Impact of *Colocasia esculenta* extract and fractions on high-fat diet-induced changes in body weight, adipose tissue and liver of rats

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Abstract: The objective of the present study was to explore on the possible protective effects of the saponins and alkaloid-rich fractions of *Colocasia esculenta* L. Schott leaves on high-fat diet-induced changes in rats. High-fat diet (HFD)-fed rats were treated orally with 10mg/kg b.wt of saponin-rich fraction [SPF], 10mg/kg bw of alkaloid-rich fraction [ALF] and 400mg/kg bw of Crude aqueous extract of *C. esculenta* [*CEAE*] for 28 days. The effects of the treatments on body weight, wet white adipose (WAT) tissue, liver marker enzymes and liver histomorphology were studied. High-fat diet induced body weight gain and increased the serum levels of liver enzymes, cholesterol and triglycerides in rats. Increased body weight changes were observed in HFD-control while the alkaloid and *CEAE* treatments significantly decreased the weight gain of treated rats. Oral treatment with *CEAE* and fractions significantly decreased all the biochemical parameters ($p<0.05$) except cholesterol levels. Microscopical examination of the WAT showed decreased adipocyte sizes upon treatments with *CEAE* and fractions while the liver histoarchitecture showed a better preservation upon treatments with SPF and *CEAE*. The outcomes from the present study suggest that *C. esculenta* leaves significantly reduced fat accumulation in adipose tissues and ameliorated HFD-induced liver damage. The alkaloids and saponins present in the plant product may be the phytoconstituents responsible for the weight attenuating and hepatoprotection activities respectively.

Keywords: *Colocasia esculenta,* Saponin and Alkaloid fractions, high-fat diet, liver, adipose tissue.

INTRODUCTION

The excessive accumulation of fat in body tissues, termed obesity, has become a global health problem (WHO, 2000). It results from an imbalance between energy intake and expenditure (Hartz *et al*, 1983). Obesity is associated with metabolic syndrome and thus is related with the development of chronic diseases such as hyperlipiemia, atherosclerosis, hypertension, type II diabetes, fatty liver and cancer (Wellen and Hotamisligil, 2005). The main factors underlying the emergence of these disease conditions are high-fat diets, sedentary lifestyles and genetic factors (Massi-Benedetti and Orsini-Federici, 2008). Weight-loss medications used in current interventions may act as lipase inhibitors or appetite suppressants but they are expensive and have relatively high side effects. However, medicinal plants used traditionally are readily available and are often assumed to have less adverse effect (Wambede, 1998).

Natural remedies have been shown to reduce body weight and also prevent diet-induced obesity *in vivo* using animal models (Shin *et al.,* 2013), but yet, many other natural products which may serve as potential weight-loss agents are still unexplored. A recent report has shown that the crude aqueous leaves extract of *Colocasia esculenta* L. Schott [family: Araceae] markedly decreased body weight gain of normal rats fed a standard diet (Azubike *et al*., 2016). *C. esculenta* is a commonly known as cocoyam and other common names are Taro, Elephant ear, and Dasheen. Traditionally, *C. esculenta* leaves have been used for the treatment of liver ailments, haemorrhoids, constipation and stomatitis (Devarkar *et al.,* 2011). The most abundant bioactive principles in Cocoyam leaves are saponins and alkaloids (Azubike *et al.*, 2012). Scientific data on the biological activities of *C. esculenta* leaves are anti-hypertensive, hypoglycemic, hypolipidemic anti-inflammatory, hepatoprotective, antifungal, antibacterial, antidiabetic, anti-cancer and antioxidant effects (Patil and Ageely, 2011; Ravikumar *et al.,* 2011; Eleazu *et al*., 2013; Azubike *et al.,* 2015). However, there is paucity of scientific literature on the effect of *C. esculenta* leaves crude extract, saponins and alkaloid-rich fractions on rats fed a high-fat diet. The present study, therefore, seeks to explore on the impact of *C. esculenta* leaves in HFD-induced changes in bodyweight, adipose tissue and liver of albino rats.

MATERIALS AND METHODS

Plant collection

Fresh *Colocasia esculenta* leaves were plucked from farms within Enugu metropolis. Plant material was identified and authenticated by comparison with the voucher specimen [UNH No.379[a]] deposited at the

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hebarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Extraction and isolation methods

Colocasia esculenta leaves were shade-dried and milled into powder using a gasoline grinding machine. Crude aqueous extract was prepared by maceration for five hours in distilled water. The homogenized mixture was filtered through clean muslin cloth and the resultant filtrate was concentrated in an evaporator so as to achieve a desired concentration. The crude aqueous extract was stored at 4±2^{o}C in a refrigerator until needed.

Milled dried leaves of *C. esculenta* (2kg) was defatted using 4 litres of Petroleum ether for 72 hours and the marc was dried and macerated with 10 litres of 95% methanol. The mixture was shaken intermittently for 48 hours and filtered. The filtrate obtained was evaporated to obtain a dark green semisolid mass which was preserved under refrigerated conditions. Conventional methods of isolating bioactive principles were employed (Cordell, 1981; Hostettmann *et al*, 1991; Sarker *et al.*, 2005) to obtain the crude saponins and alkaloid fractions of *C. esculenta* leaves.

The methanol extract was divided into two parts and one part was partitioned with n-butanol and water (1:1, v/v) and was shaken thoroughly. The n-butanol layer was separated after the mixture was allowed to stay overnight. Using aliquots of n-butanol, the aqueous partition was washed five times until it became colourless. Under reduced pressure, pooled butanol partition was evaporated to yield a residue. The n-butanolic residue was dissolved in methanol and precipitated by addition of diethyl ether in excess to yield the crude saponin fraction (Hostettmann *et al.*, 1991).

The second part of the methanol extract was used for the extraction of alkaloids using a modified version of the classic 'acid-base shakeout' method (Cordell, 1981; Sarker *et al.*, 2005). The extract was acidified with tartaric acid titrated to pH5. The mixture was partitioned with ethyl acetate pre-saturated with water. The aqueous acidic phase obtained was made alkaline with sodium bicarbonate and partitioned using ethylacetate. At the temperature of 45-50^{o}C, the ethyl acetate partition was evaporated yield the alkaloid fraction.

High Fat Diet [HFD] preparation

The High-Fat Diet (HFD) was prepared by mixing 20g lard oil with 80g commercial standard chow diet (Top Feeds® limited, Ibadan, Nigeria).

Test animals

Thirty male albino Wistar rats weighing between 90-110g were used for the study. They were divided into five groups (A - E) (n=6) according to their body weights. They were kept in clean cages in the College of Medicine Animal House, University of Nigeria, Enugu Campus. The animals were kept under standard environmental conditions and a 12:12 hr light/dark cycle. Water and commercially available rat pellets (Top Feed®) were provided for the animals *ad libitum*. The animals were allowed to acclimatize for one week at the laboratory condition prior to the experimentation. Due care and diligence were observed in handling of the animals in accordance with Institutional and International guidelines for care and use of Animals in Scientific Research.

Experimental design

After acclimatization, the animals in groups B-E were placed on high-fat diet [HFD] for 70 days (10 weeks). Groups A (Normal control) and B [HFD control respectively] were fed only normal diet and HFD respectively. On Day 42 (6th week), drug treatments commenced in test groups C, D and E [10mg/kg bw of SPF, 10mg/kg bw of ALF and 400mg/kg bw *CEAE* respectively] orally, once daily for 28 days [four weeks]

Measurement of body weight

The body weights (grams) of each rat was recorded on Day 0 and at weekly intervals (Days 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70) throughout the course of the study and the average body weights for the groups calculated.

Blood collection and biochemical analysis

The animals received the last treatment dose on Day 69. On Day 70, after an 8 hour fast, each animal was anaesthesized and blood sample (4 ml) was collected via retro-orbital sinus and placed in plain bottle for biochemical analyses. Blood samples were centrifuged at 3000rpm for 15 minutes and sera obtained were stored frozen as preparatory to biochemical assays. Serum levels of the liver marker enzymes [alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST)] were determined using commercial reagent kits. ALT and AST were analysed using the endpoint techniques of Reitman and Frankel (1957) provided by Randox Laboratories Ltd, United Kingdom. Alkaline phosphatase was estimated using the method of Roy (1970) provided by Teco diagnostics, USA. Serum levels of total cholesterol and triglycerides were also determined using enzymatic colorimetric method as described previously (Carr *et al.*, 1993)

Body fat and Organ weight measurements

The rats were sacrificed under diethyl ether anaesthesia. The wet white adipose tissues [perirenal (retroperitoneal) and epididymal], liver and kidneys of each rat were isolated and weighed immediately. The relative organ weight (ROW) for each of the samples was calculated as the ratio of organ weight and the animal's body weight (at the end of experiment) x 100] of each rat. The adiposity index and fat pad analysis was measured using the formula: sum of the total body fat pad weights/body weights x 100.

Table 1: Effect of *CE* extract and fractions treatment on experimental rats' body weight difference per week from initial body weight

Parameter	Week	GROUPS				
		A	B	C	D	E
		[Control]	HFD only	HFD + 10mg/kg ALF	HFD + 10mg/kg SPF	HFD + 400mg/kg *CEAE*
Initial body weight	0	106.00±2.45	106.00±2.45	106.00±4.00	112.00±2.00	108.00±2.00
Body weight difference (g)	1	28.00±4.90	44.00±2.45	40.00±3.16	26.00±2.45	44.00±6.78
	2	26.00±4.00	2.00±3.74*	12.00±2.00	12.00±2.00	6.00±8.72
	3	-10.00±6.32	4.00±4.00	-4.00±2.45	4.00±5.10	-10.00±7.07
	4	28.80±3.53	16.00±2.45	12.00±4.90	22.00±4.90	8.00±7.35
	5	-16.00±4.00	0.00±4.47*	8.00±2.00*	6.00±4.00*	26.00±6.00*#
	6	22.00±5.83	50.00±5.48*	24.00±2.45	30.00±8.94	8.00±2.00
	7	4.00±7.48	-26.00±2.45*	16.00±7.48	-14.00±10.77*	24.00±2.45*
	8	14.00±5.10#	32.00±4.89	-12.00±6.63*#	26.00±5.10	-26.00±4.00*#
	9	16.00±4.53	16.00±2.45	24.00±2.45	8.00±3.74	10.00±4.47
	10	28.00±3.74	10.00±3.16	10.00±4.47	16.00±5.10	0.00±5.48*

Values are expressed as Mean ± S.E; Significance when compared with normal control*= ($p<0.05$); and HFD-control#= ($p<0.05$). *ALF* – Alkaloid fraction; *SPF* – Saponin fraction; *CEAE* – *Colocasia esculenta* aqueous extract

Table 2: Effect of pre-treatment with crude aqueous extract, Saponins and Alkaloids-rich fractions of *C. esculenta* leaves on some biochemical parameters [AST, ALT, ALP, TC and TG] of rats upon feeding with High fat diet for 70 days.

Groups	Treatment	Biochemical Parameters				
		ALT(iu/l)	AST(iu/l)	ALP(iu/l)	TC (mmol/l)	TG (mmol/l)
A	Control	36.15±2.88	65.33±7.30	103.33±4.03#	2.47±0.26	0.92±0.03#
B	HFD only	43.07±1.88	76.67±1.05	139.56±4.80*	3.25±0.33	1.51±0.16*
C	HFD + 10mg/kg ALF	33.07±2.31#	51.34±2.91#*	106.67±6.71#	2.50±0.39	0.87±0.05#
D	HFD + 10mg/kg SPF	28.29±0.85#	57.33±2.67#	100.95±3.96#	3.57±0.54	1.18±0.14*
E	HFD + 400mg/kg *CEAE*	30.00±1.44#	64.00±3.23#	108.57±6.97#	1.39±0.78	0.72±0.05#
	F-ratio	8.545	11.959	8.394	2.915	9.507
	Sig.	0.000	0.000	0.000	0.047	0.000
Data						

Expressed in mean ± SEM; *$p<0.05$ when compared to normal control (Group A) and #$p<0.05$ in comparison to the HFD only-treated rats (negative control-Group B). ALT – Alanine transaminase; AST – Aspartate transaminase; ALP – Alkaline phosphatase; TC - Total cholesterol; TG – Triglyceride; ALF – Alkaloid fraction; SPF – Saponin fraction; *CEAE* – *Colocasia esculenta* aqueous extract

Table 3: Effect of treatment with crude Aqueous extract, Alkaloid fraction and Saponins fraction of *Colocasia esculenta* on organ weights of rats upon feeding with High fat diet for 70 days.

Groups	Treatment	Parameters				
		Final body weight (g)	Liver weight (g)	Liver index	Kidney weight (g)	Kidney index
A	Control	246.00±5.10	8.59±0.59	3.49±0.20	1.80±0.01	0.71±0.02
B	HFD only	254.00±17.49	9.48±0.43	3.81±0.22	1.61±0.10	0.66±0.04
C	HFD + 10mg/kg ALF	236.00±9.27	9.40±0.47	3.99±0.14	1.54±0.10	0.65±0.02
D	HFD + 10mg/kg SPF	248.00±13.56	10.25±0.40	4.16±0.20	1.61±0.17	0.64±0.04
E	HFD + 400mg/kg *CEAE*	198.00±19.85	8.38±0.80	4.40±0.45	1.49±0.12	0.78±0.04
	F – Ratio		1.917	1.985	0.513	2.433
	P- value		0.151	0.140	0.727	0.093

* = $p<0.05$ when compared with the normal control (Group A) and # = $p<0.05$ when compared with the HFD only–treated rats (Group B). *ALF* – Alkaloid fraction; *SPF* – Saponin fraction; *CEAE* – *Colocasia esculenta* aqueous extract

Table 4: Effect of pre-treatment with crude aqueous, alkaloids and saponins-rich fractions of *C. esculenta* leaves on adiposity index and adiposity diameter of treated HFD-fed rats.

Groups	Treatment	Parameters						
		Epididymal WAT weight (g)	Epididymal WAT index	Perirenal WAT weight (g)	Perirenal WAT index	Total body fat pad weights (g)	Adiposity index	Adipocytes diameter (µm)
A	Control	2.08±0.17	0.84±0.06	2.54±0.25$^{\#}$	1.04±0.10$^{\#}$	4.62±0.32$^{\#}$	1.88±0.12$^{\#}$	53.20±2.59$^{\#}$
B	HFD only	2.67±0.22	1.08±0.12	4.88±0.81*	1.95±0.32*	7.54±0.97*	3.03±0.41*	108.70±6.36*
C	HFD + 10mg/kg ALF	2.32±0.18	0.98±0.05	3.27±0.38	1.36±0.12	5.58±0.54	2.33±0.15	66.30±2.02$^{\#}$
D	HFD + 10mg/kg SPF	2.69±0.22	1.09±0.09	3.34±0.55	1.32±0.16	6.04±0.67	2.42±0.15	76.80±4.72*$^{\#}$
E	HFD + 400mg/kg *CEAE*	1.71±0.14$^{\#}$	0.89±0.04	1.67±0.25$^{\#}$	0.85±0.07$^{\#}$	3.37±0.38$^{\#}$	1.74±0.08$^{\#}$	52.40±1.93$^{\#}$

Data expressed in mean ± SEM; *p<0.05 when compared to the control (Group A) and $^{\#}$p<0.05 in comparison to the negative control (Group B). WAT – wet adipose tissue; *ALF* – Alkaloid fraction; *SPF* – Saponin fraction; *CEAE – Colocasia esculenta* aqueous extract

Histopathological study

The excised liver and adipose tissues were immediately fixed in 10% formal saline and embedded in paraffin wax prior to histological processing for light microscopical examination. Sections of 5-µm obtained from microtomy were stained with haematoxylin and eosin stain.

STATISTICAL ANALYSIS

All data were expressed as mean ± S.E.M. and the statistical evaluation was performed using SPSS software package program (SPSS, Chicago, IL; version 20.0). Hypothesis testing was conducted using one-way analysis of variance (ANOVA) followed by Tukey-highest significant difference (HSD) post-hoc test to determine the statistical significance of the differences in the parameters among the groups. Significance levels at p< 0.05 were considered to indicate statistical significance.

RESULTS

Effect on body weight

Fig. 1 and table 1 represent the effects of treatment with the crude aqueous extract [*CEAE*], alkaloid fraction [ALF] and saponins fraction [SPF] of *C. esculenta* leaves on weekly body weights and body weight differences, respectively, of rats fed with high fat diet [HFD]. Feeding the rats with a HFD containing Lard (Group B) caused a marked increase in body weight compared to a normal diet (Group A control). However, feeding a HFD plus *CEAE* (Group E) significantly reduced (p<0.05) the body weight gain induced by the HFD fig. 1. Treatment with the ALF (Group C) followed a similar pattern of body weight change as with the *CEAE* while no obvious body weight change was observed with SPF treatment when compared with the HFD-only treated group.

Effect on biochemical parameters

As shown in table 2, feeding the rats with HFD only significantly increased the serum levels of ALT, AST, ALP (p<0.05). However, treatment of HFD-fed rats with the *CEAE* and fractions significantly decreased the serum levels of the parameters (p<0.05). Furthermore, significantly raised levels of TG was observed in HFD-only and SPF-treated groups (p<0.05) when compared with the normal control. Decreased levels of TG were observed in ALF and *CEAE* treated groups (p<0.05) when compared with HFD-only fed group. TC levels were slightly raised in HFD only and SPF treated groups when compared with the normal control. TC levels of ALF-treated rats were similar to that of the normal control whereas decreased levels were obtained after treatment with the *CEAE*.

Effect on organ weights and percentage body fat

Tables 3 and 4 represent the effects of treatments with *CE* extract and fractions on organ and body fat weights respectively. All treatments for the respective rat groups had no significant effect on the liver and kidney weights (table 3). Conversely, markedly increased and decreased perirenal white adipose tissue (WAT) weight were observed for rats in groups B (HFD-only) and E (*CEAE*-treated) respectively (table 4). No significant change was observed in epididymal WAT weights in all treatment groups except for rats treated with *CEAE* which showed a statistically significant decrease (p<0.05). The mean total WAT of HFD – only fed rats was approximately 63.2% higher than in the normal control group, whereas a 27.1% decrease was observed after *CEAE* treatment (table 4). A marked increase (p<0.05) in adipocytes diameter was observed in HFD-only group when compared with the control (Group A). However, all treatment groups showed a statistically significant decrease in adipocytes diameter when compared with negative control (Group B).

Histopathological findings

Effect of C. esculenta on liver histology: (fig. 2) Histopathological examination of the liver of the control rats fed on normal diet revealed normal histological picture of the hepatic lobule which consists of the central

veins and portal tracts surrounded with normal hepatocytes (Plate 1A). Examination of the liver of rats fed HFD only showed hepatic changes consistent with moderate to severe fatty degeneration of hepatocytes (Plate 1B). Liver of rats treated with ALF of *CE* showed similar features (Plate 1C) observed in HFD-only fed group. However, marked improvement of the liver tissues was observed in the groups treated with the SPF (Plate 1D) and crude AE of *CE* (Plate 1E) with no obvious pathological lesions.

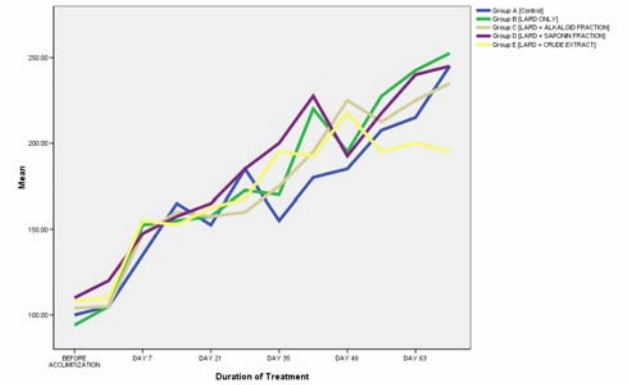

Fig. 1: Graph showing the effects of treatment with crude Aqueous extract, Alkaloid fraction and Saponin fraction of *Colocasia esculenta* leaves on weekly body weights of rats upon feeding with High fat diet for 70 days.

Effect of C. esculenta on adipose tissue histology: Photomicrographs showing adipocyte sizes of all the treatment and control groups are shown in fig. 3. Normal adipocytes of Group A-control rats showed normal histoarchitecture (Plate 1A). However, adipocytes of HFD-only treated rats were hypertrophied (Plate 1B). On the other hand, the adipose tissue histology of rats treated with ALF, SPF and *CEAE* [Plates 2C, 2D and 2E respectively] showed smaller adipocyte sizes similar with the control adipose tissues [Plate B6].

DISCUSSION

Effects of *Colocasia escukenta (CE)* leaves on rats fed a high fat diet (HFD) was studied. A HFD containing 25% fat content has been reported to show a slight increase in body weight of rats (Han *et al.*, 2005) while a 40% fat content has been shown to cause obesity (Han *et al.*, 1999). In the present study, we found that HFD of 20% slightly increased the body weight of rats. Diets containing fat are more effective for weight gain in animals than dietary carbohydrates (Portillo *et al.*, 1999). The isolation and employment of the fractions of the two most abundant phytoconstituents in *CE* leaves (saponins and alkaloids) in this study did not show similar effects on the body weight of the rats treated. The rats treated with the saponins fraction (SPF) revealed pattern of weight changes similar to the HFD-only fed rats. After one week of treatment, SPF showed a drastic reduction in body weight of rats. However, continuous treatment caused an increase in body weight of the rats as observed with HFD-only treated rats. This initial weight loss could have occurred by a mechanism that inhibits the absorption of fat, or by enhancement of excretion of fat in faeces (Portillo *et al.*, 1999). Moreover, the subsequent increase in weight contradicts previous works which have shown saponins as potent phytochemicals with weight reducing properties (Yun, 2010; Chidrawar *et al.*, 2011). Perhaps the saponins may have been overwhelmed by the high-fat diet which may have rendered the phytochemical ineffective for action. It may also be that the prolonged administration of SPF reversed the effect hence improving the weight gain of the rats. Some authors have documented that plant-derived saponins stimulate growth and increase daily live weight gain of animals (Bosler *et al.*, 1997). This infers that the effect of saponins on body weight may seem controversial.

Adipose tissue weight measurement has been used as a valid index in studies related to obesity (Atangwho *et al.*, 2012). The dynamism of adipose tissue, as an organ, is its ability to play a role in energy balance and based on the metabolic requirements of an organism, it changes in mass (Lafontan and Langin, 2009). The reduction in adipose tissue mass appears as a valid mechanism by which many anti-obesity natural products exert their effects (Roh *et al.*, 2012). In the present study, we observed that both fractions (ALF and SPF) caused a decrease in white adipose tissue (WAT) weights although this effect was not significant when compared with rats fed HFD only. However, treatment with the crude extract showed a significant decrease in WAT of the treated rats. More so, in the histological assessment of the fat pads obtained from HFD-only group, the larger sizes of adipocytes observed is similar to the works of previous researchers (Sung *et al.*, 2011 and Roh *et al.*, 2012). This increased adipocyte sizes resulted mainly from accumulation of lipids. However, treatments with *CE*, ALF and SPF showed smaller adipocytes of the perirenal WAT histology similar to that observed with control rats given normal diet. Although we do not understand the mechanism of the WAT weight reduction observed in this work, it is suggestive that treatment with the extracts from *CE* leaves has a profound inhibitory effect on the increased growth of adipocytes and on fat accumulation at large.

It is surprising that a decrease in WAT weight was observed with SPF treatment whereas no significant difference in total body weight was observed when compared with the HFD-only fed rats as earlier mentioned. Since a somewhat decrease in WAT weight was observed, it is plausible that the increased body weight of the rats may be due to muscle mass and not necessarily adipose tissue weight. Saponins are one class of plant steroids that causes muscle mass growth and fat loss (Arsyad, 1996) and many herbal products have been

claimed to produce such effects. The mechanism for improvement in muscle mass, as earlier documented, is by the elevation of the body's luteinizing hormone levels (Tsai *et al.*, 2003) which in turn stimulates the interstitial cells of Leydig in the testis to produce testosterone. Hence, testosterone induces muscle hypertrophy by multiple mechanisms (Herbst and Bhasin, 2004). It is not possible, however, to attribute the overall effect of the crude extract on WAT to either the saponin fraction or alkaloid fraction since neither of them was as effective as the crude extract in WAT weight reduction. However, it is plausible to suggest that the observed effect may be due to the combined effect of both fractions in the crude extract. Plants rich in both saponins and alkaloids which have shown anti-obesity effects have been reviewed (Santos *et al.*, 2010).

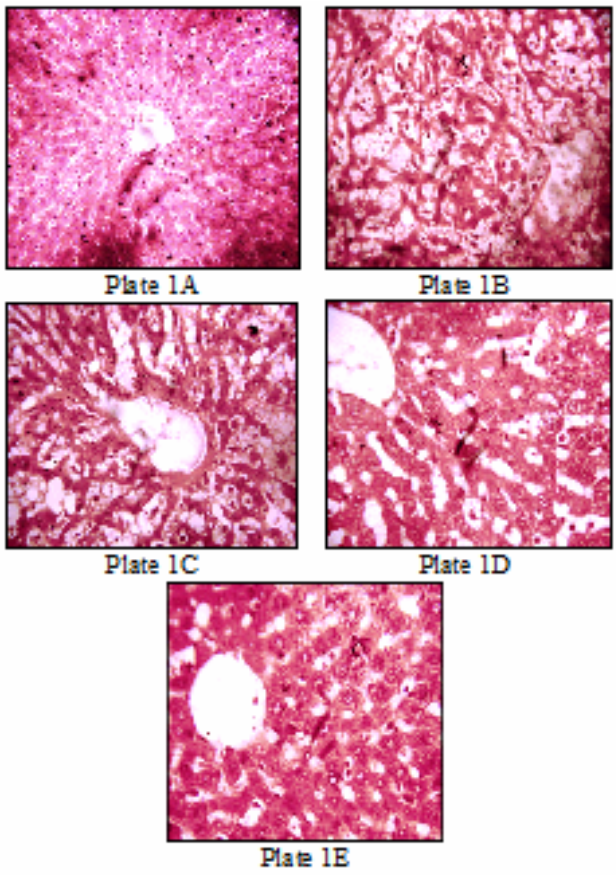

Fig. 2: Histological section photomicrograph of Liver from normal control rat, high-fat diet [HFD] only treated rat, ALF-treated high-fat model (HFM) and SPF-treated HFM and *CE*-treated HFM (Plates 1A, 1B, 1C, 1D and 1E respectively). Normal central canal, sinusoidal spaces and surrounding hepatocytes were observed in 1A. However, fatty degeneration of the hepatocytes (arrows) were noted upon treatment with HFD (1B). Degenerating tissue parenchyma with no obvious preservation of the histological features, and marked vacuolation of most hepatocytes are evident (arrows) in ALF-treated rats (1C). Better preservation of the liver parenchyma was observed with SPF and CE treatments (1D & 1E) (H&E: Magx 400).

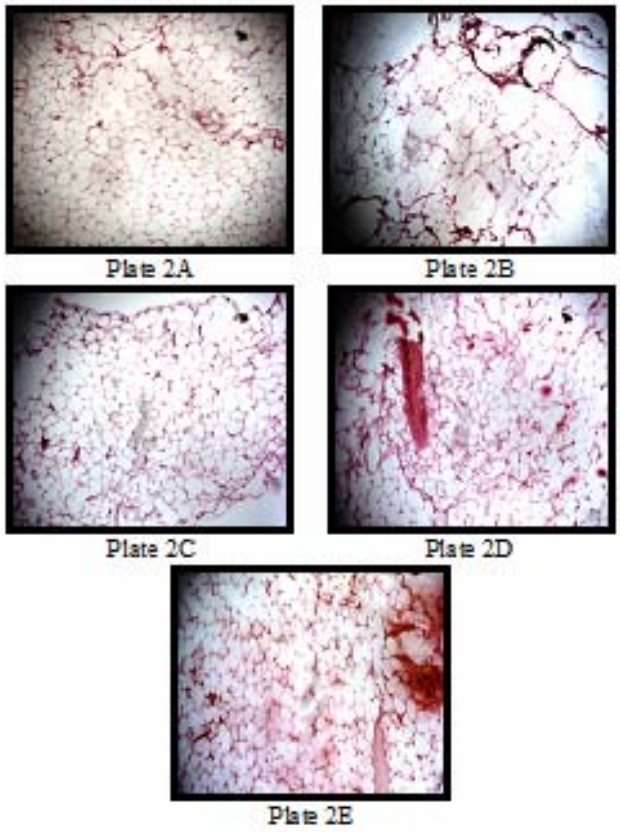

Fig. 3: Histological section photomicrograph of Perirenal white adipose tissue [WAT] from normal control rat, high-fat diet [HFD] only treated rat, ALF-treated high-fat model (HFM) and SPF-treated HFM and *CE*-treated HFM (Plates 2A, 2B, 2C, 2D and 2E respectively). Normal adipocytes sizes are observed in 2C, 2D and 2E similar to normal control (2A). However, hypertrophy of adipocytes was observed in HFD-control rats (2B). (H&E: Magx100)

Natural products with anti-obesity effects may have mechanisms of actions based on their abilities to produce decreased energy intake, lipid absorption, differentiation and proliferation of pre-adipocytes, lipogenesis or by increasing lipolysis and energy expenditure (Yun, 2010). Upon administration of *CE* extract and fractions to HFD-fed rats in the present study, it is possible that the reduction in body weights and perirenal WAT observed may be due to an inhibitory action of the test substances on pancreatic lipase. Pancreatic lipase is the key enzyme in dietary fat absorption which hydrolyses fat to monoacylglycerols and fatty acids (Verger, 1984). These products when mixed with bile salts are disseminated and conveyed to the site of fat absorption. Perhaps further studies on *C. esculenta* geared towards elucidating the percentage inhibition of pancreatic lipase *in vitro* as described by Chidrawar *et al* (2012) may provide substantial evidence. Many natural products with pancreatic inhibitory activity have been reviewed (Birari

and Bhutani, 2007) and are being developed into clinical products.

It is worthy of note that reducing body weight and body fat are important in the prevention of obesity (Hue *et al.*, 2009). Obesity, a known chronic metabolic disorder, results from imbalance between energy intake and energy expenditure. Pharmacological treatments of obesity do not appear to be effective in producing sustained long-term weight reduction (Glenny *et al*, 1997). The dissatisfaction with the high costs and potentially hazardous side effects of orthodox medicines have led to the exploration of natural products as alternative means for developing future effective, safe anti-obesity drugs (Mayer *et al.*, 2009). As deduced from the present study, the leaves of *C. esculenta*, may be harnessed as a future natural source for prevention or treatment of obesity like many other natural products which have been employed widely (Han *et al*, 2005; Rayalam *et al*, 2008).

Upon histological examination of the liver tissues of the HFD-only fed rats, some degenerative changes of the liver parenchyma which occurs as a result of hepatic lipid accumulation were observed. High-fat diet is known to affect liver metabolism, leading to steatosis, which is a complex disorder related to mitochondrial alterations and reactive oxygen species over production (Hebbard and George, 2011). Hence, the histopathological changes observed are as a result of an increase in the supply of circulating acids to the hepatic tissue or from increased endogenous liver fatty acid synthesis (Browning and Horton, 2004). Treatments with the crude aqueous extract (AE) and saponins fraction (SPF) of *CE* leaves were observed to have offered some protection to the liver tissues by the remarkable reduction of the fatty change in the liver. Conversely, no obvious change was seen upon treatment with the alkaloid fraction. The biochemical assessment of liver function supports these histopathological findings obtained. The preservation of the hepatocytes by the SPF and crude AE of *CE* leaves may have been through a mechanism that either disrupts the generation of reactive oxygen species or the cellular changes induced by HFD.

CONCLUSION

The present study demonstrates that phytoconstituents in *C. esculenta* leaves significantly reduced fat accumulation in adipose tissues and exhibited potent preservation of the liver against HFD-induced damaged.

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