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Research Article

Liver Function and Antioxidant Status of Obese Rats Treated with Ethanol Extract of *Anthocleista vogelii* Root Bark

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Abstract

This study was designed to ascertain the effect of ethanol extract of *Anthocleista vogelii* Planch root bark on liver function and antioxidant status of obese rats. Forty male wistar rats were divided into five groups of 8 rats per group. Group 1 was fed the normal pellet diet (NPD), Group 2-3 were fed high fat diet (HFD) and Group 4-5 were fed the high carbohydrate diet (HCD) with water *ad libitum* for 18 weeks. After 14 weeks, Group 3 and 5 received oral treatment of 500 mg/kg b.w *A. vogelii* extract while Group 1, 2 and 4 received the vehicle orally for 4 weeks. Weekly body weights of all the rats were measured. At the end of 18 weeks, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD) and catalase (CAT) activities were determined. Also, malondialdehyde (MDA) and reduced glutathione (GSH) levels were measured. Histopathological studies were done on sections of the liver. The results revealed that *A. vogelii* significantly decreased the body weight, ALT, AST and ALP activities compared to the HFD and HCD obese control. The *A. vogelii* extract revealed significant increase in CAT and SOD activities, and GSH level, with no significant difference in MDA level compared to the HFD and HCD obese controls. The numerous prominent fat deposits in the livers appeared relatively reduced among the group treated with the extract. In conclusion, the ethanol extract of *A. vogelii* root bark contained bioactive compounds which improved liver function and exhibited high antioxidant activities. The antioxidant activity of *A. vogelii* might be helpful in preventing the progress of various oxidative stresses, fatty liver and obesity.

Keywords: *Anthocleista vogelii*, antioxidant, fatty liver, high carbohydrate diet, high fat diet

INTRODUCTION

Overweight and obesity are often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue (WHO, 2000), arising from an imbalance between calories ingested versus calories expended (Nammi *et al.*, 2004). Overeating leads to weight gain, especially if the diet is high in fat or sugar (for example, fast food, fried food, and sweets) which has high energy density. At an individual level, a combination of excessive food energy intake and a lack of physical activity explain most cases of obesity (Lau *et al.*, 2007).

Obesity increases the risk of developing a number of chronic diseases including: insulin resistance, type 2 diabetes, hypertension, hypercholesterolemia and osteoarthritis (Ryan *et al.*, 2006). Many diseases and disorders cause the liver to function abnormally. Obesity can also lead to liver problems, as can drug and alcohol abuse. The liver function test detects the presence of liver enzymes (ALT, AST and ALP) in the blood. Under normal conditions, the level of these enzymes in the blood is low, but when liver cells are damaged, these enzymes are released into the blood and their levels increase as a result. Therefore, elevated liver enzymes in the blood are used as markers of liver disease, including non-alcoholic fatty liver disease. As some of the enzymes detected in the liver function test are also present in other tissues, it is important that results for different enzymes are combined to determine the patient's condition.

Among the different liver enzymes, ALT has been related with hepatic fat deposition and insulin resistance which plays a major role in metabolic syndrome (Wallace *et al.*, 2007). Alkaline phosphatase (ALP) is a representative brush-border enzyme functionally involved in nutrient (fat) absorption and the transport of long-chain fatty acids in the intestinal mucosa (Takase and Goda 1990; Bernard *et al.*, 1992). The Alkaline phosphatase is typically elevated when dogs have bone, bile duct, and/or liver disorders (Ryan *et al.*, 2006).

Oxidative stress is a pathologic condition resulting from either increased production of free radicals, decreased levels of antioxidants or a combination of these factors. Increased oxidative stress is a common phenomenon in obesity. Catalase (CAT) and superoxide dismutase (SOD) are enzymatic antioxidants while glutathione is a non-enzymatic antioxidant, they all exert synergistic effects in scavenging free radicals. Hyperglycemia, per se or by the promotion of lipid peroxidation of low-density lipoprotein (LDL) can result in the production of free radicals (Maritim *et al.*, 2003). Malondialdehyde which is the oxidized by-product, is often used as a reliable marker of lipid peroxidation (Yu, 1994).

Traditional medicinal plants when compared to synthetic drugs are often cheaper, locally available, and easily consumable (raw or as simple medicinal preparations). Some traditional healers have claimed that some medicinal plants in

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Nigeria could be used to manage obesity and its health risk factors. *Anthocleista vogelii* is a plant traditional healers claim could be used to manage obesity and its associated diseases. *Anthocleista* is a genus of tree- and shrub-like tropical gentians in the Gentian family (Gentianaceae), with about 50 species in the genus. The English name for *A. vogelii* is cabbage tree. It is a tall tree (6-20 m), trunk is 15–55 cm wide, twigs with spines, leaves usually 40 to 150 cm long and 24 to 45 cm wide (Burkill, 1985). The bark of *A. vogelii* is used folklorically as purgative, antidote for snake bite, healing of dropsy, swellings, oedema, gout and venereal diseases (Burkill, 1985).

Assessing the influence of *A. vogelii* on biological markers like ALT, AST, ALP, SOD, CAT, GSH and MDA, which are directly or indirectly linked with obesity or diseases associated with obesity would help to substantiate the use of the plant in the management of obesity. Therefore, this study was designed to ascertain the effect of ethanol extract of *A. vogelii* Planch root bark on liver function and antioxidant status of obese rats.

MATERIALS AND METHODS

Plant Material

The root bark of *A. vogelii* Planch was collected from the forest of Ngor-Okpala local government, Imo State, Nigeria. The plant was identified by a botanist at the Faculty of Life Science, University of Benin, Nigeria.

Experimental Animals

Forty male wistar rats weighing 80-90 g were used for this study. The rats were purchased from Anatomy Department, University of Benin, Nigeria. All animals were housed in steel cages and each cage contained 8 rats. These were kept in the animal house of Biochemistry Department, University of Benin. Nigeria. Rats were maintained under controlled temperature (± 23°C) and a 12:12 h light/dark cycle. The rats were housed for two weeks after their arrival to the animal house for acclimatization. The rats had free access to tap water and normal pellet diet (NPD) until the commencement of the experiment. The handling of animal was done in strict adherence to the stipulated guidelines of Faculty of Life Sciences at University of Benin for animal use.

Preparation of Plant Extract

The root bark of *A. vogelii* was washed, sliced into smaller pieces and air-dried at room temperature. Thereafter, the dried plant part was milled into powder and 500g of the plant powder was soaked in 1L of 95% ethanol in a container for 72 hours. Filtration was done with Whatman No. 1 filter paper, the filtrate was evaporated under reduced pressure using a rotary evaporator and then freeze dried to get powder. The yield was stored in a refrigerator at a temperature of 4 ^{o}C.

Phytochemical Screening

The root bark of *A. vogelii* was screened for bioactive compounds using standard methods of Treatise and Evans (1989) and Sofowora (1993).

Acute Toxicity (LD_{50}) of Plant

The acute toxicity of the plant extract was carried out as described by Shah *et al.* (1997) and Burger *et al.* (2005). Thirty wistar rats was divided into six groups of five (5) rats each weighing between 180-200 g. The rats were subjected to 16 hours fasting (with only water) before administration of the extract. The extract was suspended in normal saline and administered in doses of 0, 100, 500, 1000, 2000 and 5000 mg/kg body weight orally. The rats were observed for signs of toxicity and mortality for the first critical 4 hours and then for each hour for the next 12 hour, followed by 6 hourly intervals for the next 56 hour giving a total of 72 hour observations, thereafter daily for 7 days. The screening dose was taken to be 1/10 of the lethal dose of the plant (Ghosh, 2005).

Composition of Hypercaloric Diet

For laboratory animals, a high fat diet usually contains 32 to 60% of calories from fat (Gajda, 2008), while a high carbohydrate diet contains 60% and above calories from carbohydrate (Axen and Axen, 2006). In this study, the high fat diet contained 50% fat, 25% carbohydrate, 20% protein and 5% others. The high carbohydrate diet contained 5% fat, 80% carbohydrate, 10% protein and 5% others. While the normal pellet diet contained 5% fat, 60% carbohydrate, 30% protein and 5% others. Table 1 showed the composition of the formulated diet for this study.

Table 1:
Composition of Experimental Diet

Composition	Food /Supplements	NPD (%)	HCD (%)	HFD (%)
Carbohydrates	Garri	60.0	80.0	25.0
Fat	Butter	5.0	5.0	50.0
Proteins	Bonga Fish	30.0	10.0	20.0
Fiber	Afrodak	1.5	1.5	1.5
Mineral mixture	Multi-minerals	2.5	2.5	2.5
Vitamin mixture	Multi-vitamins	1.0	1.0	1.0
Energy (KCal/g)		4.095	4.095	6.345

Induction of Obesity in the Rats

The forty rats were divided into two groups, normal 8 rats and obese 32 rats. Obesity was induced in the 32 rats by feeding 16 rats with high fat diet (HFD) and the other 16 rats with high carbohydrate diet (HCD) for 14 weeks. Rats fed with HCD and HFD whose body weight was significantly increased when compared to the normal control were considered obese (Amin and Nagy, 2009; Mistry *et al.*, 2011).

Experimental Design and Animal Grouping

The rats were included in five groups of 8 rats per group after induction of obesity. Group 1 was fed the NPD, Group 2-3 was fed HFD and Group 4-5 was fed the HCD with water *ad libitum* for another 4 weeks. Group 3 and 5 received oral treatment of 500 mg/kg b.w *A. vogelii* extract while Group 1, 2 and 4 received the vehicle orally for 4 weeks. The body weight of the rats were measured weekly in grams (g).

Blood Sample Preparation

Blood samples were collected by cardiac puncture from the fasting rats after being anesthetized with chloroform into plain tubes. These were allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 minutes at room temperature for separation of serum. The clear, non-haemolysed supernatant was separated and stored at -20°C.

Tissue Sample Preparation

After abdominal incision, rat liver was immediately excised, washed in ice cold saline, blotted individually on filter paper, weighed and a part was homogenized and used to assay for catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA). The crude tissue homogenate was centrifuged at 3500 rpm for 15 minutes, and the resultant supernatant was used for the different estimations. The second part of the liver was used for histopathological studies.

Biochemical Assays

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined in a method as described by Reitman and Frankel (1957). Alkaline phosphatase was determined by an optimized standard colorimetric method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC, 1972). Catalase activity was determined by the method described by Cohen *et al.*, (1970). The superoxide dismutase (SOD) activity was determined by the method of Mistra and Fridovich (1972).

The method for MDA determination depended on the formation of MDA as an end product of lipid peroxidation which reacted with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which was measured spectrophotometrically at 532 nm (Ohkawa*et al.*, 1979). Reduced glutathione (GSH) was determined based on the reduction of 5,5dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at 405 nm (Tietz, 1990).

Histopathology

The second part of the liver was placed in 10% formal saline for histopathology studies. Sections of the liver was prepared and stained with haematoxylin and eosin following fixation. Permanent mounts were examined by light microscopy and the results obtained were compared with control (Dahiru and Obidoa, 2008).

Statistical analysis

The experimental results was expressed as the Mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by one way analysis of variance (ANOVA) followed by Duncan's multiple range test. $P<0.05$ was considered statistical significance.

RESULTS

The bioactive compounds in the ethanol root extract of *A. vogelii* contained alkaloid, saponin, tannin, steroid and cardiac glycosides (Table 2). For the acute toxicity test of *A. vogelii* extract, no death was recorded at any of the doses administered, but weakness and sluggishness were the major behavioral changes observed in the rats at 5000 mg/kg b.w. oral dose. These behavioral changes disappeared after 1 hour of observation; therefore, oral LD_{50} of *A. vogelii* was determined as ≥ 5000 mg/kg b.w in wistar rats.

Table 2:
Phytochemical constituents of the ethanol extract of *A. vogelii* root bark

S/No.	Phytochemical Constituents	*Anthocleista vogelii*
1.	Alkaloid	+
2.	Saponin	+
3.	Tannin	-
4.	Steroid	+
5.	Flavonoid	-
6.	Cardiac glycosides	+
7.	Terpenoids	-
8.	Reducing sugar	-

(+) = Presence, (-) = Absent

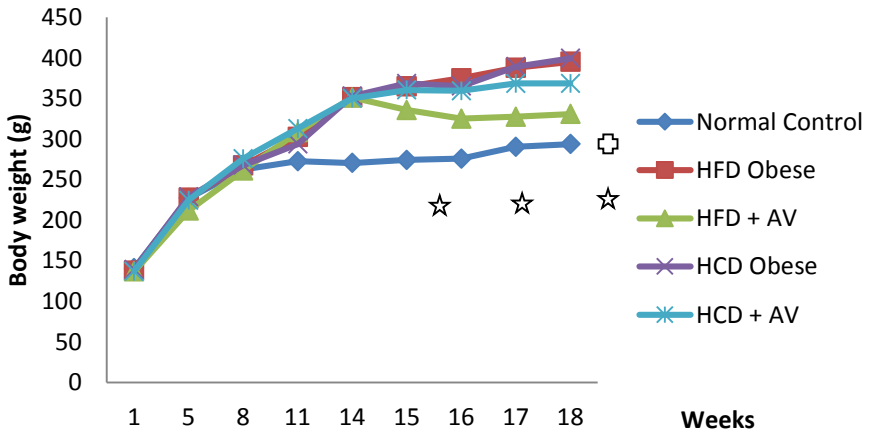
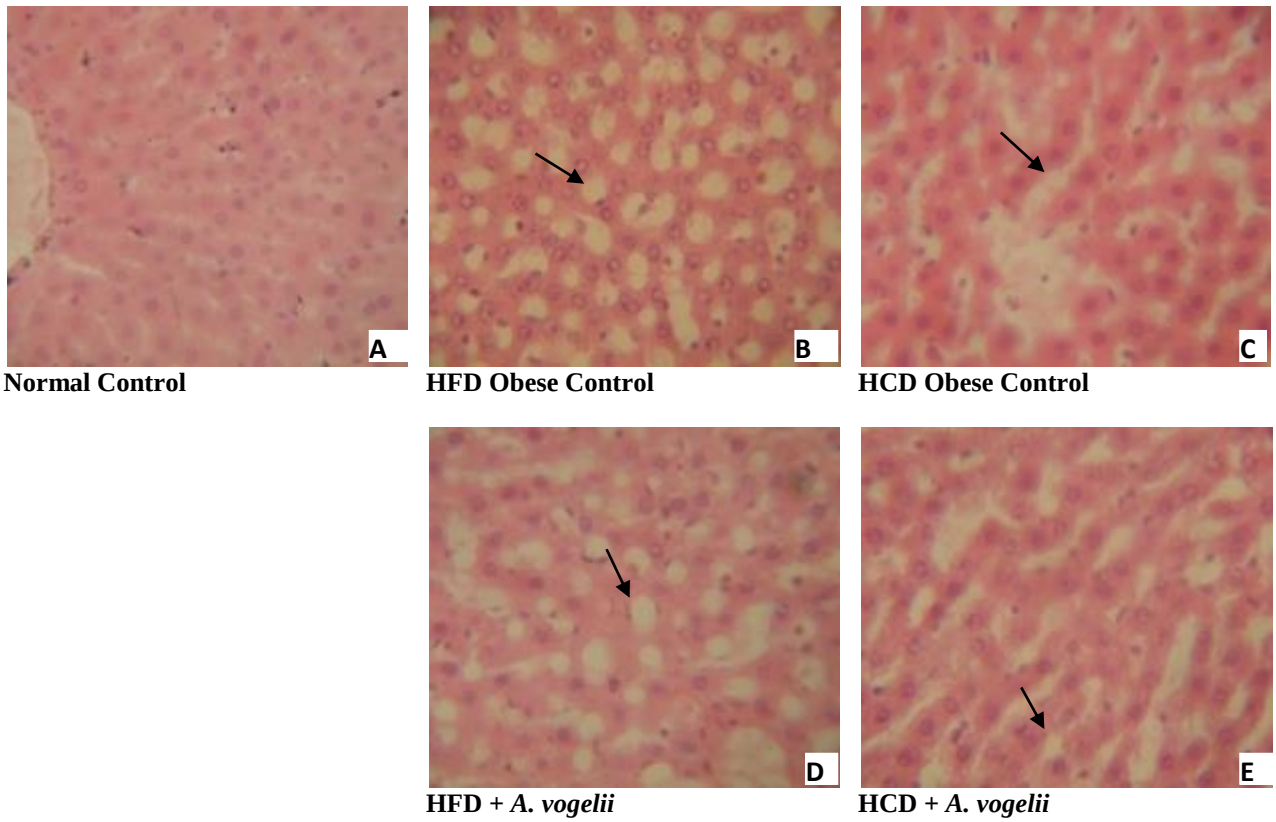


Fig. 1:
Body weight of rats during the period of obesity induction and treatment with *A. vogelii*
The symbol ( ) indicates significant difference of the HFD + AV when compared to HFD obese group, while ( ) indicates significant difference of the HCD + AV when compared to HCD obese group.



Normal Control **HFD Obese Control** **HCD Obese Control**

HFD + *A. vogelii* **HCD + *A. vogelii***

Plate 1:
Sections in liver of control (upper sections) and treated (lower sections) animals showing (↘) fat deposits (X40

The body weight of the rats was measured weekly for 14 weeks. The body weight of the rats fed with HFD and HCD showed significant increase compared to the rats that feed on NPD at the fourteenth week, which was the period of obesity induction. The body weight of the HFD and HCD obese control groups, maintained significantly increased body weight compared to the normal control throughout the treatment period. In the HFD groups, the group treated with *A. vogelii* had significant decrease in body weight compared to the HFD control. Again in the HCD group, the body weight of group treated with *A. vogelii* was significantly decreased compared to the HCD control (Fig. 1).

Table 3:
Liver function enzymes assessment of rats treated with the plant extracts

GROUP	ALT (U/l)	AST (U/l)	ALP (U/l)
Normal Control	4.18 ± 0.36^{b}	6.92 ± 0.46^{b}	2.77 ± 0.04^{d}
HFD Obese Control	4.93 ± 0.14^{a}	9.45 ± 0.33^{a}	13.39 ± 0.97^{a}
HFD + *A. vogelii*	2.73 ± 0.20^{c}	6.85 ± 0.26^{b}	4.30 ± 0.27^{cd}
HCD Obese Control	5.13 ± 0.18^{a}	9.40 ± 0.17^{a}	9.28 ± 0.88^{b}
HCD + *A. vogelii*	3.55 ± 0.12^{b}	7.43 ± 0.19^{b}	4.14 ± 0.22^{cd}

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

The ALT, AST and ALP activities were significantly elevated in the HFD and HCD obese groups compared to the normal control. Within the HFD groups, the group treated with *A. vogelii* had significantly decreased ALT, AST and ALP activities. Also, in the HCD groups, treatment with *A. vogelii* had significantly decreased ALT, AST and ALP activities (Table 3).

Feeding the rats with HFD and HCD led to significantly high hepatic MDA level. The hepatic CAT and SOD activities and GSH level were significantly lower in the HFD and HCD obese control groups when compared to the normal control. Within the HFD groups, the *A. vogelii* extract showed significant increase in CAT, SOD and GSH level and no significant decrease in MDA level. While in the HCD groups, the *A. vogelii* extract revealed significant increase in CAT, SOD and GSH level, with no significant difference in MDA level (Table 4).

Table 4:
Lipid peroxidation and antioxidant enzymes of rats treated with the plant extracts

GROUP	MDA (U/100mg)	CATALASE (K)	SOD (U/100mg)	GSH (mM)
Normal Control	23.92 ± 0.78^{c}	158.89 ± 4.37^{a}	167.40 ± 5.78^{b}	110.33 ± 2.60^{a}
HFD Obese Control	28.34 ± 0.81^{a}	142.87 ± 1.65^{c}	71.50 ± 0.64^{e}	32.67 ± 1.45^{c}
HFD + *A. vogelii*	24.08 ± 0.96^{abc}	167.70 ± 2.35^{a}	192.13 ± 4.23^{a}	51.00 ± 2.89^{b}
HCD Obese Control	25.52 ± 0.35^{ab}	146.03 ± 2.72^{bc}	140.87 ± 4.98^{c}	25.00 ± 2.89^{d}
HCD + *A. vogelii*	21.75 ± 1.11^{bc}	160.41 ± 3.59^{a}	116.67 ± 5.14^{d}	105.67 ± 2.02^{a}

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

DISCUSSION

The ethanol extract of *A. vogelii* root bark contained alkaloid, saponin, tannin, steroid and cardiac glycosides. All the parts of *A. vogelii* has been shown to have alkaloids; glycosides, saponins and steroids (Burkill, 1985). Other studies have shown that *A. vogelii* contains xanthones and some iridoid compounds were discovered (Rank *et. al.*, 2004).The oral LD_{50} of ethanol extract of *A. vogelii* root bark was determined as $\geq$ 5000 mg/kg b.w in wistar rats. In a different toxicity study on *A. vogelii*, no lethality was observed at 2000 mg/kg body weight i.p. in mice (Alaribe *et al.*, 2012).

The body weight of rats fed the HFD and HCD maintained significantly increased body weight compared to the normal control after 14 weeks, thereby establishing obesity in the rats. Obesity has been induced in rats with hypercaloric diets compounded by adding more carbohydrates (HCD) and/or more fats (HFD) to the diet composition. Gajda (2009) attested that high fats diets containing about 32 to 60% of calories from fat can be used in the laboratory to induce obesity. In the report of Amin and Nagy (2009), consumption of HFD resulted in obesity because it facilitated the development of a positive energy balance leading to an increase in visceral fat deposition which leads to abdominal obesity. Our HFD and HCD diets were found to induce obesity in Wistar rats, which was consistent with other reported works (Naderali *et al.*, 2004; Meguid *et al.*, 2004; Prunet-Marcassus *et al.*, 2003).

Certain phytochemicals are found in plants could alter appetite for food, reduce lipogenesis or promotes lipolysis which plays a role in weight loss. For examples, *Citrus aurantium* extract contains alkaloids such as p-octopamine and synephrines which exert adrenergic agonist activity (Pellati*et al.*, 2002); *Caralluma fimbriata* extracts contains pregnane glycosides which reduces lipogenesis in the adipose tissue (Preuss*et al.*, 2004; Preuss, 2004) and also reduces appetite, body weight and waist circumference in a study involving humans (Kuriyan*et al.*, 2007). *Coleus forskohlii* is a plant rich in alkaloids, contains a diterpene that acts directly on adenylate cyclase (Burns *et al.*, 1987) and in turn cAMP promotes lipolysis, increases the body's basal metabolic rate, and increases utilisation of body fat (Litosch *et al.*, 1982). Although, the mechanism of action of body weight reduction of *A. vogelii* was not determined in this study, it is suggestive that the bioactive components, like alkaloids and cardiac glycosides which it contained played important role in the decreased body weight of the rats.

ALT and AST are two classical enzymes for the assessment of liver impairment. They are enzymes known to be reliable indices for hepatotoxicity assessment (Hayes, 1989) particularly ALT which is principally found in the cytoplasm of rats liver (Benjamin, 1978; Crook, 2006). In toxic environment, the activities of the two enzymes in the blood stream are known to increase significantly (Solomon *et al.*, 1993; Crook, 2006). Among the different liver enzymes, ALT has been related with hepatic fat deposition and insulin resistance which plays a major role in metabolic syndrome (Wallace *et al.*, 2007). The ALT, AST and ALP activities were significantly increased in the HFD and HCD obese groups compared to the normal control, which is in agreement with earlier studies (Choi, 2003; Amin and Nagy, 2009). Within the HFD and HCD groups, the group treated with *A. vogelii* had significantly decreased ALT, AST and ALP activities.

Alkaline phosphatase (ALP) is a membrane-bound enzyme found in a wide variety of tissues, including liver. ALP is a TNALP isoenzyme (as are bone and kidney ALP), and it is known that TNALP is present in human preadipocytes (Ali *et al.*, 2006) and in the murine preadipocyte cell, 3T3-L1 (Ali *et al.*, 2005). The function of ALP in adipose tissue has been demonstrated in human and 3T3-L1 preadipocytes that inhibition of ALP activity blocks intracellular lipid accumulation and that ALP is localized to the lipid containing droplets of preadipocytes (Ali *et al.*, 2005; Ali *et al.*, 2006). It, therefore, has been hypothesized that ALP may be involved in the control of lipid accumulation during the maturation of preadipocytes into adipocytes.

A previous study demonstrates that total and liver but not bone and intestinal ALP serum levels are higher in obese than in lean subjects (Ali *et al.*, 2006). Thus, it is possible that the higher level of liver ALP in obese than in lean subjects is a result of ALP release from adipose tissue. This was the case in this present study, where ALP was found to be significantly increased in HFD and HCD obese rats. Also, *A. vogelii* extract was effective at reducing the ALP levels of the rats which might have been as a result of decreased adiposity.

Oxidative stress is defined as the increased generation of free radicals and/or the impaired compensatory response of endogenous antioxidant defenses (Betteridge, 2000). Oxidative stress could also result from decreased levels of antioxidants. Increased oxidative stress is a common phenomenon in obesity. This is revealed by a significant increase in the levels of MDA and a significant decrease in hepatic CAT, SOD and GSH in the obese rats. CAT, SOD and GSH are important antioxidants that are expected to counter the effects of free radicals produced by oxidative stress (Gaetani*et al.*, 1996; Chelikani *et al.*, 2004).

Numerous oxygenated compounds, mainly aldehydes such as malondialdehyde (MDA), are produced during the attack of free radicals on membrane lipoproteins and polyunsaturated fatty acids (PUFA). Assay of MDA is probably the most common method for the measurement of lipid peroxidation (Yu, 1994).

In addition oxidative stress may be increased in metabolic syndrome due to dyslipidemia resulting from increased levels of FFA and TGs that led to increased formation of foam cells, rendering LDL less dense and more vulnerable to oxidation and uptake by macrophages (Holvoet, 2008). The *A. vogelii* extract showed significant increase in CAT, SOD and GSH activity and no significant decrease in MDA in the HFD and HCD obese treated group.

The photomicrograph of HFD and HCD obese controls showed presence of numerous prominent fat deposits on the livers, and there was relatively visible reduction of the fat deposits in the livers of the rats treated with *A. vogelii* extract (plate 1). There are two histologic patterns of NAFLD: fatty liver alone (simple steatosis) and steatohepatitis (McCullough, 2002). Now there is increasing evidence that NAFLD often represents a component of the metabolic syndrome characterized by obesity, hyperinsulinemia, peripheral insulin resistance, diabetes, hypertriglyceridemia and hypertension (Cortez-Pinto *et al.*, 1999; Sharabi and Eldad, 2000). Serum ALT but not ALP activity was associated with measures of abdominal obesity. Abdominal obesity is known to be associated with an increased risk of nonalcoholic steatohepatitis (NASH) (Cortez-Pinto *et al.*, 1999; Marchesini *et al.*, 2001), leading to elevated serum levels of liver enzymes,

particularly ALT. Thus, in this present study, the HFD and HCD obese rats had significantly increased ALT which could be due to fatty liver.

A study in humans, suggested that elevated serum hepatic enzyme activities may be associated with high prevalence of fatty liver, which is observed in the subjects with elevated total body fat (Choi, 2003). Another study revealed that elevated ALT levels are due largely to the effect of abdominal obesity on liver function, whereas elevated ALP activity is not related to depot-specific adipose tissue mass but rather to whole body fat mass, which may be a source of serum ALP (Ali *et al.*, 2006). Therefore, a reduction of weight will result in decreased ALT and ALP levels and consequently prevent the risk of NASH. The three plant extracts had significantly reduced the ALT and AST levels and therefore would likely reduce the risk of NASH.

In conclusion, the ethanol extract of *A. vogelii* root bark might be useful as an antiobesity agent as it improves the liver function and antioxidant defense system, thereby suppressing oxidative stress and lipid peroxidation. Also, the plant extract reduces fat deposits on the liver that causes non-alcoholic fatty liver disease peculiar in obesity.

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