Gallic Acid Attenuates Cardiovascular and Hematological Complications in Vincristine Treatment via Hydroxyl Radical Scavenging and Endogenous Antioxidant Stimulation in Male Wistar Rats

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ABSTRACT
The menace of cancer and the nightmare of complications of cancer chemotherapy have driven researchers to explore simple but efficient combination therapy that includes antioxidants, in cancer therapy. The ability of gallic acid to correct the toxic complication of Vincristine was investigated.

Twenty adult male rats of the Wistar strain were grouped into four, randomly, consisting of five rats each. The untreated control (group A) was given only distilled water, groups B and C 0.025 mg/kg Vincristine sulfate intraperitoneally once a week for two weeks. Group C rats were thereafter administered 100 mg/kg gallic acid daily by gastric gavage for 14 days.

At 14 days, blood pressure and ECG were measured in the rats, then blood samples were obtained via the retrorbital venous plexus for determination of haematological parameters and plasma biochemistry. They were then euthanized through cervical dislocation, under ether anaesthesia, and liver, kidneys, heart, and brain samples were collected, weighed, and stored for determination of marker of oxidative stress in the post mitochondrial fractions of each organ.

Results of the study showed that rats in group B had hypertension as evidenced by elevated diastolic and systolic as well as mean arterial pressure while QT interval and corrected QT were slightly elongated. They also had lowered RBC, WBC, and granulocyte counts. Markers of oxidative stress, GSH, and SOD were also depleted while H2O2 generation increased in this group, whereas all the observed anomalies were corrected in the group C rats that were administered both Vincristine and gallic acid.

This study further showed that Vincristine, at normal recommended therapeutic dosage is toxic, causing anaemia, panleucopenia, and cardiovascular anomalies via oxidative stress and generation of hydroxyl radicals. These were however corrected by concurrent administration of gallic acid.

Keywords: Antioxidants, Cancer chemotherapy, Complications, Gallic acid and Oxidative stress.

1. INTRODUCTION
Cancer is probably the foremost cause of death throughout the entire world. It was reported to cause about 9.6 million deaths in 2018 alone [1]. Meanwhile, the treatment of cancer, irrespective of the mode employed is also a challenging feat. This ranges from the invasive nature of surgery to the damaging effect of ionizing radiation on tissues during the use of irradiation therapy; to the toxicity of chemotherapeutic agents that are used for cancer treatment. These effects have led to the development of novel and innovative concepts, such as the use of designer nanoparticles, liposomes, and other materials for direct...
delivery of drugs into cancerous tissues to prevent damage to healthy cells [2]. By nature however, most anticancer chemotherapy drugs are toxic to cells, affecting both cancerous and normal cells [3], including the rapidly dividing myeloid cells [4] immune cells in lymph nodes and other lymphoid tissues [5], and the GIT. Myelosuppression (with anaemia and panleucopenia) and GIT toxicity signs including anorexia, nausea, diarrhoea, and vomiting, are usually the early signs associated with toxicity of cancer therapy. Anaphylactic reactions, skin damage, cardiopulmonary pathologies, toxicity to the pancreas, nervous system, liver, and kidneys have been widely reported [6], [7].

In a bid to reduce the side effects of chemotherapy, clinicians and oncologists have embarked on combination therapy that includes antioxidants. Several studies have evaluated the role of antioxidants and medicinal plants with antioxidant potential on complications and side effects of anticancer drugs such as Methotrexate [8], Doxorubicin [9], [10], Cisplatin [11]. In a randomized study that evaluated the use of antioxidants therapy combined with anti-cancer drugs, it was reported that about 87% of cancer chemotherapy included one antioxidant or the other. This idea reportedly increased the efficacy of the anticancer drugs, reduced the side effects considerably, and improved the well being of affected individuals [7].

Vincristine is a vinca alkaloid synthesized from Japanese periwinkle (Vinca rosea). The vinca alkaloids generally bind to tubulin of mitotic spindle to halt cell division. Despite the popular use of vincristine in humans, leukemia in pediatric cancer patients, and transmissible venereal tumour (TVT) in dogs, there is paucity of information on the combination of vincristine with antioxidant therapy to reduce the side effects. Several antioxidants and medicinal plants with antioxidant properties have been used to ameliorate toxic effects of anticancer drugs. Notable among these antioxidants is gallic acid—a phenolic compound found in tea, especially green ones, grapes, red wine, and nuts. It can also be found as hydrolysable tannins in woody perennial plants generally [12].

However, there is paucity of information on the clinical combination of antioxidants in the use of Vincristine, especially when used in small animals. This paucity may be due to a lack of studies on the modulatory effects of antioxidants on Vincristine toxicity and side effects. This study was therefore tries to elucidate the role of the antioxidant gallic acid as it affects the toxic side effects of Vincristine Sulfate at its normal recommended therapeutic dosage.

### 2. Materials and Methods

Twenty adult male Wistar rats (130–150 g) were used in the study. The rats were housed under standard conditions of 12-hour daylight cycle and fed with normal rat chow, in the Experimental Animal Unit of the Department of Veterinary Physiology and Biochemistry, University of Ibadan. They also had access to clean potable water ad libitum. The rats were settled in, for two weeks and then divided into four groups A–D. Group A, which serve as the control was not exposed to Vincristine but given only water, group B and C were given a single weekly dose of Vincristine Sulfate at 0.025 mg/kg intraperitoneally once in a week for two weeks, while group B was given distilled water, group C was given 100 mg/kg gallic acid by gastric gavage daily for fourteen days. Group D on the other hands received only gallic acid (100 mg/kg) by gastric gavage daily for 14 days.

#### 2.1. Determination of Blood Pressure

On day 14 of the study, diastolic and systolic blood pressure and mean arteria blood pressure were measured in awake rats by tail cuff plethysmography with automatic blood pressure monitor.

#### 2.2. Electrocardiograph (ECG) Determination

The ECG was also measured in the rats using 7-lead EDAN VE-1010, ECG. From the results, heart rate, P-wave, QRS duration, PR-interval, amplitude for R, QT segment and Bazett’s correction of the QT interval (QTc) were evaluated.

#### 2.3. Haematology

At the conclusion of the experimentation, 5 ml of blood samples was collected from each rat using the retro-orbital venous plexus into tubes containing anticoagulant for determination of haematological parameters. Blood samples were spun at 4000 rev/min for 10 minutes and the plasma collected for plasma biochemistry. From the blood samples collected were determined, the packed cell volume (PCV) using the microhaematocrit method, haemoglobin concentration (Hb) by spectrophotometric method, red and white blood cell counts using the improved Neubauer’s slide while differential white blood cell count were determined in Giemsa stained slide. Erythrocytic indices—mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCH) were calculated using standard erythrocytic parameters above. Erythrocyte osmotic fragility was determined according to the method described by Azeez et al. [13], in varied phosphate-buffered sodium chloride concentrations.

#### 2.4. Plasma Biochemistry

Sodium and potassium serum levels were determined using Flame Photometry while Cl and HCO$_3$- were measured according to the methods of Schales and Schales. Urea and creatinine were determined by spectrophotometry. Alkaline phosphatase (ALP) levels were measured by the method of Bessey while ALT and AST were determined by the method of Reitman and Frankel as previously described by Oyagbemi et al. [14]. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were all measured by spectrophotometry using kits for Cobas Integra 400 plus Autoanalyzer.

#### 2.5. Determination of Indicators of Oxidative Damage

The rats were thereafter sacrificed under ether anaesthesia whilst the kidney, liver, heart, testes and brain samples were harvested. The organs were quickly removed, cleaned in cold normal saline, blotted with filter paper and

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Diastolic BP (mm/Hg) 77.47 ± Biuret method, H₂O₂ generation, GSH and Superoxide
testes and brain tissues were determined, total protein by
ative stress. From the homogenates of heart, liver, kidney,
decanted and used for determination of indictors of oxida-

Note: Data here are computed as mean ± SD. Along the rows, all values that have the same type of superscript are different significantly @ P < 0.05. Except otherwise stated, number of animals is 5 per group.

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3.1. Relative Organ Weights

The effects of vincristine treatment on the relative organ weight are shown in Table I. Relative organ weight progressively increased in the rats treated with vincristine. For example, relative weight observed for kidney, liver, liver heart, brain, and testes were slightly higher in group B that received vincristine only, though non significantly. However, group C rats that received vincristine and gallic acid had higher (P < 0.05) relative organ weight in the kidney, heart, and brain than the values in the control (A). It was also higher in the heart and brain (P < 0.05) than the values in those treated with gallic acid only (group D). The relative weight of these parenchymatous organs in group D was also similar to those of the untreated control.

3.2. Effects of Vincristine Administration on the Blood Pressure Parameters

Table II shows the blood pressure parameters of the Wistar rats following exposure to vincristine only or when combined with gallic acid therapy. The diastolic, systolic pressure as well as MAP pressure, and volume of blood flow in the cuff were elevated significantly (P < 0.05) in the vincristine only treated group (group B) than those of

Table III shows the ECG parameters in adult male Wistar rats that were administered vincristine only or combined with the antioxidant, gallic acid. The effects of vincristine treatment on the ECG parameters are shown in Table II. For example, the P wave, PR, QRS, QT, QTc, and QRS were gradually increased in group B that received vincristine only, though non significantly. However, group C rats that received vincristine and gallic acid had higher (P < 0.05) ECG parameters in the heart, liver, kidney, and brain than the values in the control (A). It was also higher in the heart and brain (P < 0.05) than the values in those treated with gallic acid only (group D). The relative weight of these parenchymatous organs in group D was also similar to those of the untreated control.

Note: Data here are computed as mean ± SD. Along the rows, all values that have the same type of superscript are different significantly @ P < 0.05. Except otherwise stated, number of animals is 5 per group.

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the untreated control (group B). The diastolic Bp, heart rate and blood volume were also elevated significantly ($P < 0.05$) in group B than the values observed in the group D rats that received only gallic acid. The heart rate in group C (vincristine + gallic acid) was also higher than that of group D significantly.

### 3.3. Effects of Vincristine on ECG Indices

As shown in Table III, the heart rate in the control that was not given any drug was lower significantly ($P < 0.05$) than that of group B, C or D. The P wave was lower in the vincristine treated groups (B and C), although non-significantly than the value in the untreated control while the QRS complex in the gallic acid only treated group was significantly lower than that of group B, C or D. The P wave was lower in group C (vincristine + gallic acid) than the values obtained in the gallic acid treated group while MCHC appears similar across the four groups.

### 3.4. Haematology

The erythrocyte and leucocyte indices as influenced by vincristine administration are shown in Tables IV and V, respectively. In a manner consistent with the observations on the ECG and blood pressure parameters above, there was observed a decline in PCV and haemoglobin concentration values in the vincristine treated groups (B and C). Similarly, RBC was lower significantly in group B, (vincristine only) than the values obtained in the gallic acid treated (group D) and group C (vincristine + gallic acid). The RBC count in group D (gallic acid only-treated) rats was also higher than that of the unexposed control (group A). Furthermore, the corpuscular volume in group B was found to be significantly higher ($P < 0.05$) than those of groups A, C, and D while the MCH value in this group (B) was also higher than that of groups C and D. Meanwhile, the MCH in group A was higher than that of the gallic acid treated group while MCHC appears similar across the four groups.

Looking at the leucocyte indices, there were generalized and considerable decreases ($P < 0.05$) in the WBC count, absolute and differential neutrophil, eosinophil, and monocyte counts when compared with the unexposed control (group A), while the differential lymphocyte count was more than the values in groups A, C and D. The total WBC, absolute neutrophil and lymphocyte counts in group C were lesser in values ($P < 0.05$) than in the control. However, the absolute eosinophil count in group C was lower than eosinophil count observed in group D rats. The erythrocyte osmotic fragility, Fig. 1 on the other hand.

### TABLE IV: Erythrocyte Parameters of the Adult Male Wistar Rats Following Exposure to Vincristine Only or Combined with the Antioxidant, Gallic Acid

<table>
<thead>
<tr>
<th>Erythrocyte parameters</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>45.80 ± 2.59</td>
<td>43.40 ± 1.14</td>
<td>43.8 ± 3.03</td>
<td>47.00 ± 3.39</td>
</tr>
<tr>
<td>RBC (x 10^6/L)</td>
<td>5.07 ± 0.71</td>
<td>4.18 ± 0.30ab</td>
<td>5.98 ± 0.70b</td>
<td>7.04 ± 0.70c</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.32 ± 2.45</td>
<td>13.18 ± 1.40</td>
<td>12.32 ± 3.24</td>
<td>12.52 ± 1.70</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>80.18 ± 11.77a</td>
<td>100.06 ± 8.14ab</td>
<td>69.32 ± 6.85b</td>
<td>67.21 ± 7.17c</td>
</tr>
<tr>
<td>MCH (g)</td>
<td>28.64 ± 5.93a</td>
<td>31.77 ± 4.70bc</td>
<td>20.86 ± 6.19b</td>
<td>18.03 ± 3.62ac</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.28 ± 5.10</td>
<td>30.44 ± 3.93</td>
<td>28.21 ± 7.41</td>
<td>26.76 ± 4.27</td>
</tr>
</tbody>
</table>

Note: Data here are computed as mean ± SD. Along the rows, all values that have the same type of superscript are different significantly @ $P < 0.05$. Except otherwise stated, number of animals is 5 per group.

### TABLE V: Leucocyte Parameters of the Adult Male Wistar Rats Following Exposure to Vincristine Alone or Combined with Gallic Acid

<table>
<thead>
<tr>
<th>Leucocyte Parameters</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x 10^3/μL) (%)</td>
<td>5.62 ± 1.14ab</td>
<td>2.16 ± 0.62a</td>
<td>3.14 ± 0.91b</td>
<td>4.11 ± 1.87 (31.0 ± 9.7)</td>
</tr>
<tr>
<td>Lymph (x 10^3/μL) (%)</td>
<td>7.27 ± 1.29a</td>
<td>6.19 ± 1.50</td>
<td>4.74 ± 0.89ab</td>
<td>5.77 ± 0.77 (45.8 ± 3.96b)</td>
</tr>
<tr>
<td>Eosinophils (x 10^3/μL) (%)</td>
<td>0.64 ± 0.34 (4.0 ± 1.87)</td>
<td>0.29 ± 0.17a (3.0 ± 1.58b)</td>
<td>0.37 ± 0.09b (3.8 ± 1.30)</td>
<td>0.83 ± 0.31ab (6.6 ± 2.30b)</td>
</tr>
<tr>
<td>Mono (x 10^3/μL) (%)</td>
<td>2.37 ± 0.93a</td>
<td>0.76 ± 0.32a</td>
<td>1.66 ± 0.56</td>
<td>1.79 ± 0.53 (14.8 ± 5.97)</td>
</tr>
<tr>
<td>Baso (x 10^3/μL) (%)</td>
<td>0.27 ± 0.18 (1.8 ± 1.48)</td>
<td>0.11 ± 0.08 (1.4 ± 1.14)</td>
<td>0.23 ± 0.11 (2.2 ± 0.83)</td>
<td>0.23 ± 0.13 (1.8 ± 0.83)</td>
</tr>
</tbody>
</table>

Note: Data here are computed as mean ± SD. Along the rows, all values that have the same type of superscript are different significantly @ $P < 0.05$. Except otherwise stated, number of animals is 5 per group.
did not show any serious variation across the group when placed beside the vincristine treated or the control groups.

3.5. Plasma Biochemistry

The plasma biochemical parameters consisting of plasma electrolytes, protein and metabolites, liver enzymes, and lipid profiles are shown in Table IX. Although many of the parameters did not show statistically significant variations, the marginal variations observed must be mentioned because of their clinical significance. For example, there was consistently lower sodium, potassium, chloride and bicarbonate values in group B, which was corrected in group C and D except plasma bicarbonate ion. Similar trend can also be observed in the total protein, albumin, globulin, urea or creatinine values whereas, total and conjugated bilirubin was slightly higher. Meanwhile, the liver ALT and GGT were slightly elevated in group B than were the control, group C and D, although non-significantly. But the plasma ALP was higher in the vincristine only treated group (P < 0.05) than the values from the control, group C and D (Table VIII). Meanwhile, lipid profile appeared relatively similar across the groups (Table IX).

3.6. Markers of Oxidative Stress

The impacts of vincristine administration on the indicators of oxidative stress, vis a vis hydrogen peroxide generation (H₂O₂), glutathione (GSH), total protein, and superoxide dismutase (SOD) in post mitochondrial fractions in the liver, kidney, heart, testes and brain samples were also investigated, as shown in Figs. 2–5, in that order. As shown in Fig. 2, the total protein and GSH values were depleted in group B (vincristine only), while H₂O₂ was elevated, but the parameters were restored to normal values in group C and D, although the decreases were not statistically significant. A similar reduction was also observed in the SOD value in group B, but the values were not restored to the observed SOD value in the normal untreated control.

In the liver samples, the GSH values in the vincristine treated groups B and C were lower significantly (P < 0.05) than the values unexposed group A, despite the treatment of the rats in group C with gallic acid. The GSH value in gallic acid only treated group (group D) was also lower (P < 0.05) than the values obtained in the control. The quantity of H₂O₂ generated in the liver of the vincristine only treated also followed the pattern observed in the

### Table VI: Plasma Electrolytes of the Wistar Rats After Exposure to Vincristine Only or Combined with the Antioxidant, Gallic Acid

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + Gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>139.6 ± 1.14</td>
<td>136.4 ± 2.30</td>
<td>139.2 ± 1.92</td>
<td>140.33 ± 0.58</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
<td>3.94 ± 0.19</td>
<td>3.70 ± 0.24</td>
<td>4.04 ± 0.15</td>
<td>3.97 ± 0.42</td>
</tr>
<tr>
<td>Cl⁻ (mmol/L)</td>
<td>107.00 ± 2.74</td>
<td>96.00 ± 2.74</td>
<td>107.20 ± 2.73</td>
<td>106.66 ± 5.77</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>31.60 ± 1.34</td>
<td>24.00 ± 1.58</td>
<td>22.60 ± 2.07</td>
<td>21.66 ± 1.53</td>
</tr>
</tbody>
</table>

Note: Data are computed as mean ± SD. Number of animals is 5 in each group.

### Table VII: Plasma Protein and Metabolites of the Wistar Rats After Exposure to Vincristine Only or Combination with the Antioxidant, Gallic Acid

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + Gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>181 ± 8.92</td>
<td>122 ± 6.06</td>
<td>122 ± 6.06</td>
<td>122 ± 6.06</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>62 ± 3.02</td>
<td>68 ± 3.70</td>
<td>68 ± 3.70</td>
<td>68 ± 3.70</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.8 ± 1.54</td>
<td>44.8 ± 1.54</td>
<td>44.8 ± 1.54</td>
<td>44.8 ± 1.54</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>129.43 ± 8.23</td>
<td>129.43 ± 8.23</td>
<td>129.43 ± 8.23</td>
<td>129.43 ± 8.23</td>
</tr>
</tbody>
</table>

Note: Data are computed, as mean ± SD. Number of animals is 5 in each group.

### Table VIII: Liver Enzymes Rats After Exposure to Vincristine Only or Combined With the Antioxidant, Gallic Acid

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + Gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>15.2 ± 2.05</td>
<td>14.4 ± 3.13</td>
<td>15.2 ± 4.43</td>
<td>13.33 ± 1.53</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>11.4 ± 1.67</td>
<td>14.2 ± 2.49</td>
<td>11.8 ± 3.03</td>
<td>9.66 ± 0.57</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>8.4 ± 1.81</td>
<td>12.6 ± 2.70abc</td>
<td>8.2 ± 2.77</td>
<td>6.00 ± 2.00</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>39.2 ± 8.92a</td>
<td>44.8 ± 0.52abc</td>
<td>37.00 ± 9.97b</td>
<td>38.00 ± 7.00c</td>
</tr>
</tbody>
</table>

Note: Data here are computed as mean ± SD. Along the rows, all values that have the same type of superscript are different significantly @ P < 0.05. Number of animals is 5 per group.

### Table IX: Lipid Profile of Adult Male Wistar Rats After Exposure to Vincristine Only and in Combination with Gallic Acid

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>Group A (Control)</th>
<th>Group B (Vincristine only)</th>
<th>Group C (Vincristine + Gallic acid)</th>
<th>Group D (Gallic acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>161.8 ± 11.69</td>
<td>161.4 ± 20.33</td>
<td>160.4 ± 12.62</td>
<td>155.33 ± 6.51</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>62.8 ± 12.23</td>
<td>60.4 ± 23.31</td>
<td>66.00 ± 14.37</td>
<td>60.67 ± 7.51</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>43.4 ± 4.03</td>
<td>41.00 ± 10.95</td>
<td>41.60 ± 5.55</td>
<td>41.67 ± 5.13</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>129.40 ± 12.74</td>
<td>124.80 ± 26.43</td>
<td>126.8 ± 13.94</td>
<td>123.33 ± 8.02</td>
</tr>
</tbody>
</table>

Note: Data computed as mean ± SD. Number of animals is 5 in each group.
kidney. There was an elevation of H$_2$O$_2$ in this group but the values in group C and D were similar to that of the control. As observed for GSH, the SOD was depleted in group B while group C and D showed slight elevations (See Fig. 3). A clearer picture of the effects of vincristine administration was observed in the heart, see Fig. 4. We also saw considerably significant decreases in the GSH and total protein while H$_2$O$_2$ was significantly elevated in group B when placed side by side with the control (GSH and H$_2$O$_2$) as well as the other groups C and D (H$_2$O$_2$). Whereas, the SOD value was only slightly reduced in the vincristine treated groups. The testes on the other had depleted total protein in group C (vincristine + gallic acid) while the other parameters, H$_2$O$_2$, GSH and SOD followed the pattern observed in the kidney, liver and heart, although non significantly. Finally, the indicators of oxidative stress evaluated in the brain did not follow the pattern observed in the other organs (Fig. 5). For example, SOD and GSH values were elevated in groups B and C but total protein did not show any observable difference, whereas the H$_2$O$_2$ was slightly elevated in the vincristine only treated group (B).

4. Discussion

Cancer chemotherapy as promising as it is, is bedeviled by complications associated with its cytotoxicity, not only to the cancerous cells but also to other cells, especially rapidly dividing myeloid cells, lymphoid tissue and cells in the GIT. Thus leading to significant complications that could hamper survival of the affected patients [6]. These anticancer drugs are mostly nonselective because they kill both rapidly dividing neoplastic tissues and those of the host. They also have low therapeutic index. A general reflection of this observation was observed in the current study on the toxic side effects of Vincristine Sulfate at normal recommended therapeutic dosage utilized in this study.
4.1. Relative Organ Weight

A generalized increase in the relative organ weight was observed in all the parenchymatous organs—kidney, liver, heart, testes, and brain that were evaluated as a result of Vincristine administration. This observed increase in relative organ weight appears to be an inflammatory response. Inflammation may have resulted in cellular infiltration as a result of the toxic effect of vincristine, in the form of an acute phase reaction to tissue damage [15]. In the presence of destructive endogenous and exogenous stimuli, the body produces several cytokines and other chemokines induced by inflammatory responses, some of which include cortisol, bradykinin, prostaglandin, etc. This is closely followed by mobilization and activation of inflammatory cells like macrophages, mast cells, and neutrophils which will also promote the acute phase reaction at the site. Because inflammatory response is a critical side effect of cancer therapy, it is a common and necessary practice to incorporate drugs with anti-inflammatory effects and opioids as an adjunct to cancer chemotherapy [16].

4.2. Cardiovascular Functions

In this study, we observed a slightly elevated QT interval while the corrected QT (QTc) was significantly higher in vincristine treated groups. Generally, by way of definition, the QT interval in an ECG is the time between depolarization of the ventricular muscle denoted as “QRS complex” and its repolarization or “T wave”. When QT is prolonged, it is a key risk factor in the pathogenesis Torsades de Pointes—a potentially deadly cardiac dysrrhythmia [17]. Wedam et al. [18] had earlier reported that prolongation of QT interval might be an indicator of cardiac toxicity from xenobiotics or an undiscovered cardiac problem or disease. Since apparently healthy rats were used for the study, these observed changes in the QT interval and QTc must have resulted from the vincristine administration.

Meanwhile, the blood pressure result also showed that there was a significantly higher blood pressure in the rats treated with vincristine only. These parameters were however corrected in the rats administered with gallic acid. The heart rate and the blood volume that traverse the cusp were...
also higher in this group. This shows that administration of vincristine in cancer therapy has the potential to cause hypertension and cardiac toxicity. Although the most common side effect of Vincristine is peripheral neuropathy [19], we can see the potential cardiac effects of the drug in the present study. However, drug induced hypertension is a common side effect of many anti cancer drugs, but hypertension in vincristine is not a prominent observation [20] unlike the one observed in the present study.

4.3. Haematology

Vincristine administration in rats in the present study to macrocytic normochromic anaemia when compared with untreated control. It also resulted in panleucopenia, in rats with significant neutropenia, eosinopenia, and monocytopenia being very prominent. However, percentage of differential lymphocytes appears higher, that is more lymphocytes were produced than neutrophils in the exposed rats. No significant changes were however noticed in the erythrocyte osmotic fragility in this study. These complications were however corrected in the rats treated with gallic acid. Our observations agree with previously documented effects of vincristine as a result of myelosuppression and disruption of rapidly dividing cells [21], [22] Vincristine acts by binding to tubulin in the mitotic spindle to prevent cell division during metaphase. It has been reported to be active in the G2 and M phases of the cell cycle, causing depolymerization of microtubules. Vincristine binding to spindle proteins also disrupts the formation of the mitotic spindle, thereby preventing alignment and segregation of chromosomes during anaphase. This singular action prevents tumour cell development during metaphase [21].

Vincristine is also known to disrupt nucleic acid and protein synthesis through prevention of the use of glutamic acid [22]. The side effect of all these activities is that the binding of vincristine to mitotic spindles and blockage of protein synthesis is not specific to tumour cells only. As stated by Fuchs-Tarlovsky [23], the haematological toxicity of antimitotic drugs generally shows in the form of leucopenia and thrombocytopenia. In fact, accumulation of these drugs in the bone marrow usually leads to destruction of myeloid cells to prevent their division and maturation. Anaemia, general myelosuppression, and neutropenia have also been widely reported in vincristine therapy in dogs [24]. Pancytopenia and desquamation of rapidly dividing cells in the GIT usually lead to death as a result of invasion of bacteria through the GIT and life threatening septicaemia following granulocytopenia in affected patients unless the patient is treated appropriately.

Gallic acid reversal of anaemia and panleucopenia in the present study, being an antioxidant has also laid credence to the oxidative effects of vincristine. Although some authors have reported that vincristine causes oxidative stress [22] its activities have been limited to its antimitotic effects mentioned earlier. Our findings as we shall soon discover also showed that vincristine administration results in significant oxidative stress, which was corrected in the rats treated with the antioxidant—gallic acid. Gallic acid is a 3,4,5-trihydroxybenzoic acid, a polyphenol widely found in nuts, green tea, hops, grapes, red wine etc. It can be said that gallic acid is one of the main plant derived phenolic compounds. Gallic has been reported to show several pharmacological properties, including antioxidant and anti-inflammatory effects, through decreased expression of cytokines and anticancer effects [8].

4.4. Plasma Biochemistry

We also evaluated the effects of vincristine administration on plasma electrolytes, plasma protein and metabolites, liver enzyme, and lipid profile in the Wistar rats. However, besides mild reduction in the electrolytes—sodium, potassium, and bicarbonate ions; total protein, globulin, urea, and creatinine, only the enzyme GGT and ALP showed some degree of elevation in the vincristine treated rats. Although many of these parameters did not show statistical significance, they are important clinically as early pointers of hepatic damage and kidney dysfunction. Because anticancer drugs follow the first-order kinetics [6] they show considerable toxic effects on the liver [25] and kidneys [26] which were corrected by antioxidants co-administration as observed in those rats administered with gallic acid in the present study. Non-catalytic enzymes such as ALT and AST, localized within the cells of numerous organs, including the liver act as a significant indicator for evaluation of kidney and liver status and tissue injury or organ dysfunction [27]. Damage to the liver kidney and other parenchymatous organs by vincristine and other anti cancer drugs have been linked to their ability to produce oxidative stress and mediators of inflammation [22].

4.5. Oxidative Stress

Vincristine administration resulted in oxidative stress as seen in elevated H2O2 generation while GSH, SOD, and total protein values were depleted in the vincristine only group. These anomalies were however corrected with concurrent administration of gallic acid. Gallic acid demonstrated its reported effects as are free radical scavengers, reducing lipid peroxidation [28]. It could also potentiate the activities of other endogenous antioxidant agents such as SOD, CAT, endothelial nitric oxide synthase and prostaglandin E2 through reduction of the expression of pro-inflammatory mediators like TNFα and inducible nitric oxide synthase, up-regulation of the proangiogenesis factors and inhibition of caspase-3 and 9 [29]. Some other authors have also reported that gallic acid disrupts intra-cellular inflammatory pathways by inhibiting the expression of nuclear transcription factors such as NFκB, STAT3, cyclooxygenase (COX)-2, and also prevent neutrophils infiltration during inflammation [30]. In some of the studies in our laboratory, gallic acid has been observed to ameliorate the hepatotoxic effects of xenobiotics by acting as an antioxidant compound that scavenges free radicals and ROS, and improve in rats [31].

Thus, we can conveniently infer from our findings and previous studies that the modulatory activities gallic acid in the present study were due to its antioxidant and anti-inflammatory activities. A similar thing can be said concerning the cardiac anomalies in the form of hypertension and prolonged QT interval and corrected QT (QTe), because gallic acid has also been observed to decrease...
the harmful oxidative consequences of myocardial infarction in the context of its antioxidant potency, either by increasing the activity of antioxidant enzymes, or by potentiation of the activities of non-enzymatic antioxidant agents including GSH [31]. All the aforementioned effects of gallic acid have the propensity to ameliorate the damage associated with vincristine and toxic side effects free radicals and ROS in several sites including parenchymatous organs and bone marrow [32] as observed in this study.

5. Conclusion

The study shows that vincristine administration causes macrocytic normochromic anaemia, granulocytopenia, especially neutropenia, cardiac dysfunction via hyper-tension, and prolonged QT interval. This may not be unconnected to its ability to induce oxidative stress via generation of hydroxyl radical and depletion of endogenous antioxidant enzymes. These complications of vincristine were however corrected by co-administration of gallic acid. It can therefore be suggested that concurrent antioxidants therapy, especially gallic acid, combined with vincristine in the treatment of cancer will significantly reduce or totally eliminate its undesirable side effects.

References