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Research Article

Role of high-density lipoprotein in the ameliorative activity of *Bryophyllumpinnatum* in male Wistar rats fed a high fat diet

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ABSTRACT

Atherosclerosis, a condition connected with fat-rich diet is a leading cause of death worldwide. It has been strongly associated with dysmetabolic, dyslipidemic and inflammatory conditions with sparingly effective available therapeutic measures. High-density lipoprotein (HDL-C), the agreeably beneficial chylomicron, has been linked to improved glycemic control, a finding being growingly investigated. *Bryophyllumpinnatum* (BP) aqueous leaf extract or its steroidal derivative is well known for antidiabetic and/or anti-inflammatory potential. This study therefore investigated the effect of BP on circulating lipids, inflammatory response markers, and glycemic control in male Wistar rats fed a high-fat diet. Twenty-four male Wistar rats (120-130 g) were randomly selected into four groups (n=6) and fed with rat chow, high-fat diet (HFD), HFD + BP (250 mg/kg), and rat chow + BP (250 mg/kg) for six weeks after two weeks of acclimatization. High-fat-fed rats showed reduced HDL-C, increased fasting glycemia, plasma triglycerides (TG), low-density-lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), and uric acid (UA). The total cholesterol was not altered in HFD group. The BP leaf aqueous extract however augmented HDL-C with accompanying improved fasting glycemia and attenuated plasma TG, TC, LDL-C, CRP, and UA in high fat-fed rats. The extract was demonstrated to confer protection against lethal accumulation of atherogenic lipids and cause the augmentation of HDL-C with a plausible resultant improvement of glycemic control and amelioration of inflammatory response in high-fat diet-fed animals.

INTRODUCTION

A High-fat diet has been shown to evoke the formation of atheromatous plaques with attendant coronary diseases in animals.[1] Atherosclerosis affects arterial blood vessels owing to a chronic inflammatory response of white blood cells in the arterial wall. This condition is promoted by low-density lipoproteins cholesterol (LDL-C) together with inadequate removal of fats and cholesterol from the macrophages by functional high-density lipoproteins cholesterol (HDL-C). It is commonly referred to as a "hardening" or furring of the arteries. It is caused by the formation of multiple atheromatous plaques within the arteries.[2] LDL-C and HDL-C are chylomicrons considered as atherogenic indices, which enable transport of lipids or fats such as cholesterol, phospholipids, and triglycerides in the extracellular fluid. LDL-C molecules transport fat molecules into the arterial wall, attract macrophages, and thus drive atherosclerosis.

Conversely, HDL molecules remove fat molecules from macrophages in the wall of arteries.[3] Removal of cholesterol from peripheral cells such as macrophages to the liver for excretion is commonly regarded as the most imperative anti-atherosclerotic action of HDL-C. However, HDL expresses a range of additional protective properties,

including inhibition of oxidation,[4] inflammation,[5] thrombosis, and vasodilatation via the release of nitric oxide.[6] Nevertheless, recent clinical and experimental evidence connecting HDL-C to glucose metabolism suggests additional beneficial action relevant to diabetes mellitus, a condition which undoubtedly elicits overt dysmetabolism. HDL-C has been suggested to reduce blood glucose levels by eliciting a variety of events, including stimulation of pancreatic β -cell insulin secretion,[7] and increased glucose uptake into skeletal muscle via activation of the AMP-activated protein kinase (AMPK) signaling pathway.[8,9]

Bryophyllum pinnatum (BP) belongs to the family Crassulaceae. It is a succulent perennial plant native to Africa, mostly Madagascar and Southern Africa, and is also available in tropical and sub-tropical areas.[10] It is recognized for its hemostatic and wound healing properties.[11] Several studies have documented that BP is antibacterial, antimicrobial, antifungal, and antiviral.[12] The aqueous and methanolic leaf extract of BP has been shown to produce dose-related and significant reductions in arterial blood pressures and heart rates of anaesthetized normotensive and hypertensive rats.[13] It was also reported that the leaf extract revealed a significant dose-dependent significant decreases in the rate and force of contractions of guinea-pig isolated atria.[14] A

phytochemical analysis of the extract has shown that it contains useful substances like alkaloids, phenols, flavonoids, saponins, tannins, carotenoids, glycosides, sitosterol, anthocyanins, malic acid, quinines, tocopherol, lectins, coumarins, bufadienolides, and lipids.[15] The extract is also a good source of ascorbic acid, riboflavin, thiamine, and niacin,[16] and this has been shown to be responsible for some of its antioxidant and anti-inflammatory properties. In a study to determine the anti-inflammatory and antinociceptive activities of BP using fresh egg albumin-induced pedal (paw) oedema, and thermally-induced nociceptive pain respectively, the extract inhibited fresh egg albumin-induced acute inflammation and nociception in rodents.[17] The BP leaf extract has also been evaluated to possess a novel anti-inflammatory and analgesic steroidal derivative formulated as Stigmast-4, 20 (21), 23-trien-3-one.[15] Interestingly and of immense importance to this present study also, is the availability of data on the antidiabetic effect of BP extract in rats. However, the distinctive mechanism of action of anti-inflammatory and antidiabetic are yet to be elucidated.[17] The aim of this study, therefore, was to investigate the role of HDL-Con the effects of BP leaf extract in high fat diet-induced dyslipidemia, glucose deregulation, and inflammatory response.

MATERIALS AND METHODS

Chemical and Assay kits

Assay kits for lipid profile analysis were obtained from Randox Laboratories Ltd, UK. All other chemicals used were of analytical grade and were prepared in standard volumetric flasks using glassware and distilled water.

Plant Materials

Fresh leaves of *Bryophyllum pinnatum* were purchased in April, 2019 from the local region of Gegele, Ilorin in Kwara State of Nigeria, and were identified and authenticated by the Herbarium unit of Department of Plant Biology, University of Ilorin, Kwara State where a voucher specimen (No. 909) was deposited.

Plant extract preparation

Fresh leaves of *Bryophyllum pinnatum* were thoroughly washed with distilled water, cut into shreds and air-dried at room temperature ($25^{\circ} \pm 2^{\circ}\text{C}$), and pulverized to powder using an electric Blender. It was then sieved to remove the larger sized particles. 500 g of powdered leaves were macerated with 1 litre of distilled water and allowed to stay for 48 hours. The resulting brownish colour liquid was obtained as the extract; the brownish colour extract was filtered, evaporated, shade-dried, scraped out, weighed as 166.0 g and stored as stock in the refrigerator to prevent absorption of moisture until administration.[18] Stock preparation was made to meet the requirement for the duration of the experiment. Low to moderate dose of BP

(250mg/kg body weight of rats) were given in distilled water per os according to the methods of Aprioku and Igbi2017.[19] High dose of BP has been shown to be toxic in a previous experimental model.[20]

Experimental rats and treatments

A total of twenty-four adult Wistar rats bred at the Animal Holding Unit of the Department of Physiology, University of Ilorin, Nigeria with a mean weight of $125 \pm 5\text{g}$ were used for the study. Throughout the experiment, the animals were housed in clean cages placed in well-ventilated housing conditions (temperature at $24\text{--}28^{\circ}\text{C}$, photoperiod of 12-hour natural light and 12-hour dark, and humidity of 45%–50%). The cages were cleaned twice daily at 12 hours interval. They were acclimatized for two (2) weeks during which they were fed *ad libitum* with pellet form of grower's mash (Vital poultry feed) with free access to water.

Animal grouping

The animals were randomized into four (4) experimental groups of six rats each which consist of Group 1, 2, 3, and 4. Group 1 (Control) rats were fed for 6 weeks with standard chow diet (2% wt/wt fat) without the extract of the plant; Group 2 rats received feed with high-fat diet (20% wt/wt fat) without the extract of the plant. Group 3 rats received feed with standard chow diet and 250 mg/kg aqueous extract of the plant; Group 4 rats received feed with high fat diet with 250 mg/kg aqueous extract of the plant. High-fat diet was prepared for the study was according to the method of Vanessa et. al.[21] The rats received the aqueous extract of the plant orally through gastric gavages.

Sample collection and preparation

The animals were fasted overnight to the morning of the sacrifice hours and were sacrificed following anaesthesia with chloroform. Blood was collected by cardiac puncture separately with a 5 ml syringe and needle into a clean Lithium Heparin bottle and centrifuged. Biochemical samples of fasting blood glucose, triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, C-reactive protein and uric acid were assayed.

Statistical Analysis

All values reported in the study were expressed as Mean $\pm$ Standard Error of Mean (SEM). Statistical evaluations were done using statistical package for social sciences (SPSS) Evaluation Version 20.0 (IBM SPSS Inc., Chicago, USA). Comparisons among the groups were made by one-way analysis of variance (ANOVA), followed by post-hoc Tukey multiple comparison test. High-fat diet treated, and BP treated groups were compared to the control group, while the groups that received combination of BP and HFD were compared to HFD-treated group. Statistical significance was considered at $p < 0.05$.

RESULTS

Table 1: Effect of *Bryophyllum pinnatum* on mean Body weight, Food Intake and Water Intake

	CTR	HFD	BP	HFD+BP
Body weight(g)				
Before	126.3±3.6	124.8±4.3	130.8±3.5	120.80±3.1
After	154.8±4.1	196.8±2.8*	175.1±3.1	178.2±5.7
Food Intake (g/kg/day)				
Before	201.5±3.5	208.7±5.6	195.2±4.3	203.5±5.9
After	220.0±4.7	227.5±4.9	223.1±5.2#	252.4±4.6
Water Intake (ml/kg/day)				
Before	87.8±4.0	90.1±3.6	80.2±4.1	89.8±3.8
After	100.4±3.0	108.8±3.0	90.5±4.81	02.4±3.9

Data were analyzed by one-way ANOVA followed by post-hoc Tukey multiple comparison test. Values are expressed as mean ± SEM of 6 rats per group (*p<0.05 vs control; #p<0.05 vs HFD)

Effect of *Bryophyllum pinnatum* Leaf Extract on Fasting Glycaemia and Triglyceride-Glucose Index in Rats Fed a High Fat Diet

High-fat diet increased fasting glycaemia and elicited slight but remarkable increase in body weight gain and triglyceride-glucose index (Figure 1). However, BP extract with or without high-fat diet reduced fasting glycaemia compared with high-fat diet group (Figure 1A) and did not alter body weight gain and triglyceride-glucose index compared with the control

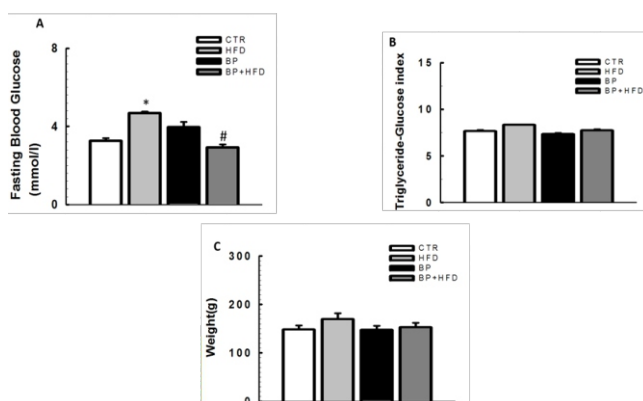


Figure 1: Effect of *Bryophyllum pinnatum* on Fasting Blood Glucose, Triglyceride-Glucose index (TYG index) and Body weight in Wistar Rats Fed a High Fat Diet; n = 6 rats per group, *p<0.05 vs control; #p<0.05 vs HFD

Effect of *Bryophyllum pinnatum* Leaf Extract on Plasma Lipids in Rats Fed a High Fat Diet

Plasma total cholesterol was unaltered in all experimental groups compared with control (Figure 2A). However, high fat diet increased plasma LDL-C and TG but reduced HDL-compared with control. BP leaf extract in normal diet-fed rats increased HDL-C but reduced LDL-C and TG compared with control and high fat diet-fed rats, whereas, BP leaf extract with high fat diet increased HDL-C and reduced LDL-C and TG compared with high fat alone. Nevertheless, HDL-C, LDL-C, and TG remained unaltered compared with control in BP with high fat diet (Figure 2B-C, D).

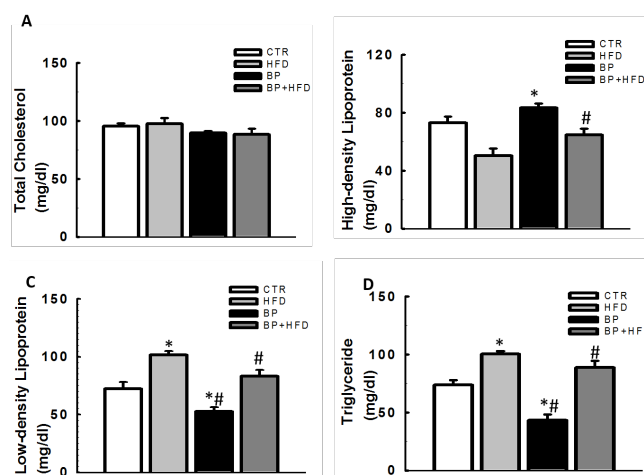


Figure 2: Effect of *Bryophyllum pinnatum* on Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Triglyceride in Wistar Rats Fed a High Fat Diet; n = 6 rats per group, *p<0.05 vs control; #p<0.05 vs HFD

Effect of *Bryophyllum pinnatum* Leaf Extract on C-Reactive Protein and Uric Acid in Rats fed a High Fat Diet

Both plasma CRP and UA increased in high-fat diet group compared with control. BP leaf extract without high-fat diet reduced plasma CRP and UA compared with control and high fat diet group. However, BP leaf extract in high-fat diet reduced plasma CRP and UA compared with high fat diet alone but did not alter CRP and UA compared with control (Figure 3A and B).

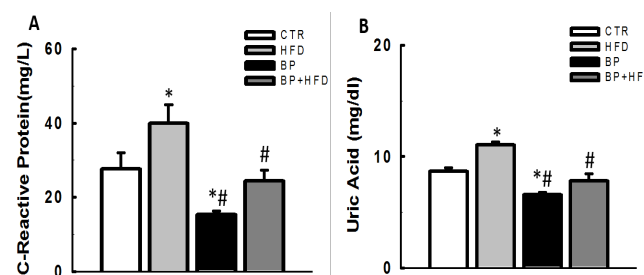


Figure 3: Effect of *Bryophyllum pinnatum* on C-Reactive protein and Uric Acid in Wistar Rats Fed a High Fat Diet; n = 6 rats per group, *p<0.05 vs control; #p<0.05 vs HFD

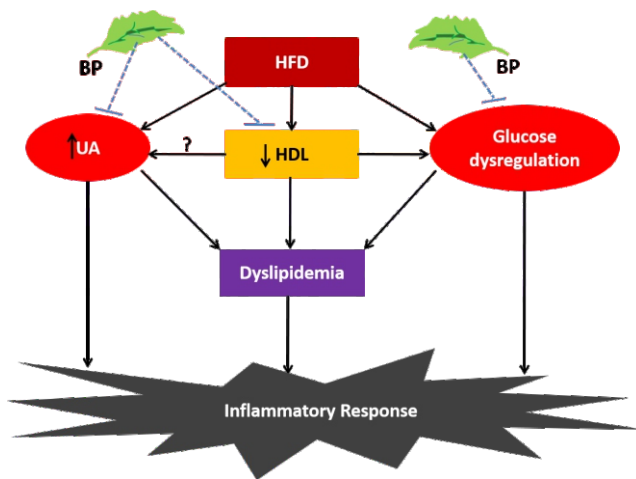


Figure 4: Graphical Abstract of the proposed mechanism.

Table 2: Diet component and percentage calories

	Normal chow (%/100g)	Calorie content (kcal/100g)	High fat feed (%/100g)	Calorie content (Kcal/100g)
Protein	27.5%	110	16.2	64.8
Carbohydrate	60.3%	24.1	30.28	121.2
Fat	10%	90	50.5	454.5
Vitamin	0.28%		0.28	
Minerals	2.6%		2.68	
Total(Kcal/100g)		224.1		640.5
Protein	g/kg		g/kg	
Casein	80.0		100.0	
Corn gluten meal	65.0		40.0	
Rice bran	50.78		90.0	
Corn bran	100.0		30.0	
Methionine	2.00		3.0	
CHO				
Corn starch	27.0		200.0	
Sucrose	91.38		85.0	
Oat meal	20.22		30.0	
Wheat	180		40.0	
Fat				
Corn oil	70.0		81.0	
Butter oil	30.0		100.38	
Soy bean oil	10.0		100.0	
Meat fat	30.0		100.0	
Vitamin				
Grower				
premix	0.10		0.03	
Vit C	0.18		0.01	
Mineral				
Oyster shell	1.1		0.25	
Bone shell	1.0		0.3	
Salt	0.5		0.03	

DISCUSSION

The present study demonstrates that high fat diet caused glucose deregulation with accompanying dyslipidaemia, hyperglycaemia, evidence of inflammatory response, and oxidative stress, which are indicated in obesity-associated diseases with increased body weight following the

6-week high fat diet. However, there is no significant increase in food intake following HFD consumption despite the increased body weight, an indication of possible calorogenic effect (Table 1). *Bryophyllum pinnatum* leaf aqueous extract also reduces food intake and protects high fat diet-fed rats against these dysmetabolism. Insights into this BP intriguing beneficial effects may culminate in the characterization of therapeutic candidates with greater efficacy and safety in the treatment of obesity-associated diseases and prevention of hyperlipidaemia-related cardiovascular diseases like atherosclerosis. In line with afore-stated thought, the present study revealed that BP leaf extract with and without high fat diet augmented HDL-C in rats compared with high fat diet groups and control, respectively. This outcome might be the plausible mechanism of BP anti-dyslipidaemia and interestingly antidiabetic effects.

High fat diet and Obesity is considered to elicit low-grade inflammation and possibly the development of obesity-associated diseases.[22] Obesity-associated diseases are often preceded by glucoregulation failure, a manifestation of insulin resistance (IR) and consequent hyperglycaemia and diabetes mellitus. However, the present study demonstrates that a 5-week high fat diet-induced hyperglycaemia as indicated by increased fasting blood glucose (Figure 1A) without overt obesity-related weight phenotypes(Figure 1). The rats herein placed on high fat diet had increases in weight compared with control, but the increase was not significant. This tendency towards obesity/overweight might have provided an obesity-like insulin resistance milieu with attendant elevated fasting blood glucose independent of overt excess adiposity. The outcome here might be due to the length of the high fat diet exposure, 5 weeks, which may not be enough to elicit excess adiposity but can lead to metabolic derangement. Metabolic derangement without excess adiposity presages the deleterious effect of excess free fatty acid (FFA) on tissues. Moreover, when adipose tissue reaches the storage threshold, excess FFA induces lipotoxicity through ectopic deposition in non-adipose tissue, especially the liver resulting in local and systemic insulin resistance.

The increased fasting blood glucose in this study alludes to the plausibility of a concurrent or preceding insulin resistance. Although, as a limitation, this study did not assess insulin concentration, the data on plasma lipids and inflammatory markers in high fat diet-fed rats show a profile with appreciable atherogenic tendencies (Figure 2) and inflammatory response (Figure 3), which are obtainable in insulin resistance condition. Also, TyG, a surrogate marker of insulin resistance, was slightly elevated in HFD group.(Figure 1B)These show that diminished insulin sensitivity in the high fat diet-fed animals could have led to heightened fasting glycaemia with associated lipid dysmetabolism and inflammatory responses as already accepted.[23] Congruently, a previous study has reported high fat diet-induced elevated blood glucose and atherogenic risk in offspring (pups) of normotensive and hypertensive rats.[24] Taken together, it is conceivable that high fat diet is a significant risk factor for metabolic syndrome and may predispose to both juvenile and adult metabolic diseases by initiating an atherogenic status and low-grade inflammation.

The involvement of low-grade inflammation and oxidative stress in the pathogenesis of high fat-diet-induced dysmetabolism is conclusive. In corroboration of the afore-

stated thought, data herein show that high fat diet-induced glucose deregulation is associated with elevated plasma CRP (Figure 3A), an acute phase protein which correlates with inflammatory responses. Congruently, it has been shown in a previous study that in pancreatic β cells, high fat diet evokes inflammation, endoplasmic reticulum stress and abortive adjustments to peripheral insulin resistance.[25] The present study also revealed that high-fat diet led to elevated plasma UA levels (Figure 3B). UA is thought to be two-edged in its impact on biological systems. However, it is more deleterious than beneficial when elevated. Hyperuricaemia has been linked to inflammatory responses via two mechanisms: activation of inflammasomes by UA crystals and production of ROS by xanthine oxidase activity.[26] Thus, this study shows that high-fat diet might induce UA-associated inflammation and/or oxidative stress, which can contribute immensely to the identified metabolic disorder.

However, the unique molecular event in high fat diet-engendered deleterious effect is worthy of note. The present study investigated the effect of high fat diet on HDL-C and data herein advances the possibility that alteration in the concentration of HDL-C molecule might play pivotal roles in high fat diet-induced inflammation and glucose dysregulation. More precisely, this study revealed that reduction in HDL-C by exposure to high fat diet leads to glucose dysregulation with a consequent hyperglycaemic-related expression of inflammatory mediators and aggravation of detrimental metabolic events such as increased UA production also leading to inflammation (Figure 4). In addition to beneficial modulation of glucose metabolism via insulin and non-insulin-dependent mechanisms,[7,8] several studies have verified that HDL-C reduces the activity of transcription factors and prevents transcription of inflammatory genes.[27-29] Other studies have documented the anti-inflammatory effects of HDL-C in immune cells,[28] and progenitor cells.[30] Therefore, low HDL-C levels as realized in the present study with high fat diet (Figure 2B) is not just passively associated with metabolic syndrome as a symptomatic outcome but plays an active role in promoting dysmetabolism and overt inflammation in multiple cell types with deleterious impact on both insulin secretion and sensitivity.

Therapy for a multi-factorial disease requires a pharmacological target of the ubiquitous mechanism involved in the pathophysiology. Some pharmacological agents in the treatment of obesity-associated diseases are not effectively producing effects because the target for most of the drug is not a universal mechanism for the multiple factors associated with the disease. Therefore, studies that can culminate in the characterization of safe and effective therapeutic candidates for the treatment of multi-factorial obesity-related diseases/metabolic syndrome should become the focus of collaborative studies in medical and paramedical fields. Interestingly, it is agreeable that a low HDL-C level is ubiquitously evident in a spectrum of co-morbid diseases of the, metabolic syndrome.[31] Therefore, insights into the effect of pharmacological agents on HDL-C molecules in the treatment of metabolic syndrome might assist in determining the efficacy of such an agent.

Interestingly, the augmentation and normalization of HDL-C in high fat diet-fed rats and elevation of HDL-C in normal diet-fed rats by BP treatment compared with control

rats in this study are novel findings and thought as being an underlying mechanism of BP beneficial effects in high fat diet-induced derangement. BP normalized fasting glycemia, LDL-C, TG, CRP, and UA in high fat diet-fed rats compared with high fat diet-fed rats which were not treated with BP (Figures 1-3). This outcome attests to both antidiabetic and anti-inflammatory effects of BP, which could be partly due to the antioxidant and anti-inflammatory constituents of the extract like tocopherol,[32] and ascorbic acid,[33] and partly due to its novel HDL-C increasing property. However, the fact that UA and CRP were reduced compared with control in normal diet-fed rats (which were normoglycaemic) treated with BP (Figure 3) is fascinating and shows that BP in healthy individuals might inhibit UA production and consequent inflammatory responses. This outcome could become eventful since inflammation and UA production are normal physiological processes with unique roles in biological systems. Therefore, caution is required if healthy individuals must ingest BP for plausible disease prevention.

In conclusion, BP leaf aqueous extract augmented HDL-C with accompanying improved fasting glycaemia and attenuated plasma TG, TC, LDL-C, CRP, and UA in high fat-fed rats. This beneficial anti-glycaemic and anti-inflammatory effect is linked to its characteristic HDL-C increasing property and this outcome may play an important role in the development of safe and effective therapy for obesity/high fat diet-associated diseases. It is, therefore, crucial to focus considerable research expertise on the study of BP to further validate this intriguing finding.

Conflict of interest

There is no known conflict of interest that could affect the outcome of this work.

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