

## Preliminary Phytochemical and Lipoprotein Studies of *Desmodium velutinum* Aqueous Leaves Extract on Albino Wistar Rats

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**Abstract:** Studies on phytochemical analyses and aqueous leaves extract of *Desmodium velutinum* was carried using albino Wistar rats. Twenty eight (28) albino wistar rats used for this study were randomly distributed into four groups (I to IV) of seven (7) rats each. Test animals in groups II, III, and IV were initially fed with a high fat diet (10mg/kg) for 12 days. Group II animals remained untreated. The groups III and IV animals were later treated with 5 mg/kg of atorvastatin and 5 mg/kg of *D. velutinum*, respectively for 4 days. However, the group I rats were fed with normal feed for 12 days and served as the control. The serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels were determined from blood samples of the various groups of rats. The result showed that there was a significant ( $p < 0.05$ ) reduction in the serum TC, HDL-C, LDL-C, and TG concentrations of test animals following the administration of the atorvastatin and the extract. Alkaloids, tannins, flavonoids and reducing sugars were found to be highly present in the crude extract. Other phytochemicals present in the extract were saponins, carbohydrate, steroids, cyanide and terpenoid. These findings indicate that the use of the extract lowered the serum lipid profile of albino wistar rats and may be of clinical importance to individuals at risk of cardiovascular disease.

**Keywords:** Hypolipidaemic, phytochemicals, *Desmodium velutinum*, atorvastatin, lipid profile.

### INTRODUCTION

Hyperlipidaemia promotes oxidative stress which leads to the development of atherosclerosis, coronary artery diseases and other complication of obesity [1]. In recent years, there has been a rapid interest in the use of herbal medicines all over the world including plant parts like seeds, berries, leaves, roots, barks or flowers for development of drugs that serve therapeutic purposes [2]. This study was carried out to determine the phytochemical composition and hypolipidaemic effect of *Desmodium velutinum* leaves on albino wistar rats. *Desmodium velutinum* (DV) serves many therapeutic purposes such as anti-diarrhoea and anti-pyretic [3], anti-inflammatory, anti-nephrolithic, and anti-bacterial [4]. Other traditional users claim that it serves as anti-tumour, anti-ulcer, antilipidaemic, analgesic, anti-malarial and most importantly as an aphrodisiac. *D. velutinum* is an erect perennial shrub which grows all over tropical Africa. In Nigeria, it spreads across the South Eastern part where it is referred to as "Ikeagwu-ani". The phytochemical

composition of the plant has not been fully studied, but ethanol extract of the leaves has demonstrated the presence of resins, tannins, saponins and flavonoids [3]. It is these phytochemicals which are non-nutritive chemicals that are believed to have the protective or disease preventive properties [5].

Foods with high content of low density lipoprotein lead to increase of cholesterol in human system. However, cholesterol is not harmful in small amount but its excess leads to heart disease by clogging of arteries. It is therefore recommended to consume more foods like fish and walnuts with high density lipoprotein (HDL) which is the good cholesterol [6], whole grains [7] and oils rich in omega-3-fatty acids as they remove bad cholesterol (LDL).

Indigenous herbs like the Nigerian *Spondias mombin* [8] and the Indian *Phyllanthus reticulatus* [1] are some examples of plant products with hypolipidaemic activity.

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The aim of this work is to investigate the possible effect of ingestion of the water extract of *D. velutinum* on some lipid parameters in healthy subjects using albino wistar rats. Lipoprotein parameters were investigated following their involvement in atherosclerosis.

## MATERIALS AND METHODS

### Identification and Extraction

Fresh and healthy leaves of *D. velutinum* were obtained from a bush in Umueze Awkunanaw at Nkanu-West Local Government Area of Enugu State, Nigeria. The plant was identified by Prof. J. C. Okafor of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria. The fresh leaves were air-dried for twenty days. Dried leaves were pulverized into fine powder with the aid of a clean dry electric grinder (Moulinex optiblend 2000, France). One hundred and fifty grams (150 g) of the pulverized leaves were soaked in 150 ml of distilled water for 24 hours. The mixture was later extracted by hot-continuous percolation method in a Soxhlet apparatus and the aqueous extract concentrated with the aid of a rotary evaporator. The concentrated extract was weighed and placed in two sterile containers labeled A and B and stored at 4°C in a refrigerator. The extract in container A was used for phytochemical analysis while the extract in container B was used for experimental animal model.

### Phytochemical analysis of aqueous extract of *Desmodium velutinum* leaves

The phytochemical analysis of the concentrated aqueous extract of *D. velutinum* leaves stored in Container A was carried out based on procedures outlined by Trease and Evans [9].

### Experimental Animal Model

Twenty eight (28) apparently healthy male wistar albino rats were obtained from Veterinary Medicine Department, University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were randomly distributed into four (4) different groups (I to IV) of seven (7) rats each. They were housed separately and allowed to acclimatize. Group I rats were fed only growers mash (Guniea feed, Nigeria) and water for twelve (12) days. Group II rats were fed with 10.0 mg/kg of high fat diet twice a day (morning and evening) for also 12 days. Group III rats were also fed with 10.0 mg/kg of the high fat diet for 12 days (morning and evening) and later were administered with daily dose, 5.0 mg/kg, of the hypolipidaemic drug (Atorvastatin) for the following four (4) days. Group IV rats were also fed with 10.0 mg/kg of the high fat diet

for 12 days (morning and evening) and later were administered with daily dose, 5.0 mg/kg, of the aqueous extract of *D. velutinum* leaves for the following 4 days. The high fat diet used was cow's brain and the feeding of the high fat diet and administration of the drug and extract were done orally. All the animals were allowed access to feed and water throughout the period of study.

### Blood Samples Collection

The collection of blood samples from the rats in each group was done by application of mild anaesthesia with chloroform, followed by simple dissecting of the rats and then cardiac puncture. About 7.0 to 9.0 ml of blood samples were collected in EDTA tubes from each group using a medical syringe. The blood samples were centrifuged to separate plasma from the blood which was used for the lipid analysis. Blood samples were collected from group I and II rats on the following day after the 12th day of feeding. Blood samples were collected from groups III and IV rats on the following day after the 4th day of administration of atorvastatin and the concentrated aqueous extract of *D. velutinum* leaves respectively.

### Lipid Profile Analysis

The total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triacylglycerol were determined using the method of Richmond [10].

### Statistical Analysis

The data obtained in this study were evaluated using statistical package for social science (SPSS). Significant levels were at  $P < 0.05$  and values were expressed as means of triplicate determinations  $\pm$  standard deviation (SD).

## RESULTS

The result of the preliminary phytochemical investigation on the aqueous extract of the sample is as presented in Table-1. Phytochemical result of the water extract of *D. velutinum* leaves indicated a greater composition of reducing sugar (321.74 mg/100 g) than steroid (0.63 mg/100 g). Flavonoids have been considered rich source of antioxidants and leaves of *D. velutinum* showed a higher content of flavonoid (3.82 mg/100 g). Tannin (2.87 mg/100 g) was present in lesser concentration compared to alkaloid (3.82 mg/100 g). The concentration of soluble carbohydrate (1.43 mg/100 g) in the aqueous extract of *D. velutinum* is higher when compared to its concentration of terpenoid (0.28 mg/100 g). In addition, 1.05 mg/100 g concentration of saponin was estimated to

**Table-1: Quantitative Phytochemical Composition of Aqueous Extract of *Desmodium velutinum* Leaves in mg/100g**

Phytochemical constituents	Quantitative composition (mg/100 g)
Soluble carbohydrate	1.43 ± 0.003
Reducing sugar	321.74 ± 0.003
Saponin	1.05 ± 0.003
Tannin	2.87 ± 0.004
Flavonoid	3.82 ± 0.003
Alkaloid	3.82 ± 0.003
Steroid	0.63 ± 0.004
Terpenoid	0.28 ± 0.005
Hydrogen cyanide	0.63 ± 0.003

**Table-2: Lipid Profile Analysis of Rats Blood Samples**

Groups	Cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Triacylglycerol (mg/dl)
Group I (normal feed)	140.00 ± 0.14	30.00 ± 1.41	125.00 ± 0.14	95.00 ± 1.41
Group II (high fat diet)	145.00 ± 0.14	40.00 ± 1.41	165.00 ± 0.14	105.00 ± 1.41
Group III (high fat diet + atorvastatin)	110.00 ± 0.14	13.00 ± 1.41	100.00 ± 0.14	40.00 ± 1.41
Group IV (high fat diet + extract)	125.00 ± 0.00	25.00 ± 0.00	115.00 ± 0.00	50.00 ± 0.00

Be present in the aqueous extract of *D. velutinum*. The result of the total lipoprotein assay of the rat's blood is as indicated in Table 2. The serum cholesterol of rats fed on high fat diet and later administered with 5 mg/kg of the crude extract of *D. velutinum* leaves lowered (125.00 mg/dl) significantly ( $P < 0.05$ ) when compared with rats in group II (145.00 mg/dl) fed with high fat diet alone. Also there was a significant reduction ( $P < 0.05$ ) in the serum cholesterol concentration (110.00 mg/dl) of rats fed with high fat diet and administered 5 mg/kg of atorvastatin in group III compared with those in group II (145.00mg/dl). Table 2 showed that the concentration high density cholesterol (HDL-cholesterol) of rats in the various groups varied significantly. Group II rats have their HDL-cholesterol (40.00 mg/dl) increased significantly when compared with those in group III (13.00 mg/dl) and group IV (25.00 mg/dl) administered 5 mg/kg of atorvastatin and 5 mg/kg of the crude extract, respectively. Atorvastatin significantly ( $P < 0.05$ ) lowered the LDL-cholesterol (100.00 mg/dl) of rats in group III when compared with the group IV rats (115.00 mg/dl) administered with 5 mg/kg of *D. velutinum* crude extract and group II rats (165.00 mg/dl) fed on high fat diet without the administration of any drug. The serum triacylglycerol of group II rats (105.00 mg/dl) remained significantly higher than those of rats in other groups. The administration of 5 mg/kg of atorvastatin significantly reduced the serum LDL-cholesterol of group III rats (40.00 mg/dl) in comparison with the administration of 5 mg/kg of the crude extract of *D. velutinum* leaves in the group IV rats (50.00 mg/dl).

## DISCUSSION

The investigations into secondary plant metabolites have led to important breakthroughs in pharmacology which has improved in the continuity of medicinal use of plant parts in management and treatment of diseases. These medicinal plants have continued to be a major source of commercially consumed drugs and consequently most synthetic drugs have their origin from natural plant products [11]. These secondary metabolites exhibit varied biochemical and pharmacological actions in animals when ingested [12]. Thus, this study shows in Table 2, the high content of tannin, alkaloid, flavonoid and reducing sugar observed in aqueous extract of leaves of *D. velutinum* plant. Tannin, alkaloid and flavonoid are types of phytochemicals that had been detected as constituents of plant extract that have lipid lowering effect [8]. Table-1 indicates higher alkaloid content than tannins; however alkaloid and flavonoid are significantly (level of significance) the same. The combined effect of these secondary metabolites has been associated with antioxidant, diuretic, antispasmodic, anti-inflammatory, analgesic and anti-microbial [13]. They can as well be responsible for the LDL lowering effect of the extract. Flavonoid, which is an antioxidant, is observed in the water extract of *D. velutinum* leaves. This antioxidant may have contributed in lowering the LDL-C in the animal models [14].

The presence of saponin as observed in Table-2, may have contributed to the lipid-lowering potential of aqueous extract of DV leaves as saponin is a known anti-nutritional factor that can reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intra-luminal physiochemical interactions or other yet unidentified [15]. The low presence of steroids ( $0.63 \pm 0.004$ ) as observed in

Table-1, also may have contributed to the lipid-lowering effect of aqueous extract of DV leaves as plant sterols are said to be effective in blocking the absorption of cholesterol by intestinal cells. As sterols inhibit HMG-CoA reductase [16], saponins increase the LDL receptor activity and excretion of bile acids [17]. These two constituents may have acted in synergy. The presence of hydrogen cyanide (HCN) in aqueous extract of *D. velutinum* leaves as observed in Table 2 possibly may have been introduced into the leaves through environmental activities. HCN prevents intracellular oxygen use causing aerobic energy production to cease. However, its presence was found in very small quantity ( $0.63 \pm 0.003$ ) and therefore poses no threat to individual's health. It is hypothesized that HDL can remove cholesterol from the arteroma within arteries and transport it back to the liver for excretion or re-utilization [8]. Thus, high level of HDL-C protects against cardiovascular and coronary artery disease. On the other hand, both the extract and atorvastatin, at their particular doses, significantly reduced the LDL-C concentration. Cholesterol transported to the arteries by LDL is retained by the arterial proteoglycans forming plaques [8]. When this LDL-C enters the endothelium and becomes oxidized it poses risk for cardiovascular and other vascular diseases, since the oxidized form is more easily retained by the proteoglycans. Therefore this observed LDL-C lowering effect of the extract indicates that the extract will be of clinical importance in prevention or reduction of cardiovascular risk factors like heart attacks and stroke and also in atherosclerosis and peripheral vascular diseases which are all associated with increased levels of LDL-C [18]. However, the increase in the extracts dose and duration of administration according to body weight could be significant in reducing LDL-C concentration. The observed significant changes in total cholesterol and triglyceride produced by the extract indicate its antilipidaemic potential and portend its remedy for cardiovascular, vascular and coronary artery disease.

## CONCLUSION

The aqueous extract of *D. velutinum* leaves has a general lipid lowering potential which is considered to be clinically beneficial to individuals at risk of cardiovascular, coronary artery and vascular diseases and even to obese individuals due to the high content of important secondary metabolites.

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