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Antioxidant status, antihyperlipidemic activity, and serum electrolyte levels in dexamethasone-induced oxidative stress in Wistar rats treated with vitamin E

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ABSTRACT

Objectives: Dexamethasone (Dex) is an anti-inflammatory drug with serious side effects. The aim of this study was to investigate, antioxidants status, antihyperlipidemic activity, and serum electrolyte levels of rats treated with Vitamin E in Dex-induced oxidative stress.

Materials and Methods: Wistar rats of both sexes were assigned to four groups (n = 6). The control group was fed with standard rat feed and water, while Dex group received 30 µg/kg of Dex. Vitamin E group received 300 IU/kg of vitamin E, while Dex + Vitamin E group received 30 µg/kg of Dex and 300 IU/kg of vitamin E. After 14 days of administration, blood samples were taken through cardiac puncture for biochemical analysis and the liver for histological analysis.

Results: Triglyceride, low-density lipoprotein-cholesterol, and malondialdehyde levels were significantly (P < 0.05) raised, while high-density lipoprotein (HDL)-cholesterol and superoxide dismutase (SOD) were reduced in Dex only group when compared with control and vitamin E only groups. The treatment with vitamin E significantly (P < 0.05) reduced these biochemical parameters and increased HDL-cholesterol and SOD level when compared with Dex group. Electrolyte levels in Dex only and vitamin E-treated groups were comparable with the control group.

Conclusion: Vitamin E can ameliorate hyperlipidemia and antioxidant imbalance caused by Dex in rats.

Keywords: Dexamethasone, electrolytes, lipid peroxidation, oxidative stress, Vitamin E

INTRODUCTION

Oxidative stress (OS) is an imbalance between the production of reactive oxygen species (ROS) and the detoxification of their reactive intermediate. It is an imbalance between oxidants and antioxidants in favor of the oxidants which can result in excessive production of destructive free radicals.[1] Free radicals are product of aerobic metabolism that is raised in pathological conditions. Oxidants can modulate a number of cell signaling pathways and also regulate the expression of several genes in eukaryotic cells which include mRNA stability, transcriptional, and transduction levels.[2] The negative impact of OS can be minimized by antioxidants.

Antioxidant is any substance when present at low concentration, prevents oxidation of a particular substrate.[3] Humans and animals possess an antioxidant defense mechanism which is made up of enzymes such as catalase (CAT), superoxide dismutase (SOD) glutathione (GSH) reductase, and GSH peroxidase and also non-enzymatic antioxidant including vitamins and non-protein thiols, mostly GSH. OS occurs when these defense mechanisms are not sufficient to minimize or neutralize the negative impact of ROS in the body.[4] Vitamins are non-enzymatic anti-oxidants that renders increase free radicals inactive in the tissues. One of such vitamins is vitamin E.

Vitamin E is the term given to a group of tocotrienols and tocopherols, they include alpha (α) delta (δ) and gamma (ϒ) tocopherols, among these groups of vitamin E, alpha tocopherol has the highest biological activities.[5] Due to active
Electrolytes, fluids, and acid-base balance are tightly regulated in the body by the kidney. Imbalance in these electrolytes which include sodium, chloride, potassium, bicarbonate, and calcium can result in pathological conditions. For instance, hyponatremia can result in seizures and neurologic symptoms, while hypokalemia can cause cardiac arrhythmia and elevated levels of chloride and decreased bicarbonate are seen in acidosis. In OS condition, increase production of ROS decreases kidney function and can result in electrolyte imbalance that is characterized by elevated serum sodium, calcium, and potassium.

Lipids are a heterogeneous group of compounds that are soluble in non-polar solvents and insoluble in aqueous solutions, they are important constituents of diets. The importance of lipid is evidenced in the ability to modify the biophysical and physiological state of the cell membrane and accumulation of lipid in the body poses a great danger to tissues. Lipids undergo peroxidation, a process in which reactive oxygen species attack membrane lipid, particularly polyunsaturated fatty acids which is evidence in oxidative stress diseases. A study has also shown that accumulation of lipid in the body predisposes an individual to obesity and hyperlipidemia which are risk factors of cardiovascular and renal dysfunction.

The liver plays an important role in inflammatory cascade and can become dysfunctional in OS condition. The Kupffer cells in the liver produce inflammatory cytokines and accumulation of these pro-inflammatory cytokines in the body induces liver injury. In inflammatory conditions, glucocorticoids are commonly prescribed for medication globally due to their wide anti-inflammatory properties. Recently, the study has shown that prolonged use of corticosteroid is associated with serious adverse effects in the liver including hepatic injury and steatohepatitis.

Dexamethasone (Dex) is a member of glucocorticoid class of hormone. Its use is strictly controlled due to its serious side effects such as insomnia, hypertension, hypokalemia, and OS. Although it is use as ant-inflammatory drug and in treatment of autoimmune diseases, the study has shown that long-term use of Dex can reduce the antioxidant capacity of renal tissue and increase ROS level in the body. Dexamethasone suppresses endothelium-dependent vasodilatation of resistance arterioles by inhibiting endothelium nitric oxide synthase (eNOS), a potent enzyme that helps in dilatation of blood vessels. Inhibition of eNOS could result in an increase in blood pressure due to vasoconstriction. In the liver, long-term Dex exposure reduces liver iron content, by inhibiting hepcidin synthesis which is closely associated with down-regulated hepatic transferrin receptor 1 protein expression. Dexamethasone influences the Kreb cycle and causes oxidation of fatty acids and deposition of fat in the body, particularly in the liver. It reduces food intake and weight gain by down-regulating the hypothalamic appetite center and might cause osteoporosis and hyperglycemia. Electrolyte imbalance and OS are common causes of altered physiological functions. Since vitamin E an antioxidant and Dex are frequently used drugs, this study was carried out to investigate antioxidants, antihyperlipidemic, and electrolytes regulatory activity of vitamin E in Dex-induced OS in rats.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

Vitamin E, Dex, chloroform, and formalin were purchased from Sigma Aldrich Chemical Co, St Louis, MO, USA. The lactate dehydrogenase, malondialdehyde (MDA), and SOD enzyme kits were purchase from Cayman Chemical Company (Ann Arbor, MI, USA). Serum triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein kits were purchased from Sclavo Diagnostics (Siena, Italy).

**Experimental Animals**

Ethical approval for this study was obtained from the Faculty of Basic Medical Sciences Animal Research Ethics Committee, University of Calabar (Approval NO: 019PY20317). Twenty-four healthy Wistar rats of both sexes (12 males and 12 females) weighing 180–200 g were assigned into four groups of six rats each namely: Group 1 – control group, Group 2 – Dex only, Group 3 – Vitamin E (Vitamin E) only, and Group 4 – Dex + Vitamin E (DEX + Vitamin E), respectively. The rats were allowed to acclimatize for 1 week and kept at the animal house of the Faculty of Basic Medical Sciences University of Calabar at room temperature of 28 ± 2°C with 12-h light/dark cycle. All animals were fed ad libitum with normal rat feed and tap water.

**Induction of OS**

OS was induced using Dex following a method reported by Afuafua et al. Briefly, Dex was administered at 30 μg/kg/day subcutaneously in the morning (single dose) for 2 weeks to animals in Dex only and Dex + Vitamin E-treated group.

**Administration of Vitamin E**

Vitamin E was administered to Vitamin E and Dex + Vitamin E-treated groups orally using orogastric tube at a dose of 300 IU/kg for 14 days following a method reported by Yildiz et al. Briefly, vitamin E was dissolved in 1 ml olive oil and then administered together with Dex to the Dex + Vitamin E group.

**Collection of Blood Sample**

At the end of 14 days of administration, animals were allowed to fast for 6 h before collection of blood sample. All animals were euthanized with chloroform anesthesia and each rat was quickly dissected and blood was collected through cardiac puncture into plain sample bottles. The blood samples were allowed to stand for 2 h and then centrifuged at 300 g for 10 min to obtain the serum. The
serum was then collected and stored at -20°C for subsequent biochemical analysis.

**Determination of OS Biomarkers**

Serum SOD activity was assessed using the method of Uked et al. using an assay kit (Cayman Chemical Company, USA) following the manufacturer's instruction. Serum MDA level was determined using an assay kit (Cayman Chemical Company, USA) according to the manufacturer's protocol as reported by Ahmed Amar et al.  

**Determination of Serum Electrolytes**

Sodium and potassium were determined by flame photometry using flame emission photometer (Sherwood Flame Photometer, USA). Estimation of bicarbonate was done using the back titration method. Briefly, standardized dilute H2SO4 was added in excess to the serum. The CO2 released from HCO3 was taken as an equivalent amount of H+ removed for the formation of water (H2O). The excess standardized H2SO4 was titrated against 0.01N NaOH using natural red as indicator. A pink color indicated the endpoint. The chloride concentration in serum was measured using the titrimetric mercuric nitrate method.

**Determination of Lipid Profile**

Triglyceride, total cholesterol, and HDL-cholesterol were measured using an enzymatic colorimetric assay kits (Scavo diagnostic, Sovicille, Italy) following the manufacturer's instruction. Low-density lipoprotein-cholesterol was estimated using the equation of Friedewald.

**Histopathological Analysis**

After collecting blood samples from the rats, the liver was rapidly excised, trimmed, and fixed in 10% buffered formalin. The tissues were soaked in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope and photomicrographs were taken. This was done following a method reported by Wang et al.

**Determination of Body Weight**

Before commencement of the experiment, the mean body weight of all rats in the control and treated groups was determined using a digital weighing balance. The body weights of all the animals in each group were recorded throughout the duration of the experiment. The final mean body weight was then determined from the total body weight and analyzed appropriately for each group.

**Statistical Analysis**

Results were expressed as mean ± standard error of mean. Data obtained were analyzed using one-way analysis of Variance followed by Tukey's post hoc test using GraphPad Prism software version 7.0 for Windows (GraphPad Software, San Diego, California, USA). For all statistical analysis, results were considered significant at P < 0.05.

**RESULTS**

**Effect of Dex on Lipid Profile and Antioxidant Levels in Control and Treated Groups**

The effect of Dex on lipid profile and antioxidant levels in the control and test groups are presented in Table 1. Dex increased total cholesterol level, though not significant when compared with the control group. Administration of vitamin E (vitamin E only) significantly (P < 0.05) reduced serum total cholesterol level when compared with the control and Dex only group. The treatment with vitamin E in Dex-treated group (Dex + Vitamin E) significantly decreased (P < 0.05) serum total cholesterol level when compared with Dex only group. No significant change was observed in the control group when compared with Dex-treated group.

Administration of Dex increased (P < 0.05) serum triglyceride level when compared with the control group. A significant (P < 0.05) increase was also observed in Dex only group when compared with vitamin E only group. However, the treatment with vitamin E in Dex-treated group (Dex + Vitamin E) significantly (P < 0.05) reduced serum triglyceride level when compared with Dex only group. No significant change was observed in Dex-treated group when compared with the control group.

The serum low-density lipoprotein (LDL)-cholesterol level was raised significantly (P < 0.05) in Dex only group when compared to the control and vitamin E only group. However, the treatment with vitamin E (Dex + Vitamin E) significantly decreased (P < 0.05) serum LDL-cholesterol level when compared with Dex only group. No significant change was observed in Dex-treated group (Dex + Vitamin

<table>
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<tr>
<th>Parameters</th>
<th>Control</th>
<th>Dexamethasone Only</th>
<th>Vitamin E Only</th>
<th>Dexamethasone+Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mMol/l)</td>
<td>2.0±0.06</td>
<td>2.2±0.06</td>
<td>1.7±0.08b</td>
<td>1.9±0.04b</td>
</tr>
<tr>
<td>Triglyceride (mMol/l)</td>
<td>0.5±0.02</td>
<td>0.7±0.044</td>
<td>0.3±0.04b</td>
<td>0.4±0.04b</td>
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<tr>
<td>LDL-Cholesterol (mM/l)</td>
<td>1.1±0.1</td>
<td>1.4±0.055</td>
<td>0.7±0.03b</td>
<td>1.1±0.03b</td>
</tr>
<tr>
<td>HDL-Cholesterol (mM/l)</td>
<td>0.7±0.04</td>
<td>0.7±0.04</td>
<td>0.9±0.04b</td>
<td>0.7±0.03</td>
</tr>
<tr>
<td>SOD (IU/l)</td>
<td>0.3±0.08</td>
<td>0.1±0.011</td>
<td>0.4±0.03b</td>
<td>0.3±0.04b</td>
</tr>
<tr>
<td>MDA (IU/l)</td>
<td>0.2±0.02</td>
<td>0.7±0.034</td>
<td>0.2±0.04b</td>
<td>0.3±0.04b</td>
</tr>
</tbody>
</table>

Table 1: Effects of dexamethasone on lipid profile and antioxidant levels in control and treated groups. a=p<0.05 compared with the control group, b=p<0.05 compared with DEX only group. LDL-cholesterol (Low density lipoprotein cholesterol), HDL-cholesterol (High density lipoprotein cholesterol), SOD (Superoxide dismutase), MDA (Malondialdehyde).
Okon, et al.: Vitamin E protects against dexamethasone-induced oxidative stress

E) when compared with the control group. Although no significant change was observed in serum HDL-cholesterol level in Dex only group when compared with the control group, administration of vitamin E significantly ($P < 0.05$) increased HDL-cholesterol level in vitamin E group when compared with the control, Dex only and Dex + Vitamin E treated group.

SOD activity decreased significantly ($P < 0.05$) following Dex administration when compared with control group. However, administration of vitamin E significantly increased ($P < 0.05$) SOD activity in Dex-treated group (Dex + Vitamin E) and vitamin E only group. No significant change was observed in Dex-treated group when compared to the control group.

Figure 1: Effects of dexamethasone on serum electrolyte levels in control and treated groups. (a) serum sodium level; (b) serum potassium level; (c) serum chloride level; and (d) serum bicarbonate serum.

Figure 2: Photomicrograph of the liver in dexamethasone and Vitamin E-treated Wistar rats (a) Control; (b) DEX only; (c) Vitamin E; (d) Dex + Vitamin E Arrows indicate significant lesion. (H&E $\times 400$). HP (Hepatocytes), SS (sinusoidal spaces), PV (Portal vein), BD (Bile duct), CV (Central vein), HA (Hepatic artery).
Dex administration triggered lipid peroxidation as observed in raised serum MDA level. The MDA level increased significantly ($P < 0.05$) in Dex only group when compared with the control group and vitamin E only group. The treatment with vitamin E resulted in a significant ($P < 0.05$) decrease in serum MDA level in Dex + Vitamin E group when compared with Dex only group.

**Serum Electrolyte Levels in Control and Treated Groups**

Serum electrolyte levels in control and treated groups are presented in [Figure 1a-d]. The result for serum sodium electrolyte concentration is presented in Figure 1a. No significant change was observed in the Dex only and vitamin E only groups when compared with control group. Furthermore, the result showed no significant change in Dex + Vitamin E treated group when compared with Dex only group. Potassium ion concentration showed no significant difference in all the groups, as presented in Figure 1b. The serum chloride level is presented in Figure 1c. The serum chloride level in the Dex only group was comparable with the control, vitamin E only, and Dex + Vitamin E treated group. Administration of vitamin E showed no significant change in Dex + Vitamin E treated group when compared with vitamin E only group. The serum bicarbonate level did not show any significant difference in all the groups, as presented in Figure 1d.

**Histological Analysis of the Liver**

The photomicrographs of the liver are shown in [Figure 2a-d]. In the control group (2A), the result showed plates of hepatocytes radiating outward from the central vein having abundant cytoplasm with prominent nuclei. The cells were separated by sinusoidal spaces and the portal area contained the hepatic artery, bile duct, and portal vein with scanty inflammatory cells and an intact limiting plate hepatocytes. In the Dex induced OS rats (2B), the section of the liver showed enlarged hepatocytes with prominent nuclei and microvesicular and macrovesicular steatosis within abundant cytoplasm. The hepatic cells were separated by narrowed sinusoidal spaces and the portal area contained the hepatic artery, bile duct, and portal vein with mild inflammatory cells with intact limiting plate hepatocytes. Finding showed fatty change resulting from sublethal injury to the liver. In the liver of vitamin E only rats (2C), section showed plates of hepatocytes radiating outward from the central vein. The hepatocytes were enlarged with prominent nuclei and abundant cytoplasm. The hepatic cells were separated by dilated sinusoidal spaces and the portal area contained the hepatic artery. Finding showed no hepatotoxicity. In the Dex+ Vitamin E-treated rats (2D), section of the liver showed a preserved architecture with plates of hepatocytes radiating outward from the dilated and congested central vein. The hepatocytes were enlarged with prominent nuclei that were pushed to the periphery and there were signs of microvesicular and macrovesicular steatosis within the abundant cytoplasm. The hepatic cells were separated by dilated sinusoidal spaces and the portal area contain the hepatic artery, bile duct, and portal vein with scanty inflammatory cells and an intact limiting plate hepatocytes. These finding showed minimal fatty change resulting from sublethal injury to the liver.

**Effect of Dex on Body Weight in Control and Treated Groups**

The result for body weight gained in the control and treated animals is presented in Figure 3. There was a significant ($P < 0.05$) weight loss in Dex only group when compared to the control and other groups. The weight gains in the control and vitamin E-treated groups were comparable. However, the treatment with vitamin E to Dex-induced OS rats significantly ($P < 0.05$) improved their body weight when compared with Dex only group.

**DISCUSSION**

The aim of this study was to investigate the role of vitamin E, an antioxidant in ameliorating OS, hyperlipidemia and electrolyte imbalance induced by Dex. The results of this study showed that administration of vitamin E significantly increased serum HDL-cholesterol level and decreased total cholesterol, triglyceride, and LDL-cholesterol levels in Dex-induced OS rats, while no significant change was observed in serum electrolytes level in the control and treated animals. The results showed a significant decrease in SOD activity with an increase in MDA level in Dex induced-OS that was ameliorated by vitamin E administration.

The increase in total cholesterol, triglyceride, and LDL-cholesterol levels in Dex-induced OS rats observed in this study could be due to OS induced by Dex. The study has shown that glucocorticoids can increase lipolysis in diseased condition. Free radicals can also induce lipid peroxidation and reduce antioxidants activities in OS models. This result is in agreement with a previous study that reported hyperlipidemia and lipid peroxidation in Dex-treated rats. The protective effects of vitamin E against hyperlipidemia could be attributed to its ability to reduce oxidative damage which can result in pathological disorders such as diabetes mellitus, hypertension, and stroke. Furthermore, the decrease in HDL-cholesterol as shown in dex-induced OS rats indicates its decreased availability which helps in the transport of LDL-cholesterol to the liver for detoxification. Similar results were observed.
in several animal studies that considered alterations in lipid profile as predictors of cardiovascular complications.\(^{39,40}\)

In the present study, Vitamin E treatment showed a reduction in total cholesterol, triacylglycerol and LDL-cholesterol, and elevation of HDL-cholesterol levels. This could be attributed to the efficacy of vitamin E to protect the body against hyperlipidemia and membrane-bound lipoprotein lipase against lipid peroxidation.\(^{41}\) Administration of vitamin E has ameliorative effects with a positive correlation on total cholesterol, LDL-cholesterol, and triglycerides levels in the body\(^{42}\) and protects against atherosclerosis and related cardiovascular disorders caused by increase in lipid peroxidation.\(^{43}\) The mechanism of Vitamin E could be due to alteration in cholesterol transport through cholesterol acyltransferase (LCAT) activity and the rate of cholesteryl ester transfer within HDL lipoprotein\(^{44}\) to the liver.

SOD enzyme, as an antioxidant, is greatly involved in elimination of ROS, through dismutation of superoxide radicals.\(^{45}\) Increased production of ROS raises lipid peroxidation and depletes endogenous antioxidants such as SOD. In the present study, lipid peroxidation (MDA) increased, while SOD activity decreased in Dex-induced OS rats. These results are consistent with other reports of an increase in lipid peroxidation in OS subjects.\(^{46}\) The decrease in antioxidant enzyme could be due to glycation of this SOD enzyme and reduced activity.\(^{47}\) Administration of vitamin E attenuated the increase in lipid peroxidation accompanied by an increase in SOD activity which is consistent with the beneficial effects of vitamin E on antioxidant enzyme activity in OS model.\(^{48}\) Since increase use of Dex a glucocorticoid is associated with increased oxidative damage as a result of lipid peroxidation and production of ROS,\(^{39}\) supplementation with vitamin E could have ameliorative or protective effect against oxidative damage caused by prolong use of glucocorticoid.

Electrolytes play a role in many physiological functions in the body. The electrolyte profiles of dex-induced oxidative group were comparable with those of Vitamin E and non-OS group. Contrary to other findings, the results showed no significant difference which could reveal that there were no disturbances in water and electrolytes balance in Dex-induced OS. Dex has a minimal or insignificant mineralocorticoid activity\(^{49}\) by suppressing aldosterone release from the adrenal cortex.\(^{50}\) This could be the case in this study in addition to the its down-regulatory effect on bicarbonate and sodium co-transport.\(^{51}\)

The infiltration of inflammation observed in the histological examination of the liver in Dex-induced OS rats showed that there was a marked inflammation. Prolonged use of Dex can cause damage the liver.\(^ {20}\) This inflammation was minimal in vitamin E-treated group which suggests hepatoprotective properties of vitamin E.\(^ {48}\) Although the markers of liver damage were not assessed, the histology points to hepatocellular injury due to OS that was ameliorated by vitamin E.

The decrease in body weight observed in this study supports our findings on the negative effect of long-term use of glucocorticoids. Prolonged use of Dex induces peripheral insulin resistance and down-regulates the appetite center of the hypothalamus through the release of neurotransmitters and neuropeptides, this causes a reduction in food intake and loss in body weight.\(^ {25}\) The loss in body weight in this study may be due to the inhibitory effects of Dex on the hypothalamic appetite center. This is in agreement with previous study that reported a decrease in body weight in Dex-treated rats.\(^ {26}\) The study has shown that vitamin E improved body weight by protecting the cell membrane against lipid peroxidation and by enhancing growth and muscle performance.\(^ {132}\) The improved body weight in Dex-treated animals with vitamin E also supports the benefit of vitamin E in ameliorating OS condition.

In conclusion, the administration of vitamin E led to decrease in total cholesterol, triglyceride, and LDL-cholesterol levels and a significant increase in HDL-cholesterol level. Furthermore, administration of vitamin E decreased MDA level and increased SOD antioxidant activity. While electrolytes concentration was normalized in both vitamin E-treated groups and Dex-induced OS group, body weight was improved due to vitamin E. Thus, vitamin E can correct lipid metabolism disorders and increase antioxidant activities as well as maintain homeostatic functions of the electrolytes in Dex-induced OS in rats.

**Clinical Significance of the Study**

Prolong use of glucocorticoids results in elevated lipid peroxidation. Lipid peroxidation is associated with cardiovascular diseases. Administration of vitamin E, a potent antioxidant, resulted in a reversed hyperlipidemia and OS caused by the glucocorticoid, Dex. Thus, vitamin E is recommended for coadministration with glucocorticoids used for the treatment of inflammatory disorder.

**Data Availability**

All data generated from this study are available from the corresponding author on request.

**Disclosure of Interest**

The authors declare that there are no conflicts of interest.

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