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CARDIOPROTECTIVE AND ANTIOXIDANT POTENTIAL OF TERMINALIA CATAPPA (ETHANOL LEAVE EXTRACT) IN AUGMENTIN-INDUCED WISTAR RATS

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ABSTRACT

Over the years, the sustained high and leading rate of mortality from cardiovascular disease (CVD) globally has been a source for research concern, thus this investigation considered cardioprotective potential of ethanol leave extract of *Terminalia catappa* in an experimental rat model of augmentin - induced cardiac damage. Random male wistar rats weighing within 150 and 200 grams were designated and administered as follows; A Group, B Group both administered 10ml/kg normal saline, C Group and D Group administered low dose (200 mg/kg) and high dose (400 mg/kg) of the extract respectively. E Group received 200 mg/kg dose of Vitamin E from day 1 to day 14. B, C, D and E Groups were then administered 30 mg/kg bwt of Augmentin daily by oral route on the 15th to 21st days. All the animals, 6 in each group were sacrificed on day 22, and blood samples collected by cardiac puncture for analysis, using standard protocol to analyze lipid profile. 2g of heart portion was used to prepare the homogenates for lipid peroxidation profile (TBA) and antioxidant analysis. The results showed that in the Augmentin treated rats, there was significant increase in concentrations of Cholesterol - TC (104.9±10.8), Triglyceride - Trigs (8.7±0.52), Lactate dehydrogenase - LDH (46.6±1.9), Creatine kinase - CK (45.7±2.04) and a non-significant increase in the lipid peroxidation, TBA (4.2±0.11) compared to normal control (A group). There was a significant decrease in concentration of HDL (32.3± 2.16) in B group relative to rats in A group. Meanwhile, treatment with 200mg/kg bwt and 400mg/kg bwt of *Terminalia Catappa extract* showed a significant ($p<0.05$) dose dependent decrease in serum concentration of TC (76.9±7.85 and 70.5±4.41), Trigs (6.8±0.37 and 5.9±0.37), LDH (25.2±2.14 and 11.6±5.12), Ck (24.5±1.42 and 9.5±1.43), and a significant ($p<0.05$) dose dependent increase in serum concentration of HDL (43.3 ±1.48 and 50.5±1.23) respectively, relative to the Augmentin-treated B groups. The results also shows that Augmentin caused a significant ($p<0.05$) decrease in the activities and functions of antioxidant enzymes in positive control relative to normal rats groups; SOD (3.7±1.06), GSH (2.3±0.51) and CAT (2.3±0.41) . However treatment with *Terminalia Catappa* doses of 200 mg/kg and 400 mg/kg body weight respectively showed a significant ($p<0.05$) dose dependent increase in the Antioxidant enzymes and functions as follows; SOD (5.9±1.28 and 8.6±1.62), CAT (3.4±0.26 and 4.1±0.14), GSH (4.1±0.69 and 4.4±0.31) and a non-significant decrease in the lipid peroxidation values, TBA (3.2±0.67 and 3.3±0.09), compared to Augmentin treated group. These findings are indication that ethanol leave extract of *Terminalia Catappa* may have constituents that potentially offer Cardio - protective benefits for Wistar Rats exposed to Augmentin-induced cardiac damage.

KEYWORDS: *Terminalia Catappa,* CVD, Antioxidant, Augmentin, lipid profile.

INTRODUCTION

For over one decade, deaths due to CVD had been reported as the highest, worldwide in the burden of diseases.[1] In lieu of this it is a relevant research concern. Meanwhile, it is common knowledge that medicinal plants and human civilizations have developed *pari passu,* with the exploration of curative values of plants in developing traditional, as well as orthodox medications.[2,3] The use of plants for various purposes that range from food, shelter, to economic and therapeutic is widespread.[4] In the last decade, Sule and his team of researchers have been investigating how medicinal plants may have beneficial health effects on some specific vital organs which include cardio - protective benefits among others.[5,6]

In recent time, one plant that is reportedly used for therapeutic purpose is the *Terminalia catappa* also called

Indian almond, "being native to India, found in Asian continent commonly as well as parts of Africa and within the Niger Delta Region of Nigeria".[7,8] The phytochemical constituents of this plant are numerous - gallic acid, ellagic acid, triterpinoids like ursolic acid, tannins such as punicalin, punicalagin and tergallagin among others have been isolated from it.[9,10]

The heart primarily helps in pumping blood around the whole body, through blood vessels to supply oxygen and nutrients that the cells and tissues require for metabolism.[11] Some adverse conditions could affect the heart thereby resulting in dysfunction, disability or fatality if not detected and treated appropriately.[11] Therefore, "intervention made in attempt to reduce the risk of developing detrimental cardiovascular ocurrence, and preserve the normal function of the heart, can be described as cardio-protective".[11]

Generally, there are certain cellular reactions in the body that can predispose cells and tissues to damage, for instance those that tend to release free radicals; whereas, there are some activities that may preserve cells and tissues from damage, for instance the activities of antioxidants.[12] Antioxidants are substances that hinder the process of oxidation, thus inhibiting production of free radicals. They are predominantly seen in plants as natural molecules.[12]

There are enzymes associated with highly potent antioxidant activities in the body, which may be specific or non-specific for certain organs or tissue. Some antioxidant enzymes include superoxide dismutase (SOD), Gluthatione (GSH), Creatine Kinase (CK) and Catalase. They can be used in heart studies.[15]

In this current study, the researchers' aim was to investigate the effects of ethanol leave extract of *Terminalia Catappa* on wistar rats, exposed to cardiac damage by augmentin administration relying on the assay of serum HDL, LDH, TC, Ck levels and antioxidant enzymes activity.

METHODOLOGY

Terminalia catappa sample was obtained from Niger Delta University, Bayelsa state, Nigeria, and promptly identified by Professor Kola Ajibeshin of the Department of Pharmacognosy, Niger Delta University. The fresh leaves were allowed to dry for two weeks at room temperature, and then ground into powder form. 2 litres of ethanol was used to dilute 500g of the powder and allowed to stand for 48 hours while stirring at intervals. 110 mm size of Whattman filter paper was used to obtain the filtrate, which was evaporated at 40 ^{0}C with a rotary evaporator. Distilled water was then used to re-dilute the powdery residue.

Five (5) plastic cages customized for rats' standard were designated A to E groups, each of which was used to house six (6) rats selected randomly from total of 30 male albino wistar rats, weighing between 150 and 200 grams. The entire groups of rats were acclimatized for 14 days under standard laboratory conditions and allowed to access grower's mash (Delta Feeds), water *ad libitum* and fresh air with an equal light and darkness cycle within 24 hours. At the onset of the study days 1 to 14, **A and B Groups** were administered 10ml/kg bwt normal saline daily (normal control) and (positive control) respectively. **C Group** was given 200mg/kg bwt of the extract (test group 1) while **D Group** was given 400mg/kg bwt of the extract (test group 2). **E Group** was administered 200 mg/kg Vitamin E (standard group).

On day 15 of the study, rats in B, C, D and E Groups were administered 30mg/kg bwt of augmentin orally, for seven days after which the animals were anaesthetized with chloroform and sacrificed on the 22nd day. Blood samples were aspirated through cardiac puncture into plain bottles, allowed to stand for 30 minutes to coagulate, and then centrifuged for 10 minutes at 2000 RPM. The supernatant was collected for biochemical analysis in which HDL, LDH, Total cholesterol, Triglycerides and Ck were parameters used to assess the cardio-protective activities. Also, 2g of heart portion was used to prepare the homogenates for lipid peroxidation and antioxidant analysis. The various analyses were done following the guideline provided in the standard biochemical kits, (Randox product).

Estimation of total cholesterol was by spectrophotometric method as described by.[16]

Triglycerides measurement was also done according to the method described by.[17]

Assay for High-density lipoprotein (HDL) was performed by following standard protocol as described by.[18]

Lactate Dehydrogenase (LDH) was measured by spectrophotometry in line with standard procedure in the kit as outlined by.[19]

Creatine Kinase (CK) Activity was measured spectrophotometrically and CK activity was calculated using the formula CK activity (U/l) = 8095 x ΔA 340nm/min.[20]

Lipid peroxidation was performed by estimation of thiobarbituric acid reactive substances (TBARS) formed during lipid peroxidation.[21]

The antioxidant enzymes analysis, the level of SOD activity was determined by the method of [22] and for reduced glutathione (GSH) the method described by.[23]

Statistical analysis

The parameters measured were presented as Mean ± Standard deviation. The statistical significance was evaluated using Graphpad InStat, [one way Analysis of

Variance (ANOVA) under Turkey Kramer Multiple Comparison Test]; and values were considered statistically significant at $p < 0.05$.

RESULT ANALYSIS

The results of the study are presented in Tables:

Table 1: Protective role of *terminalia catappa* on augmentin treated wistar rats.

Experimental group	HDL mg/dl	LDH mg/dl	Total cholesterol mg/dl	Creatine kinase mg/dl	Trigs mg/dl
Normal control with normal saline (A group)	57.3±3.72[a]	6.3±1.82[b]	65.7±3.88[a]	6.3±1.83[b]	4.3±0.58[b]
Positive control with 30 mg/kg Augmentin(B group)	32.3±2.16[c]	46.6±1.9[c]	104.9±10.8[a]	45.7±2.04[b]	8.7±0.52[b]
C group with 200 mg/kg extract and 30 mg /kg Augmentin	43.3±1.48[c]	25.2±2.14[d]	76.9±7.85[a]	24.5±1.42[d]	6.8±0.37[b]
D group with 400 mg/kg extract and 30 mg/kg Augmentin	50.5±1.23[a]	11.6±5.12[b]	70.5±4.41[a]	9.5±1.43[b]	5.9±0.37[b]
Standard control with 200 mg /kg Vitamin E and 30 mg /kg Augmentin (E group)	51.1±1.01[a]	5.1±1.84[b]	68.8±4.72[a]	8.7±1.01[b]	5.0±0.51[b]

Data are expressed as Mean ± SD (n=6), means with the same column carrying same superscripts are not significantly ($p<0.05$) different.

Results in the table 1 shows that Augmentin caused a significant increase in the concentration of Cholesterol (104.9±10.8), Triglycerides (8.7±0.52), LDH (46.6±1.9), Creatine kinase (45.7±2.04) and a significant decrease in concentration of HDL (32.3± 2.16) in positive control relative to normal control. However, treatment with *Terminalia Catappa* doses of 200 mg/kg and 400 mg/kg bwt showed a significant ($p<0.05$) dose dependent decrease in serum concentration of Cholesterol (76.9±7.85 and 70.5±4.41), Triglycerides (6.8±0.37 and 5.9±0.37), LDH (25.2±2.14 and 11.6±5.12), Creatine kinase (24.5±1.42 and 9.5±1.43), but a significant ($p<0.05$) dose dependent increase in serum concentration of HDL (43.3 ±1.48 and 50.5±1.23), respectively compared to the Augmentin-treated group.

Table 2: The Antioxidant role of *Terminalia Catappa* on Augmentin treated Wistar Rats:

Experimental group	GSH (U/mg protein)	SOD(U/mg protein)	TBA (U/mg protein)	CAT(U/mg Protein)
Normal control with normal saline (A group)	5.1±0.42[a]	9.4±1.35[a]	2.8±0.77[b]	4.8±0.30[b]
Positive control with 30 mg/kg Augmentin (B group)	2.3±0.51[b]	3.7±1.06[b]	4.2±0.11[b]	2.3±0.41[b]
C group with 200 mg/kg Extract and 30mg/kg Augmentin	4.1±0.69[b]	5.9±1.28[a]	3.2±0.67[b]	3.4±0.26[b]
D group with 400 mg/kg Extract and 30 mg /kg Augmentin	4.4±0.31[b]	8.6±1.62[a]	3.3±0.09[b]	4.1±0.14[b]
Standard control with 200 mg/kg of Vitamin E and 30 mg/ kg of Augmentin (E group)	4.2±0.20[b]	9.1±0.83[a]	3.3±0.12[b]	4.5±0.31[b]

Data was expressed as the Mean ± SD(n=6) means with the same column carrying same superscripts ($p<0.05$) difference.

Results in Table 2 shows that Augmentin caused a significant ($p<0.05$) decrease in the activities and functions of antioxidant enzymes in positive control relative to normal rats groups; SOD (3.7±1.06), GSH (2.3±0.51) and CAT (2.3±0.41) and a non-significant increase in the lipid peroxidation values, TBA (4.2±0.11). However treatment with *Terminalia Catappa* doses of 200 mg/kg and 400 mg/kg bwt respectively showed a significant ($p<0.05$) dose dependent increase in the Antioxidant enzymes and functions as follows; SOD (5.9±1.28 and 8.6±1.62), CAT (3.4±0.26 and 4.1±0.14), GSH (4.1±0.69 and 4.4±0.31) and a non-significant decrease in the lipid peroxidation values, TBA (3.2±0.67 and 3.3±0.09), compared to Augmentin treated group.

DISCUSSION

The burden of cardiovascular disease (CVD) globally is overwhelming, being the leading cause of death worldwide for more than a decade; with a reported 17.3 million deaths of almost equal spread amongst men and women in 2008. It accounts for 30% of all deaths globally, with underdeveloped countries having the huge 80% chunk of CVD deaths globally which has been projected to increase to 23.3 million annually by 2030 if there is no intervention or interruption in the current spate.[24,25]

Generally, medicinal plants have continued to receive wide spread use among populations globally by 80%, along with their role in the enhancement of orthodox medication in which 70 – 95% of developing nations' primary health care are reliant on medicinal plants.[26,27] In the current study, ethanol extract of the plant – *Taminalia catappa* was investigated for cardio-protective activities in an experimental rat model of augmentin-induced damage, following standard protocols.

From the results, it was observed that augmentin administration significantly ($p<0.05$) raised the concentration of Cholesterol, Trigs, LDH, Ck and lipid peroxidation (TBA) but reduced the concentration of HDL in the positive control relative to normal control rats. The implication of such lipid profiling is that, the rats treated with augmentin are predisposed to heart disease or damage. On the other hand, pretreatment of the rats with *Terminalia Catappa* low and high doses presented a significant ($p<0.05$) dose dependent decrease in serum concentration of Cholesterol, Trigs, LDH, and Ck but a significant ($p<0.05$) dose dependent increase in serum concentration of HDL, relative to the Augmentin-treated group.

This apparent reversal in the lipid profile suggests that the extracts may have potentially offered protection to the heart of the *Terminalia catappa* treated rats. Worth noting is the interference in the activities of those enzymes such as LDH and CK that also have established antioxidant activities. It was reported that improving plasma lipid profile and reducing the levels of TAGs are other cardioprotectve mechanisms of phytochemicals.[28]

Earlier reports by,[29] found that the extract of *Urtica Parviflora* leafs (400 mg/kg daily for 3 weeks) significantly lowered plasma levels of TAGs and LDL in DOX-intoxicated rats. More so, following treatment with augmentin, there was significant reduction of antioxidant functions and activities (SOD, GSH, CAT) in positive control relative to normal rats and a non-significant increase in the lipid peroxidation profile (TBA). Troyano and his team reported several mechanisms suggested for DOX-induced cardiotoxicity, such as oxidative stress, produced by decreased levels of antioxidants e.g. glutathione (GSH).[30] This oxidative stress causes acceleration of lipid peroxidation that damages the cell membrane and other cellular components.[31,32]

However, when the low and high doses of the plant extract was administered, there was significant dose dependent increase in antioxidant function and activities and non-significant decrease in the lipid peroxidation profile (TBA), in the test groups compared to augmentin treated group; thus establishing the antioxidant activity of the extract. It has been reported that reddish brown leaves of *Terminalia Catappa* contain flavonoid apigenin 6-c-(2 - galloyl)- L-Dglycoside, apigenin 8-c-(2 - galloyl)- L-Dglycoside, isovitexin, vitexin, isoorienthin, rutin and tannin; gallic acid, ellagic acid, puricalagin, punicalin which are reported for good antioxidant property.[33] More so, the HPLC analysis of the extracts *Terminalia Catappa* indicated the presence of the same antioxidant and isolation work for the compound identified as ellagic acid which showed strong antioxidant activity in the assay systems used. These phyto-compounds may have been responsible for the cardio-protective action conferred on the hearts of the rats induced with augmentin as reported in our present study.

CONCLUSION

This investigation provides empirical evidence, premised on the current experimental model, that ethanol extract of *Terminalia Catappa* leaves may possess a dose-dependent cardio-protective and antioxidant therapeutic property that can provide health benefits in male wistar rats exposed to Augmentin-induced cardiac damage; corroborating its potential use in the management of cardiovascular diseases.

Recommendations

Considering the enormousness of the burden of cardiovascular diseases (CVDs), and the projected trajectory of CVD mortality from 17.3 million in 2008 to 23.3 million by 2030,[25] expedited, concerted efforts to harness the cardio-protective medicinal value of this plant are recommended.

Comparative investigation of different models of this experiment is encouraged.

Further studies to elucidate the mechanism of action will be relevant.

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