

Proceedings

# Modulation of Hydrogen Peroxide-Induced Oxidative Stress in Rats by Deep Root Herbal Mixture<sup>®</sup> – A Nigerian Branded Polyherbal Drug<sup>†</sup>

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**Abstract: Background:** Oxidative stress has been implicated in many chronic diseases and the use of natural antioxidants has been suggested to be beneficial in the prevention and management of some chronic diseases. Deep Root<sup>®</sup> herbal mixture (DRHM) is a branded Nigerian polyherbal drug composed of *Cymbopogon citratus* (17%), *Carica papaya* leaves (16%), *Magnifera indica* bark (15%), *Moringa oleifera* leaves (14%), *Citrus limonia* (12%), *Psidium guajava* (11%), *Zingiber officinale* root (9%) and *Allium sativum* (6%). The potential of DRHM in modulating hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced oxidative stress in rats was assessed in this study. **Methods:** Healthy Wistar rats were divided into six groups (n = 5) with group 1 serving as normal control while groups 2–6 were intoxicated (3 mL/kg b.w of 5% v/v of H<sub>2</sub>O<sub>2</sub> i.p). Group 2 served as H<sub>2</sub>O<sub>2</sub> control, groups 3–5 received 1, 2 and 3 mL/kg/d b.w. p.o of DRHM, respectively while group 6 was given silymarin (100 mg/kg/d. b.w. p.o) for 14 days. **Results:** H<sub>2</sub>O<sub>2</sub> elevated aspartate and alanine aminotransferases activities, and malondialdehyde and total bilirubin levels ( $p < 0.05$ ). Conversely, H<sub>2</sub>O<sub>2</sub> decreased superoxide dismutase, catalase and glutathione peroxidase activities, and antioxidant vitamins and reduced glutathione levels ( $p < 0.05$ ). However, DRHM dose-dependently attenuated oxidative damage to hepatic tissues likely by enhancing antioxidant defense system. The polyherbal drug was shown to be tolerable up to 10 mL/kg. b.w. dose. **Conclusion:** DRHM has hepatoprotective, antioxidant and anti-lipid peroxidation properties that may be attributed to its phytoconstituents.

**Keywords:** oxidative stress; hepatotoxicity; polyherbal drug; antioxidant; silymarin; lipid peroxidation

## 1. Introduction

The excessive generation of free radicals beyond the level in which the natural antioxidant defence of the body can neutralize results in oxidative stress is linked with pathogenesis of cardiovascular diseases, ageing and diabetes [1]. These damages usually occur via inactivation of important metabolic enzymes and damages to vital cellular macromolecules, often resulting in devastating consequences [2]. The liver helps in the detoxification and removal of xenobiotics, some

of which are potentially toxic [3]. This renders the liver susceptible to injury or impairment of its functions in the presence of toxicants. The burden of liver diseases is high because damage to this important metabolic organ has serious implications to the entire health and wellbeing [4]. One major mechanism of xenobiotic-induced liver damage is radical species-mediated, leading to oxidative damage to the hepatocytes. Hepatotoxicity is usually characterized by necrosis of hepatocytes, elevation in lipid peroxidation and decrease in reduced glutathione (GSH) concentration. The serum bilirubin and lipid profile and activities of transaminases and alkaline phosphatase are increased during liver damage [5]. In addition, supplementation with antioxidants-rich agents as a remedy to radical species-related assaults on cells has been suggested [6,7]. The use of herbal remedies all over the world for the management of diseases is increasing [8,9]. There is a widespread perception that herbal drugs have little or no side effect [10], making them a first consideration in some African and Asian populations for treating many diseases [11]. Traditional remedies are usually made up of only one part of the plant. However, accumulation of therapeutic experience and the search for improved health outcomes by herbal practitioners over time have resulted in a shift from the use single plant or plant parts to combining different plants or plant parts for enhanced therapeutic potentials [12]. This involves the use of specific proportions of leaves, stem, seeds and roots of different plants in water, alcohol or other non-toxic solvents. It is believed that the active principles in these plants work synergistically or in combination to produce enhanced therapeutic effect [13]. Nigerian drug stores are enriched with several branded polyherbal formulations. One of such polyherbal formulations which have flooded Nigerian drug market is DRHM, a product of FESCO Herbal Mixtures Nigeria Limited. According to the manufacturer, the drug is composed of extracts of *Cymbopogon citratus* (17%), *Carica papaya* leaves (16%), *Magnifera indica* bark (15%), *Moringa oleifera* leaves (14%), *Citrus limonia* (12%), *Psidium guajava* (11%), *Zingiber officinale* root (9%) and *Allium sativum* (6%) blended in water. It is acclaimed by the manufacturer to be a potent detoxifier and blood boost, and also effective in the management of disease conditions such as malaria and typhoid, hepatitis, gonorrhoea, fibroid, syphilis, *E. coli*, menstrual problems, low sperm count, among others. The effects of Deep Root® herbal mixture on H<sub>2</sub>O<sub>2</sub>-induced hematological and biochemical aberrations in rats were assessed in this study.

## 2. Experiments

**Phytochemical analyses of Deep Root® herbal mixture:** The method of Harborne [14] and Trease and Evans [15] were used for the detection and quantification of the phytochemical constituents of DRHM.

**Determination of acute toxicity profile of Deep Root® herbal mixture:** This was done using twenty healthy male Wistar mice of body weight range 25–30 g. They were sourced from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. After 7 days of acclimatization to laboratory environment, the mice were fasted overnight and the body weights were measured, thereafter, they were then divided into four groups of five mice each. Mice in groups 1–4 were treated with 1, 3, 5 and 10 mL/kg b.w. of DRHM, respectively. The experimental mice were monitored for 24 h for neurological, behavioural and morphological signs of toxicity. Body weights of the mice were measured 24 h-post DRHM-treatment to evaluate if there is any significant body weight change.

**Study design for animal study:** Thirty healthy male Wistar albino rats (180–200 g) used for this study were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were maintained under standard husbandry conditions of light (12 h) and darkness (12 h), room temperature of 26 ± 2 °C, and with free access to commercial rat chow (Vital Grower Feed Nigerian Limited) and portable water *ad libitum*. The animals were ethically handled according to standard institutional, national and international protocols. After acclimatization, the experimental rats were divided into 6 groups of 5 rats each: Group 1 served as normal control (NC). Group 2–6 were intoxicated by single intraperitoneal administration of 3 mL/kg b.w of 5% v/v of H<sub>2</sub>O<sub>2</sub> on day 0; group 2 that served as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) control (HC) was not treated. Groups 3–6 received 1, 2

and 3 mL/kg/d b.w. of DRHM while group 6 received 100 mg/kg/d b.w. of silymarin (Livergad Forte®, Laborate Pharmaceuticals, India) p.o. from days 1 to 14. The rats were sacrificed under mild chloroform anaesthesia on day 15. An overnight fasting blood sample was collected from each rat through the jugular veins in anticoagulated bottle as well as plain tube. Samples collected in anticoagulated tubes were subjected to hematological analyses while samples collected in plain tubes were allowed to clot for 15 min and thereafter centrifuged at 3,000 g for 10 min. Serum from each sample was subjected to biochemical analyses of liver, lipid peroxidation and antioxidant status.

**Determination of biochemical parameters:** The parameters evaluated and methods used were: Serum activities of aspartate (AST) and alanine (ALT) aminotransferases [16], catalase (CAT) [17], glutathione peroxidase (GPx) [18] and superoxide dismutase (SOD) [19], serum concentrations of total bilirubin [20], reduced glutathione [21], malondialdehyde (MDA) [22], and vitamins A, C and E [23,24].

**Statistical analysis:** Statistical analysis of primary laboratory data was performed by one-way ANOVA using statistical products and service solutions (SPSS), version 18 and the results presented as mean  $\pm$  standard deviation (SD) in Tables. Test of significance were set at  $p < 0.05$ .

### 3. Results and Discussion

#### 3.1. Phytochemical Constituents of Deep Root® Herbal Mixture

The presence of important secondary metabolites such as alkaloids (3.50%), steroids (1.00%), terpenoids (1.00%), glycosides (0.50%), anthocyanins (0.46%), anthraquinones (0.43%), saponins (0.40%), flavonoids (0.18%), tannins (0.03%), phenols (0.22%) and carotenoids (0.11%) in DRHM (data not shown), suggest that it could be of medicinal value to human health. Alkaloids have been reported to exhibit anticancer and antimalarial effects [25]. Flavonoids, tannins, phenolics, saponins, and anthocyanins have been reported to possess antimicrobial, antioxidant and anti-inflammatory [26]. The presence of these phytochemicals may support the manufacturer's claim that DRHM is effective in the treatment/management of hepatitis and venereal diseases. The result of the phytochemical analysis compares well with the findings of Khawaya et al. [26] and Capasso [26] which reported the presence of flavonoids in *Zingiber officinale*, and *Allium sativium* respectively. Similarly, Ghosh [29] and Ahmad et al. [30] also reported the presence of flavonoids, alkaloids and anthraquinones in *A. sativium* and *Moringa oleifera* which are all components of DRHM.

#### 3.2. Acute Toxicity Profile of Deep Root® Herbal Mixture

The polyherbal formulation was tolerable up to 10 mL/kg b.w since there was no significant behavioural and body weight change within the study period (data not shown). This finding indicates that the lethal dose is higher than 10 mL/kg b.w.

#### 3.3. Effects of DRHM on the Liver Status of H<sub>2</sub>O<sub>2</sub>-Intoxicated Rats

From the findings of this study, administration of H<sub>2</sub>O<sub>2</sub> caused significant ( $p < 0.05$ ) hepatic damage as seen in the elevated serum activities of AST and ALT, and concentration of serum total bilirubin (Table 1). Most toxin-induced liver injuries involve oxidative stress as a mechanism of cellular injury, and may be identified by rapid increase in serum aminotransferases (ALT and AST) activities over days [3], followed by increases in serum bilirubin and alkaline phosphatase [31]. The increase in serum activities of these liver marker enzymes indicates damage to hepatic cell membrane and leakages of intrahepatic enzymes into circulation. Treatment of intoxicated rats with DRHM and silymarin normalized the hepatic status by reducing the serum activities of liver marker enzymes towards normal rats. This decrease in liver enzymes' activities is indicates stabilization of hepatocyte membrane by DRHM, preventing the release of intracellular content of liver cells to circulation. This effect could be attributed to the phytochemical content of DRHM. The result of this study is consistent with the findings of Varsha et al. [32] which reported a decrease in AST and ALT activities in rats

treated with *M. oleifera* leaves, one of the components of the polyherbal formulation. Bilirubin is a breakdown product of hemoglobin in liver cells, spleen and bone marrow. As the liver becomes stressed, serum bilirubin level becomes elevated, indicating hepatic damage or bile duct damage within the liver itself [33]. In this study, treatment of intoxicated rats with DRHM significantly ( $p < 0.05$ ) reduced the total bilirubin level when compared to intoxicated and untreated group. Justin et al. [26] reported that plant secondary metabolites are effective in protecting against oxidative stress-related diseases. The observed improvement in hepatic function could therefore, be attributed to the phytochemicals present in DRHM.

**Table 1.** Effect of DRHM on the liver status of H<sub>2</sub>O<sub>2</sub>-intoxicated rats.

Groups	AST (IU/L)	ALT (IU/L)	Total Bilirubin (mg/dL)
Normal control (NC)	86.75 ± 2.87 <sup>b</sup>	54.25 ± 2.75 <sup>a</sup>	1.38 ± 0.15 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> control (HC)	108.75 ± 7.89 <sup>c</sup>	93.25 ± 3.10 <sup>d</sup>	2.23 ± 0.17 <sup>c</sup>
H <sub>2</sub> O <sub>2</sub> + 1 mL/kg DRHM	89.50 ± 4.80 <sup>b</sup>	70.00 ± 4.32 <sup>c</sup>	1.45 ± 0.13 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> + 2 mL/kg DRHM	88.50 ± 3.11 <sup>b</sup>	61.50 ± 2.75 <sup>b</sup>	1.08 ± 0.10 <sup>a</sup>
H <sub>2</sub> O <sub>2</sub> + 3 mL/kg DRHM	87.50 ± 3.56 <sup>b</sup>	53.00 ± 3.37 <sup>a</sup>	1.00 ± 0.18 <sup>a</sup>
H <sub>2</sub> O <sub>2</sub> + 100 mL/kg silymarin	81.50 ± 3.42 <sup>a</sup>	54.00 ± 2.16 <sup>a</sup>	1.23 ± 0.13 <sup>a</sup>

Data are mean ± standard deviation (SD) (n = 5). Values with different superscripts in a column are significantly different at  $p < 0.05$ . AST = Aspartate aminotransferase; ALT = alanine aminotransferase.

#### 3.4. Effect of DRHM on Antioxidant and Lipid Peroxidation Status of H<sub>2</sub>O<sub>2</sub>-Intoxicated Rats

Malondialdehyde (MDA) is a common biomarker of lipid peroxidation status [32,33]. Elevation in MDA level indicates the degree of injury in the hepatocytes [36,37]. It was observed that MDA level increased in serum of HC when compared with NC. Meanwhile, treatment of intoxicated rats with DRHM significantly ( $p < 0.05$ ) reversed these changes (Table 2). This suggests that the mechanism of hepato-curative effect of DRHM seen above is probably due to its anti-lipid peroxidation effect. SOD, CAT and GPx are part of the hepatic antioxidant defense system that contributes to the regulation of redox homeostasis and mitigation of oxidative attacks on cells [38]. DRHM significantly ( $p < 0.05$ ) increased SOD, CAT, and GPx activities in the treated rats (Table 2). H<sub>2</sub>O<sub>2</sub> control (HC) had a significantly ( $p < 0.05$ ) lower GSH concentration than NC. Meanwhile, treatment of intoxicated rats with graded doses of DRHM significantly ( $p < 0.05$ ) increased the GSH concentration compared with HC, in a manner comparable to that of intoxicated rats treated with silymarin (Table 2). The GSH is an intracellular antioxidant which is also a co-factor to GPx and glutathione reductase. A low GSH concentration exacerbates oxidative assaults, resulting in increased membrane and cell damage. The lower GSH level observed in the intoxicated and untreated rats when compared with normal control might be responsible for the reduced GPx activity. Similarly, a decrease in CAT activity leads to reduced H<sub>2</sub>O<sub>2</sub> decomposition. H<sub>2</sub>O<sub>2</sub> reacts with free iron leading to production of hydroxyl radical that is very toxic to the cells [39]. Treatment of intoxicated rats with DRHM significantly ( $p < 0.05$ ) increased the GSH concentration and this could be responsible for the overall improvement in antioxidant status of the treated rats.

**Table 2.** Effect of DRHM on antioxidant and lipid peroxidation status of H<sub>2</sub>O<sub>2</sub>-intoxicated rats.

Groups	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)	MDA (mmol/L)	GSH (mmol/L)
Normal control (NC)	10.53 ± 0.26 <sup>c</sup>	0.93 ± 0.15 <sup>b</sup>	12.50 ± 0.88 <sup>c</sup>	4.70 ± 0.70 <sup>a</sup>	6.73 ± 0.17 <sup>c</sup>
H <sub>2</sub> O <sub>2</sub> control (HC)	7.00 ± 0.29 <sup>a</sup>	0.59 ± 0.28 <sup>a</sup>	5.53 ± 0.49 <sup>a</sup>	7.85 ± 0.43 <sup>d</sup>	2.68 ± 0.78 <sup>a</sup>
H <sub>2</sub> O <sub>2</sub> + 1 mL/kg DRHM	9.58 ± 0.81 <sup>b</sup>	1.11 ± 0.07 <sup>b</sup>	8.71 ± 1.21 <sup>b</sup>	5.98 ± 0.10 <sup>c</sup>	5.65 ± 0.21 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> + 2 mL/kg DRHM	10.85 ± 0.53 <sup>c</sup>	1.32 ± 0.08 <sup>c</sup>	12.28 ± 0.99 <sup>c</sup>	5.33 ± 0.17 <sup>b</sup>	8.80 ± 0.37 <sup>d</sup>
H <sub>2</sub> O <sub>2</sub> + 3 mL/kg DRHM	12.25 ± 0.10 <sup>d</sup>	1.45 ± 0.08 <sup>d</sup>	15.05 ± 0.94 <sup>d</sup>	4.77 ± 0.08 <sup>a</sup>	9.48 ± 0.46 <sup>e</sup>
H <sub>2</sub> O <sub>2</sub> + 100 mL/kg silymarin	10.88 ± 0.48 <sup>c</sup>	0.98 ± 0.07 <sup>b</sup>	11.21 ± 1.08 <sup>c</sup>	5.10 ± 0.56 <sup>a</sup>	6.05 ± 0.93 <sup>c</sup>

Data are mean  $\pm$  standard deviation (SD) (n = 5). Values with different superscripts in a column are significantly different at  $p < 0.05$ . SOD = superoxide dismutase; GPx = glutathione peroxidase; CAT = catalase, GSH = reduced glutathione; MDA = malondialdehyde.

### 3.5. Effect of DRHM on Antioxidant Vitamins Concentrations of H<sub>2</sub>O<sub>2</sub>-Intoxicated Rats

H<sub>2</sub>O<sub>2</sub>-injection significantly ( $p < 0.05$ ) decreased the concentrations of vitamins A, C and E in HC when compared with NC. Meanwhile, when intoxicated rats were treated with graded doses of DRHM, there were significant ( $p < 0.05$ ) increases in the concentrations of vitamins A, C and E in groups 3–5 compared with HC. A similar result was obtained when intoxicated rats treated with silymarin was compared with NC and HC (Table 3). Vitamin E is a very important antioxidant in lipid medium; it scavenges free radicals and possibly up-regulate the expression of antioxidant enzymes. Similarly, vitamin A also scavenges free radicals in lipid medium and helps maintain pro-oxidant/antioxidant balance. Vitamin C also neutralizes reactive species in the aqueous medium prior to their initiation of lipid peroxidation [40]. It takes part as a co-factor in many enzymatic reactions, and also acts as a plasma localized antioxidant [41]. In addition, vitamin C helps in regeneration of vitamin E to protect the cells in lipid medium. The increase in antioxidant vitamins concentrations observed in the intoxicated and DRHM-treated rats could be attributed to their presence in some of the plant components of DRHM. For example, studies have shown that *Moringa* leaf extract is rich in vitamins and carotenoids [42]. In addition, findings from this study suggest that DRHM is non-lethal up to 10 mL/kg body weight dose. This implies that DRHM is relatively safe for human consumption at the doses studied.

**Table 3.** Effect of DRHM on reduced glutathione and antioxidant vitamins concentrations of H<sub>2</sub>O<sub>2</sub>-intoxicated rats.

Groups	Vitamin A (mg/dL)	Vitamin C (mmol/L)	Vitamin E (mmol/L)
Normal control (NC)	8.88 $\pm$ 0.51 <sup>d</sup>	3.90 $\pm$ 0.08 <sup>d</sup>	0.51 $\pm$ 0.01 <sup>d</sup>
H <sub>2</sub> O <sub>2</sub> control (HC)	4.03 $\pm$ 0.33 <sup>a</sup>	1.90 $\pm$ 0.08 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>
H <sub>2</sub> O <sub>2</sub> + 1 mL/kg DRHM	5.88 $\pm$ 0.51 <sup>b</sup>	2.30 $\pm$ 0.52 <sup>b</sup>	0.47 $\pm$ 0.02 <sup>c</sup>
H <sub>2</sub> O <sub>2</sub> + 2 mL/kg DRHM	7.33 $\pm$ 1.12 <sup>c</sup>	3.18 $\pm$ 0.24 <sup>c</sup>	0.83 $\pm$ 0.01 <sup>e</sup>
H <sub>2</sub> O <sub>2</sub> + 3 mL/kg DRHM	8.50 $\pm$ 0.86 <sup>d</sup>	4.70 $\pm$ 0.18 <sup>e</sup>	1.15 $\pm$ 0.03 <sup>f</sup>
H <sub>2</sub> O <sub>2</sub> + 100 mL/kg silymarin	5.70 $\pm$ 0.46 <sup>b</sup>	2.45 $\pm$ 0.21 <sup>b</sup>	0.40 $\pm$ 0.01 <sup>b</sup>

Data are mean  $\pm$  standard deviation (SD) (n = 5). Values with different superscripts in a column are significantly different at  $p < 0.05$ .

## 4. Conclusions

The results of the present study support the existing knowledge that hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induces cellular oxidative stress via enhancing the production and attack of free radicals on cells and weakening of body's antioxidant defense system. The results further added that treatment of H<sub>2</sub>O<sub>2</sub>-intoxicated rats with Deep Root<sup>®</sup> herbal mixture (DRHM) reverses the associated biochemical aberrations. The above beneficial bioactivities might be attributed to wide varieties of phytochemicals detected in DRHM. This makes the herbal drug a potential candidate for the treatment/management of oxidative-stress related conditions. The polyherbal formulation was not toxic up to 10 mL/kg b.w. dose. However, further studies are needed to evaluate the long-term effects of using this polyherbal formulation.

**Author Contributions:** I.U.O.; C.C.C. and J.C.N. conceived and designed the experiments; I.U.O. and C.C.C. performed the experiments; I.U.O.; A.P.O.; E.C.A. and C.C.C. analyzed the data; J.C.N. and E.C.A. contributed reagents/materials/analysis tools; I.U.O., J.C.N., C.C.C., A.P.O. and C.C.C. wrote the paper.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

DRHM:	Deep Root® herbal mixture;
SOD:	superoxide dismutase;
GPx:	glutathione peroxidase;
CAT:	catalase;
GSH:	reduced glutathione;
MDA:	malondialdehyde;
AST:	Aspartate aminotransferase;
ALT:	alanine aminotransferase

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