Trop J Nat Prod Res, January 2021; 5(1):135-139

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Distinctive Roles of Butylated Hydroxytoluene and Ascorbic Acid in Lead-Instigated Oxidative Stress in Wistar Rats

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ARTICLE INFO

Article history:
Received 01 October 2020
Revised 10 December 2020
Accepted 24 January 2021
Published online 03 February 2021

ABSTRACT

Overwhelmed antioxidant defense system of exposed biological system to a toxicant maybe relieved using *in vitro* antioxidant source. Here, the study evaluated the distinctive roles of ascorbic acid (AA) and butylated hydroxytoluene (BHT) in lead (Pb)-instigated oxidative stress in Wistar rats, where group I rats received 1.0 mL of distilled water, group II rats received a single dose of 30 mg $PbCl_2$ per kg b.w. of rats by intraperitoneal injection, groups III and IV dosed with the toxicant received 25 mg/kg of AA and BHT, and group V dosed with the toxicant received 25 mg/kg of AA and BHT orally daily for 28 days. There was a significant increase ($p < 0.05$) in serum, hepatic and renal MDA levels for group II rats relative to the group I rats, but this was reversed in tissues of groups III-V rats relative to group II rats. No significant changes ($p > 0.05$) occurred in tissue MDA levels for group III and IV rats. SOD activity decreased significantly for group II rats, as compared with group I rats, but group III-V rats showed enhanced SOD activity in tissues assessed in relation to group II rats at $p<0.05$. CAT activity was significantly inhibited in tissues of group II rats relative to group I rats, which was significantly improved in group III-V rats relative to group II rats at $p<0.05$. Therefore, Pb-mediated oxidative stress was better improved by oral administration of AA than BHT, which suggested the exogenous use of natural antioxidants in lead toxicity.

Keywords: Antioxidant, Ascorbic acid, Butylated hydroxytoluene, Lead toxicity, Oxidative stress.

Introduction

The susceptibility of biological systems to environmental stressors may not be unrelated to over-exposure and compromise of their antioxidant defense system. Lead (Pb) is a known heavy metal pollutant among other environmental stressors, whose sources of exposure are chiefly occupational with the industrial manufacturing of lead products.[1,2] Upon absorption into biological systems, especially humans, Pb is distributed through the bloodstream to various tissues/organs of the body, mainly the hepatic, renal and reproductive tissues, etc., where it bioaccumulates to detrimental levels while a small amount of the toxicant appears in urine: the accumulated toxicant at this point was reported to affect biological systems at the physiological, metabolic and molecular levels, which may evidently cause temporary or permanent morphological alterations.[3] Pb toxicity was observed in all tissues, especially the renal, hepatic, hematological, central nervous, and reproductive systems with the onset of pathologies characterized by oxidative stress.[4] Therefore, the mediation of oxidative stress by Pb was explained as one of the mechanisms employed by the toxicant to excessively generate reactive oxygen species (ROS).[4,5]

Under normal physiological conditions, ROS shows no detrimental effect, but its over-expression down-regulates diverse physiological and metabolic functions, especially in the inhibition of both enzymatic and non-enzymatic defense systems leading to the onset of diseases.[3,4] The chronic onset of Pb-mediated oxidative stress may be associated with insufficient production of endogenous antioxidants, which may not curtail the excessive synthesis of ROS and the aftermath consequences are often massive to explain oxidative alterations of various components of cells as well as oxidative changes occurring in biomolecules thereby resulting in the development of different dysfunctions in organ systems.[5,6] Reports have shown that Pb-associated dysfunctions in organ systems include: respiratory system leading to pulmonary inflammation, reproductive system with the development of testicular degeneration, hemorrhagia, sterility, etc., skeletal system impairments such as mental retardation, jerky movements, ataxia to mention but a few, excretory system with the onset of tubular necrosis, impairment of cardiovascular system leading to increased blood pressure and arteriosclerosis, and development of anemic condition in Pb- related effect on blood cells.[6-8]

In order to reduce or prevent Pb toxicity to tissues, there may be a need to enhance the endogenous antioxidant defense system of exposed biological systems. Reports have shown that exogenous antioxidant supplementation was a therapeutic strategy, other than known chelation therapy with great consequences, to stimulate and improve the compromised endogenous antioxidant system.[4,9] To this end, the consideration of synthetic and natural antioxidants was emphasized in scientific reports.[10,11] This development necessitated the selection of ascorbic acid (AA) and butylated hydroxytoluene (BHT), as natural and synthetic antioxidants, in the present study. AA, otherwise called vitamin C, is a chelating agent with excellent antioxidant potential with the capacity to mitigate against Pb toxicity through the reduction of bioaccumulated metal in tissues and enhanced elimination in urine respectively.[12] AA is widely distributed in nature among fruits, vegetables and some animal sources in which the recommended dietary allowances (RDAs) for children and women were stated as 45 and 90 mg/kg respectively. Studies have shown that AA deficiency in biological systems may be associated with the onset

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Citation: Obiwulu ENO, Apiamu A, Ugbebor G. Distinctive Roles of Butylated Hydroxytoluene and Ascorbic Acid in Lead-Instigated Oxidative Stress in Wistar Rats. Trop J Nat Prod Res. 2021; 5(1):135-139. doi.org/10.26538/tjnpr/v5i1.19

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

of anaemia, scurvy, deterioration of muscle tissues, infectious diseases, and atherosclerosis.[13,14] It plays a metabolic role in wound healing through modulation of collagen synthesis and in the biosynthesis of neurotransmitters.[15,16] The functional role of AA during the oxidation of metals as well as the regeneration of other antioxidants, particularly β-carotene and α-tocopherol were reported in literature, where the industrial value (as it pertains to preservation of aroma, flavour, optimization of nutrient content and prolonged shelf life of processed foods) and medicinal uses (as a therapeutic requirement for diabetes, inflammatory disorders, obesity, stroke, cancer, cataracts and glaucoma) of AA were evidently highlighted.[13,15,17]

As a synthetic phenolic antioxidant, however, BHT is used as food additive at considerable dose in fat-rich foods and cosmetic products to act against lipid oxidation reactions with the aim of reducing deterioration.[18,19] Although the use of BHT as an antioxidant in foods and cosmetics was identified, but its LD_{50} was well established with a numerical equivalence that is >2970 mg/kg during oral administration in rats. The exposure of rats to high dose of BHT was associated with the inhibition of prothrombin in the hepatocytes thereby leading to hemorrhagic death.[18] Primarily, the cytochrome p450-dependent metabolism of BHT occurs in the liver with the formation of BHT-quinone derivative, which has increased affinity for cysteine-rich molecules thereby leading to hepatotoxicity.[18,20] Scientific reports have shown the efficacy of BHT against a broad spectrum of lipid-coated viruses, where it may function as a promoter or anti-promoter agent during carcinogenic process.[21] As a concern of novelty, the comparative distinction between BHT and AA with synthetic and natural antioxidant origins during Pb-instigated oxidative changes in tissues of exposed rats was central in this study.

Materials and Methods

Chemicals

All chemical reagents used in this study were of analytical grade and obtained from Sigma Aldrich, USA.

Animal models used in presents study

A total of thirty (30) male Wistar rats (140-182 g, 3 months old) were procured from the Department of Biochemistry, University, Benin, where animals were kept in standard metabolic cages for two weeks of acclimatization. Based on the experimental design, the rats were subjected to treatment for Twenty-eight (28) days, following standard experimental protocols such as nutritional status (regular rat chows & water *ad libitum*) and environmental conditions (like 26 ± 1°C, 12-12 h light/dark cycle & 46 ± 1% R.H) respectively. The study adheres strictly to international ethics for handling and caring for experimental animals, as highlighted by the European Community Act of 1986.

Randomized experimental block design

By randomized block design, five experimental groups labelled I-V with six rats (n = 6) each were obtained. Groups I and II served as negative and positive controls respectively. Based on the report presented by Obiwulu[19] that a single dose administration of 30 mg of $PbCl_2$ per kilogram body weight instigated oxidative stress in Wistar rats by intraperitoneal injection. This was administered to group II-V rats, where group III rats received 25 mg/kg of AA per day for 28 days via oral route. Group IV rats received 25 mg/kg of BHT dissolved in corn oil per day for 28 days through oral administration. In each case, 25 mg of AA and BHT were administered orally per kg body weight of rats daily for 28 days to group V rats. All oral administration was done by means of a gavage

Post-experimental sampling

At the end of 28 days treatments, blood samples were obtained by heart puncture, after overnight fast of rats, by means of hypodermal syringe into EDTA bottles and transported immediately to the laboratory for centrifugation to collect supernatants at 2,500 revolution per minute (rpm) for 10 min. The hepatic and renal tissues were excised, washed with pre-chilled normal saline, where 10 % of each tissue (i.e. liver and kidney) were homogenized in 10 mL of 0.05 M phosphate buffer (pH 7.4) and was centrifuged using Search-Tech Laboratory Centrifuge at 15,000 rpm for 20 min for supernatants. All supernatants were collected and refrigerated at 4°C for further biochemical analyses.

Biochemical analyses

Protein content in each tissue can be determined following the assay protocol outlined by Lowry *et al*.[22] using bovine serum albumin.[22] As a biomarker of oxidative stress, thiobarbituric acid reactive substances (TBARS) in terms of malondialdehyde (MDA) levels were measured according to the method of Shafiq-ur-Rehman *et al.*[23] based on colour formation. The pink colour obtained during the assay was measured spectrophotometrically at 535 nm. Enzymatic assays, which involves superoxide dismutase (SOD) activity and catalase (CAT) activity, were measured to monitor oxidative changes in respective tissues. Thus, SOD activity in tissues was measured in accordance with the assay procedure put forward by Madesh and Balasubramanian [24] on the basis of photo-oxidation of nitroblue tetrazolium (NBT). The enzyme activity was monitored at 560 nm and expressed in units/mg protein. Based on the decomposition of hydrogen peroxide (H_2O_2), CAT activity was evaluated in accordance with the procedure outlined by Aebi[25] such that the change in absorbance was monitored at 240 nm and its activity was expressed as units/mg protein.

Statistical analysis

Experimental data were subjected to critical statistical analysis, where mean results were represented by bars and the standard error of mean (SEM), in each case, was depicted by error bars for six determinations (n = 6). One-way analysis of variance (ANOVA) for the experimental groups was compared and the Schefe's test of significance was applied. Bars with different letters differ significantly at $p < 0.05$.

Results and Discussion

Pb toxicity among heavy metal pollutants occupies a central reference of environmental stressors. Therefore, the present study revealed changes in MDA level, as a biomarker of oxidative stress, in tissues (blood, liver and kidney) of Wistar rats (Figure 1) during treatment with Pb compound, AA, and BHT. Consequently, the single intraperitoneal administration of 30 mg PbCl2 per kg body weight (b. wt) provoked the development of oxidative stress in blood, hepatic and renal tissues of rats with a marked significant increase ($p < 0.05$) in group II rats relative to group I (negative control). However, the oral administration of 25 mg/kg b.wt of AA (group III rats), BHT (group IV rats) and combined effect (group V rats) revealed a significant reduction ($p<0.05$) in blood, hepatic and renal MDA levels in relation to group II rats (positive control), which was a clear indication of improvement on Pb-instigated oxidative stress in rats. This observation further consolidates the fact that 30 mg/kg b. wt of Pb compound has the capacity to induce oxidative stress.[18,26] The observed increase in MDA level in the respective tissues of group II rats conform with earlier reports that Pb toxicity has the capacity to deplete endogenous antioxidant pool due to excessive production of ROS, hence the marked induction of oxidative stress. Consequently, the observed Pb-induction of oxidative stress in group II rats shows the likelihood of toxicity with disruption of hematological, hepatic and renal functions due to tubular necrosis of the tissues.[6,27] However, group III, IV and V rats (Figure 1), which showed a significant reduction ($p < 0.05$) in MDA levels as compared with group II rats, was a function of AA and BHT with the capacity to scavenge ROS.[28,29] Washio *et al*.[30] revealed that AA initiates direct antioxidant capacity to scavenge Pb-induced excessive synthesis of free radicals thereby protecting biological cells from oxidative damage. Similarly, Obiwulu[19] reported BHT antioxidant capacity to stimulate depleted endogenous antioxidant capacity to significantly reduce Pb-induced ROS. These up-regulatory measures by the supplementation with exogenous antioxidants showed that the tissues of treated rats, particularly group V rats in relation to groups III and IV rats were adequately preserved despite their exposure to toxic effects of Pb.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The postulation of Pb-instigated oxidative stress reflects its basic mechanism to exerts toxicity in tissues of exposed biological systems, which can be marked with increased MDA levels and exhausted activities of endogenous antioxidant defense systems regardless of their nature.[27,31] SOD activity is one of such endogenous enzymatic antioxidants affected by Pb toxicity. Figure 2 clearly explains the changes associated with SOD activity in tissues of rats exposed to the toxic effect of Pb and exogenous supplementation with natural and synthetic antioxidants (AA and BHT) respectively. Here, there was an inhibitory effect on serum SOD activity in group II rats relative to group I rats at $p < 0.05$ on exposure to Pb. Although no significant difference ($p > 0.05$) was observed in serum SOD activity for group III and IV rats exposed to the toxic action of Pb, but AA and BHT supplementation led to significant increase ($p < 0.05$) in serum SOD activity for group III and IV rats, as compared with group II rats. Most importantly, group V rats exposed to the toxic action of Pb showed a pronounced increase ($p < 0.05$) in serum SOD activity when supplemented with combined action of AA and BHT (when compared with groups III and IV rats) relative to group II rats. Similar changes were observed in SOD activity for the respective groups in hepatic and renal tissues at $p < 0.05$. Basically, exposure to Pb toxicity generates the free radical, "superoxide anion (O_2^-)" as a byproduct of oxygen metabolism, which can be further disproportionated into hydrogen peroxide (H_2O_2) by SOD activity.[4,5,24,32] However, the biological function of SOD activity, as mentioned above, is distorted or truncated by exposure to the toxic effect of Pb, which has been reported to displace SOD's cofactors (Cu and Zn).32 Therefore, the inhibitory effect exerted by Pb on SOD in tissues of group II rats may be attributed to the ability of the toxicant to dislodge the enzyme's cofactors, as earlier reported.[19,27,32] Again, the protection of tissues of exposed rats in groups III, IV and V from oxidative damage , as against group II rats, may not be unconnected with the free hydroxyl (OH) groups present in AA and BHT, which may pair up with the unpaired electron of superoxide anion thereby converting it to a non-radical, as indicated in available literature.[27] Finally, the observed protection of AA on group III rats exposed to Pb toxicity may be attributed to its capacity to regenerate reduced glutathione (GSH), capable of quenching lipid peroxidation chain reaction.[32,33]

CAT, an antioxidant enzyme known to catalyze the decomposition of H_2O_2 into metabolic water (H2O) and molecular oxygen under the influence of Zn cofactor.[25,27,34] In this empirical survey, exposure to Pb toxicity and co-treatment with different exogenous antioxidant types (AA and BHT) shows the trending mosaic of CAT activity in various tissues of Wistar rats (Figure 3). In group II rats treated with a single dose of Pb by intraperitoneal injection, significant reductions ($p < 0.05$) in serum, hepatic and renal CAT activities were observed when compared with group I rats. This may be a sign of inhibition of CAT activity in respective tissues of Wistar rats due to over-expression of ROS instigated by Pb toxicity, as a mark of conformity to earlier reports.[4,19,27,31] Pb toxicity has been reported to retard heme biosynthesis and displacement of the enzyme cofactor (Zn), which may eventually inactivate CAT activity over time: the biological function of CAT activity in the tissues is disrupted with subsequent accumulation of H_2O_2 that predisposes the host tissues to health complications and induction of diverse pathologies such as neurodegenerative disorders, cancer and diabetes characterized by oxidative stress.[35,36] However, the inhibitory action of Pb toxicity on serum, hepatic and renal CAT activities was relieved during supplementation of groups III, IV and V rats with levels of AA, BHT and their combinations, as shown in Figure 3. There was significant stimulation ($p < 0.05$) of serum, hepatic and renal CAT activity for groups III, IV and V rats in relation to group II rats, as an indication of truncating Pb-induced synthesis of ROS, and this may be attributed to the proton-donating capacity of AA and BHT.[27] It should be noted that though there were no significant variations ($p > 0.05$) in serum CAT activity for groups III and IV rats, but hepatic and renal CAT activity was significantly higher ($p < 0.05$) in group III rats than group IV rats, which may suggest that AA is a better antioxidant relative to BHT. Despite the toxic action of Pb, the observed stimulating effect of the exogenous antioxidants (AA and BHT) agrees with earlier reports that antioxidants of these sorts stimulate and improve overwhelmed endogenous antioxidant defense system associated with Pb toxicity.[4,9]

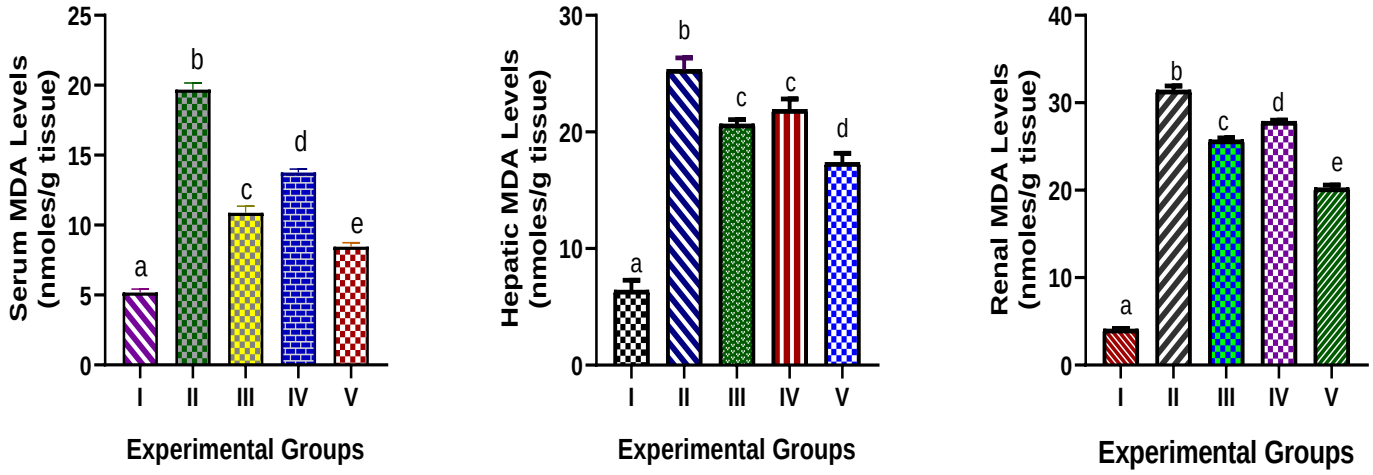

Figure 1: MDA levels in tissues of Wistar rats exposed to Pb, AA and BHT treatments. Each bar designates mean ± SEM (standard error of mean) for n = 6, where bars with different letters differ significantly at $p < 0.05$

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

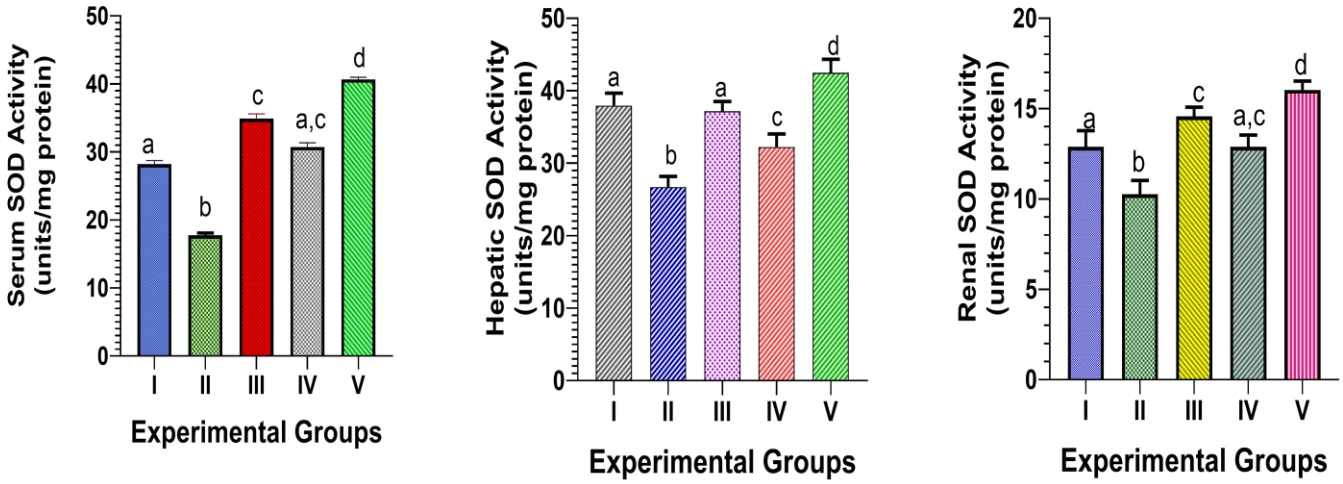

Figure 2: SOD activity in tissues of Wistar rats exposed to Pb, AA and BHT treatments. Each bar designates mean ± SEM (standard error of mean) for n = 6, where bars with different letters differ significantly at $p < 0.05$

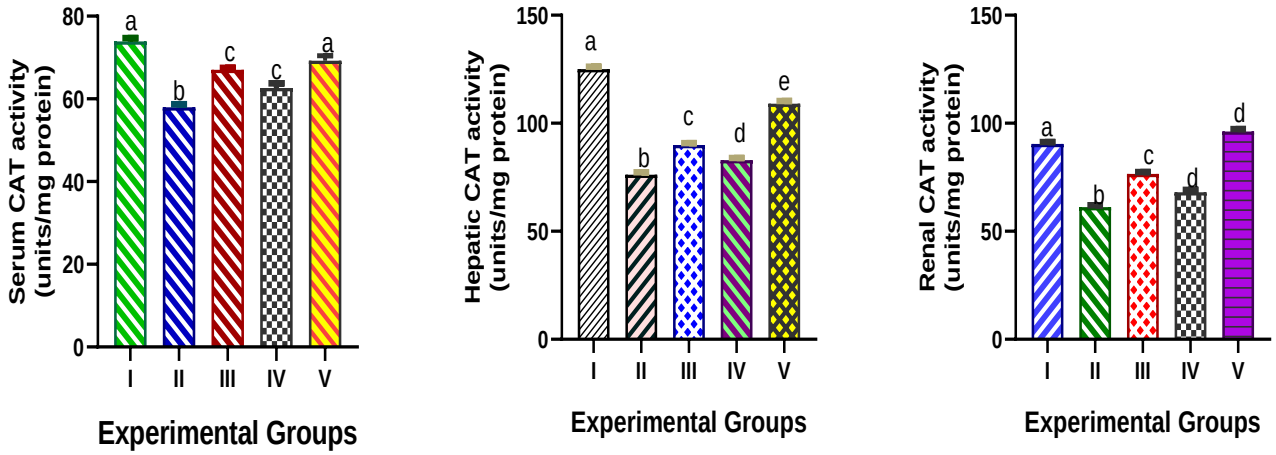

Figure 3: CAT activity in tissues of Wistar rats exposed to Pb, AA and BHT treatments. Each bar designates mean ± SEM (standard error of mean) for n = 6, where bars with different letters differ significantly at $p < 0.05$

Conclusion

Exposure of biological systems to Pb toxicity certainly instigated oxidative stress in which the onset of different degrees of cellular injuries and pathologies were marked by the compromized SOD and CAT activities. This development may call for exogenous antioxidants to supplement the overwhelmed endogenous antioxidants, as observed in this study. The use of AA and BHT during exposure of Wistar rats to Pb toxicity showed a clear-cut amelioration of induced oxidative stress, where AA was a better antioxidant than BHT. The present study hereby culminates that natural antioxidant may be recommended as a better antioxidant source relative to synthetic antioxidant source to relieve Pb toxicity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors to thank Delta State University for supporting the research work.

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