



Effect of *Cnidoscopus aconitifolius* (Family Euphorbiaceae) Aqueous Leaf Extract on Some Antioxidant Enzymes and Haematological Parameters of High Fat Diet and Streptozotocin Induced Diabetic Wistar Albino Rats.

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KEYWORDS: Phytochemical, vitamin, *Cnidoscopus aconitifolius*, SOD, CAT, TBARS, PCV, Hb

ABSTRACT: This study was carried out to evaluate the oxidative and haematologic effects of aqueous extract of *Cnidoscopus aconitifolius* (CA) in high fat diet (HFD) and streptozotocin (STZ) induced diabetes in Wistar albino rats. Diabetes was induced by feeding the rats with HFD that consisted of 20% sucrose and 20% lard for 4 weeks, followed by a single dose intraperitoneal injection of STZ (40mg/kg body weight (BW)). The aqueous leaf extract of CA was administered orally and daily at 400, 600 and 800mg/kg BW from 7days after induction of diabetes and lasted for 12 weeks, while the normal control and diabetic control rats received regular diet and HFD respectively. Metformin (50mg/kg BW) was used as a standard antidiabetic drug. The animals were anaesthetized and sacrificed to obtain blood by cardiac puncture. Preliminary phytochemical analysis of CA showed the content of tannins (5.72±0.00), saponins (12.49±0.021), alkaloids (17.45 ±0.65), flavonoids (23.72 ±0.02), cyanogenic glycosides (0.75 ±0.10) and phytate (1.97 ±0.06). The six vitamins analysed, showed the concentration of vitamin A (5.24mg/kg), vitamin B₃ (1.40mg/kg), vitamin B₆ (37.23mg/kg), vitamin B₁₂ (15.98mg/kg), vitamin C (382.00mg/kg) and vitamin E (18.28mg/kg). There was significantly (p<0.05) reduced activity of catalase (CAT), significantly (p<0.05) increased level of TBARS, and non significantly (p>0.05) reduced activity of superoxide dismutase (SOD), Packed Cell Volume (PCV) and haemoglobin (Hb) concentration in HFD/STZ induced diabetic rats. CA significantly (p<0.05) increased the SOD and CAT activities, PCV, and Hb concentrations of the treated diabetic rats (TDR) while the TBARS was significantly (p<0.05) decreased compared to the diabetic control (DC). Our findings suggest that CA exhibited reversal effects on these selected oxidative and haematologic markers in rats which were previously damaged by HFD and STZ. © JASEM

<http://dx.doi.org/10.4314/jasem.v19i2.5>

Introduction

Free radicals can be defined as atoms or group of atoms with unpaired electrons that are capable of interacting with molecules thereby causing serious damage to living tissues. The presence of free radicals within the body has been shown to play a significant role in the development and progression of many disease processes such as heart disease, congestive heart failure, hypertension and diabetic complications (Chen *et al.*, 2002). There is increasing evidence that oxidative stress induced by the generation of free radicals is associated with complications related to diabetes (Hussein, 2008). Hyperglycaemia causes glucose autooxidation, protein glycation, and advanced glycation end products (AGE) formation which could lead to the development of diabetic complications including retinopathy, neuropathy, and macro- and microvascular damage (Jack and Wright, 2012). In

diabetes, oxidative stress has been found to be mainly due to an imbalance between radical-generating and radical-scavenging systems with increased production of oxygen free radicals and a sharp reduction of antioxidant defences or both (Maritim *et al.*, 2003; Oberley, 1988). Oxidation of macromolecules such as proteins, lipids, carbohydrates and DNA is elevated in oxidative stress. The harmful effects of free radicals are neutralized by the enzymatic antioxidant defenses including the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Antioxidants are substances that can safely interact with the free radicals and significantly delay, scavenge or inhibit these radicals from their deleterious effects on the tissues. Hence, compounds with antioxidative properties would be useful antidiabetic agents (Hussein, 2008). Numerous natural antioxidants have already been isolated from

different varieties of plant materials such as leafy vegetables, fruits, seeds, cereals and algae (Pokorny, 1991). They have been shown to have free radical scavenging and lipid peroxidation preventive effects (Atawodi, 2005; Aqil *et al.*, 2006). The protection can be explained by the capacity of the antioxidants phenolics, flavonoids and polypropanoids in the plants and plant products to scavenge free radicals. Thus, there is need for more medicinal plants with excellent antioxidant and haemopoietic properties which would serve as natural therapies for the treatment of diseases associated with oxidative stress and also boost the systemic blood flow. *Cnidoscopus aconitifolius* (family Euphorbiaceae), commonly called chaya is a leafy perennial shrub native of Yucatan peninsula of Mexico in Central America (Kolterman *et al.*, 1984; Ranhotra *et al.*, 1998). The plant which is also called spinach tree is consumed as vegetable in soups, salads and therapeutically used for a number of ailments such as diabetes, arteriosclerosis, gallstone and high cholesterol (Kuti and Torres, 1996). Chaya is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants (Kuti and Konuru, 2004) and have a possible antidiabetic effect (Kuti and Torres, 1996).

This study investigated the effect of *Cnidoscopus aconitifolius* on some haematologic and oxidative stress parameters, that is, activity levels of antioxidant enzymes (SOD and CAT) and the amount of Lipid peroxidation product in the plasma of diabetic rats; and some phytochemical and vitamin constituent of this plant extract were also investigated.

MATERIALS AND METHODS

Collection and authentication of plant materials: *Cnidoscopus aconitifolius* was collected from Woji town in Obio-Akpor Local Government Area of Rivers State, South-South Nigeria. The plant material was identified by Prof. B.E. Okoli of the Department of Plant Science and Biotechnology, University of Port Harcourt. The leaves were removed, cleaned and air dried at 28°C for about 28days before use.

Determination of phytochemical composition: Quantitative phytochemical analysis to determine the level of alkaloids, tannins, saponins, phytate, flavonoid and cardiac glycosides using standard methods as described by Trease and Evans (1985), Harbone (1984) and Sofowora (2008) were carried out.

Determination of vitamin composition: The vitamin composition was determined using UV-visible

spectrophotometer. To 10mls of methanol was added 0.5g of sample. It was filtered and poured into a cuvette for analysis using uv-visible speed for various vitamins based on their standard calibration curves.

Procurement of animals and preparation of the aqueous leaf extract: Wistar albino rats (weighing 180-230g) were obtained from the animal houses of the Departments of Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State and Nnamdi Azikiwe University, Awka, Anambra State respectively. The leaves were air dried and ground into powder. The powder was freshly soaked in boiled water for ten minutes after which the resultant mixture was filtered and administered to the rats. The extract was quantified by drying 1 ml of the homogeneous filtrate (by controlled heating i.e. in an oven kept at 40°C) in a pre-weighed watch glass.

Experimental design: The animals were housed in plastic metal top cages in the animal house of the Department of Biochemistry, University of Port Harcourt. The rats were fed standard diet and allowed access to clean water for a period of one week acclimatization. Animals were then weighed and randomly divided into six groups with twelve rats in each group:

Normal control (NC): non diabetic: received normal diet and distilled water vehicle only.

Diabetic control (DC): diabetic: received distilled water vehicle only. Metformin (met): diabetic: treated with standard antidiabetic agent, metformin at 50 mg/kg body weight. Groups A1, A2 and A3: diabetic: treated with *Cnidoscopus aconitifolius* leaf aqueous extract at 400 mg/kg, 600 mg/kg and 800 mg/kg body weight, respectively.

Induction of diabetes: Rats in groups 2 to 6 were fed with high fat diet (20% sucrose +20% lard + 60% standard diet) for four weeks and then injected at third week of feeding with 40% Streptozotocin in distilled water to induce diabetes. The development of hyperglycemia in rats was allowed for 6 days after STZ injection after which the blood glucose level was analyzed and the treatment was started, which lasted for twelve (12) weeks. The DiabetimTM (metformin) and extracts were administered daily by intra-gastric gavage.

Blood collection: At the end of weeks 3, 6, 9 and 12, three rats from each group were fasted overnight and anaesthetized by exposure to chloroform and then sacrificed. Blood was collected from each rat into EDTA, heparinized and plain tubes for the determination of haematologic and serum enzyme levels respectively.

Estimation of Superoxide dismutase Activity: Superoxide dismutase activity was assayed according to the method by Misra and Fridovich (1972). The assay procedure involves inhibition of epinephrine autooxidation to adrenochrome in an alkaline medium (pH 10.2), which was markedly inhibited in the presence of SOD.

Estimation of Catalase Activity: Catalase activity was determined according to the method of Beers and Sizer (1952). Catalase enzyme activity was determined on the basis of hydrogen peroxide decomposition.

Estimation of Lipid peroxidation product: Lipid peroxidation (LPO) was estimated by assessing the

concentration of thiobarbituric acid reactive species (TBARS) in the plasma of the rats. TBARS was analyzed and expressed as the amount of MDA formed according to the method of Fraga *et al.* (1988).

Assay of haematologic parameters: The haemoglobin (Hb) concentration and packed cell volume (PCV) were analyzed according to the standard techniques described by Schalm *et al.*, 1975 and Cheesbrough (2006).

Statistical Analysis: All data are expressed as Mean \pm SEM. Statistical differences were evaluated by analysis of variance (ANOVA) and values with $p < 0.05$ are considered as significant.

RESULTS AND DISCUSSION

Table 1: Phytochemical composition of the leaves of *Cnidoscopus aconitifolius*.

Parameter	Composition (%) Dry weight <i>Cnidoscopus aconitifolius</i>
Tannin	5.72 \pm 0.00
Phytate	1.97 \pm 0.06
Saponin	12.49 \pm 0.021
Alkaloid	17.45 \pm 0.65
Flavonoid	23.72 \pm 0.02
Cyanogenic Glycoside	0.75 \pm 0.10

The phytochemical composition of the leaves of *Cnidoscopus aconitifolius* was analysed and the results presented in Table 1. The data shows that concentration of tannins is 5.72 \pm 0.00, saponins is 12.49 \pm 0.021, alkaloids is 17.45 \pm 0.65, flavonoids is 23.72 \pm 0.02, cyanogenic glycosides is 0.75 \pm 0.10 and phytate is 1.97 \pm 0.06.

The vitamins composition of the leaves of *Cnidoscopus aconitifolius* was analysed and the results presented Table 2. The data shows that concentration of vitamin A is 5.24 mg/kg, vitamin B₆ is 37.23 mg/kg, vitamin B₁₂ is 15.98 mg/kg, vitamin C is 382.00 mg/kg and vitamin E is 18.28 mg/kg.

Table 2: Vitamin composition of the leaves of *Cnidoscopus aconitifolius*

Vitamins	Composition(mg/kg) Dry weight <i>Cnidoscopus aconitifolius</i>
Vitamin A	5.24
Vitamin B ₃	1.40
Vitamin B ₆	37.23
Vitamin B ₁₂	15.99
Vitamin C	382.00
Vitamin E	18.28

Effect of *C. aconitifolius* on SOD activity of HFD/STZ induced diabetic rats.

Figure 1 shows the effect of the aqueous extract of the leaves of *C. aconitifolius* on SOD activity of HFD/STZ induced diabetic rats. In all the weeks, the SOD activity of the DC decreased, but non significantly ($P > 0.05$) when compared to that of the NC. Met, A2 and A3 in week 3, A1 and A2 in week 6 and A1 in weeks 9 and 12 significantly ($P < 0.05$) increased the SOD activity of the treated diabetic rats (TDR) compared to that of the DC. In week 9, A1 significantly ($P < 0.05$) increased the SOD activity compared to that of met.

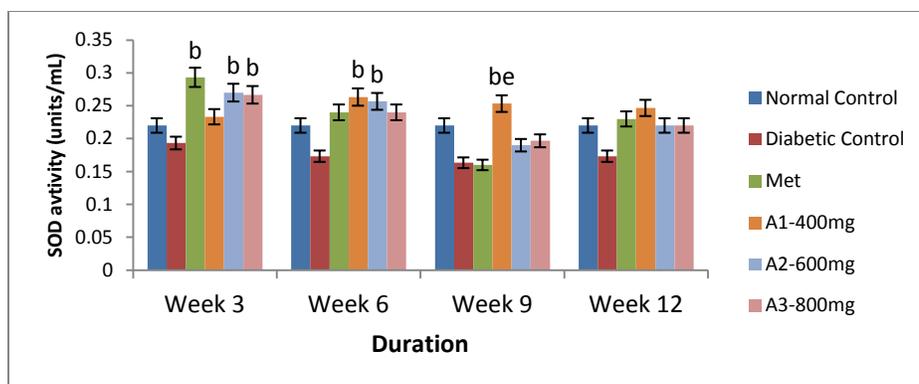


Fig 1: Effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on SOD activity of hfd/stz induced diabetic rats.

Data represent Mean \pm S.E.M., n=3 per group. Superscript a, b and e, indicate significant difference ($p < 0.05$) compared to normal control, diabetic control & met respectively.

Effect of *C. aconitifolius* on CAT activity of HFD/STZ induced diabetic rats.

Figure 2 shows the effect of the aqueous extract of the leaves of *C. aconitifolius* on CAT activity of

HFD/STZ diabetic rats. In all the weeks, the CAT activity of the DC decreased significantly ($P < 0.05$) compared to NC. Met, A1, A2 and A3 in week 3, Met and A3 in week 6, A1, A2 and A3 in week 9, Met, A1, A2 and A3 in week 12 significantly ($P < 0.05$) increased the CAT activity of the TDR compared to that of DC. A3 in week 3 and A2 in week 9 significantly ($P < 0.05$) increased the CAT activity when compared to that of met.

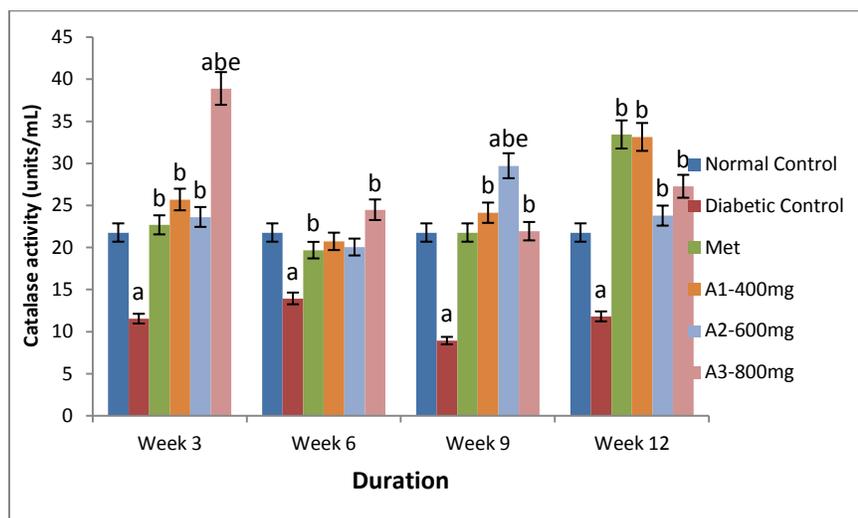


Fig 2: Effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on CAT activity of hfd/stz induced diabetic rats

Data represent Mean \pm S.E.M., n=3 per group. Superscript a, b and e, indicate significant difference ($p < 0.05$) compared to normal control, diabetic control & met respectively.

Effect of *C. aconitifolius* on TBARS concentration of HFD/STZ induced diabetic rats. Figure 3 shows the effect of the aqueous extract of the leaves of

Cnidoscopus aconitifolius on TBARS concentration of HFD/STZ induced diabetic rats. In all the weeks, the TBARS concentration of the DC increased significantly ($P < 0.05$) compared to that of NC. A2 in week 6, Met, A1, A2 and A3 in week 12 significantly ($P < 0.05$) lowered the TBARS concentration of the TDR compared to that of DC. In week 12, A1, A2

and A3 significantly ($P < 0.05$) lowered the TBARS concentration compared to that of met

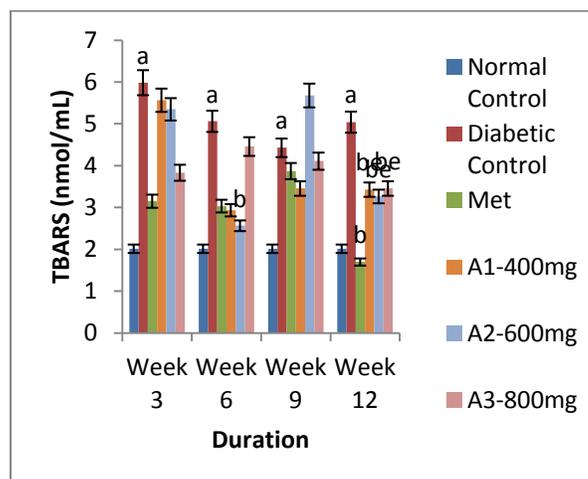


Fig 3: Effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on TBARS concentration of hfd/stz induced diabetic rats.

Data represent Mean \pm S.E.M., n=3 per group. Superscript a, b and e, indicate significant difference ($p < 0.05$) compared to normal control, diabetic control & met respectively.

Effect of *C. aconitifolius* on PCV of HFD/STZ induced diabetic rats.

Figure 4 shows the effect of the aqueous extract of the leaves of *C. aconitifolius* on PCV of HFD/STZ

induced diabetic rats. The PCV of the DC decreased non significantly ($P > 0.05$) compared to the normal control in all the weeks. A2 in week 3, A1 and A2 in week 9, A1, A2 and A3 in week 12 significantly ($P < 0.05$) increased the PCV of the TDR compared to that of DC.

In week 12, A3 significantly ($P < 0.05$) increased the PCV of the rats compared to that of met.

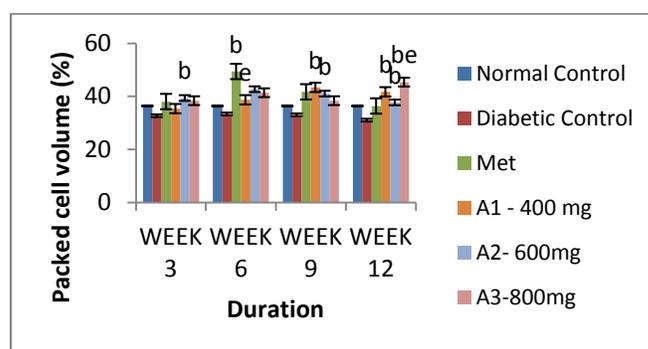


Fig 4: Effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on packed cell volume of hfd/stz induced diabetic rats

Data represent Mean \pm S.E.M., n=3 per group. Superscript a, b and e, indicate significant difference ($p < 0.05$) compared to normal control, diabetic control & met respectively.

Effect of *C. aconitifolius* on haemoglobin concentration of HFD/STZ induced diabetic rats.

Figure 5 shows the effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on haemoglobin concentration of HFD/STZ induced diabetic rats. The haemoglobin concentration of the DC decreased non significantly ($P > 0.05$) compared to the NC in all the weeks. A2 in week 3, A1 and A2 in week 9, A1, A2 and A3 in week 12 significantly

($P < 0.05$) increased the haemoglobin concentration of the TDR compared to that of DC. In week 12, A3

significantly ($P < 0.05$) increased the haemoglobin concentration of the rats compared to that of met.

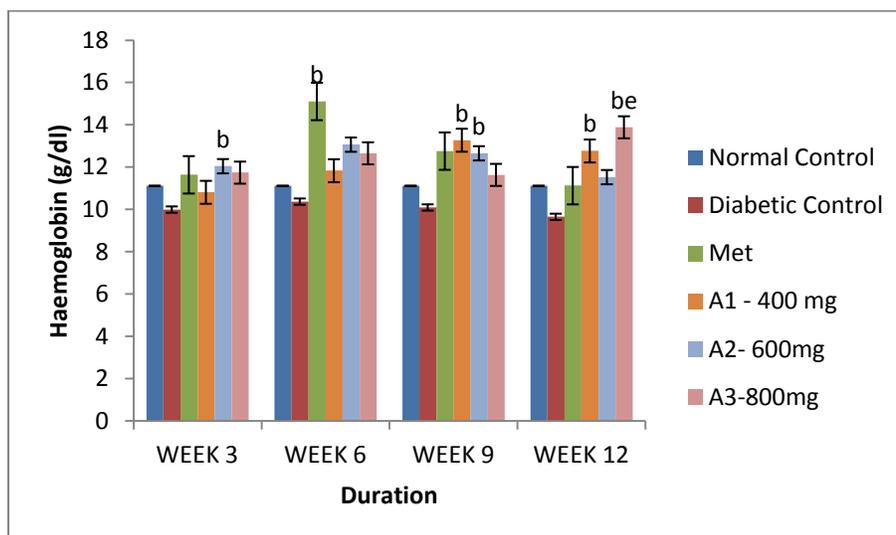


Fig 5: Effect of the aqueous extract of the leaves of *Cnidoscopus aconitifolius* on haemoglobin concentration of hfd/stz induced diabetic rats.

Data represent Mean \pm S.E.M., $n=3$ per group. Superscript a, b and e, indicate significant difference ($p < 0.05$) compared to normal control, diabetic control & met respectively.

Phytochemical constituents are responsible for medicinal activity of plant species. In this present study, preliminary phytochemical analysis revealed a large amount of flavonoids, phytate, saponin, alkaloid, tannins and cyanogenic glycosides present in aqueous leaf extract of *Cnidoscopus aconitifolius*. The results of the phytochemical suggest that the plant possess potential anti-inflammatory, antimicrobial and antioxidant properties. Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols. The flavonoids show antioxidant activity and have strong anti-cancer activity (Salah *et al.*, 1995). Flavonoids exhibit their antioxidative properties through several mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995; Kessler *et al.*, 2003), they also inhibit microbes which are resistant to antibiotics (Linuma *et al.*, 1994). Tannin is a complex moiety with wide pharmacological activities and is produced by majority of plants as protective substance. Tannin has astringent property, hastens the healing of wounds and inflamed mucous membrane and has

been used since past as tanning agents. Tannin has received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties (Killedar and More, 2010; Asquith and Butter, 1986). Saponins protect against hyperglycaemia, hypercholesterolaemia, hypertension (Trease and Evans, 1985), have antibiotic properties and anti-inflammatory property and aid healing (Krishnaiah *et al.*, 2009). Phytate has been linked to the prevention of kidney stone, dental decay and calcification of blood vessels. Alkaloids have been reported to be powerful pain relievers, exert an anti-pyretic, antihypertensive, antifungal, antiinflammatory, antifibrogenic effect (Awoyinka *et al.*, 2007), stimulating, anaesthetic action (Edeoga and Enata, 2001) and inhibiting activity against most bacteria (Al-Bayati and Sulaiman, 2008). The cardiac glycoside has been used for over two centuries as stimulating cases of cardiac failure and diseases (Olayinka *et al.*, 1992).

Vitamin A is important for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions (Lukaski, 2004). Vitamin C is a potent antioxidant that facilitates the transport and uptake of non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. Its

deficiency includes fragility to blood capillaries gum decay, scurvy (Achikanu *et al.*, 2013). Vitamin E is a powerful antioxidant which helps to protect cells from damage by free radicals and it is vital for the formation and normal function of red blood cells and muscles (Lukaski, 2004). Adequate supply of dietary antioxidants may prevent or delay diabetes complications including renal and neural dysfunction by providing protection against oxidative stress (Bartlett and Eperjesi, 2008). B vitamins are essential for growth, development, and a variety of other bodily functions. They play a major role in the activities of enzymes (American Cancer Society, 2013).

Hyperglycaemia increases the production of free radicals and reduces antioxidant defence systems of the body (Vincent *et al.*, 2004; Pop-Busui *et al.*, 2006). These imbalances lead to tissue oxidative stress. Oxidative stress is associated with the peroxidation of cellular lipids, which is determined by measurement of Thiobabutaric acid reactive substances (TBARS). Many studies have shown that hyperglycaemia was able to increase tissue oxidative stress via activating the polyol pathway, nonenzymatic protein glycosylation, and autooxidation of glucose leading to increased production of reactive oxygen species (ROS) which include superoxide radical ($O^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}). Experimentally induced diabetic rats were known to have high levels of tissue oxidative stress as characterized by high amount of LPO products, MDA, which indirectly reflects intensified free radical production (Uyanik *et al.*, 2012). The concentration of lipid peroxidation products may reflect the degree of oxidative stress in diabetes. The end product of lipid peroxidation are found to cause damage to the proteins, lipids, and DNAs and is associated with a variety of diseases, such as atherosclerosis and brain damage (Borek, 2001; Valko *et al.*, 2006). It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification (Pandey and Rizvi, 2010). In the present study, there was significant increase in the plasma concentration of TBARS of diabetic rats when compared to the non diabetic rats. However, the oral administration of *Cnidoscopus aconitifolius* to the diabetic group of rats significantly ($p < 0.05$) lowered the TBARS concentration of TDR which could be as a result of improved antioxidant status.

The enzymatic antioxidants such as SOD and catalase are components of the body antioxidant defense systems which are involved in detoxification of free radicals formed during oxidative stress. Superoxide

dismutase (SOD) is a metalloprotein and is the first enzyme involved in the antioxidant defence which scavenges the superoxide ions produced as cellular by products. Catalase (CAT) is a hemoprotein, localized in the peroxisomes or the microperoxisomes which catalyses the decomposition of H_2O_2 to H_2O and O_2 and thus protecting the cell from oxidative damage by H_2O_2 and OH (Naganuma *et al.*, 1990). The decreased activities of SOD and CAT in diabetic rats have been reported (Miyazaki *et al.*, 2007), resulting in the accumulation of superoxide radicals and H_2O_2 respectively. These enzymes prevent generation of hydroxyl radicals and protect the cellular constituents from oxidative damage (Pari and Amadi, 2005). SOD and CAT activities in the plasma of DC were lower than NC. Oral administration of *Cnidoscopus aconitifolius* to the diabetic group of rats significantly ($p < 0.05$) increased the SOD and CAT activities of the TDR compared to the DC. The increased activities of SOD and CAT in *Cnidoscopus aconitifolius* TDR may be attributed to free radical scavenging activities of *Cnidoscopus aconitifolius* leaf extract. These antioxidant properties of the extract can be attributed to the presence of flavonoids and vitamins C and E in the plant known to possess antioxidant activities (Akah and Okafor, 1992; Ong *et al.*, 2011).

Non significant ($P > 0.05$) reduction in PCV and haemoglobin values was observed in DC compared to NC. PCV is a measure of erythrocytes in the blood and increment connotes production of red blood cells and in turn an increase in blood volume (Adebayo *et al.*, 2006). Oral administration of *C. aconitifolius* increased these parameters in TDR compared to DC suggesting that the plant has blood boosting effects and can be used in management of anaemia. The increase in these parameters could be attributed to their phytochemical contents.

Conclusion: Our findings showed that the leaves of *C. aconitifolius* consist of useful antioxidant compounds, such as flavonoids and vitamins C and E. It also suggest that *C. aconitifolius* exhibited reversal effects on oxidative stress and haematologic parameters in the plasma of TDR, thus making the plant a potential source of natural antioxidants and blood boosters.

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