Hepatoprotective Effect of Ethanolic Leaf Extract of \textit{Vernonia amygdalina} and \textit{Azadirachta indica} against Acetaminophen-Induced Hepatotoxicity in Sprague-Dawley Male Albino Rats

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Abstract  Acetaminophen (paracetamol) is a commonly and widely used analgesic and antipyretic agent, but at high dose it leads to undesirable side effects, such as hepatotoxicity. The study investigate the hepatoprotective effect of ethanolic leaf extract of \textit{Vernonia amygdalina} and ethanolic leaf extract of \textit{Azadirachta indica} against acetaminophen-induced hepatotoxicity in Sprague-Dawley male albino rats. Male albino rats were randomly divided into six groups each consisting of five albino rats. Group A rats served as the normal control and were given water daily for a period of 14 days. Hepatotoxicity was induced in-vivo to all animals of Groups B, C, D, E, and F orally by administering 2g/kg body weight of paracetamol once a day for a period of 14 days. Group C, D, E and F were orally administered silymarin (100 mg/kg B.W), Vitamin C (100 mg/kg B.W), ethanolic leaf extract of \textit{Vernonia amygdalina} (300 mg/kg B.W) and \textit{Azadirachta indica} (300 mg/kg B.W) respectively daily for a period of 14 days. Group B animals served as the paracetamol control and they were not treated. The result of this study shows that animals treated with silymarin, \textit{Vernonia amygdalina} and \textit{Azadirachta indica} extracts significantly (P<0.05) have reduced WBC count compared to paracetamol control group. HGB, RBC and HCT values in all the groups administered with silymarin, Vitamin C, \textit{Vernonia amygdalina} and \textit{Azadirachta indica} extracts were significantly (P<0.05) increased when compared to the paracetamol-intoxicated animals without treatment. Oral administration of acetaminophen caused marked liver damage as noted by the significant increased (P<0.05) in activities of plasma AST, ALT, ALP and GGT as well as the level of cholesterol, triglyceride and a reduction in plasma total protein. The drug also resulted to a significant increase (P<0.05) in liver MDA content, decrease in liver GSH content, decrease in SOD and CAT activities. Treatment with silymarin, Vitamin C, \textit{Vernonia amygdalina} and \textit{Azadirachta indica} extracts showed effective hepatoprotective effect as evidenced in the decrease in the plasma levels of liver biomarker enzymes and reduction in oxidative stress parameters. Histopathological evaluation of the liver architecture also revealed that all the treated animals have reduced the incidence of paracetamol-induced liver lesions.

Keywords: acetaminophen, antioxidant, \textit{Azadirachta indica}, haematological parameters, hepatoprotective effect, histopathology, liver biomarker enzymes and \textit{Vernonia amygdalina}


1. Introduction

Liver is the major organ for detoxification and removal of endogenous substances. It is continuously and widely exposed to hepatotoxins, xenobiotic substances and chemotherapeutic agents that lead to impairment of its functions. Liver diseases are mainly caused by excess consumption of alcohol, toxic chemicals, infections and autoimmune disorders. Liver diseases often progress from subclinical icteric hepatitis to necroinflammatory hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [1,2]. Documented evidence has been reported that reactive oxygen species (ROS), including singlet oxygen, superoxide, and hydroxyl radicals, are known to play an
Vitamin C may prevent certain types of hepatic cellular injury by increasing the total glutathione (GSH) and glutathione peroxidase (GSH-Px), thus decreasing lipid peroxidation [3,4,5].

Paracetamol also known as acetaminophen is chemically named N-acetyl-p-aminophenol.

Acetaminophen is a commonly and widely used analgesic (pain reliever) and antipyretic (fever reducer) drug. This drug has been highly abused in Nigeria (West Africa) due to ignorance. This research shows that at high dose, it leads to undesirable side effects, such as liver necrosis and hepatic injury, production of reactive oxygen species (ROS) that lead to oxidative stress. At the therapeutic levels, paracetamol is primarily metabolized in the liver by glucuronidation and sulphation; however, a small proportion reacts with cytochrome P450 -mediated bioactivation to N-acetyl-p-benzoquinimine (NAPQI), which is rapidly quenched by glutathione [6]. After an overdose of paracetamol, elevated levels of the toxic NAPQI metabolite are generated, which extensively deplete hepatocellular glutathione (GSH) and covalently modify cellular proteins resulting in hepatocyte death [7,8].

Silymarin a well known hepatoprotective agent, is a flavonoid obtained from the plant Silybum marianum or milk thistle and is composed of three isomers: silybinin, silydianin and silychristin, of which silybinin is the most well recognised sources of fibrogenic mediators [10, 11]. Silybinin has been shown to inhibit the function of Kupffer cells, which are quantitatively the most important [9]. Silybinin has been shown to inhibit the function of Kupffer cells, which are well recognised sources of fibrogenic mediators [10, 11]. In addition, silymarin stabilizes the lipid structures in the hepatocellular membrane, which may generally apply to all cell membranes and even outside the liver [11]. Clinical trials have shown that Silymarin exerts hepatoprotective effects in acute viral hepatitis, poisoning by Amanita phalloides, ethanol, carbon tetrachloride and paracetamol. Many studies have demonstrated the beneficial hepatoprotective effects when treated with Silymarin [12].

Vitamin C (ascorbic acid) is a water-soluble micronutrient required for multiple biological functions [13]. It is found intra- and extracellularly as ascorbate, and is well absorbed from the gastrointestinal tract [14,15]. Vitamin C may prevent certain types of hepatic cellular damage [16, 17]. Ascorbic acid is a natural antioxidant that prevents the increase production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues [18].

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine. Vernonia amygdalina (V. amygdalina) is a shrub that grows predominantly in the tropical Africa. Leaves from this plant serve as food vegetable and culinary herb in soup [19]. V. amygdalina commonly known as bitter leaf is a shrub or small tree of 2.5 m tall, belonging to the family Asteraceae. It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste. The taxonomic classification of Vernonia amygdalina is as follows: Kingdom: plantae, Division: Angiosperms, Order : Asterales, Family: Asteraceae, Genus: Vernonia, Species: V. amygdalina, Botanical Name: Vernonia amygdalina. It is commonly called Bitter leaf in English language, Onugbu in Igbo language, it is called Etidot in Efik, Ibibio, Ewuro in Yoruba language, Oriwo in Edo and Chusa-doki in Hausa [20]. In many parts of Nigeria, the plant has been domesticated and used in the treatment of various infection and diseases. In our previous studies, we show that the leaf contains different phytochemicals like: tannins, Phlobatanin, alkaloids, saponins, flavonoids, anthraquinone, glycoside, polyphenols, proanthocyanidins, terpenoid etc.

Azadirachta indica (A. indica) commonly known as Neem, is found in Nigeria and in most of the tropical and subtropical countries and is widely distributed in the world. The taxonomic classification of Neem is as follows: Kingdom :Plantae, Order: Rutales, Suborder: Rutinae, Family: Meliaceae, Subfamily: Melioideae, Genus : Azadirachta, Species: indica [21]. All parts of the plant are useful and have been used in treatment of diseases ranging from teeth decay, swollen liver, ulcers, dysentery, diarrhea, malaria etc. [22,23]. The plant has great medicinal uses and has been used for the treatment of bacterial infections. In our previous studies, we also show that A. indica contain some phytochemicals like: tannins, saponins, alkaloids, flavonoids, terpenoids etc. In the present study, male albino rats were orally treated with silymarin ( a standard drug), Vitamin C, ethanolic leaf extract of Vernonia amygdalina and ethanolic leaf extract of Azadirachta indica daily accompanied by paracetamol administration once a day for 14 days. The haematological parameters of the treated and untreated groups were determined. Hepatic MDA, SOD, GSH and CAT levels, as well as plasma activities of AST, ALT, ALP and GGT and total protein level, cholesterol and triglyceride were measured to monitor liver injury. The extent of paracetamol-induced liver injury was also analyzed through histopathological observations.

2. Materials and Methods

2.1. Collection and Identification of Plants Materials

The leaves of Vernonia amygdalina and Azadirachta indica were obtained from Ikorodu in Lagos State, Nigeria. The plants were authenticated by Miss Shokefun a botanist from Science Laboratory Technology Department, Environmental biology Unit, Lagos State Polytechnic, Ikorodu.

2.2. Preparation of Ethanolic Leaf Extract of Vernonia amygdalina and Ethanolic Leaf Extract of Azadirachta indica

The leaves of Vernonia amygdalina and Azadirachta indica were washed separately, air dried under shade in the Biochemistry Laboratory, pulverised separately into coarse power using blender. Extraction was carried out by
dispersing 250g of the grounded *Vernonia amygdalina* and *Azadirachta indica* plant material separately into 1L of 70% ethanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40°C. The concentrated extracts were dried to complete dryness in an aerated oven at 40°C for 48 hours. The extracts were latter stored in a refrigerator at 4°C.

2.3. Experimental Animals

Sprague-Dawley male albino rats with body weight ranging from 150 to 200g were obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. They were acclimatized for one week to Laboratory condition 23 ±2 °C. They were kept in plastic cages and fed with commercial rat chow and supply with water *ad libitum*. The rats were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

2.4. Grouping of Experimental Animals

The animals were randomly divided into six groups each consisting of five rats. Group A rats served as the normal control and were given water daily for a period of 14 days. For inducing hepatotoxicity (in vivo), animals of Group B, C, D, E, and F were orally administered 2g/kg body weight of paracetamol once a day for a period of 14 days. After paracetamol intoxication, Group B rats served as the paracetamol control (negative control). Group C animals served as the positive control and were orally administered silymarin (100 mg/kg) daily for a period of 14 days. Groups D, E and F animals were orally administered Vitamin C (100mg/Kg B.W) and ethanolic leaf extract of *Vernonia amygdalina* (300 mg/kg B.W) and ethanolic leaf extract of *Azadirachta indica* (300 mg/kg B.W) respectively daily for a period of 14 days. Liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible, and then they were immediately stored at -20°C until analysis. An extra sample of each liver was excised and fixed in a 10% formalin solution for histopathological analysis.

2.5. Collection of Blood Samples

The albino rats were sacrificed by cervical decapitation after 24 hours fasting on the fourteen day. Blood were collected from the male albino rats by ocular puncture into EDTA tubes for hematological analysis and the remaining blood were collected in an heparinised tubes and centrifuge at 3000 rpm for 20 minutes using a centrifuge and the plasma stored at -20°C.

2.6. Determination of Haematological Parameters

The haemoglobin concentration (HGB), total red blood cell (RBC), white blood cell count (WBC), Hematocrit (HCT), and other haematological parameters were determined in the whole blood using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

2.7. Measurement of Plasma AST, ALT, ALP, GGT, CH and TG levels

Liver damage was assessed by the estimation of plasma activities of aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) using commercially available test kits from Randox Laboratories Ltd. (UK). The results were expressed as units/liter (IU/L). In addition, the plasma levels of total protein, cholesterol (CH) and triglyceride (TG) were estimated in the experimental animals using kits produced by Randox Laboratories Ltd. (UK).

2.8. Hepatic Antioxidant Enzyme Activities

2.8.1. Preparation of Liver Homogenate

The Liver tissues of some of the sacrificed albino rats were excised and the liver samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 50 minutes. The supernatant obtained was later used for assay of thiobarbituric acid reactive substances (TBARS) content, superoxide dismutase, catalase and reduced glutathione.

2.8.2. Estimation of Lipid Peroxidative (LPO) Indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Nicahaus and Sameulsson [24].

2.8.3. Estimation of Superoxide Dismutase (SOD)

The liver homogenate was assayed for the presence of SOD by utilizing the technique of magwere et al [25] with slight modification.

2.8.4. Estimation of Catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620 nm and expressed as μmoles of H₂O₂ consumed/min/mg protein as described by sinha, [26].

2.8.5. Estimation of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellma [27].

2.9. Histopathological Studies

The histopathological analyses were assayed in the Department of Anatomy, college of Medicine, University of Lagos, Idi-Araba, Surulere, Lagos, Nigeria. The albino rats were sacrificed and their abdomens were cut open to remove the liver. Some of the livers were fixed in Boucin’s solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hours, and then embedded in paraffin using conventional methods [28]. They were cut into 5μm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.
3. Statistical Analysis

Data analysis was done using the Graph Pad prism computer software version 5. Students' t-test and one-way analysis of variance (ANOVA) were used for comparison. A P-value < 0.05 was considered significant.

4. Results

Table 1. Haematological parameters of paracetamol intoxicated rats treated with silymarin, Vit C, V. amygdalina and A. indica extracts.

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3/L)</td>
<td>8.3±1.2</td>
<td>12.3±1.1**</td>
<td>6.9±2.91*</td>
<td>13.1±2.90</td>
<td>9.9±3.50*</td>
<td>10.2±2.60*</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.250 ± 0.102</td>
<td>0.450 ±0.132</td>
<td>0.198±0.031</td>
<td>0.236 ±0.127</td>
<td>0.220±0.140</td>
<td>0.295±0.151</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>7.1 ±1.01</td>
<td>8.4 ±1.10</td>
<td>7.2±1.33</td>
<td>7.8 ±1.92</td>
<td>6.9 ± 1.12</td>
<td>7.2±1.21</td>
</tr>
<tr>
<td>PDW</td>
<td>16.67±1.1</td>
<td>16.59±1.10</td>
<td>15.20±1.6</td>
<td>15.72±1.40</td>
<td>15.2±1.90</td>
<td>15.32±1.31</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>11.13±2.1</td>
<td>7.84 ±3.11**</td>
<td>9.1±2.4*</td>
<td>11.2±2.7*</td>
<td>10.58±2.5*</td>
<td>11.5±2.40*</td>
</tr>
<tr>
<td>RBC (10^12/L)</td>
<td>6.47±1.4</td>
<td>4.24 ±1.11**</td>
<td>5.07±1.11*</td>
<td>5.85±1.41*</td>
<td>5.79±1.20*</td>
<td>6.78±1.40*</td>
</tr>
<tr>
<td>HCT(%)</td>
<td>37.43±3.10</td>
<td>29.6±1.81**</td>
<td>31.5±2.03*</td>
<td>33.2±2.00*</td>
<td>34.98±2.59*</td>
<td>39.5±3.52*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>62.23±2.4</td>
<td>61.30±1.70</td>
<td>58.30±1.2</td>
<td>55.11±1.1</td>
<td>54.20±3.2</td>
<td>52.12±2.32</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>19.68±2.1</td>
<td>20.1±1.5</td>
<td>21.78±1.5</td>
<td>20.51±1.3</td>
<td>19.98±1.9</td>
<td>18.24±1.4</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.6±1.5</td>
<td>30.2±1.6</td>
<td>32.5±1.4</td>
<td>33.3±1.32</td>
<td>31.1±1.7</td>
<td>32.4±2.71</td>
</tr>
<tr>
<td>RDW-CV (%)</td>
<td>19.3±1.3</td>
<td>19.9±1.4</td>
<td>17.8±1.5</td>
<td>17.2±1.4</td>
<td>19.6±1.6</td>
<td>17.6±1.1</td>
</tr>
<tr>
<td>RDW-SD (fl)</td>
<td>40.3±2.9</td>
<td>41.7±1.9</td>
<td>37.5±2.5</td>
<td>36.3±2.5</td>
<td>34.8±2.52</td>
<td>32.5±2.6</td>
</tr>
</tbody>
</table>

All values are mean ± S.D (n = 5). * indicate significant difference (p < 0.05) compared with paracetamol control group (Group B). ** indicate significant difference (p < 0.05) compared with normal group (Group A). White blood count (WBC), Haemoglobin (HGB), Red blood count (RBC), Haematocrit (HCT), Mean cell volume(MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration(MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD), Platelet count (PCT), Mean platelet volume (MPV), and platelet Distribution Width (PDW).

Table 2. Effects of silymarin, Vit C, V. amygdalina and A. indica extracts on plasma AST, ALT, ALP, GGT, CH, TG and TP in paracetamol intoxicated albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>10.10±4.50</td>
<td>49.10±9.11**</td>
<td>12.50±2.5*</td>
<td>26.30±2.4*</td>
<td>18.30±3.0*</td>
<td>21.10±4.0*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>12.50±3.20</td>
<td>55.10±10.10**</td>
<td>16.20±4.80*</td>
<td>29.30±7.68*</td>
<td>20.80±4.00*</td>
<td>24.50±7.0*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>40.22±6.46</td>
<td>66.26±5.66**</td>
<td>44.62±7.42*</td>
<td>55.42±6.31*</td>
<td>49.26±6.46*</td>
<td>51.22±8.46*</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>10.12±3.40</td>
<td>40.42±7.32**</td>
<td>19.51±5.71*</td>
<td>29.82±4.32*</td>
<td>22.26±5.10*</td>
<td>20.52±4.29*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>72.48±6.23</td>
<td>155.34±16.45**</td>
<td>90.74±8.48*</td>
<td>128.94±13.78*</td>
<td>94.32±7.59*</td>
<td>98.28±9.44*</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>59.66±5.50</td>
<td>102.54±10.49**</td>
<td>72.69±8.41*</td>
<td>97.18±8.62*</td>
<td>84.28±6.56*</td>
<td>87.21±9.49*</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>50.10±8.20</td>
<td>19.50±5.10**</td>
<td>39.10±5.50*</td>
<td>29.20±4.30*</td>
<td>44.20±7.10*</td>
<td>40.22±8.46*</td>
</tr>
</tbody>
</table>

All values are mean ± S.D (n = 5). * indicate significant difference (p < 0.05) compared with paracetamol group (Group B). ** indicate significant difference (p < 0.05) compared with normal group (Group A).

Table 3. Effects of silymarin, Vit C, V. amygdalina and A. indica extracts on liver MDA, SOD, CAT, and GSH in paracetamol intoxicated albino rats

<table>
<thead>
<tr>
<th>S/N</th>
<th>Oxidative Stress Parameters.</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lipid Peroxidation (x10^3 MDA/mg protein)</td>
<td>4.32±2.24</td>
<td>13.42±5.69**</td>
<td>6.20±2.46*</td>
<td>8.10±2.30*</td>
<td>8.86±3.84*</td>
<td>9.10±3.80*</td>
</tr>
<tr>
<td>2</td>
<td>SOD (units/ mg protein)</td>
<td>8.20±1.28</td>
<td>4.31±0.86**</td>
<td>7.60±1.14*</td>
<td>6.20±1.02*</td>
<td>7.11±0.92*</td>
<td>6.75±1.60*</td>
</tr>
<tr>
<td>3</td>
<td>GSH (mg/mg protein)</td>
<td>0.34±0.08</td>
<td>0.13±0.03**</td>
<td>0.30±0.04*</td>
<td>0.21±0.02*</td>
<td>0.29±0.05*</td>
<td>0.26±0.02*</td>
</tr>
<tr>
<td>4</td>
<td>CAT (μmol/min/mg protein)</td>
<td>33.75±4.42</td>
<td>19.13±3.20**</td>
<td>29.40±3.00*</td>
<td>24.13±2.90*</td>
<td>30.20±3.40*</td>
<td>31.82±2.20*</td>
</tr>
</tbody>
</table>

All values are mean ± S.D (n = 5). * indicate significant difference (p < 0.05) compared with paracetamol group (Group B). ** indicate significant difference (p < 0.05) compared with normal group (Group A).

4.1. Histopathological Examination

Histopathological studies also provided important evidence supporting the biochemical analysis and liver antioxidant status in the normal control, paracetamol-control and animals treated with silymarin, vitamin C, V. amygdalina and A. indica extracts after paracetamol intoxication.
Figure 1. Photomicrographs of liver sections stained with hematoxylin and eosin (H&E). The slides were examined at a magnification of × 40 under a light microscope. The slides show the effect of the extracts on liver architecture in paracetamol-induced hepatotoxicity in Sprague Dawley male albino rats. (A) Normal control, (B) paracetamol control (negative control), (C) paracetamol + silymarin, (D) paracetamol + Vit C (100mg/Kg B.W), (E) paracetamol + V. amygdalina (300mg/Kg B.W), (F) paracetamol + A. indica (300mg/Kg B.W)

5. Discussion

In our previous studies, we showed that the methanolic leaf extract of Vernonia amygdalina contain phytochemicals like: tannins, Phlobatanin, alkaloids, saponins, flavonoids, anthraquinone, glycoside, polyphenols, proanthocyanidins, terpenoid etc [29]. We also showed that the methanolic leaf extract of Azadirachta indica contains some phytochemicals like: tannins, saponins, alkaloids, flavonoids, terpenoids etc [30]. Both extracts contain phenol, the antioxidant effects of phenolic compounds are related to a number of different mechanisms, such as free radical-scavenging, singlet oxygen quenching, metal ion chelation, hydrogen-donation and their action as substrates.
for free radicals such as superoxide anion and hydroxyl radical [31,32].

Hematological and biochemical indices have been reported to be a reliable parameter for the assessment of the health status of animals [33,34]. The primary reason for assessing the RBC is to check the level of anaemia and to evaluate normal erythropoiesis. HGB level shows the amount of intracellular iron present, while HCT, indicates the volume of RBC in 100ml of blood and it helps to determine the degree of anaemia or polycythemia [35]. The study shows that there is a significant decrease (p<0.005) in the level of blood HGB, RBC and HCT of the paracetamol intoxicated animals (Group B) compared to the healthy animals and the animals treated with silymarin, Vit C, *V. amydalina* and *A. indica* extracts respectively (Table 1). The significant reduction (P < 0.05) in these haematological parameters in group B animals may be attributed to the cytotoxic effects and suppression of the erythropoiesis caused by constituents of the paracetamol. WBC helps the body to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least to transport and distribute antibodies in immune response. The result of this study shows that animals treated with silymarin, *V. amydalina* and *A. indica* extracts significantly (P<0.05) have reduced WBC count compared to paracetamol control group (Table 1). There were significant increase (P<0.05) in the WBC number in the animals administered with acetaminophen. Leukocytosis observed in group B animals may be due to leukemia, lymphoma, bone marrow infection and inflammatory disease of the rats administered with acetaminophen. Other haematological parameters like PCT, MPV, PDW, MCH, MCHC, RDW-CV, RDW-SD and MCV showed no significant differences in the entire groups. The results obtained from this study showed clearly that ethanolic leaf extracts of *V. amydalina* and *A. indica* does possess haematopoietic activity and is not haematoxic.

The result from this study showed significant decrease (P<0.05) in the plasma levels of AST and ALT (Table 2) values in animals of the control group, animals treated with silymarin, ascorbic acid, *V. amydalina* and *A. indica* extracts respectively when compared to paracetamol intoxicated rats (group B). Treatment with silymarin, *V. amydalina* and *A. indica* extracts markedly inhibits paracetamol induced liver damage as evidenced by decreased level of plasma activities of AST and ALT, and reduced plasma concentration of TG and cholesterol (Table 2). The biochemical observations are supported by the histopathological examination of the rat’s liver. These transaminases (AST and ALT) have high concentrations and can be liberation from the hepatocyte cytoplasm; they are sensitive indicators of necrotic lesions within the liver [36,37]. Hence, the marked release of transaminases into the circulation is indicative of severe damage to hepatic tissue membranes during paracetamol intoxication [38,39]. There were significant increase (P<0.05) in the ALP and GGT values of group B rats compared to the healthy rats and the treated groups. This may imply that damage occur in the liver cells of the animals administered with paracetamol since the activities of these enzymes are reported to be increased in liver damage. The significant increase in these liver biomarker enzymes (AST, ALT, ALP and GGT) in the plasma of these animals is an indication of the hepatotoxicity of the liver in the animals administered with paracetamol. Several researchers have reported elevations in serum transaminases following administration of toxic doses of paracetamol in rats [36,40,41]. We observed significant decrease (P < 0.05) in plasma transaminase activities in the silymarin, Vitamin C, *V. amydalina* and *A. indica* extracts treated groups and these indicate their hepatoprotective effects against paracetamol damage. The significant decrease (P<0.05) in the total protein value of animals administered with paracetamol without treatment compared to healthy animals and treated animals signify malnutrition, malabsorption, liver disease and kidney disease.

Oxidative stress is caused by the presence of reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity. Many studies have showed that ROS can damage lipids, proteins and DNA, thus altering the structure and function of the cell, tissue, organ and system respectively. SOD is an effective defence enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide (H₂O₂). Catalase is an haemeprotein in all aerobic cells that catalyses the conversion of H₂O₂ into oxygen and water and protects the tissue from oxidative damage by highly reactive oxygen free radicals and hydroxyl radicals. GSH is an extremely efficient intracellular buffer for oxidative stress and GSH acts as a non-enzymatic antioxidant that reduces H₂O₂, hydroperoxides (ROOH) and xenobiotic toxicity. Lipid peroxides or other ROS easily inactivate these antioxidant enzymes, which results in reduced activities of these enzymes in paracetamol toxicity. In the present study, paracetamol administration was significantly (P <0.05) accompanied by increased lipid peroxidation, reduced SOD, reduced CAT and depletion in GSH activity in the liver. It is well documented that liver tissue contains relatively high content of polyunsaturated fatty acids (PUFAs), which are sensitive to peroxidative damage, [42,43] and lead to an increase in lipid peroxidation in the group of animals intoxicated with paracetamol without treatment compared to other treated groups. The observed hepatoprotective effect of silymarin against lipid peroxidation could be related to its antioxidant effects which assist in the preservation of membrane integrity. Silymarin can chelate transition metal ions such as iron and copper, rendering them effective antioxidants [44]. Previous studies have shown that silymarin has an inhibitory effect on NF-kB/Rel activity in a human hepatoblastoma-derived cell line and human histiocytic lymphoma cells [45]. Activation of NF-kB/Rel transcription factor leads to the increased production of inflammatory mediators, including IL-1β and tumor necrosis factor-alpha (TNF-α) [46,47]. Soon et al [48] demonstrated that NF-kB/Rel DNA binding is completely blocked by silymarin treatment in RAW264.7 cells. These results may suggest that inhibition of IL-1β production by silymarin is mediated, at least in part, by the inhibition of the NF-kB/Rel transcription factor. In addition, silymarin by virtue of increasing GSH content in some tissues might protect these tissues against the toxicity of GSH depletors such as paracetamol [49]. Hepatotoxic doses of acetaminophen deplete the normal levels of hepatic glutathione, when NAPQI covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts [50]. The glutathione protects hepatocytes by
combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins [51]. It has been generally accepted that cytochrome P450-dependent bio activation of paracetamol is the main cause for potentially fulminant hepatic necrosis upon administration or intake of lethal doses of paracetamol [52,53]. NAPQI is initially detoxified by conjugation with reduced GSH to form mercapturic acid [54]. Under conditions of NAPQI formation, following toxic paracetamol doses, GSH concentrations become very low in the centrilobular cells [55,56], which could account for the observed depletion in liver GSH stores. Increased lipid peroxidation, as evidenced by the elevated levels of MDA in hepatic tissues demonstrated in the present study after administration of paracetamol could be expected owing to the depletion in GSH concentration.

Histopathological studies also provided important evidence supporting the biochemical analysis and liver antioxidant status. Hepatic tissue of normal control Sprague-Dawley male rats showed normal hepatic cells with well preserved cytoplasm and central vein (Figure 1). The administration of 2g /kg body weight of paracetamol induces severe histopathological damage in the liver with significant degeneration of cells, moderate to severe hepatocyte necrosis and swelling around the central vein region and mild inflammatory cell infiltration. The damage extended to the majority of the hepatic lobule with marked loss of its normal pattern. These changes positively correlated with the noted increases in transaminase activities in paracetamol-induced hepatotoxicity. Hepatic tissue of silymarin (100 mg/kg) treated rats (group C), exhibited significant liver protection against paracetamol-induced liver damage, as evident by the presence of slight to milder degree of hepatocyte necrosis and swelling around the central vein region and a few to milder degree of inflammatory cell infiltration. Vitamin C treated rats showed mild degeneration of cells, moderate hepatocyte necrosis and swelling around the central vein region and milder inflammatory cell infiltration. Furthermore, histopathological changes (fibrosis) occurred in paracetamol-intoxicated rat livers and their treatment with V. amygdalina and A. indica extracts were observed to show mild to moderate degrees of fibrosis, improved generation of cells, milder degree of hepatocyte necrosis and swelling around the central vein region and milder degree of inflammatory cell infiltration. V. amygdalina extract treatment, gave a better histopathological results compared to A. indica extract.

6. Conclusion

In conclusion, the results of this study demonstrate that silymarin, Vitamin C, V. amygdalina and A. indica extracts were effective in reducing the hepatic damage caused by paracetamol in Sprague Dawley male albino rats.

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Conflict of Interest

The authors declared that there is no conflict of interest.

References

Department of Biochemistry, Michael Opara University of Agriculture Umudike, Nigeria. 2010.


