, without any noticable side effects (Kumar et al., 2011).

Obesity is associated with a number of metabolic syndrome abnormalities including type 2 diabetes mellitus, cardiovascular disease, increased incidence of certain cancers, musculoskeletal disorders and pulmonary diseases (Kruger et al., 2002; Wolf, 2003). The chronic inflammation of obesity is considered the likely culprit factor responsible for these macrovascular and neoplastic lesions which are mediated by accelerated atherothrombotic process (Yudkin et al., 2000; Samocha-Bonet et al., 2008). During obesity, the white adipose tissue is infiltrated and populated by macrophages which promote a low-grade chronic inflammation, by producing pro-inflammatory cytokines such as tissue necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby exerting some local effects on the adipose tissue physiology and also systemic effects on other organs (Bastard et al., 2006; Sun et al., 2012). The systemic effect may explain the indentified relationship between faulty blood cells count, particularly white blood cells (WBC) and platelets, and obesity reported earlier by Pratley et al. (1995) and more recently by Charles et al. (2007). Interestingly, it has been observed that weight loss can attenuate the macrophage population or infiltration of the adipose tissue and improve the inflammatory profile of the obese subject (Bastard et al., 2006).

Similarly, several reports have noted a strong positive correlation between obesity and liver dysfunction and failure (Wang et al., 2003). Infact, fat-induced liver disease is said to have overtaken alcohol and viral infection as the commonest cause of liver disease in Europe and North America (BBC Health News, 2008). In a study with 732 apparently healthy adults, Choi (2003) clearly established a strong association between elevated serum hepatic enzyme activity and total body fat, and that this elevation in hepatic enzyme activity was significantly higher in high total body weight fat subjects with associated fatty liver, compared to those without fatty liver. The kidneys are equally affected by the the chronic inflammation mediated pathology of obesity (Zoccali et al., 2003; Abrass, 2004). A systemic review and meta-analysis on the association between obesity and kidney disease indicated a positive association between body mass index (BMI) and risk for kidney disease, and according to the report, over weight individuals had elevated risk, while obese individuals were at much higher risk for kidney disease (Wang et al., 2008).

In line with this view, reducing body mass is thought to reverse associated clinical features of obesity including hypertension and proteinuria in patients with diabetes and nephropathy (Hollenberg, 2007). The anti-obesity action of diet supplemented with *Vernonia amygdalina* leaves was earlier demonstrated in diet induced obese rat models (Atangwho et al., 2012). The study noted successive reduction in weight gain, attenuation of serum and hepatic lipids, as well as white adipose tissue, suggesting a potential for use of VA leaves for obesity management. As a follow up, the present study was carried out to investigate the effect of this potential obesity therapy on blood parameters and liver and kidney functions in diet induced rat models.

MATERIALS AND METHODS

Experimental diet

Cafeteria diet (CD): The fattening diet, so-called cafeteria diet was formulated according to the method of Kumar et al. (2011) with some modifications. The CD is comprised of three sets of diets A, B, and C formulated as below. For every 100 g CD A, B and D were comprised of:

A = Condensed milk (24 g) + whole grain bread (24 g) + rat pellet (52 g) – 48% replaced.
B = Chocolate (9 g) + biscuits (8 g) + dried coconut (18 g) + rat pellet (55 g) – 45% replaced.
C = Cheese (24 g) + boiled potato (30 g) + rat pellet (46 g) – 54% replaced.

Each of diets A, B and C was then supplemented with VA at 5% or 15% by weight and fed in succession to the animals.

Plant material and standard drug

Mature leaves of *V. amygdalina* Del. obtained from a research farm, University of Calabar (UNICAL) were authenticated by Pastor Frank, a botanist in the Department of Botany, and a voucher specimen (ERU/2011/188) was deposited in the herbarium of the same department. The leaves were air-dried at room temperature 25 to 29°C for two weeks and thereafter milled into coarse powder, which was stored in air-tight plastic containers from where aliquots were weighed out for supplemented CD formulation. Fresh leaf samples were prepared every week until end of study. Orlistat, an anti-obesity drug (Xenical Pharmaceuticals, Japan) obtained from Karmel Pharmacy, 112 Goldie Street, Calabar, Nigeria, was used

Table 1. Animal grouping and treatment schedule showing the experimental design.

Group	Treatment (obesity induction, 6 weeks)		Treatment (4-week supplementation)	
1	NC	Normal rat pellets only	NC	Normal rat pellets only
2	CD1	Cafeteria diets A, B, C	CDC	Cafeteria diets A, B, C only
3	CD2	Cafeteria diets A, B, C	STDC	Cafeteria diets A, B, C + orlistat (5.14 mg/kg b.w., p.o.)
4	CD3	Cafeteria diets A, B, C	VAS 1	Cafeteria diets A, B, C + 5% VA
5	CD4	Cafeteria diets A, B, C	VAS 2	Cafeteria diets A, B, C + 15% VA

CD: Cafeteria diet, NC: Non obese control, CDC: Obese contro, STDC: Standard control orlistat, VAS: *Vernonia amygdalina* supplemented.

as standard or positive control in the study. It was administered via oral gavage at 5.14 mg/kg body weight, a dose simulated from human regimen.

Animals and housing conditions

Thirty healthy weaning Wistar rats (51 to 58 g) obtained from the Animal Resource of Department of Zoology and Environmental Biology, UNICAL, were used for this study. The rats were allowed to acclimatize in the animal house of Department of Biochemistry, College of Medical Sciences, UNICAL, where the experiment was conducted under controlled temperature (25 to 29°C). The animals were allowed free access to food and water, and to 12-h light/dark cycle. The protocol was in line with the guidelines of the National Institute of Health (NIH) publication (1985) for Laboratory Animals Care and Use, and was approved by the College of Medical Sciences' Animal Ethics Committee, UNICAL.

Experimental protocol

After acclimatizing the animals to the experimental conditions, the rats were divided into five groups of 6 rats each. The average body weights of rats in the six groups were similar at onset of the experiment (54.82 ± 3.25 g). Group 1, normal control (NC), was fed rat pellet obtained from Vital Feeds Ltd, Jos, Plateau State, Nigeria, throughout the 10 weeks of study. Group 2, cafeteria-diet-fed control (CDC), was also fed CD only for the 10 weeks of study, whereas groups 3 to 5, STDC, VAS 1 and VAS 2 were fed CD only for the first 6 weeks of the study and thereafter in the last 4 weeks, CD with oral administration of orlistat (5.14 mg/kg, b.w), 5% VA supplemented CD and 15% VA supplemented CD, respectively (Table 1). The three CDs A, B, C alone or supplemented were presented to the animals on days 1, 2 and 3 and then repeated in succession until the end of study (Kumar et al., 2011). Fresh diet for each group was compounded every day, to avoid spoilage, particularly the milk products. The food intake was recorded by measuring the difference between the pre-weighed diet presented to the rats and the weight of leftover 24 hourly. Food spillage was also recorded and adjusted for, in calculation of food intake. Body weight was measured two to three times per week, but consistently.

Sample collection and pretreatment

At the end of the experiment, final body weights were recorded and the rats killed by euthanesia. Whole blood was collected by cardiac puncture and immediately divided into two portions each: 1/5 into heparinized tubes for full blood count, and 4/5th into plain tubes from where serum was prepared and used for biochemical assays.

Full blood count

Full blood counts including packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet count, differential WBC (lymphocytes and mixed), and red cell indices [mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)], were estimated using the Sysmsex Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan. The procedure is as described in our earlier work (Eyong et al., 2011).

Biochemical analyses

Serum chemistry: amino transferase activity, alkaline phosphatase activity, total protein, albumin, electrolytes and urea and creatinine, were evaluated using commercial analytical kits obtained from Randox company, United Kingdom.

Statistical analysis

Data were expressed as the mean ± SD. Analysis of variance was used to test the data, followed by LSD post hoc test, using statistical package for social sciences (SPSS) software version 17. Differences were considered significant at $P < 0.05$.

RESULTS

Haematological indices

Table 2 and Figure 1 show measured blood indices of obese rats fed VA supplemented diet or orlistat p.o. for 4 weeks. It was observed that whereas cafeteria diet (CD) alone did not alter leucocyte counts, 4-week supplemented feeding with 15% VA lowered counts by 31.75% ($P < 0.05$), indicative of anti-inflammation. Compared to pellet fed rats, pletelet cell population decreased in CD fed group (56.17%, $P < 0.05$), and intervention with orlistat and 5 and 15% VA diets, respectively increased the counts by 40.70, 51.34 and 97.63% of CDC. However, only the increase caused by the 15% VA supplemented diet was statiscally significant. Haemoglobin concentration (HB), haematocrit (PCV) and erythrocyte counts (RBC) which increased only slightly by CD (7.29, 9.56 and 11.40%, respectively) were modulated by the 3

Table 2. Effect of 28-day treatment with extracts or orlistat on some blood indices of diet-induced obese rats.

Group	RBC (× 10^6/µl)	HB (g/L)	PCV (%)	MCV (f/l)	MCH (pg)	MCHC (g/L)	LYM (× 10/µl)
NC	6.37±0.38	12.45±0.85	41.9±3.37	65.53±1.85	19.50±0.20	29.83±0.58	74.38±4.19
CDC	7.19±0.45	13.43±0.78	46.33±3.72	64.30±2.51	18.73±0.50	29.20±0.71	78.63±1.69
STDC	6.72±0.51	12.63±0.98	41.88±3.89	62.10±1.08	18.78±0.08	30.28±0.49	77.40±3.41
VAS1	6.75±0.60	13.25±0.87	43.85±3.44	65.23±1.31	19.80±0.61	30.35±0.49	76.80±0.74
VAS2	6.14±0.25	12.38±0.37	42.08±1.53	68.55±2.50$^{\beta}$	20.20±0.54*$^{\beta}$	29.50±0.74	75.93±4.36

Values are the mean ± sem, n = 4 – 6, * = $P < 0.05$ vs. CDC, β = $P < 0.05$ vs. STDC.

Table 3. Effect of 4-week feeding with VA supplemented diet or orlistat on serum liver enzymes activities.

Group	Serum (U/L)			
	ALT	AST	AST/ALT	ALP
NC	27.88±1.72	58.93±5.56	2.12±0.19	343.98±46.60
CDC	41.35±1.76$^{\alpha}$	73.38±9.40	1.81±0.39	425.39±17.25
STDC	38.13±2.40	84.60±5.20	2.22±0.08	385.05±17.44
VAS 1	24.25±2.84*	60.33±7.55	2.88±0.12	447.76±29.83
VAS 2	30.13±1.60*	69.17±3.24	2.33±0.21	371.78±16.62

Values are the mean ± sem, n = 4-6, α = $P < 0.05$ vs. NC, * = $P < 0.05$ vs. CDC.

treatments to values comparable to the control group fed pellets only. The effect on HB was also mildly reflected in mean cell haemoglobin (MCH) as 5 and 7.28% reduction in 5 and 15% VA diet fed groups, respectively.

Liver function

Serum indices of liver function are shown on Table 3 and Figure 2. Measured alanine amino transferase activity (ALT) was higher in CD fed rats (32.58%) than the pellet fed rats ($P < 0.05$). However, 5 and 15% VA diet fed for 4 weeks lowered the activity by 41.35 and 27.13%, respectively ($P < 0.05$). Decrease caused by oral orlistat was not significant (7.79%). Similarly, aspartate amino transferase (AST) activity raised by CD feeding (19.69%), decreased by 17.78 and 5.74% with 5 and 15% VA supplemented feeding, respectively. Oral orlistat further increased the activity by 13.26% of CD control. The ratio of AST:ALT lowered by CD alone (14.62%), was restored by the vegetable supplementation and anti-obesity drug. Measured alkaline phosphatase (ALP) activity which increased by 19.14% in CD fed group, only appreciably decreased in 15% VA supplemented diet fed rats. The VA supplemented diets as well as orlistat failed to up regulate serum total protein concentration, which decreased upon obesity induction by 29.84% ($P < 0.05$). Similar to positive control, 15% VA diet exerted a 33.33% decrease in serum albumin level ($P < 0.05$) raised by CD diet alone (18.93%).

Kidney function

Changes in measured serum indices of kidney function are shown in Table 4 and Figure 3. Compared to NC, CD fed group suppressed serum sodium concentration by 58.24% ($P < 0.05$), suggesting hyponatremia. However, the anti-obesity drug, and 5 and 15% VA supplemented diets upregulated the sodium concentration by 29.63, 39.63 and 33.39%, respectively ($P < 0.05$). Intervention with the standard drug and VA supplemented diet at 15%, respectively increased serum chloride concentration (9.42 and 7.57%, $P < 0.05$) and calcium concentration (38.15%, $P < 0.05$ and 22.00%) with respect to CD control. Obesity induction also depressed serum urea levels by 58.48% relative to NC, but the 4-week intervention with orlistat and 5 and 15% VA supplemented diet, respectively increased the concentration by 26.69, 18.84 and 40.00%. Contrariwise wise, serum creatinine which increased with obesity induction, was not modulated by any of the 3 treatments. Also, there was no observed impact on measured serum potassium levels.

DISCUSSION

Several reports and evidence from basic and cross

Table 4. Effect of 4-week feeding with VA supplemented diet or orlistat on some selected serum electrolytes of diet-induced obese rats.

Group	Na (mEq/L)	Cl (mg/dl)	K (mEq/l)	Ca (mg/dl)
NC	106.54±4.15	66.19±1.79	6.38±0.21	3.58±0.97
CDC	44.49±3.13$^{\alpha}$	69.01±3.35	6.38±0.93	4.75±1.53
STDC	63.22±6.36*	76.19±1.97$^{\alpha}$	6.59±0.30	7.68±0.44$^{\alpha}$*
VAS 1	73.69±6.85*	73.64±3.19	6.96±0.15	4.27±0.50
VAS 2	66.79±2.31*	74.68±3.31$^{\alpha}$	6.24±0.08	6.09±0.57

Values are the mean ± sem, n = 4-6, α = $P < 0.05$ vs. NC, * = $P < 0.05$ vs. CDC.

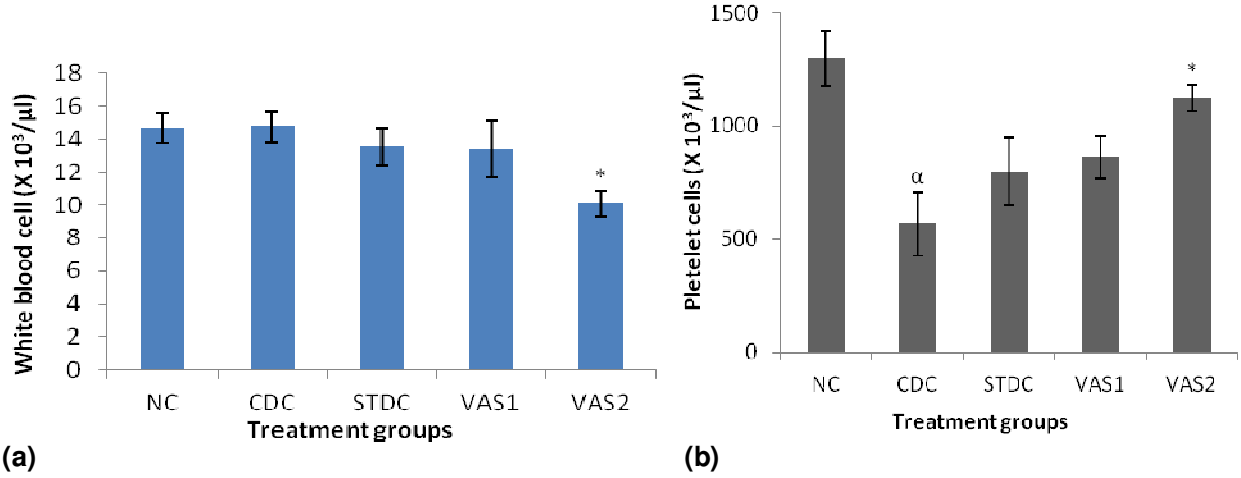

Figure 1. (a) Effect of 4-week feeding with VA supplemented diet or orlistat on white blood cells counts; n = 4-6, mean ± sem, * = $P < 0.05$ vs. CDC. (b) Effect of 4-week feeding with VA supplemented diet or orlistat on pletelets cell count; n = 4-6, mean ± sem, α = $P < 0.05$ vs. NC, * = $P < 0.05$ vs. CDC.

sectional studies have continuously strengthened the association between obesity and faulty blood parameters and liver and kidney functions. The present study therefore evaluated the possible impact of a 28-day VA supplemented diet, a potential anti-obesity diet, on the full blood counts and indices of liver and kidney functions in diet-induced obese rat models. To our knowledge, the result showed for the first time, a dose dependent decrease in WBC count, better than orlistat, a standard anti-obesity drug. The WBC cell population defines the effectiveness of the immune system of the body, and has also been used as markers of inflammation (Charles et al., 2007). In a particular study, it was reported that individuals with central fat had higher levels of several inflammatory markers, including 17% higher WBC counts, compared to those with normal body fat distribution (Panagiotakos et al., 2005). The suppressive action of dietary VA on WBC may imply an anti-inflammatory action in obesity, a mechanism employed by some anti-obesity agents. For instance, it has been reported of recent that curcumin, a dieferuloylmethane, isolated from *Curmuna longa* exert its anti-obesity action via suppressing inflammation, a so-called new mechanism (Shehzad et al., 2011). Infact, anti-inflammatory nutrition has been advocated as a pharmacological approach to treat obesity (Sears and Ricordi, 2010).

Additionally, platelet cell count seen to decrease with dietary obesity, were up regulated dose-dependently at the end of the study. Reports on the association of platelet cell counts with obesity in the literature have rather been contoversial. Two studies only, carried out in Israel and the United States to establish this relation in obese subjects, indicated an association with obesity in women, but not in obese men, as well as a no association of obesity with increased platelet activation (Charles et al., 2004; Samocha-Bonet et al., 2008,). This

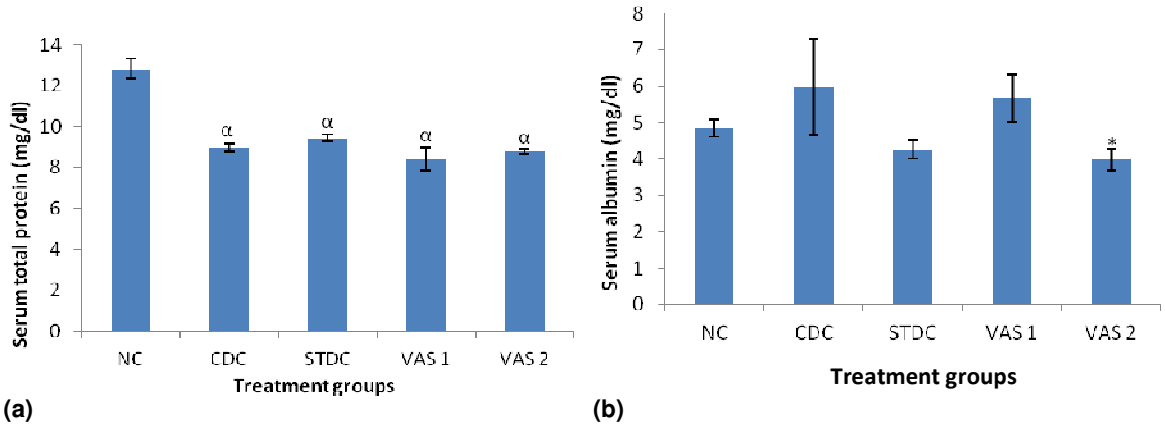

Figure 2. (a) Effect of 4-week feeding with VA supplemented diet or orlistat on serum total protein of obese rats; n = 4 to 6, mean ± sem, α = $P < 0.05$ vs. NC. (b) Effect of 4-week feeding with VA supplemented diet or orlistat on serum albumin of obese rats; n = 4 to 6, mean ± sem, * = $P < 0.05$ vs. NC.

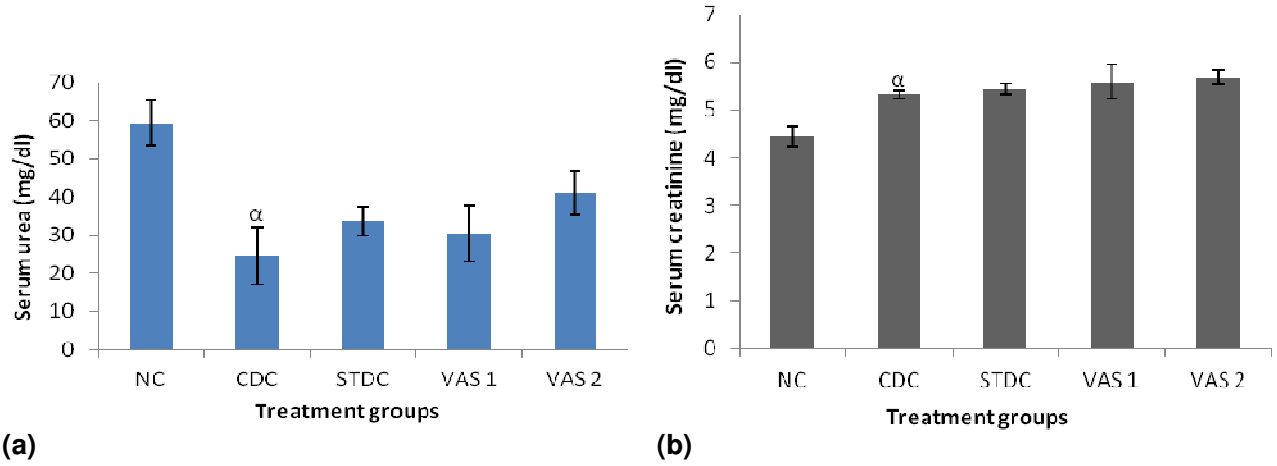

Figure 3. (a) Effect of 4-week feeding with VA supplemented diet or orlistat on serum urea of obese rats; n = 4-6, mean ± sem, α = $P < 0.05$ vs. NC. (b) Serum creatinine of obese rats fed VA supplemented diet or orlistat for 4 weeks; n = 4-6, mean ± sem, α = $P < 0.05$ vs. NC.

not withstanding, there are reports of decreased platelet cell counts in diabetic humans (Hekimsoy et al., 2004) and streptozotocin-induced diabetic rats models (Eyong et al., 2011), a condition akin to obesity in terms of metabolic pathologies, where in the latter study, the authors attributted the decrease to aggregation, typical of atherosclerotic disorder also associated with obesity.

Increased platelet aggregation in favour of thrombosis has extensively been discussed by Cowell and Nexto (2003). The up regulatory action of VA diet in the present study is in line with our recent report, where extracts from leaves of this vegetable up regulated hitherto decreased platelet cell counts in streptozotocin-induced diabetes (Eyong et al., 2011). The other measured haematological indices were non significantly impacted at the end of the study, implying a non haematotoxic effect of the intervention protocol. However, this attests to the fact that the observed changes in WBC and platelets could be more related to inflammation rather than bone marrow function.

Alteration in hepatic enzymes and hence liver dys-function are frequently found in obesity or hyperlipidemic

subjects (Luyckx et al., 1998; Bruckert et al., 2002). The present study therefore evaluated serum liver enzyme activities with the aim of ascertaining the impact of the potential obesity therapy on liver function. Results showed elevated levels of serum amino transaminases and alkaline phosphatase activities, but a decrease AST:ALT ratio in the obese subjects compared to the non obese, implying an associated liver dysfunction risk. This agrees with the reported association between elevated serum hepatic enzyme activity and total body fat in obese humans (Golik et al., 1991; Choi, 2003) and a positive association of liver enzyme activity with BMI (Doi et al., 2007). Twenty-eight-day VA supplemented diet exerted a reversive effect on the activities of these enzymes. The events in the hepatocytes were also in agreement with the serum changes, indicating an amelioration of potential hepatotoxicity. Although this ameliorative action of VA in diet-induced obesity, to best of our knowledge, is reported for the first time, the hepatoprotective effect of extracts from leaves of this plant had been demonstrated in diabetic rat models (Atangwho et al., 2007a). Also, terpenoid extract from the leaves of the vegetable, VA, have been shown to reverse carbon tetrachloride (CCL_4) induced damage in the liver (Babalola et al., 2001). Both in diabetes and CCL_4 injury, liver cell damage is mediated by imbalance in oxidant-antioxidant flux in favour of oxidative stress, a situation also reported in obesity (Vincent et al., 1999). VA in the supplemented diet may mediate the anti hepatotoxic action via its well known and reported antioxidant action (Ebong et al., 2011). The distorted and fatty hepactic cells were restored at the end of the study, similar to its reported action in diabetic liver risk (Atangwho et al., 2012). The weight reduction property of the VA supplemented diet also could have contributed to the hepatoprotection, since of all 15 cases reviewed by Wang et al. (2003), there was overall improvement in liver outcomes after weight reduction in patients with non alcoholic fatty liver.

Serum indices of kidney function – creatinine, urea and selected electrolytes were measured in this study. Creatinine levels, the most sensitive and realiable marker of kidney function, was found to increase abnormally in the obese rats compared to the non obese, suggesting some form of kidney risk. This agrees in part with the work of Pahl et al. (1988) who reported a transient rise in serum creatinine levels following a rapid weight loss with supplemented fasting. In that study, the rise in creatinine was not sustained to the end of the programme, unlike the present study. This difference can be attributed to the contrast in weight reduction protocol. Weight reduction upon feeding VA suplemented diet was rather gradual and successive. Moreover intermittent fasting was not employed, as the animals eat at will throughout the 28 days of the study. The supplemented diet as well as orlistat, a standard anti-obesity drug was however not able to attenuate the serum rise in creatinine. Duration of intervention could be responsible for this delayed effect, since the intervention lasted for 28 days only compared to the obese control which received the fattening diet for 10 weeks. The less sensitive indices of kidney function, serum urea and sodium were seen to decrease in obese rats compared to the non obese.

The interventions in this study partially up regulated the levels of these indices. Though similar or related reports have not been found in the literature, the action of VA in up regulating sodium and urea levels contrasts with our earlier observation in diabetic rats treated with extracts from this vegetable (Atangwho et al., 2007b). This appears difficult to explain, but it is probable that the leaves of this vegetable when fed dietarily allow for dynamic interaction among intact components of the plant compared to components in extracts whose chemistry must have been affected by the extraction environment and procedure. Moreover, bioavailability of the components in the animal system may influence results. Whereas, extracts are administered in pharmacological doses, in this experiment, the leaves were rather incorporated in the diet and the animals allowed to eat freely. The concomittant rise in serum chloride levels in these intervention groups similar to orlistat, justifies to a large extent the rise in sodium, since always sodium and chloride are similarly affected.

Conclusion

Besides its anti-obesity action which may be mediated by anti-inflammatory mechanism, this study also suggests that *V. amygdalina* leaves when supplemented in diet can ameliorate the hepatotoxic and nephrototoxic effects usually associated with obesity. Further research is however needed to establish these findings.

REFERENCES

Abrass CK (2004). Overview: Obesity: What does it have to do with kidney disease? Frontiers in Nephrology. J. Am. Soc. Nephrol. 15:2768-2772.

Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M (2012). Synergistic antidiabetic activity of *Vernonia amygdalina* and *Azadirachta indica*: Biochemical effects and possible mechanism. J. Ethnopharmacol. 141(3):878–887.

Atangwho IJ, Ebong PE, Eteng MU, Eyong EU, Obi AU (2007b). Effects of *Vernonia amygdalina* Del. leaf on kidney function of diabetic rats. Int. J. Pharmacol. 3:143-148.

Atangwho IJ, Ebong PE, Egbung GE, Eteng MU, Eyong EU (2007a). Effect of *Vernonia amygdalina* Del. on liver function in alloxan-induced hyper glycemic rats. J. Pharm. Bioresour. 4:25-31.

Atangwho IJ, Edet EE, Uti DE, Obi AU, Asmawi MZ, Ahmad M (2012). Biochemical and histological impact of *Vernonia amygdalina* supplemented diet in obese rats. Saudi Journal of Biol. Sci. 19:385–392.

Babalola OO, Anetor JI, Adeniyi FAA (2001). Amelioration of carbon tetrachloride induced hepatotoxicity by terpenoid extract from leaves

of *Vernonia amygdalina*. Afr. J. Med. Med. Sci. 30:91-93.
Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur. Cytokine Netw. 17(1):4-12.BBC Health News (2008). "Obesity fuelling liver disease". Retrieved: November 01, 2011. http://news.bbc.co.uk/2/health/7109596.stm
Bruckert E, Giral P, Ratziu V, Poynard T, Chapman MJ, Opolon P, Turpin G (2002). A constellation of cardiovascular risk factors is associated with hepatic enzyme elevation in hyperlipidemic patients. Metabol. 51:1071-1076.
Charles LE, Fekedulegn D, McCall T, Burchfiel CM, Andrew, ME, Violanti JM (2004). Obesity, white blood cell counts and platelets counts among police officers. Obesity 15:2846-2854.
Choi JW (2003). Association between elevated serum hepatic enzyme activity and total body fat in obese humans. Ann. Clin. Lab. Sci. 33:257-264.
Cowell JA, Nesto RW (2003). The platelets in diabetes: focus on prevention of ischemic events. Diabet. Care 26:2181– 2188.
Doi Y, Kubo M, Yonemoto K, Ninomiya T, Iwase M, Tanizaki Y, Shikata K, Lida M, Kiyohara Y (2007). Liver Enzymes as a predictor for incident diabetes in a Japanese population: The Hisayama Study. Obesity 15:1841–1850.
Ebong PE, Atangwho IJ, Eyong EU, Egbung GE, Ikpeme EV (2011). Effect of co-administration of extracts of *Vernonia amygdalina* and *Azadirachta indica* on lipid profile and oxidative stress in hepatocytes of normal and diabetic rats. Agric. Biol. J. North Am. 2:1087-1095.
Eyong EU, Atangwho IJ, David-Oku E, Agiang MA, Ebong PE (2011). Haematological and immunological effects of co-administration of extracts of *Vernonia amygdalina* and *Azadirachta indica* in normal and diabetic rats. Afr. J. Biotechnol. 10:10258–10262.
Golik A, Rubio A, Weintraub M, Byrne L (1991). Elevated serum liver enzymes in obesity: a dilemma during clinical trials. Int. J. Obes., 15: 797-801.
Hekimsoy Z, Payzin B, Ornek T, Kandogan G (2004). Mean platelet volume in type 2 diabetic patients. J. Diabet. Compl. 18:173–176.
Hollenberg NK (2007). Obesity and the kidney: Why is the kidney at risk? Kid. Int. 71:187-188.
Hsu YW, Chu DC, Ku PW, Liou TH, Chou P (2010). Pharmacotherapy for Obesity: Past, Present and Future. J. Exp. Clin. Med. 2(3):118–123.
Ioannides-Demos LL, Piccenna L, McNeil JJ (2011). Pharmacotherapies for Obesity: Past, Current, and Future Therapies. J. Obesity Volume 2011, Article ID 179674.
Kruger J, Bowles HR, Jones DA, Ainsworth BE, Kohl HW (2002). Health-related quality of life, BMI and physical activity among US adults (18 years): national physical survey and weight loss survey. Int. J. Obes. (Lond) 31:321-327.
Kumar S, Alagawadi KR, Rao MR (2011). Effect of *Argyreia speciosa* root extract on cafeteria diet-induced obesity in rats. Ind. J. Pharmacol. 43:163-167.
Luyckx FH, Desaive C, Thiry A, Dewe' W, Scheen AJ, Gielen JE, Lefebvre PJ (1998). Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int. J. Obes. Relat. Metab. Disord. 22:222–226.
Pahl MV, Vaziri ND, Akbarpour F, Afrasiabi A, Friis R (1988). Effect of rapid weight loss with supplemented fasting on serum electrolytes, lipis, and blood pressure. J. Nat. Med. Assoc. 80:803-809.
Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysohoou C, Stefanadis C (2005). The implication of obesity and central fat on markers of chronic inflammation: the ATTICA study. Atheroscl. 183:308–315.
Pratley RE, Wilson C Bogardus C (1995). Relation of the white blood cell count to obesity and insulin resistance: effect of race and gender. Obes. Res. 3:563–571.
Samocha-bonet D, Justo D, Rogowski O, Saar N, Abu-Abeid S, Shenkerman G, Shapia I, Berliner S, Tomer A (2008). Platelet counts and platelet activation markers in obese subjects. Mediat. Inflammat. Article ID 834153.
Sears B, Ricordi C (2011). Anti-Inflammatory nutrition as a pharmacological approach to treat obesity. J. Obes. Article ID 431985.
Shehzad A, Ha T, Subham F, Lee YS (2011). New mechanism and the ant-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. Eur. J. Nutr. 50:151-161.
Sun S, Ji Y, Kersten S, Qi L (2012). Mechanisms of Inflammatory Responses in Obese Adipose Tissue. Annu. Rev. Nutr. 32:261–86.
Vincent HK, Powers SK, Stewart DJ, Shanely RA, Demirel H, Naito H (1999). Obesity is associated with increased myocardial oxidative stress. Int. J. Obes. Relat. Metab. Disord. 23:67-74.
Wang RT, Koretz RL, Yee HF (2003). Is weight reduction an effective therapy for non alcoholic fatty Liver? A systematic review. Am. J. Med. 115:554–559.
Wang Y, Chen X, Song Y, Caballero B, Cheskin LJ (2008). Association between obesity and kidney disease: a systemic review and meta-analysis. Kid. Int. 73:19-33.
Wolf G (2003). After all those years: renal consequences of obesity. Nephrol. Dial. Transpl., 18: 2471-2474.
Yudkin JS, Kumar IM, Humphries SE, Mahammed-Ali V (2000). Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? Atheroscl. 148:209-214.
Zoccali C, Mallamaci F, Tripepi G (2003). Adipose tissue as a source of inflammatory cytokines in health and disease: focus on end stage renal disease. Kid. Int. 63(84):S65-S68.