Antioxidant Effect of *Curcuma longa* on Some Haematological Parameters in Alcohol Induced Toxicity in Female Rats

Eteng OE*, Moses CA¹, Joe-Enobong E¹, Akamo AJ², Akinloye DI¹, Ugbaja RN¹ and Akinloye OA¹

¹Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
²Department of Biochemistry, University of Calabar, Calabar, Nigeria

*Corresponding Author:* Eteng OE, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

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**Abstract**

This study aimed to evaluate the effects of ethyl acetate extract of *Curcuma longa* Linn on the plasma antioxidant status and haematological parameters in alcohol-induced toxicity in female wistar rats. Thirty female wistar rats were divided randomly into six groups (n = 5). Group I (control) was given normal saline, Group II was administered with 20% ethanol only, Group III was given 100 mg/kg b.w of the extract + 5.22 ml per kg b.w of 20% ethanol, Group IV was given 200 mg/kg b.w of the extract + 5.22 ml per kg b.w of 20% ethanol, Group V was given 400 mg/kg b.w of the extract + 5.22 ml per kg b.w of 20% ethanol and Group VI was administered with 350 mg/kg b.w of *Curcuma longa* extract orally for 14 days. Plasma antioxidants activities and haematological parameters were evaluated using standard methods. Ethanol significantly (p < 0.05) decreased the levels of some of the blood parameters (RBC, HB, WBC, HCT, PLT and PCT) when compared to the control. The pretreatment with *Curcuma longa* L. significantly (p < 0.05) increased the levels of RBC, HB, WBC, HCT, LYMP, PLT and PCT when compared to the group given only ethanol. Oral administration of *Curcuma longa* L. significantly decreased the levels of GSH, GPx, MDA, SOD and CAT activities in the plasma as compared to the group administered ethanol only. In conclusion, these observations show that pretreatment with ethyl acetate extract of *Curcuma longa* Linn exerts its protective effect on haematological parameters and decreases lipid peroxidation as well as improving plasma antioxidant status.

**Keywords:** *Curcuma longa* Linn; Alcohol; Antioxidants; Haematological Parameters

**Introduction**

Alcohol occupied an important place in the history of humankind for at least 8000 years [1]. Alcohol when taken in moderate amounts relieves anxiety and fosters a feeling of euphoria [2]. However, chronic consumption of alcohol has been known to be the cause of over 60 different types of disease and condition including injuries, mental and behavioral disorder, gastrointestinal conditions, cancers, cardiovascular diseases, immunological disorders, lung diseases, skeletal and muscular diseases, reproductive disorders and pre-natal harm including an increases risk of prematurity and low birth weight [3]. People who abuse alcohol are also at risk of alcohol-related haematological complications, including those affecting the blood cells as well as proteins present in the blood plasma and the bone marrow, where the blood cells are produced. Alcohol has direct and indirect adverse effects on the blood building, or hematopoietic system. The direct consequences include toxic effects on the bone marrow; the blood cell precursors; and the mature red blood cells (RBCs), white blood cells (WBCs) and platelets. Alcohol’s indirect effects include nutritional deficiencies that impair the production and function of various blood cells [4]. The consumption of alcohol increases the production of free radicals and the concomitant development of oxidative...
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stress, which is directly related to the products of ethanol oxidation [5]. In recent years, overwhelming evidence has confirmed that both the volume of lifetime alcohol use and the combination of frequency of drinking increase the risk of alcohol-related disease [6]. Alcohol poses a risk by acting as an immunosuppressant increasing the risk of communicable diseases.

Ethanol is primarily metabolized in the body by alcohol dehydrogenase and aldehyde dehydrogenase enzyme systems, in which acetaldehyde and acetate are generated as products [7]. Acetaldehyde generated is toxic to the mitochondria and triggers or aggravates their oxidative stress by binding to reduced glutathione and promoting its leakage from the cell [8]. Another metabolic pathway that plays a significant role in alcohol toxicity is the cytochrome P450 system (CYP2E1) of liver microsomes [9]. The by-products generated are more dangerous than the alcohol itself and contribute to the alcohol-induced liver damage. It was discovered that the generation of acetaldehyde, induction of CYP2E1 by ethanol, generation of ROS by CYP2E1 with dysfunctioning of the mitochondrial electron transport chain, impairment of antioxidant mechanisms, and the resultant oxidative stress trigger inflammatory responses and cell death by either necrosis or apoptosis of the hepatocytes. The ability of alcohol to promote oxidative stress and the role of free radicals in alcohol-induced tissue injury clearly are important areas of research in the alcohol field, particularly because such information may be of major therapeutic significance in attempts to prevent or ameliorate alcohol's toxic effect. Thus, these findings could result in the development of more effective and selective new medications capable of blocking the actions of reactive oxygen species (ROS) and consequently, the toxic effects of alcohol [10]. Therefore, the continuous search for a more natural, effective, affordable and readily available plant that can mitigate the toxic effects of alcohol informed this research. Turmeric (Curcuma longa L.) is a medicinal plant extensively used in mostly India and China medicine as home remedy for various diseases [11]. It is botanically related to ginger (Zingiberaceae family) and it is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis [11-13]. In the last few decades there has been considerable interest in the active compounds in turmeric called curcuminoids. The major curcuminoid is called curcumin (diferuloylmethane), which makes up approximately 90% of the curcuminoid content in turmeric, followed by demethoxycurcumin and bisdemethoxycurcumin [14]. Hundreds of in vitro and animal studies have been published describing the antioxidant, anti-inflammatory, antiviral, and antifungal properties of curcuminoids [15]. The curcuminoids also give turmeric its bright yellow color. While this plant is rather an important spice in Iran, it is also an important component of curries to which it gives the yellow color in Malaysia, India, China, Polynesia, and Thailand, and the mustard and sauces in the West [16]. Turmeric is also used to add flavor and color to rice, pasta, meat and vegetable dishes, and salads. It is stated that turmeric has been widely used for medical treatments of various diseases for at least 2500 years in Asian countries mostly [16,17] and it has many benefits for prevention and treatment of many diseases in Ayurveda and traditional Chinese medicine. The importance of turmeric in medical treatment primarily stems from the orange-yellow colored curcumin, the most active component. Curcumin is a lipophilic polyphenol substance [18], which constitutes the 2 - 5% of turmeric. With the studies about curcumin, it has been determined that the chemical structure of this polyphenol substance shows antioxidant, antimicrobial, anti-inflammatory, antiangiogenic, antimitogenic, and antiplatelet aggregation property. It is stated that, curcumin has a protective and preventive effect against various diseases such as cancer, autoimmune, neurological, metabolic, lung, liver, and cardiovascular diseases (CVDs).

Aim of the Study

This study aimed to determine the ameliorative effects of the ethyl acetate extract of Curcuma longa Linn on the hematological parameters and blood plasma antioxidant status in female wistar rats.
Materials and Methods

Materials

Reagent

Sodium hydroxide solution (0.04M), 0.1M phosphate buffered saline (pH 7.4), hydrochloric acid (0.1M) and 20% ethanol.

Equipments and apparatus

Weighing balance, centrifuge, micropipette, cotton wool, tissue paper, heparinised tubes, beakers, eppendorf tubes, measuring cylinder, conical flask, disposable gloves, dissecting sets, needles and syringes (5 ml and 10 ml), spectrophotometer (Spectrum23A), centrifuge, homogenizer, measuring cylinder, needle and syringe, cotton wool, quartz cuvette, water bath, cannula, stop-watch, spectrophotometer, vortex mixer and wooden animal cages.

Methods

Plant collection

Curcuma longa Linn rhizomes were harvested from its natural habitat in Ajasa farms, Ile-Ise Awo, Abeokuta. The plant specimen was authenticated by the Department of Botany, Federal University of Agriculture, Abeokuta, as Curcuma longa L. (Family: Zingiberaceae). The plant specimen matches with the Herbarium specimen number: FUNAAB H-0065

Plant preparation and extraction

The rhizomes were rinsed properly, cut into small sizes and air-dried to remove moisture present in them. After they were dried, the rhizomes were ground into powdery form using a mechanical blender. The powdery turmeric was weighed (1000 mg) and treated with 2000 ml of ethyl acetate. The mixture was left to stay for 3 days. It was subsequently filtered using Whitman paper and evaporated using a rotatory evaporator. The extracts were stored for subsequent biochemical analysis.

Citation: Eteng OE, et al. “Antioxidant Effect of Curcuma longa on Some Heamatological Parameters in Alcohol Induced Toxicity in Female Rats”. EC Pharmacology and Toxicology 8.6 (2020): 43-56.
Experimental animals and study design

Thirty (30) female Wistar rats weighing between 150 - 220g were obtained from Tayo farm, Ajibode, University of Ibadan, Ibadan. The animals were housed in well-ventilated wooden cages at room temperature (28 - 30°C), light and humidity. They were allowed free access to maximum feeding of animal feed and water. They were acclimatized for two weeks before the commencement of the experiment. The animals were randomly distributed into six groups of five animals each as shown in the table below. Administration was done with the use of oral cannula.

<table>
<thead>
<tr>
<th>Groups (n = 5)</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (normal saline)</td>
</tr>
<tr>
<td>2</td>
<td>20% ethanol (Kanbak., et al. 2001)</td>
</tr>
<tr>
<td>3</td>
<td>100 mg/kg body weight of extract + 20% ethanol</td>
</tr>
<tr>
<td>4</td>
<td>200 mg/kg body weight of extract + 20% ethanol</td>
</tr>
<tr>
<td>5</td>
<td>400 mg/kg body weight of extract + 20% ethanol</td>
</tr>
<tr>
<td>6</td>
<td>350 mg/kg body weight of extract</td>
</tr>
</tbody>
</table>

*Table 1: Animal grouping.*

Sacrifice

Feed and water were withdrawn from the animals 24 hours before the sacrifice. The rats were slightly anaesthetized with diethyl ether in a desiccator and then sacrificed.

Measurement of hematological indices

The haematocrit (HCT), haemoglobin (HB) concentration, red blood cell (RBC), white blood cell (WBC), granulocyte (GRAN), lymphocytes (LYMP), platelet (PL) counts and plateletcrit (PCT) were measured using an auto-analyzer machine (SFRI blood cell Counter, H18 light, France).

Biochemistry analyses

Assay protocol for malondialdehyde (MDA) concentration

Lipid peroxidation was determined by the methods at 535 nm. The reagents used are 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid (TBA) and 0.25N hydrochloric acid (HCl). The reagent composition is TCA-TBA-HCl in ratio 1:1:1. 20 µl of the sample was added to 400 µl of the TCA-TBA-HCl reagent in the eppendorf tubes. The content is boiled for 15 minutes and cooled over ice. It was then centrifuged at 5000 rpm for 10 minutes. The absorbance of the clear supernatant is measured using blank to zero the spectrophotometer. The blank was prepared by adding 400 µl of TCA-TBA-HCl reagent to 20 µl of distilled water.

Calculation

Malondialdehyde concentration was calculated using Beer-Lambert's law.

\[ A = \text{absorbance, } b = \text{pathlength, } a = \text{extinction coefficient (155nM·cm}^{-1}) \text{, } c = \text{concentration.} \]
Antioxidants assay

Assay protocol for glutathione peroxidase (Gpx) activity in the plasma

Glutathione peroxidase (Gpx) was measured by the method described by Rotruck, et al. (1973). Briefly, the reaction mixture contained 0.2 ml 0.4M phosphate buffer (pH 7.0), 0.1 ml of 10 mM sodium azide, 0.2 ml tissue homogenized in 0.4M phosphate buffer saline, 0.2 ml reduced glutathione, 0.1 ml of 0.2 mM hydrogen peroxide. The contents were incubated for 10 minutes at 37°C, 0.4 ml of 10% TCA was added to terminate the reaction and centrifuged at 3200*g for 20 minutes. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg of 5,5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate). The absorbance of each sample was read 412 nm against the blank (which contained 50 µl of distilled water in place of the sample) within 5 minutes of adding the DTNB. The activities were expressed as µg of GSH consumed/min/mg protein. One-unit activity of GxP activity is defined as the amount of enzymes required to convert 1 µmol of substrate per minute under the above assay condition.

Assay protocol for glutathione

Glutathione in blood plasma was determined using the spectrophotometric method described by Ellman (1959), in which 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) complexes with glutathione in the buffered (pH 8.0) test sample and the colour formation of glutathione conjugate at 25°C, which developed rapidly after two minutes was monitored and absorbance read at 420 nm within 20 minutes.

Procedure

To a clean test tube was pipetted 0.02 ml whole blood, 9 ml of 50% acetone-water (1:1) mixture, and 1 ml of 0.1M phosphate buffer pH 8 and thoroughly mixed. 3 ml of the mixture obtained above was placed into a cuvette and read as blank. To another 3 ml of the mixture was added 0.02 ml of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.01M), mixed, left for 2 minutes for colour to develop and the absorbance read at 420 nm within 20 minutes of preparation.

Glutathione concentration was obtained using the following equation: Glutathione Concentration (mM) = Absorbance x 36.8.

Preparation of reagents used in the determination of glutathione

1. **DTNB**: 3.96 mg (0.0369g) was dissolved in 10 ml of 0.1 ml pH 7.0 phosphate buffer and stored in amber bottle.
2. **50% Acetone**: 50 mls of Acetone was added to 50 mls of distilled water and stored in amber bottle.
3. **Phosphate buffer of 0.1M, pH 7.0**: 30 ml of 0.2 mol/l KOH was added to 50 ml of 0.2 mol/l KH₂PO₄ and diluted to 100 ml.
4. **Phosphate buffer of 0.1M, pH 8.0**: 47 ml of 0.2 mol/l KOH was added to 50 ml of 0.2 mol/l KH₂PO₄ and was diluted to 100 ml.

Assay protocol for superoxide dismutase assay

Superoxide dismutase was determined according to the modified [19] method of Xican Li (2012). Two test tubes marked blank (B) and test (T) were arranged in a test tube rack. 100 µl of potassium phosphate buffer (0.05M; pH 7.4), 830 µl of distilled water and 50 µl of diluted blood plasma (400 fold dilution using phosphate buffer 0.05M; pH 7.4) was pipetted into tube T while 150 µl of buffer and 830 µl of distilled water was pipetted into tube B. The tubes were incubated for 10 minutes at 25°C and 20 µl of 0.01M pyrogallol (prepared in 0.01M HCl) was added to both tubes. They were mixed by inversion and absorbance at 420 nm was recorded at 30 seconds interval for 3 to 5 minutes. The difference between the initial and final absorbance and average absorbance difference was calculated (ΔA 420/min). Super-
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Oxidase dismutase activity was calculated as:

\[
\% \text{ inhibition} = \frac{(\Delta A_{420\text{nm}} \text{ /minute of blank} - \Delta A_{420\text{nm}} \text{ /minute of sample}) \times 100}{\Delta A_{420\text{nm}} \text{ /minute of blank}}
\]

Units of SOD = \[
\frac{\% \text{ inhibition}}{100 - \% \text{ inhibition}}
\]

Units/mL = Units \times \text{ dilution factor (400 fold for whole blood)}

Units/mg protein = \[
\frac{\text{Units/mL}}{\text{mg protein/mL}}
\]

### Assay protocol for catalase (CAT) activity

Catalase was determined [20]. The disappearance of peroxide is followed spectrophotometrically at 240 nm. One unit decomposes one micromole of H\(_2\)O\(_2\) per minute at 25°C and pH 7.0 under the specified conditions. The reagents used for the assay are 0.05M Potassium phosphate, pH 7.0, 30% Hydrogen peroxide (60 µL) in 0.05M potassium phosphate (10 ml), pH 7.0, this is the substrate. 200 µL of the enzyme (sample) and 100 µL of the substrate was pipetted into the cuvette. The substrate solution is pipetted directly into the enzyme solution. Readings are taken at 10 seconds interval for 70 seconds.

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**Reagents**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Blank</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Phosphate buffer (0.05M; pH 7.4)</td>
<td>100 µl (50 µl)*</td>
<td>100 µl (50 µl)*</td>
</tr>
<tr>
<td>Doubly Distilled H(_2)O</td>
<td>830 µl (415 µl)*</td>
<td>830 µl (415 µl)*</td>
</tr>
<tr>
<td>Potassium Phosphate buffer (0.05M; pH 7.4)</td>
<td>50 µl (25 µl)*</td>
<td>-</td>
</tr>
<tr>
<td>Sample (400 fold Diluted with PO(_4) buffer 0.05M; pH 7.4)</td>
<td>-</td>
<td>50 µl (25 µl)*</td>
</tr>
<tr>
<td>Incubate at 25°C for 10 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogallol (0.06M) in 0.01M HCl</td>
<td>20 µl (10 µl)*</td>
<td>20 µl (10 µl)*</td>
</tr>
</tbody>
</table>

*Volumes for 800 µl microcuvette.*

Potassium Phosphate buffer (0.05M; pH 7.4) = Acid (KH\(_2\)PO\(_4\)) - 1.69 g/600 ml Salt (K\(_2\)HPO\(_4\)) - 4.2 g/600 ml adjust pH to 7.4 with KOH. Dilute samples with the above buffer, 400 fold (3.990 µl H\(_2\)O + 10 µl plasma).

0.01M HCl - 99.97 ml Distilled H\(_2\)O + 0.03 ml (30 µl) Conc. HCl (measure 100 ml, pipette out 30 µl of water and replace with 30 µl of Conc. HCl) and add Pyrogallol (0.06M). Mass Conc. = Molarity X Molar Mass = 0.06 X 126.11 = 7.5666g/L

= 0.075666 g/10 ml of prepared 0.01M HCl OR (0.038 g/5 ml of prepared 0.01M HCl) OR 0.04 g/5 ml of HCl.

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Calculation:

\[
\text{Unit/ml} = \frac{\Delta \text{Abs/min} \times 1000 \times \text{dilution} \times 3}{43.6 \times 2}
\]

Where 43.6 = molar absorbance index for H₂O₂ at 240 nm in a 1 cm cuvette.

Statistical analysis

All values were expressed as the mean ± standard error of mean (SEM). The data were analyzed, using the one-way analysis of variance (ANOVA), followed by post hoc Duncan's test by statistical software package SPSS 20.0. p < 0.05 was considered statistically significant. Statistical analysis of data were carried out using the method of Duncan Multiple Range Test, mean, standard error of mean and statistical analysis of variance (ANOVA) at p < 0.05 level of significant. All these were done using SPSS statistical package (version 16).

Results

Haematological parameters

Alcohol administration caused as slightly significant decrease (p < 0.05) in the HB and RBC and a significant decrease (p < 0.05) in the HCT, WBC, PLT and PCT levels when compared with that of the normal control rats. However, pretreatment of the rats in groups 3, 4 and 5 with the ethyl acetate extract of *Curcuma longa* caused a dose dependent slightly significant increase (p < 0.05) in the RBC and HB levels when compared to the ethanol treated control group. Treatment of the rats in group 6 with the extract alone caused a significant increase (p < 0.05) in the HB, RBC, HCT, WBC, PLT and PCT when compared to the ethanol treated group (Table 2). No significant difference was observed in the LYMP and GRAN levels in all the groups.

<table>
<thead>
<tr>
<th>Groups (n = 5)</th>
<th>HB (g/dl)</th>
<th>RBC (10⁶/µl)</th>
<th>HCT (%)</th>
<th>WBC (10³/µl)</th>
<th>LYMP (%)</th>
<th>GRAN (%)</th>
<th>PLT (10³/µl)</th>
<th>PCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.90 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.04 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.58 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.38 ± 1.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.05 ± 3.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.10 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>720.00 ± 68.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>9.13 ± 2.46&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.03 ± 1.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.23 ± 7.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.85 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.70 ± 8.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.38 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>457.25 ± 59.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>12.14 ± 1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.46 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.88 ± 3.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.02 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.78 ± 3.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>491.80 ± 94.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>12.58 ± 0.98&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.79 ± 0.98&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>38.45 ± 2.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.07 ± 1.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>89.32 ± 2.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.52 ± 0.54&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>552.80 ± 59.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.37 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>12.80 ± 0.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.96 ± 0.46&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>38.70 ± 2.85&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.18 ± 1.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>90.24 ± 0.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.78 ± 0.69&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>524.25 ± 40.87&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.37 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>13.10 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.27 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.64 ± 2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.42 ± 1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.73 ± 1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.15 ± 5.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>667.60 ± 43.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Table 2: Effects of ethyl acetate extract of Curcuma longa Linn on the haematological parameters.*

Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences based on one-way ANOVA followed by Duncan’s multiple comparison test.

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Table 3 shows the effects of ethanol and ethyl acetate extract of Curcuma longa on the plasma MDA level and antioxidants status of the female Wistar rats. Based on the investigation of oxidative stress biomarkers, there was a significant increase in lipid peroxidation levels in the animals treated with ethanol alone, as evidenced by the significant increase (p < 0.05) in plasma MDA levels when compared to the control group. However, Curcuma longa conferred a protective effect as animals pre-treated with the extract had a dose dependent significant lower MDA levels when compared to the ethanol treated group.

<table>
<thead>
<tr>
<th>Groups (n = 5)</th>
<th>GSH (µmol/g protein)</th>
<th>GPx (pg GSH/g protein)</th>
<th>SOD (U/min/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>MDA (µmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.72 ± 0.01b</td>
<td>0.63 ± 0.02b</td>
<td>15.89 ± 0.65a</td>
<td>0.04 ± 0.01a</td>
<td>7.20 ± 0.48a</td>
</tr>
<tr>
<td>2</td>
<td>1.03 ± 0.04d</td>
<td>0.81 ± 0.02d</td>
<td>23.18 ± 0.68c</td>
<td>0.16 ± 0.01d</td>
<td>23.33 ± 0.81d</td>
</tr>
<tr>
<td>3</td>
<td>0.84 ± 0.01c</td>
<td>0.73 ± 0.04c</td>
<td>20.09 ± 0.49b</td>
<td>0.13 ± 0.01c</td>
<td>13.8 ± 0.82c</td>
</tr>
<tr>
<td>4</td>
<td>0.82 ± 0.04c</td>
<td>0.61 ± 0.05b</td>
<td>17.45 ± 0.36c</td>
<td>0.09 ± 0.01b</td>
<td>8.73 ± 0.54c</td>
</tr>
<tr>
<td>5</td>
<td>0.73 ± 0.03b</td>
<td>0.61 ± 0.03c</td>
<td>16.87 ± 0.40c</td>
<td>0.05 ± 0.00b</td>
<td>8.01 ± 0.36c</td>
</tr>
<tr>
<td>6</td>
<td>0.63 ± 0.02a</td>
<td>0.50 ± 0.03a</td>
<td>14.69 ± 0.35a</td>
<td>0.03 ± 0.00a</td>
<td>7.15 ± 0.33a</td>
</tr>
</tbody>
</table>

Table 3: Effects of ethyl acetate extract of Curcuma longa Linn on the plasma enzymatic antioxidant activities and MDA concentration.

Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences based on one-way ANOVA followed by Duncan’s multiple comparison test.

GPx, GSH, SOD and CAT plasma activities were significantly increased in animals which received ethanol only when compared with normal control. However, co-administration with the extract to the ethanol treated rats significantly decreased (p < 0.05) the levels of GPx, GSH, SOD and CAT enzymes when compared with rats treated with ethanol only treated group.

A significant decrease (p < 0.05) in the MDA levels was observed in the rats given only the extract (group 6) in comparison to the ethanol treated group (group 2).

GPx, GSH, SOD and CAT plasma activities also showed significant decrease (p < 0.05) in their activities in the animals which received the extract only when compared to the ethanol treated group.

Figure 2: Effects of ethyl acetate extract of Curcuma longa Linn on the plasma SOD activity.

Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences (p < 0.05) based on one-way ANOVA followed by Duncan’s multiple comparison test.

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**Figure 3:** Effects of ethyl acetate extract of *Curcuma longa* Linn on the plasma CAT activity.
Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences (p < 0.05) based on one-way ANOVA followed by Duncan’s multiple comparison test.

**Figure 4:** Effects of ethyl acetate extract of *Curcuma longa* Linn on the plasma GPx activity.
Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences (p < 0.05) based on one-way ANOVA followed by Duncan’s multiple comparison test.

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**Figure 5:** Effects of ethyl acetate extract of *Curcuma longa* Linn on the plasma GSH activity. Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences (p < 0.05) based on one-way ANOVA followed by Duncan’s multiple comparison test.

**Figure 6:** Effects of ethyl acetate extract of *Curcuma longa* Linn on the plasma GSH activity. Data are expressed as mean ± SD of five animals per group. The mean values with different superscripts (a, b, c) on the same column denote significance differences (p < 0.05) based on one-way ANOVA followed by Duncan’s multiple comparison test.

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## Discussion

Plant phenolics, flavonoids and ascorbic acids are important constituents that contribute to the functional quality, color and flavor of natural products. They serve as powerful antioxidants due to their electron-donating ability to arrest the production of free radicals following oxidative stress [22]. To counteract the potential hazards of oxidative damage, dietary consumption of antioxidants may be regarded as the first line defense against highly reactive toxicants [12]. The significant increase in the HB of the extract treated groups, when compared to the ethanol only treated group, could be as a result of the role curcumin, the main active ingredient of *Curcuma longa*, plays in the synthesis of succinyl-CoA. Curcumin can accelerate the emulsification of fats by stimulating the production of bile [23] so that the digestion of fat will be more optimal. This indirectly increases the provision of substrates for β-oxidation which eventually enhances the production of succinyl-CoA through Kreb’s cycle [24]. Considering succinyl-CoA is one of the materials needed for hemoglobin (heme in particular) synthesis [25], enhanced succinyl-CoA production can therefore be attributed to the increase in hemoglobin synthesis. The significant decrease (p < 0.05) in the RBC count of the rats in group 2 when compared to the control group could be as a result of the toxic effects of alcohol on the synthesis of viable RBCs in the bone marrow. This is supported by [26] in which it was discovered that chronic alcohol intake suppresses blood cell production (hematopoiesis). Impaired hematopoiesis usually occurs in people with severe alcoholism, who also may suffer from nutritional deficiencies of folic acid and other vitamins that play a role in blood cell development. Chronic excessive alcohol ingestion reduces the number of blood cell precursors in the bone marrow and causes structural abnormalities in these cells, resulting in fewer than normal or non-functional matured blood cells. As a result, alcoholics may suffer from moderate anemia, characterized by enlarged, structurally abnormal RBC [26,27]. However, the pretreatment of the rats in groups 3, 4 and 5 with the extract showed a dose-dependent significant increase (p < 0.05) in the RBC count when compared to the ethanol only treated group. The significant increase in the RBC count in the rats treated with the extract might be due to the presence of iron which is an essential co-factor for cytochrome oxidase enzymes at cellular level metabolisms and required for red blood cell production [28]. This might suggest that *Curcuma longa* is a rich source of iron. According to the significant decrease (p < 0.05) in the WBC count observed in the alcohol treated rats could be as a result of immunodeficiency associated with chronic alcohol consumption. The total WBC count showed a dose-dependent significant (p < 0.05) increase in all the extract treated groups as compared to the ethanol group. This increase might be due to the immunomodulatory effect of *Curcuma longa* [29]. This suggests that *Curcuma longa* helps in activation of immune responses and increasing the WBC count [29]. It was also observed that there was a significant decrease (p < 0.05) in the PLT count and PCT in the group 2 rats when compared to the control group. This is as a result of impaired synthesis of the platelets. Reduced platelets level may be due to impaired production or destruction of platelets in the circulation. When either of this occur the normal function of platelets among which; maintaining the integrity of vascular endothelium, performing the primary haemostatic plug following vessel injury, activation of blood coagulation system, producing mediators involved in vessel wall repair and regulation of vascular toxicity, as well as inflammatory reaction and producing growth factor [30] will be affected. According to Dailey [31] impaired platelet production may occur during heavy alcohol ingestion, though, this usually disappears after 3 - 7 days [32] also said that the inhibition of bone marrow by alcohol can result in a low number of circulating platelets. Acquired immune deficiency syndrome (AIDS), alcoholism probably is the leading cause of thrombocytopenia i.e. decrease in platelet count. Moreover, alcohol-related thrombocytopenia generally is transient and platelet counts usually return to normal within one week of abstinence. Alcohol affects not only platelet production but also platelet function. Thus, patients who consume excessive amounts of alcohol, can exhibit a wide spectrum of platelet abnormalities. These abnormalities include impaired platelet aggregation, decreased secretion or activity of platelet-derived proteins involved in blood clotting and prolongation of bleeding [33]. The role alcohol plays in the elucidation of oxidative stress can’t be overemphasized. In this study, the levels of GSH activity were increased significantly in the alcohol treated group when compared to the control group, during alcohol ingestion indicating oxidative stress. GPx has a well-established role in protecting cells against oxidative injury and it’s significant increase (p < 0.05) in the ethanol treated group as compared to the control group upon ethanol metabolism suggested oxidative stress [34]. The significant increase (p < 0.05) in catalase activity in the ethanol treated group when compared to the control group may be either due to leakage of the enzyme

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from tissues to the plasma or due to oxidative stress response of alcohol metabolism to eliminate the accumulated hydrogen peroxides in the plasma [35]. The significantly increased (p < 0.05) in superoxide dismutase activity when compared to the control group also in the ethanol treated group may also be due to a compensatory mechanism to cope with excess generation of superoxide radicals in the plasma. The significantly increased (p < 0.05) levels of plasma MDA observed in the ethanol treated group as compared to the control group was as a result of ethanol metabolism. MDA is a highly reactive compound that forms adducts with proteins and DNA, leading to cell damage and mutagenicity. But upon administration of C. longa, the levels of MDA decreased and improved plasma antioxidant status. This antioxidant capacity of C. longa may include scavenging or neutralizing the free radicals, oxygen quenching and making it less available for oxidative reaction and inhibition of oxidative enzymes like cytochrome P450.

Conclusion

In conclusion, the results of our recent study clearly demonstrate that Curcuma longa Linn confers protection on haematological parameters and decreases the damage of lipid peroxidation, against oxidative stress in rats, thus confirming its antioxidant status.

Conflict of Interest

The authors declare no conflict of interest about this work.

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