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Full Length Research Paper

Antioxidant Potential of *Vitex Doniana* Ethanol Leaf Extract In Triton X-100induced Hyperlipidemic Wistar Albino Rats

[1*]Okpala, C.O; [1]Igwilo, I.O and [1]Eze F.

[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State.

[*]Email: co.okpala@unizik.edu.ng

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ABSTRACT

This study was undertaken to investigate the antioxidant potential of ethanol leaf extract of *Vitex doniana* against Triton X-100 induced hyperlipidaemia in rats. The percentage yield of the leaf was 9.21% w/w. The phytochemical constituents of the extract were identified using the standard method of AOAC. *In vitro* antioxidant activity of the extract (0.1–0.5 mg/mL) was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and nitric oxide radical. *In vivo* antioxidant potential of the extract was evaluated in triton X-100 induced hyperlipidemic and normal rats. The LD_{50} of the leaves extract was greater than 5000mg/kg body weight. Quantitative analysis of the plant secondary metabolites showed the presence of tannin (32.40±0.02 mg/g), flavonoid (16.87±0.27 mg/g), alkaloid (0.50±0.07 mg/g), cyanogenic glycosides (0.06±0.00 µg/g) and saponin (0.83±0.10mg/g). The result of proximate composition showed the presence of carbohydrate (41.85±0.73 %), protein (19.26±0.12%), fats (12.75±0.41%), moisture (12.04±0.17 %), crude fibre (11.82±0.19 %) and ash (11.90±1.00 %). The extract scavenged DPPH and nitric oxide radical concentration dependently. A total of thirty (30) adult male albino rats were divided into six (6) groups (A, B, C, D, E, and F), with five (5) animals in each group**.** Group A and B received distilled water only; Group C received 10 mg/kg b. w of atorvastatin while groups D, E, and F received an oral administration of 200, 400 and 600 mg/kg b.w. of *Vitex doniana* leaf extract, respectively. After 21 days, their blood serums were analysed. A significant ($p<0.05$) decrease in the concentration of malondialdehyde (MDA) was observed in groups that received atorvastatin and extract when compared to the hyperlipidemic untreated group. The activities of superoxide dismutase (SOD), and catalase (CAT) showed a dose dependent significant ($p<0.05$) increase when the groups that received extract and atorvastatin were compared to the hyperlipidemic untreated group.

Keywords: antioxidant, *Vitex doniana*, triton X-100, atorvastatin

INTRODUCTION

Raasch (1988) described hyperlipidemia as the elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides, and phospholipids. According to Saunders (2007), it is well established that elevated blood lipid levels (hyperlipidemia) constitute the primary risk factor for atherosclerosis. Van Horn (1997) reported that the risk of coronary artery diseases is influenced by dietary factors, nutritional habits, and genetic origin.

The role of oxidative stress in the pathogenesis of several degenerative or chronic diseases, such as atherosclerosis, cardiovascular diseases (CVD), stroke, diabetes, Parkinson's disease, renal disease, AIDS, and cancer is very crucial. Consistent increase of oxidative stress (Jung *et al*., 2004) as well as decreased antioxidant defense system (Wang *et al.,* 1998; Landmesser *et al*., 2000) in cardiovascular disease has been reported.

Medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from plants, many based on their use in traditional medicine (Hostettmann *et al.,* 2000). Plants continue to play a vital role in health care, with about 90% of the world's inhabitants dependent on them for their primary health care (Hostettmann *et al.,* 2000). Cook and Samman (1996) mentioned that the antioxidant activity of plants might be due to their phenolic compounds.

Vitex doniana is a deciduous tree, with a dense rounded crown, usually 4-8m high, light grey bark with numerous vertical fissures with the leaflets usually widest towards the tip, hairless. It is extremely widespread in tropical Africa and commonly known as Black Plum or African olive according to Glew *et al., (*1997), Dinya (Hausa), Galbihi (Fulani), Oori-nla (Yoruba), Ucha coro (Igbo), and is widely distributed in the southwestern Nigeria as a perennial tree. Earlier reports have shown that plant extracts can be used for the treatment of anaemia (Abdulrahman *et al.,* 2010), diarrhoea, dysentery (Kilani, 2006) and for the treatment of liver disorder (James *et al.,* 2010). The present study investigated the antioxidant potential of ethanol *Vitex doniana* leaf extracts in triton x-100 hyperlipidemia-induced rats.

MATERIALS AND METHODS

Collection of plant material and identification

The fourth fully opened *Vitex doniana* leaves were harvested from its natural habitat at Nnamdi Azikiwe University, Awka, Anambra State in the month of August 2019, and authenticated at the Herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka.

Experimental animals

A total of thirty (30) male albino rats were used for the study while twelve (12) male albino rats were used for acute toxicity study of the plant leaf extract. All the apparently healthy male wistar albino rats weighing between 100 to 150g were obtained from Anagonye Animal Farm, Awka, Anambra State, Nigeria. The rats were kept in well aerated cages and allowed to acclimatize for two weeks before the commencement of experiment. They were maintained on a commercial mash (Grower

feed) and water *ad libitum* throughout the experiment.

Chemicals and reagents

All assays' kits were from Randox laboratories, UK. Chemicals and reagents used were of analytical grade unless otherwise stated.

Sample Preparation and extraction

The leaves were air dried at room temperature for 21 days. The dried sample was pulverized with a Corona manual grinder into a fine powder and was stored in an airtight container till further use.

Four hundred grams (400g) of the ground sample was soaked in 4L of 70% ethanol and was allowed to stand for 48hours at room temperature with intermittent stirring.

The mixture was filtered through Whatman paper No. 4 with the aid of a vacuum filter and the filtrate was concentrated at a reduced pressure using rotary evaporator at 40^{o}C. The dried residue was weighed and reconstituted in 70% ethanol at a concentration of 10mg/ml and stored at 4^{o}C in a refrigerator till further use.

Percentage yield

The percentage yield of the extracts was determined as percentage of the weight of the extracts to the original weight of the dried sample used, using the formula below:

Percentage yield = $weight\ of \frac{extract}{sample} \times 100$

Quantitative phytochemical composition

The saponin content was determined using the method of Obadoni and Ochuko (2002).The flavonoid, total phenols, beta-carotene, and lycopene contents were determined by using the method described by Barros *et al.* (2007). The cardiac glycoside and tannin contents of the samples were determined by the method of the AOAC (1990). Oxalate content was determined according to Osagie (1998).The phytate contents were determined using the method of Young and Greaves (1940).

Proximate analysis

Proximate analysis was determined according to the method described by AOAC(1999).

***In vitro* screening of the extracts**

DPPH scavenging activity was carried out using the method of Ebrahimzadem, *et al*., (2009).The reducing power was determined according to the method of Barros *et al,* (2007).Inhibition of Lipid peroxidation activity was determined by the method of Barros *et al.* (2007).Nitric oxide scavenging activity was determined using the method of Rozina *et al.* (2013).

Acute toxicity study

The median lethal dose (LD_{50}) of the plant leaf extract was conducted to determine a suitable dose for the evaluation of effects of the extract. This was done using the method described by Lorke (1983).

Animal grouping

A total of 30 male wistar albino rats were randomly divided into 6 groups of 5 rats each.

Group A: Normal Rats control given feed and water only. This served as the normal control group.

Group B:Rats induced with hyperlipidemia and given feed and water only. This served as the hyperlipidemic control group.

Group C: Rats induced with hyperlipidemia and treated with 20 mg/kg b.w. Atorvastatin according to Kumar *et al*.,(2010).

Group D:Rats induced with hyperlipidemia and treated with 200mg/kg b.w. ethanolic extract of leaves of *Vitex doniana.*

Group E: Rats induced with hyperlipidemia and treated with 400mg/kg b.w. ethanolic extract of leaves of *Vitex doniana.*

Group F: Rats induced with hyperlipidemia and treated with 600mg/kg b.w. ethanolic extract of leaves of *Vitex doniana.*

Induction of hyperlipidemia

Hyperlipidemia was induced in rats with the method described by Thanga *et al.*, *(*2013). 100mg/kg dose of Triton x-100 was introduced intraperitoneally. To confirm the induction of hyperlipidemia, blood samples were collected from the tails of rats 24 hours after induction and assayed for; triacylglycerol (TAG) and total cholesterol (TC). TC and TAG levels above borderline of (200-239) mg/dl and (150-199) mg/dl respectively were considered hyperlipidemic.

Preparation of standard drug

Atorvastatin (Pfizer, Ireland) was purchased in a tablet form at a strength of 20 mg. The tablets were dissolved in distilled water and administered orally.

Collection and preparation of blood samples

At the end of 21 days of treatment, the rats were sacrificed by cardiac puncture after using chloroform anesthesia and blood samples collected from the heart wound in plain tubes. Blood samples collected were allowed to clot and the serum separated by centrifugation using Labofuge 300 centrifuge (Heraeus) at 3000 rpm for 10 minutes and the supernatant (serum) collected was subjected to biochemical screening.

***In vivo* antioxidant activity**

Serum lipid peroxidation was assessed by malondialdehyde (MDA) formation (Ohkawa *et al.,* 1979).Superoxide dismutase (SOD) was determined by a method described by Fridovich (1989).Catalase activity was determined using the method described by Sinha (1972).

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The data was analyzed by the analysis of variance (ANOVA) using SPSS program (version 16.0 SPSS Inc., USA). The difference between the various extracts and animal groups were compared using the Duncan's Multiple Range Test (1955) and ($p< 0.05$) was considered significant.

RESULTS

Percentage yield of *Vitex doniana* extract.

The extraction of 400g (0.4kg) of *V.doniana* leaves with 70% ethanol gave an ethanol extract yield of 36.84g which represented 9.21% of the starting material.

Phytochemical constituent of *Vitex doniana* leaves.

The number of phytochemicals found in the leaf extract was quantitatively determined by standard procedures and is presented in Table 1.

Table 1: Phytochemical composition of the ethanol extract of the leaf of *Vitex doniana*

Phytochemical	Composition
Total Phenol (mg/g)	74.21±1.51
Flavonoid (mg/g)	16.84±0.27
Tannin (mg/g)	32.40±0.02
Cyanogenic glycoside (µg/g)	0.06±0.00
Oxalate (mg/g)	6.21±0.38
Beta-Carotene (mg/g)	6.33±0.00
Lycopene (mg/g)	0.72±0.00
Phytate (mg/g)	0.48±0.02
Alkaloid (mg/g)	0.5±0.07
Saponin (mg/g)	0.83±0.10

Proximate composition of *Vitex doniana* leaves

The proximate compositions of *V.doniana* leaf extract are presented in Table 2.

Table 2: Proximate composition of *Vitex doniana* leaf in % dry weight (%DW)

Parameters	Composition
Ash (%)	12.55±0.01
Moisture (%)	12.46±0.01
Fibre (%)	11.90±0.01
Carbohydrate (%)	41.71±0.02
Crude Protein (%)	9.18±0.03
Total lipids (%)	13.60±0.00

Mean ± SEM, n = 2

***In vitro* Antioxidant activity of *Vitex doniana* leaf extract**

Free Radical Scavenging Activity

The result showed an increase ($p<0.05$) in radical scavenging activity of ethanol leaf extract of *Vitex doniana* with an increase in concentration when compared to standard antioxidant (BHA) with maximum activity at 500µg/ml. The graded concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) of the ethanol leaf extract of *V.doniana* showed a dose dependent increase in radical scavenging activity of between 2.34%and 56.04% in the same pattern as the graded concentrations(100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) of the standard antioxidant (BHA) which was however showing between 48.8%and 90.48% radical scavenging activity respectively.

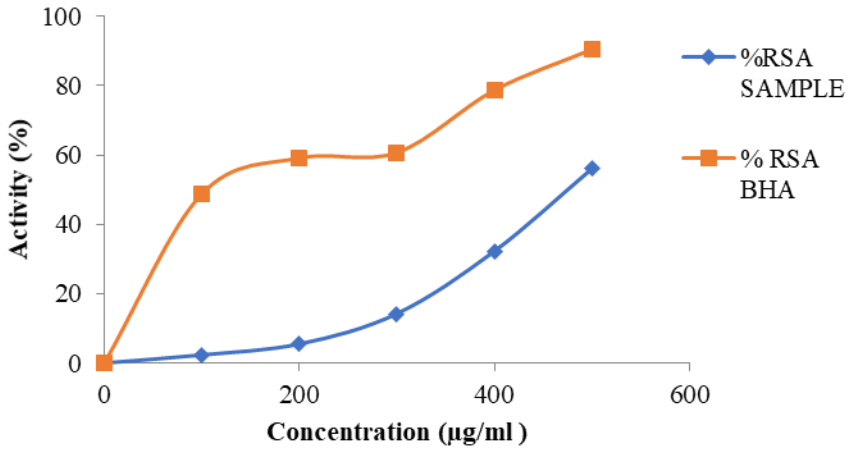


Figure 1: Free Radical Scavenging Activity of Ethanol leaf extract of *Vitex doniana* and standard antioxidant (BHA).

Inhibition of Lipid Peroxidation

The result showed an increase in inhibition of lipid peroxidation of ethanol leaf extract of *Vitex doniana* with an increase in concentration with maximum inhibition at 500µg/ml. It compared favourably to that of the standard antioxidant (BHA).The graded concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) of the ethanol leaf extract of *V.doniana* showed 5.07%, 22.09%, 23.28%, 51.94% and 55.52% inhibition of lipid peroxidation respectively , while the graded concentrations(100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml) of the standard antioxidant (BHA) showed 30.75%, 60.00%, 71.64%, 72.54% and 73.43% inhibition of lipid peroxidation respectively.

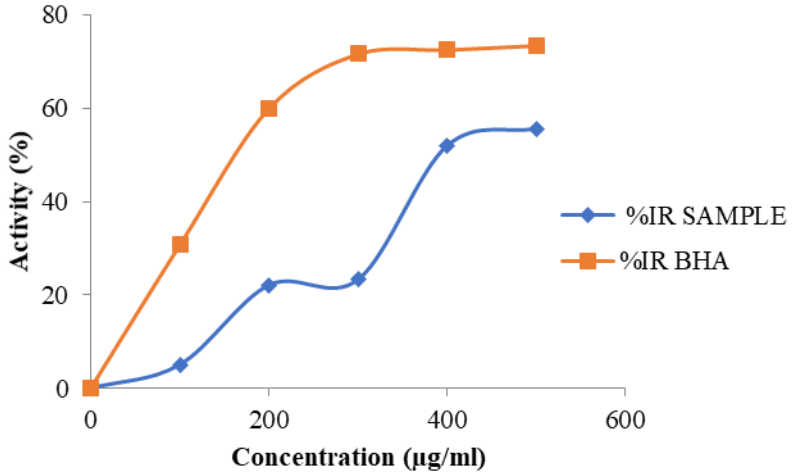


Figure 2: Inhibition of lipid peroxidation of Ethanol leaf extracts *Vitex doniana* and standard antioxidant (BHA).

Nitric oxide scavenging Activity

The result showed an increase ($p<0.05$) in Nitric Oxide scavenging activity of ethanol leaf extract of *Vitex doniana* with an increase in concentration when compared to standard antioxidant (BHA) with maximum activity at 500mg/ml. The graded concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) of the ethanol leaf extract of *V.doniana* showed 14.90%, 17.02%, 17.02%, 27.69% and 56.21% radical scavenging activity respectively ,while the graded concentrations(100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) of the standard antioxidant (BHA) showed 53.44%, 62.93%, 67.34%, 72.13% and 76.54% radical scavenging activity respectively.

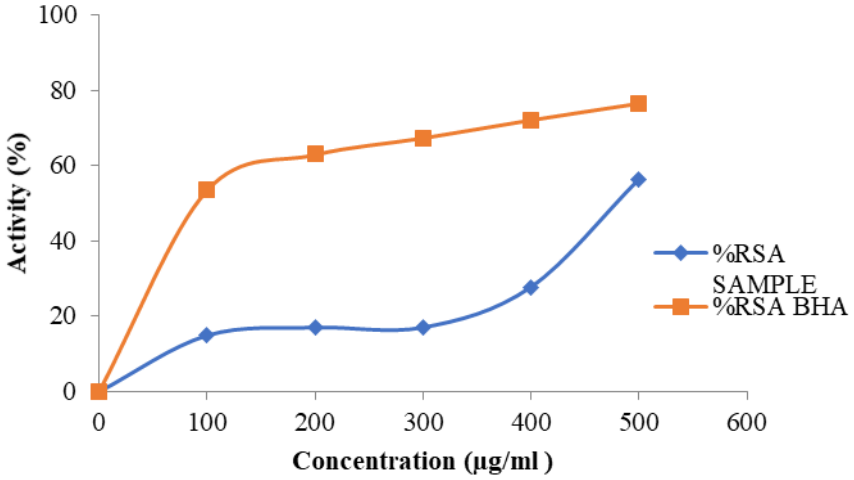


Figure 3: Nitric oxide scavenging activity of Ethanol leaf extract of *Vitex doniana* and standard antioxidant (BHA).

Reducing Power Capacity assay

The result showed an increase ($p<0.05$) in reducing power capacity of ethanol leaf extract of *Vitex doniana* with an increase in concentration when compared to standard antioxidant (BHA) with maximum activity at 500µg/ml as presented in Figure 4.

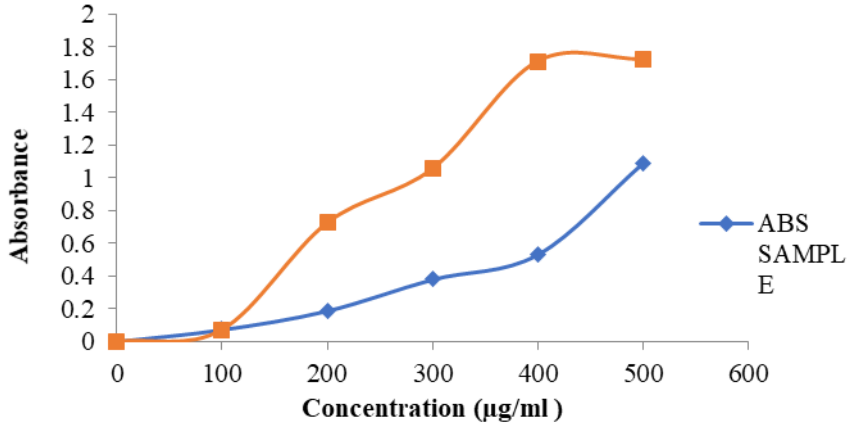


Figure 4: Reducing power activity of Ethanol leaf extract of *Vitex doniana* and standard antioxidant (BHA).

IC_{50} of Radical Scavenging Activity, Inhibition of Lipid peroxidation and Nitric Oxide Scavenging Activity.

The result showed an increase in EC_{50}/IC_{50} of Radical Scavenging Activity, Inhibition of Lipid peroxidation and Nitric Oxide Scavenging Activity of ethanol leaf extract of *Vitex doniana* when compared to that of the standard. The IC_{50} of Radical scavenging activity, lipid peroxidation inhibition and nitric oxide scavenging activity of ethanol leaf extract were found to be 595.23µg/ml, 561.79µg/ml and 458.71µg/ml respectively while that of the standard antioxidant were found to be 246.30µg/ml, 261.78µg/ml and 268.81µg/ml respectively.

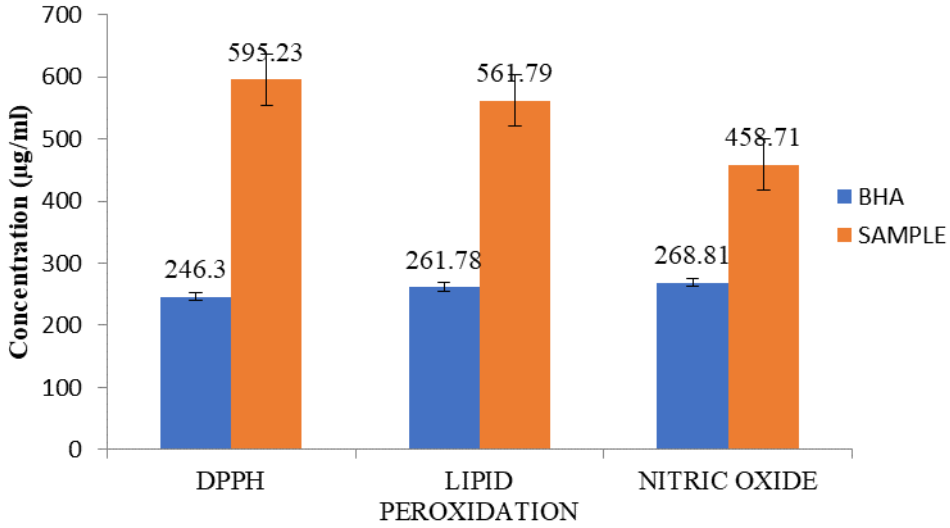


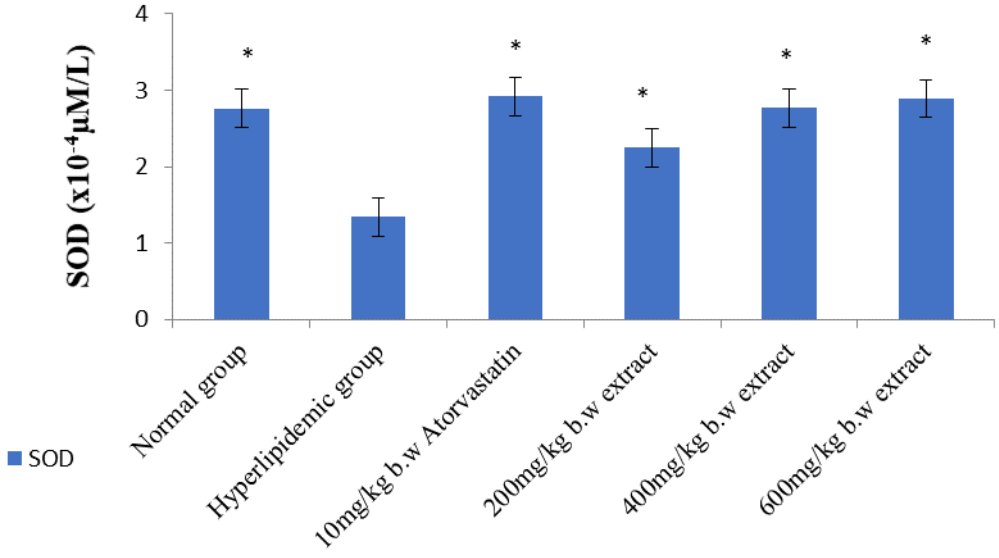
Figure 5: IC_{50} of Radical Scavenging Activity, Inhibition of Lipid peroxidation and Nitric Oxide Scavenging Activity.

In vivo* antioxidant activity of ethanol leaf extract of *Vitex doniana

The results of the effect on superoxide dismutase (SOD) and catalase activities were shown in Figures 6 and 7 respectively. The activity of catalase significantly ($p<0.05$) decreased from 0.19 ± 0.00 u/l in the normal group to 0.10 ± 0.04 u/l in the hyperlipidemic group.

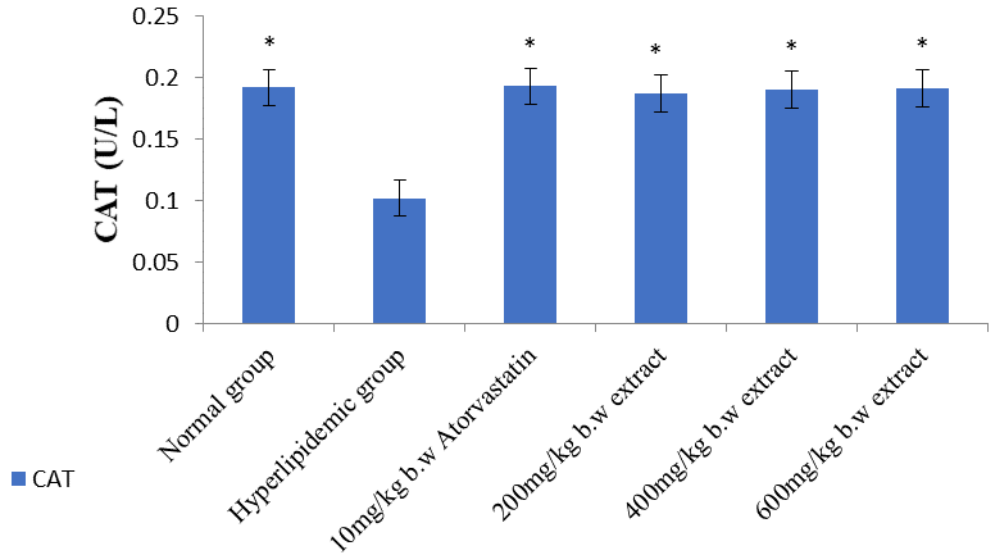
Concurrently, the activities of SOD also reduced significantly ($p<0.05$) from 2.76 ±0.04 u/l in normal group to 1.34 ± 0.07 u/l in the hyperlipidemic group. Figures 6 and 7 also showed that treatment of the animals with different doses of the leaf extract for seven (21) days, significantly ($p<0.05$) attenuated the effect of the triton x-100 on the enzymatic antioxidant in a dose dependent manner. The activities of all the tested enzymatic

antioxidants were significantly increased by the leaf extract at the different doses. These increases in the activities of CAT, and SOD were higher (though not significant) in rats administered with 600 mg/kg b.w. extract compared to that recorded for atorvastatin.



*significantly different when compared to Triton X-100 induced hyperlipidemic rats

Figure 6: Effect of ethanol leaf extract of *Vitex doniana* on Superoxide Dismutase activity in normal and Triton X-100 induced hyperlipidemic rats.



*significantly different when compared to Triton X-100 induced hyperlipidemic rats

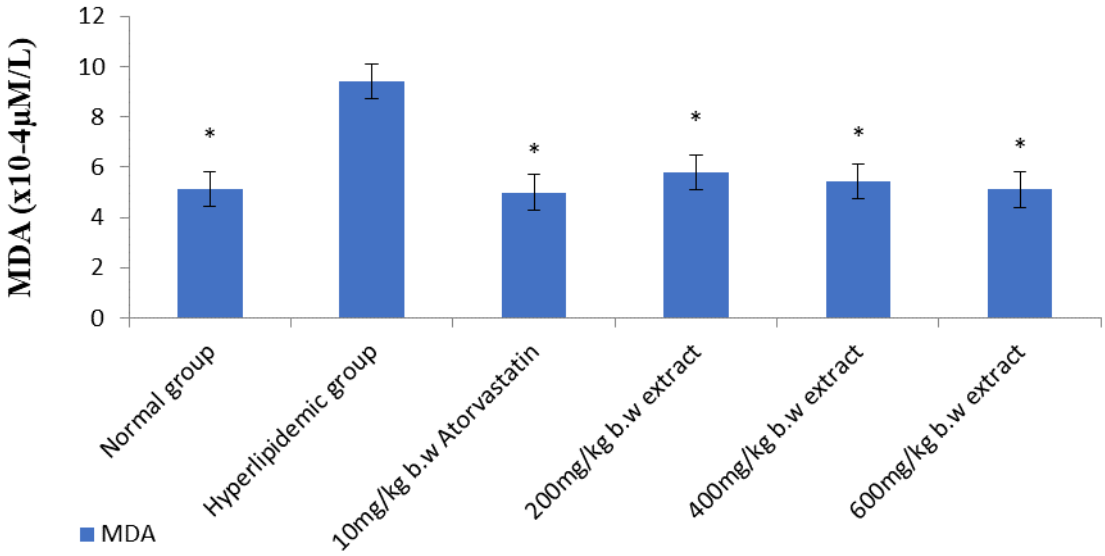
Figure 7: Effect of ethanol leaf extract of *Vitex doniana* on Catalase activity in normal and Triton X-100 induced hyperlipidemic rats.

Effect of Extract on serum lipid peroxidation

Figure 8 shows that induction of hyperlipidemia in rats with triton x-100 induced the elevated generation of significant amounts ($p<0.05$) of malondialdehyde (MDA)

from 5.12 x10^{-4}µM/L in the normal control to 9.4 x10^{-4}µM/L mg/ml in the hyperlipidemic group.

Administration of the plant leaf extract significantly ($p < 0.05$) inhibited the generation of MDA in a dose dependent manner. The highest tested dose of *Vitex doniana* leaf extracts (600 mg/kg b.w) inhibited the generation of a stable product of lipid peroxidation (MDA) from 5.12 ±0.21 x10^{-4}µM/L in the normal control to 5.10 ± 0.07 x10^{-4}µM/L. Also, the 600 mg/kg extract showed a significant ($p < 0.05$) inhibition of lipid peroxidation compared to that recorded for atorvastatin (4.99 ± 0.69 x10^{-4}µM/L).



* significantly different when compared to Triton X-100 induced hyperlipidemic rats

Figure 8: Effect of ethanol leaf extract of *Vitex doniana* on Malonaldehyde concentration in normal and Triton X-100 induced hyperlipidemic rats.

DISCUSSIONS

Alkaloids, flavonoids, saponins, tannins, and cardiac glycosides which were present in substantial quantities in *Vitex doniana* ethanol leaf extract as shown in Table 1 agreed to the report of James *et al.*, (2014) on the phytochemical composition of *Vitex doniana* leaf extract. Boumerfeg *et al*, (2009) stated that the free radical scavenging ability by various constituents of plants may be due to phenolic acids and flavonoid. The proximate composition of the leaf extract presented in Table 2are slightly higher than the results obtained by Frances *et al*., (2013) which were moisture (12.04 %), protein (19.26 %), crude fat (12.75 %), crude fibre (11.82%), ash content (11.90%) while the value obtained by Frances *et al.*, (2013) for carbohydrate (57.50 %) was higher. The crude fibre content (11.82%) and ash content (11.90%) obtained for the leaf of *Vitex doniana* were relatively low compared to reports on some medicinal plant stem barks such as *Cassianigricans* whose values were found to be 41.36 % and 15.98 % respectively (Ayo, 2013).Araya *et al.* (2003), reported that the consumption of leafy vegetables, comparatively high in crude fibre can decrease serum cholesterol

level, risk of coronary heart diseases and hypertension, and boost glucose tolerance and insulin sensitivity.

The major groups of antioxidants, polyphenols, have generated a great interest among Scientists for the development of natural cure for cardiovascular diseases (Pietta, 2000). Antioxidant activity of a compound has been ascribed to the mechanism exerted by polyphenols (Anoosh and Fatemeh 2010). Scavenging radical species such as ROS/RNS, suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production and upregulating or protecting antioxidant defense are the various mechanisms proposed by (Cotelle, 2001) for an effective antioxidant property. DPPH is an unstable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH was determined by the decrease in its absorbance at 520 nm, which is induced by antioxidants. According to Pattanayak *et al.*,(2012), a higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. The result shows that the ethanol leaf extract IC_{50} value is significantly ($p<0.05$) higher when compared with the standard antioxidant (Fig. 5).

Increased oxidative stress and overproduction of reactive oxygen species according to (Zalba *et al.*, 2001) are associated with hyperlipidemia. Free radical attack on membrane polyunsaturated fatty acids initiates lipid peroxidation, leading to their transformation and fragmentation to alkanes and reactive aldehyde compounds. The observed increase in lipid peroxidation (TBARS) in hyperlipidemic control rats is consistent with experimental studies which have shown that Triton X-100 induced hyperlipidemia leads to increased lipid peroxidation (Dutta and Bishayi, 2009). Treatment with the extracts of *Vitex doniana* significantly ($p<0.05$) reduced the MDA concentration (Fig. 8). The ability of the extracts to inhibit the process of lipid peroxidation in hyperlipidemic rats may be due to the presence of flavonoids, phenols and tannins found in the preliminary phytochemical screening as it has been previously reported that steroids (Ghule *et al.*,2009), and flavonoids (Patel *et al.*,2009) possess antihyperlipidemic activity.

Free radicals-induced damage is neutralized by the body's defense mechanism, and this is achieved by a set of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase. Antioxidant enzymes such as superoxide dismutase catalyse the conversion of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and O_2 (Oyedemi, 2010) and catalase further detoxifies H_2O_2 into H_2O and O_2 (Fridovich, 1986) under normal conditions. Nonetheless, imbalance between the formation of reactive oxygen species and their elimination occasioned by hypercholesterolemia has been implicated in oxidative-induced diseases.

In this study, the endogenous antioxidant enzymes (SOD, CAT) activities significantly ($p<0.05$) decreased in rats induced with hyperlipidemia compared to

those in normal control group (Figures 6 and 7). The decrease in the activities of these enzymes could be ascribed to the over utilization of these enzymes in inactivating the free radicals generated due to the induced hyperlipidemia (Ma *et al.,* 2011) or insufficient availability of GSH. Our results indicate that *V. doniana* has a free radical scavenging activity which probably provides organ protection from hypercholesterolemia. The reduction of MDA levels and increased endogenous antioxidant enzyme activities in rats treated with *V. doniana* ethanol leaf extracts may be related to lipid-lowering ability because its activity compared favorably with atorvastatin, a lipid lowering drug (Ozansoy, 2001). It can be put forward as a hypothesis that the administration of *V. doniana* ethanol leaf extract increases the activities of endogenous antioxidant enzymes in response to hypercholesterolemia-enhanced free radical production.

CONCLUSION

The results from this study suggest that hydroethanolic extract of *V. doniana* leaves possess *in vitro* antioxidant properties as indicated by the scavenging of DPPH and nitric oxide radicals. It lowered serum lipid peroxidation (MDA) and elevated levels of the endogenous antioxidant enzymes, which is probably due to its antioxidant property of scavenging Triton X-100 induced free radicals. Therefore, the study draws a relation between the traditional use of *V. doniana* leaves as ethnomedicine and the scientific validation of its properties. More work should be done on the ethanol leaves extract to ascertain the mechanism of action of the extract.

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