Cardioprotective Effects of Aqueous Leave Extract of *Desmodium adscendens*

**Seriki SA** and **Odetola AO**

**1Department of Human Physiology, College of Medicine, Bingham University, Nigeria**
**2Department of Human Physiology, College of Medical Sciences, Nnamdi Azikwe University, Awka, Nigeria**

*Corresponding Author: Seriki SA, Department of Human Physiology, College of Medicine, Bingham University, Nigeria.

Received: December 11, 2018; Published: January 09, 2019

**Abstract**

*Desmodium adscendens* is an ethno-medicinal plant with tremendous health benefits. It is effective against asthma, epilepsy, bronchitis, vaginal infections, central nervous system disorders among many others. This study was conducted to elucidate the effect of *D. adscendens* on some biochemical parameters in rats. Forty-eight (48) male Wistar rats were randomly assigned into four (4) groups, n = 12. Group A (control) received normal rat feeds and water. Group B received rat feeds and low dose of the extract (300 mg/kg body weight). Group C received rat feeds and median dose of the extract (450 mg/kg body weight). Group D was treated with high dose of the extract (600 mg/kg body weight) and was also fed with rat feeds. The administration was done orally and once daily for four (4) weeks. Blood samples were obtained via cardiac puncture after rats were put under chloroform anaesthesia. Results show that the LD50 of *D. adscendens* was 1,342.32 mg/kg. The extract has higher levels of polyphenols, flavonoids and reducing sugar (all antioxidants) compared to other phytochemical constituents like tannins, saponins, glycosides and alkaloids. The extract also increased triglycerides, HDL, and VLDL concentrations significantly (p < 0.05), but caused a significant (p < 0.05) decrease in total cholesterol and LDL concentrations.

**Keywords:** Triglycerides; Density Lipoprotein (DL); Low Density Lipoprotein (LDL)

**Introduction**

*Desmodium adscendens* is among notable medicinal plants in Africa and indeed the world. It’s of the Family - “Fabaceae”; and genus - *Desmodium*. Common names of *Desmodium adscendens* are Beggar-lice, Beggar weed, Tick Clover, Tick trefoil [1] and *Mbansang Ekpo* in Efik language, South-south, Nigeria.

It is a rainforest herb which has been traditionally used by the natives for a wide variety of medical conditions including: muscle cramp, tendon, spinal pain, bronchitis, epilepsy and some central nervous system disorders. Other uses include rheumatism, jaundice, hepatitis, protection of liver from cirrhosis, asthma (owing to its bronchial-dilating effects), allergic symptoms and eczema. It is also a very potent natural antispasmodic agent [1].

Other studies have revealed that *Desmodium adscendens* could also be used in women to manage leucorrhoea (a thick yellowish vaginal discharge usually caused by oestrogen imbalance), vaginal infections, and ovarian inflammations [2]. Also, disorders of the ovary and reproductive tract infections have been treated using extracts from the herb [3]. It has been known to also promote lactation in women. *Desmodium adscendens* has also been used in treating wounds and sores [4,5].

*Citation:* Seriki SA and Odetola AO. "Cardioprotective Effects of Aqueous Leave Extract of *Desmodium adscendens*". *EC Cardiology* 6.2 (2019): 130-139.
The therapeutic phytochemicals in *Desmodium adscendens* include alkaloids of the family of indolic, alkaloids flavonoids (such as astragalin, cosmosin), soyasaponins (such as dehydrosoyasaponin), and bioamine (tyramine). The plant contains about 4mg/kg of alkaloids expressed in tryptamine. Fatty acids are present to a concentration of about 3%, which is relatively rich in unsaturated acids.

The triterpenoid glycosides (and other phytochemicals such as beta-phenylethylamines and tetrahydroisoquinolines) found in *Desmodium adscendens* are very potent potassium channel agonists. They activate the calcium-dependent potassium ion channels; when potassium ions cross the cell membranes, while the tone in the smooth muscles is maintained.

The phytochemicals in the plant have no effects on the central nervous system (CNS), so drowsiness or impaired judgment does not occur with the administration of the *D. adscendens*. It reduces anaphylactic contractions, interfere with histamine-induced contractions, and reduce the amount of smooth muscle stimulating substances released from lung tissue of guinea pigs.

Biochemical analysis refers to a set of methods, assays, and procedures to elucidate chemical substances found in living organisms and the chemical reactions underlying life processes. Some of the parameters resulting from such analysis are; Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), protein, albumin, globulin, triglycerides, total cholesterol, urea and creatinine concentrations.

By biochemical analysis, the various changes in the blood, enzymes and electrolytes associated with the use of *Desmodium adscendens* are determined.

The leaves extract of *Desmodium adscendens* has been known to be useful in the management and treatment of several disease conditions. Knowledge of the biochemical parameters, as well as its effects on the histology of the heart has added to the existing body of knowledge about the plant.

To determine the effect of the extract on lipid profile: Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL).

**Material and Methods**

Fresh leaves of *Desmodium adscendens* were collected from the forest in Akpabuyo Local Government of Cross River State, Nigeria. The leaves were thoroughly rinsed in tap water twice to remove debris and were thereafter air dried for about 2 weeks under shade, away from direct sunlight to avoid possible damage to their phytochemical constituents. It was then cut into small pieces and crushed into coarse powder using an electric blender. The powder form of *Desmodium adscendens* was stored in a sealed bucket until required.

**Experimental animals**

A total of forty 48 adult male albino Wistar rats weighing between 120 - 160g were used for the experiment. The experimental animals were handled in accordance with the principles guiding the use and handling of experimental animals in Nigeria. The rats were maintained on standard rat feed (growers feed) and tap water available all through the period of experiment. The animals were maintained at an ambient temperature between 28 - 30°C, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 hours of light (06:30 hour - 18:30 hour) alternating with approximately 12 hours of darkness (18:30 hour - 06:30 hour). The rats were allowed to get familiarized with the environment for a period of 7 days before treatments commenced.
1. Control (Received normal rat chow and tap water).
2. Low dose treated group (Received low dose of extract (300 mg/kg)
3. Median dose treated group (Received middle dose of extract (450 mg/kg)
4. High dose treated group (Received high dose of extract (600 mg/kg).

Treatments lasted for a period of four (4) weeks, all animals had free access to feeds and water ad libitum. Extract administration was done orally with the aid of an orogastric cannula.

Collection of blood samples
At the end of treatment period, animals from all the experimental groups were sedated and made unconscious using chloroform anaesthesia. Blood samples from each rat was collected via cardiac puncture [6] into EDTA and plain sample bottles for the estimation of haematological and biochemical parameters.

Analysis of serum
Serum from the different groups was analyzed for biochemical analysis (Lipid Profile); Total cholesterol (TC), Triglyceride (TG), Low density lipoproteins (LDL-c), High density lipoproteins (HDL-c), and very low density lipoproteins (VLDL-c).

Determination of Lipid profile

***Triglyceride (TG) Level by GPO-PAP method

**Principle:** The triglycerides are determined after hydrolysis with lipase. The glycerol formed as a product of the reaction goes through a series of reaction shown below to ultimately produce a by-product, hydrogen peroxide which is quantified via an indicator system. The indicator is quinone imine formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase. The concentration of H\textsubscript{2}O\textsubscript{2} is directly proportional to the intensity of the colour and hence the triglycerides.

\[
\text{Triglycerides} + \text{H}_2\text{O} \xrightarrow{\text{LPL}} \text{glycerol} + \text{fatty acid}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{DHAP} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + 4\text{-chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCl} + 4\text{H}_2\text{O}
\]

The serum level of triglycerides (TG) was estimated by the GPO-PAP method using reagent kit method of USA Randox Laboratories San Francisco).

**Procedure:** Three set of tubes were cleaned, dried and labelled B (blank), S (standard) and T (test), to each set of tubes was added 1.0 ml of reconstituted (working) reagent. 10 μl (0.01 ml) o serum sample, distilled water and standard were respectively pipette into the B, S and T-tubes respectively and allowed to mix and incubate for at 20 - 25°C. At 546 nm, the absorbance of the standard (A\textsubscript{std}) and test samples (A\textsubscript{samples}) was read against the blank (B\textsubscript{blank}) with a Mindray Chemistry Analyzer BS-120.
Calculation

Triglyceride concentration (mg/dl)

\[
\text{Triglyceride} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{std}} - A_{\text{blank}}} \times C_{\text{std}}
\]

\(A_{\text{sample}}\) = Absorbance of sample
\(A_{\text{std}}\) = Absorbance of standard
\(A_{\text{blank}}\) = Absorbance of blank
\(C_{\text{std}}\) = Concentration of standard

***Total Cholesterol (TC) - CHOD-PAP method

**Principle:** Cholesterol esters are hydrolysed into free cholesterol and fatty acids by cholesterol esterase. The free cholesterol is then oxidized to 4-cholesten-3-one and hydrogen peroxide. The hydrogen peroxidase then combines with phenol and 4-aminoantipyrine catalyzed by peroxidase to produce a red coloured quinonimine which is read colourimetrically at 546 nm. The intensity of the colour produced is directly proportional to the total cholesterol concentration of the sample.

**Procedure:** Three sets of tubes were cleaned, dried and labelled A (Blank), S (standard) and T (test). To each of the tubes was added 1000 ml (0.1 ml) of the working reagent while 10 μl (0.01 ml) of the serum and standard were respectively added to T-tubes and S-tubes. The contents of the tubes were mixed and incubated for 10 minutes at 20 - 35°C (room temperature). At 346 nm, the absorbance of test samples and standard was read against the reagent blank within 60 minutes.

Calculation

Total serum cholesterol (mg/dL)

\[
\text{Total Cholesterol} = \frac{A_{S_{\text{sample}}} - A_{S_{\text{blank}}}}{A_{S_{\text{std}}} - A_{S_{\text{blank}}}} \times C_{S_{\text{std}}}
\]

\(A_{S_{\text{sample}}}\) = Absorbance of sample
\(A_{S_{\text{std}}}\) = Absorbance of standard
\(A_{S_{\text{blank}}}\) = Absorbance of blank
\(C_{S_{\text{std}}}\) = Concentration of standard

***High-Density Lipoprotein-cholesterol (HDL-c) concentration

**Principle:** Low-density lipoproteins (LDL and VLDL) and chylomicrons fractions were precipitated quantitatively from the serum samples by the addition of phosphotungstic acid in the presence of magnesium ions (MgCl₂). The sample was then centrifuged. After centrifugation, the cholesterol concentration in the HDL (high-density lipoprotein) fraction, which remained in the supernatant, was determined according to the mono-reagent enzymatic colourimetric end point method (CHOD-PAP assay) for total cholesterol determination as previously described. Aliquots (200 μl or 0.2 ml) of samples (semi-microquantities) were used for this determination.

**Procedure:** Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remained in the supernatant was determined. 200 ml (0.2l) of sample and 200 μl of standard were respectively pipetted into centrifuge tubes containing 500 μl (0.5 ml). On mixing, each tube was allowed to sit for 10 minutes at room temperature. The mixture was then centrifuged for 10 minutes at 4000 rpm. The clear supernatant was separated off within 2 hours and its cholesterol content determined by the CHOD-PAP described for cholesterol above.

---

*Citation:* Seriki SA and Odetola AO. “Cardioprotective Effects of Aqueous Leave Extract of *Desmodium adscendens*. EC Cardiology 6.2 (2019): 130-139.
Cardioprotective Effects of Aqueous Leave Extract of *Desmodium adscendens*

**Calculation**

HDL-cholesterol (mg/dL) in supernatant

\[
\text{HDL-cholesterol} = \frac{A_{\text{Sample}}}{A_{\text{St}}} \times C_{\text{Std}}
\]

**Very Low Density Lipoprotein cholesterol (VLDL-c) concentration**

The VLDL-cholesterol concentration was obtained by dividing the serum triacylglyceride value by 5 (for values mg/dl) or by 2.2 (for values in mmol/dl). The factor “5” for values in mg/dl is based on the assumption or understanding that in fasting subject with triacylglyceride concentration of 400 mg/dl or 176 mmol/L, the VLDL to plasma triacylglyceride ratio is fixed relatively at 1:5 or 1:2.2 respectively.

**Calculation:** VLDL-cholesterol (mmol/L) = TG/2.2

VLDL-Cholesterol (mg/dl) = TG/5

**Low-Density Lipoprotein-cholesterol concentration**

According to the Friedewald’s relationship, low-density lipoprotein-cholesterol (LDL-C) is calculated from the difference between the total serum cholesterol and the sum of HDL-cholesterol (HDL-c) and very low-density lipoprotein cholesterol (VLDL-c).

**Calculation:** LDL-cholesterol = Total cholesterol - (HDL-c+ VLDL-c)

**Results**

**Comparison of lipid profile in the different experimental groups**

**Total cholesterol (TC)**

Comparison of Total Cholesterol Concentration in the different experimental groups is shown in figure 1. Result shows that all the three groups given low, median and high doses of *Desmodium adscendens* showed significant (p < 0.05) decrease in total cholesterol concentration when compared with the control (3.40 ± 0.06 mmol/L) group at p < 0.05.

![Figure 1: Comparison of total cholesterol concentration in the different experimental groups.](image)

Values are expressed as mean ±SEM, n = 6.

* = Significantly different from control at p < 0.05.

a = Significantly different from low dose at p < 0.05.

*Citation:* Seriki SA and Odetola AO. “Cardioprotective Effects of Aqueous Leave Extract of *Desmodium adscendens*. EC Cardiology 6.2 (2019): 130-139.
Triglyceride concentration (TG)

Comparison of Triglyceride concentration in different experimental groups is as shown in figure 2. Result shows that all the three groups given low, median and high dose of extract showed significant (p < 0.05) increase in triglyceride concentration when compared with the control (0.89 ±0.01 mmol/L) group at p < 0.05. Median and high doses groups showed significant (p < 0.05) decrease when compared with the group given low dose (1.07 ± 0.05 mmol/L) at p < 0.05.

![Figure 2: Comparison of triglyceride concentration in the different experimental groups. Values are expressed as mean ±SEM, n = 6.
* = Significantly different from control at p < 0.05.
a = Significantly different from low dose at p < 0.05.
b = Significantly different from median dose at p < 0.05.](image)

High density lipoprotein cholesterol concentration (HDL-c)

Comparison of High Density Lipoprotein Cholesterol concentration in the different experimental groups is as shown in figure 3. Result shows that all the three groups given low, median and high doses of extract showed significant (p < 0.05) increase in HDL-c at p < 0.05 when compared with control (0.84 ± 0.01 mmol/L).

Median and high doses groups also showed significant increase in HDL-c when compared with low dose group at p < 0.05.

Low density lipoprotein cholesterol concentration (LDL-c)

Comparison of Low Density Lipoprotein Cholesterol concentration (LDL-c) in the different experimental groups is as shown in figure 4. Result shows that all the three groups given low, median, and high doses of extract showed significant (p < 0.05) decrease in LDL-c compared to control (2.17 ± 0.05 mmol/L).

_Citation:_ Seriki SA and Odetola AO. “Cardioprotective Effects of Aqueous Leave Extract of Desmodium adscendens”. _EC Cardiology_ 6.2 (2019): 130-139.
Figure 3: Comparison of high density lipoprotein cholesterol concentration in the different experimental groups. Values are expressed as mean ±SEM, n = 6.

* = Significantly different from control at p < 0.05.
a = Significantly different from low dose at p < 0.05.
b = Significantly different from median dose at p < 0.05.

Figure 4: Comparison of low density lipoprotein cholesterol concentration in the different experimental groups. Values are expressed as mean ±SEM, n = 6.

* = Significantly different from control at p < 0.05.
a = Significantly different from low dose at p < 0.05.
b = Significantly different from median dose at p < 0.05.

Citation: Seriki SA and Odetola AO. "Cardioprotective Effects of Aqueous Leave Extract of Desmodium adscendens". EC Cardiology 6.2 (2019): 130-139.
The groups given median and high doses of extract also showed significant (p < 0.05) decrease in LDL-c when compared with the low dose group.

**Very low-density lipoprotein cholesterol concentration (VLDL-c)**

Comparison of Very Low-Density Lipoprotein Cholesterol concentration (VLDL-c) in the different experimental groups is as shown in figure 5. Result shows significant (p < 0.05) increase in VLDL-c in all the three groups given low, median and high doses of the extract when compared with the control (0.40 ±0.00 mmol/L) group at p < 0.05.

Figure 5: Comparison of very low density lipoprotein cholesterol concentration in the different experimental groups.

Values are expressed as mean ±SEM, n = 6.

* = Significantly different from control at p < 0.05.

a = Significantly different from low dose at p < 0.05.

b = Significantly different from median dose at p < 0.05.

Median and high doses groups showed significant (p < 0.05) decrease in VLDL-c when compared with low dose group.

**Discussion**

The results show that extract of Desmodium adscendens increased triglycerides, HDL, and VLDL concentrations significantly (p < 0.05), but caused a significant (p < 0.05) decrease in total cholesterol, and LDL concentrations, meaning extract favours the good cholesterol (HDL-c) which helps remove the bad cholesterol (LDL-c) from the blood vessels. So, it has the potential to protect the cardiovascular system (blood vessels and heart).

This may be attributed to the presence of polyphenols, flavonoids, and saponins in D. adscendens leaves.

Polyphenols, which are in high concentration in D. adscendens leaves reduce the build-up of low density lipoprotein (LDL) or bad cholesterol in the blood vessels by increasing high density lipoprotein (HDL) or good cholesterol which mops up the LDL and prevents blood dots thereby reducing the risk of cardiovascular congestion. The cholesterol-lowering effect of saponins has been known for decades.

**Citation:** Seriki SA and Odetola AO. “Cardioprotective Effects of Aqueous Leave Extract of Desmodium adscendens”. EC Cardiology 6.2 (2019): 130-139.
Administering a certain saponin extract to rats with high cholesterol has been reported to reduce LDL (the bad cholesterol) without affecting HDL (the good cholesterol). These may have accounted for the cardio-protective benefits of *D. adscendens*.

Flavonoids have been shown to inhibit coagulation, thrombus formation or platelet aggregation, reduce risk of atherosclerosis, reduce arterial blood pressure and risk of hypertension, reduce oxidative stress and related signalling pathways in blood vessel cells, modify vascular inflammatory mechanisms, improve endothelial and capillary function, modify blood lipid levels, regulate carbohydrate and glucose metabolism [9]. *D. adscendens* leaves have very high concentration of flavonoids and may have accounted for its cardio-protective benefits.

The features of the histology of the heart tissues in the control are quite similar, with normal size cardiac myocytes which are ovoid in shape, and with a clear zone at the poles. There are also inter-digitation of muscle fibres and thin walled blood vessel in both the control and treated groups. This implies that the extract may not cause any toxic effect to the heart tissues.

*D. adscendens* leaf extract increased high density lipoprotein, triglycerides, and VLDL concentrations significantly (p < 0.05), but also caused a significant (p < 0.05) decrease in total cholesterol and LDL-c concentrations, indicating that extract has cardio-protective potentials.

**Figure 6:** Photomicrograph of a section cardiac muscle in; a) control; b) low dose; c) median dose group and d) high dose extract fed groups. x400. **MF:** Muscle Fibres; **N:** Nucleus.

---

*Citation:* Seriki SA and Odetola AO. “Cardioprotective Effects of Aqueous Leave Extract of Desmodium adscendens”. *EC Cardiology* 6.2 (2019): 130-139.
**Conclusion**

*Desmodium adscendens* protects the heart and the entire cardiovascular system by the presence of the good Cholesterol which mops up the bad cholesterol for the cardiovascular system and also reduces clotting tendencies.

**Bibliography**