Hepatocurative effect of aqueous extract of *Hibiscus sabdariffa* on some antioxidants and haematological indices of acetaminophen-challenged Wistar albino rats

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*Hibiscus sabdariffa* is among the medicinal plants which have been shown to possess several medicinal properties. The present study was conducted to investigate the antioxidant and haematological properties of the aqueous leaf extract of *H. sabdariffa* on acetaminophen-challenged liver using rat model. Twenty (20) Wistar albino rats were used for this study and were divided into 4 groups of 5 rats each. Group 1 rats were the normal control; group 2 (positive control) rats were administered acetaminophen only, at a dose of 750 mg/kg b.w. ip. Group 3 rats were administered mid dose (400 mg/kg b.w) of the extract after acetaminophen-induction while group 4 rats received high dose (600 mg/kg b.w) of the extract after acetaminophen-induction. Group 2 rats showed a significant (p<0.05) decrease in the activities of the enzymes, catalase (CAT), superoxide dismutase (SOD) and vitamin C concentrations when compared with group 1 rats. However treatment with the extract caused a significant (p<0.05) increase in the activities of the enzymes, catalase (CAT), superoxide dismutase (SOD) and vitamin C concentrations when compared with group 2 animals. More so, group 2 rats treated with acetaminophen only, showed significant increase (p<0.05) in white blood cell, neutrophil and lymphocytes counts when compared with the group 1 rats. Conversely, a significant decrease (p<0.05) was observed in packed cell volume, red blood cell count and haemoglobin concentration of the group 2 rats when compare with the group 1 rats. Treatment with the aqueous extract of *H. sabdariffa* caused a dose-dependent significant increase (p<0.05) in the pack cell volume, red blood cell count and haemoglobin concentration of the treatment groups (groups 3 and 4) when compared with the group 2 rats. Conclusively, the results of this study suggest that *H. sabdariffa* possess antioxidant properties and could be potent in boosting the blood level in a disease state.

**Key words:** *Hibiscus sabdariffa*, antioxidant, haematological properties, acetaminophen-challenged, liver.

**INTRODUCTION**

The liver plays a central role in transforming and clearing both endogenous and exogenous chemicals and is susceptible to the toxicity from these agents (Zahra et al., 2012). The liver performs its function through two identified processes described as phases I and II detoxification pathways. In the phase I mechanism, the liver either directly neutralizes a toxin or transforms it into an activated metabolite which is subsequently neutralized
in the phase II (Monira et al., 2012). The phase II process involves the addition (conjugation) of the toxin with certain substances so as to make the toxin more water soluble and thus easy to excrete. Drugs are an important cause of liver injury (Bray et al., 2000). More than 900 drugs and toxins have been reported to cause liver injury even at therapeutic dose and it is the most common reason for a drug to be withdrawn from the market (Laura et al., 2003). However, hepatotoxicity is a direct liver injury which can be caused by an overdose of acetaminophen and consequently the actions of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Alexander and Glyn, 2005). Its ability to cause liver damage at an overdose has made it one of the most preferred drug used in studying hepatotoxicity in animal models.

A number of drugs or chemicals such as melatonin, vitamin E and N-acetyl-cysteine have been used to prevent acetaminophen-induced hepatic and renal injury (Bray et al., 2000). Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore, there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable (Sharida et al., 2012). The use of the leaf extracts of *H. sabdariffa* is among such natural resources. *H. sabdariffa* belongs to a family of herbal plants called malvaceae. Phytochemical analysis showed that there are some plant chemicals present in the extract such as alkaloids, tannins, saponins, glycosides, phenols and flavonoids and quantitative result revealed their presence as follows: Tannins (17.0%), saponins (0.96%), phenols (1.1%), glycosides (0.13%), alkaloids (2.14%) and flavonoids 20.08%) (Okereke et al., 2015). Furthermore, HPLC analysis revealed two phenolic acids, 16 flavonoids and four anthocyanins in petal of *H. sabdariffa*. The major compounds were gossypetin, hibiscetin, quercetin and sabdarin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins (Obouayeba et al., 2014).

Previous works on *H. sabdariffa* suggests that it could inhibit lipid peroxidation by maintaining the levels of antioxidants in the serum of animals treated with acetaminophen (Kolawole and Maduenyi, 2004). Studies have also shown that *H. sabdariffa* can offer protective effects against paracetamol-induced hepatotoxicity in rats (Mukesh and Ashok, 2011). Low doses of ascorbic acid found in the leaves of *H. sabdariffa* were able to prevent lipid peroxidation following acetaminophen induction in rats (Norina and Hazlin, 2004).

Thus, this present study was set out to investigate the hepa-to-regenerative properties of an aqueous extract of *H. sabdariffa* on some antioxidants and haematological indices of acetaminophen-challenged Wistar albino rats

**MATERIALS AND METHODS**

Fresh leaves of *H. sabdariffa* were purchased from Ogige market, Nsukka, Enugu State of Nigeria and were identified by Mr. Alfred Ozioko of the herbarium Botany Department, University of Nigeria, Nsukka. The leaves were air-dried separately at room temperature and ground to powdery form using electrical grinding machine.

**Animals**

Adult male Wistar albino rats of 10 to 16 weeks and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for a duration of 7 days under standard environmental conditions with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

**Chemicals/reagents/samples**

All chemicals used in this study were of the analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Teco (TC), USA; Biosystem Reagents and Instruments, Spain.

**Preparation of acetaminophen (paracetamol) sample**

The stock concentration of acetaminophen was prepared by dissolving 600 mg of the standard drug in 2 ml of distilled water bringing the stock concentration to 60 mg/ml. Paracetamol was induced intraperitoneally at the dose of 750 mg/kg b.w. (Hiroshini et al., 1987).

**Extraction of the active agents of *H. sabdariffa***

Large quantities of the leaves of *H. sabdariffa* were purchased from Ogige market in Nsukka, Enugu State of Nigeria and were identified by Mr. Ozioko of the herbarium Botany Department, University of Nigeria, Nsukka. The leaves of *H. sabdariffa* were air-dried separately at room temperature (25-30°C), then into powdery form using electrical grinding machine. The ground samples extracted with aqueous solvent (H2O), using cold maceration techniques for 48 h. The samples were filtered using Whatman filter paper No 1. The filtrates (that is, the active agents of the extract) concentrated using rotary evaporator, which then become the stock sample of the aqueous leaf extract which were used for the analysis. These extracts were stored in the refrigerator compartment to prevent microbial growth.

**Experimental design**

Twenty (20) male albino Wistar rats were acclimatized for seven

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days at room temperature and vital animal feeds were used for all the rats. The rats were divided into four (4) groups of five (5) rats each as shown below: Group 1: Normal/negative rats (Control); Group 2: positive control (Acetaminophen-induced untreated rats); Group 3: Acetaminophen-induced + 400 mg/kg b.w. of the extract H. sabdariffa; Group 4: Acetaminophen-induced + 600 mg/kg b.w. of the extract H. sabdariffa.

After the experiment, the animals were sacrificed at the end of the experiment and blood was collected for biochemical analysis.

**Determination of malondialdehyde concentration**

Lipid peroxidation assay was done by determining the concentration of malondialdehyde (MDA) formed using the method of Varshney and Kale (1990).

**Determination of vitamin C**

The concentration of vitamin C (ascorbic acid) was determined according to the method of Baker et al. (1971).

**Assay of superoxide dismutase activity**

Superoxide dismutase (SOD) activity was assayed using the method as described by Fridorich (1989) as contained in Randox commercial kit.

**Assay of catalase activity**

Catalase activity was assayed using the method of Aebi (1983).

**Total white blood cell count (WBC)**

This was done using standard techniques as described by Ramnik (2003).

**Red blood cell count (RBC)**

This was done using standard method as described by Daice and Lewis (2000).

**Hemoglobin estimation**

Hemoglobin concentration was determined by the method described by Dacie and Lewis (2000).

**Packed cell volume (PCV)**

This was done using standard technique as described by Ochei and Kolharta (2008).

**White blood cell differential count**

The differential WBC counts was obtained using a coulter counter in a well standardized commercial laboratory.

**Statistical analysis**

The results were expressed as Mean±SEM and test of statistical significance was carried out using one-way analysis of variance (ANOVA). The means were separated using Duncan multiple test. The statistical packaged used was the statistical package for social sciences (SPSS), version 17.

**RESULTS AND DISCUSSION**

Acetaminophen belongs to a subgroup of analgesics called aniline analgesics and is very effective in relieving pain and mild fever. At therapeutic doses, it causes no discomfort. However, an overdose of the drug can result to severe hepatic damage (Linda et al., 2009). It undergoes metabolic activation by hepatic microsomal cytochrome P<sub>450</sub> mixed function oxidase system (especially the enzyme CYP2E1) to N-acetyl-P-benzoquinone imine (NAPQI). The active metabolite (being highly electrophilic) quickly binds to intracellular proteins, causing a change in their structure and hence their function (Monira et al., 2012). The NAPQI is the active metabolite involved in virtually all the metabolic disorders experienced during an overdose of acetaminophen.

From Figure 1 and Table 1, intraperitoneal (ip) induction of Acetaminophen at the dose of 750 mg/kg b.w. caused a significant increase (p<0.05) in the malondialdehyde (MDA) concentration which is a product of lipid peroxidation of the group 2 animals as compared to the normal control group 1. This increase in MDA concentration is as a result of increase in lipid peroxidation by the actions of the toxic metabolite NAPQI. However, treatment with H. sabdariffa aqueous extract caused a significant decrease (p<0.05) in the MDA concentration of the treatment groups (groups 3 and 4) as compared to the untreated group 2 animals. Thus, these decreases could be as a result of the actions of the phytochemical constituents of the extract such as flavonoids in inhibiting the actions of the toxic metabolite NAPQI and also stabilizing the cell membranes of the intracellular proteins and other compounds. This result is consistent with the finding of Bray et al. (2000) who observed a decrease in the above mentioned parameters following treatment with ethanoic stem extracts of H. sabdariffa after acetaminophen-induction.

From Figure 2 and Table 2, induction of acetaminophen (ip) caused a significant decrease (p<0.05) in vitamin C concentrations, superoxide dismutase (SOD) activity and catalase activity of the group 2 rats when compared with the normal control group (group 1). This may be attributed to the metabolite, N-acetyl-P-benzoquinone imine (NAPQI), which induces lipid peroxidation and free radical generation. However, the free radicals generated caused the significant decrease (p<0.05) in the vitamin C concentration, SOD activity and catalase activity of the group 2 animals as compared to the normal control group 2.
Figure 1. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on malondialdehyde and vitamin C concentrations against acetaminophen-induced liver damage in Wistar Albino rats.

Table 1. Effect of aqueous leaf extract of *H. sabdariffa* on malondialdehyde and vitamin C concentrations against acetaminophen-induced liver damage in Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (mg/dl)</th>
<th>VIT C (ml/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>6.00 ± 0.42*</td>
<td>2.33 ± 0.18*</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>7.60 ± 0.87</td>
<td>0.90 ± 0.24</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>6.40 ± 0.34*</td>
<td>1.07 ± 0.31</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>6.16 ± 0.45*</td>
<td>1.03 ± 0.21</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

Figure 2. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on superoxide dismutase and catalase activity against acetaminophen-induced liver damage in Wistar Albino rats.
Table 2. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on superoxide dismutase and catalase activity against acetaminophen-induced liver damage in Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (IU/L)</th>
<th>CATA (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>14.53 ± 1.82*</td>
<td>8.60 ± 0.54*</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>10.50 ± 1.16</td>
<td>7.79 ± 0.41</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>16.30 ± 1.50*</td>
<td>9.05 ± 0.58*</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>17.27 ± 0.82*</td>
<td>10.12 ± 0.38*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

Table 3. Effect of aqueous leaf extract of *H. sabdariffa* on packed cell volume and hemoglobin concentration against acetaminophen-induced liver damage in Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCV (%)</th>
<th>HB (G/DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>44.33 ± 4.45*</td>
<td>12.33 ± 3.50*</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>33.33 ± 3.76</td>
<td>8.50 ± 2.67</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>52.66 ± 4.74*</td>
<td>13.67 ± 3.43*</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>53.00 ± 2.67*</td>
<td>13.33 ± 4.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

group 1. Administration with both low (400 mg/kg b.w) and high (600 mg/kg b.w) doses of the extract after acetaminophen-induction however significantly increased (p<0.05) the activities of CAT and SOD as compared to the untreated group 2 animals. However, there was a slight non-significant (p > 0.05) increase in the Vitamin C concentration of the treated group as compared to the untreated group 2 animals. This is probably due to the antioxidant components of the extract. A high level of vitamin C is a good body mechanism for fighting not only free radicals but also diseases.

These antioxidants include beta-carotene, vitamin C and niacin. SOD, being an enzyme which converts superoxide radical (O$_2^-$) to hydrogen peroxide and molecular oxygen. CAT, an enzyme which decomposes the hydrogen peroxide generated during lipid peroxidation, is also decreased following acetaminophen-induction without treatment with extract. However, the increase experienced could be as a result of the antioxidant and vitamin contents of the extract being able to mop up the free radicals generated as a result of overdose of acetaminophen. This result is consistent with the findings of Monira et al. (2012) who observed an increase in vitamin C concentration following carbon tetrachloride-induction and the findings of Sharida et al. (2012) who observed a restoration of the above parameters to their normal value following treatment with methanol leaf extract of *H. sabdariffa* after cyclophosphamide-induction Table 3.

Administration of acetaminophen alone to all the groups significantly increased (p<0.05) the white blood count cell (wbc) of the group 2 rats when compared with the normal control of rats of group 1 as can be seen in Table 4 and Figure 3. Acetaminophen overdose initiates fast mobilization of total white blood cells for the initial defense against drug toxicity, this is in response to the metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which causes reactive metabolite formation, GSH depletion and mitochondrial oxidant stress, which contributes directly to the mitochondrial membrane permeability transition pore opening and collapse of the membrane potential and indirectly through release of inter-membrane proteins, to nuclear DNA damage. Treatment with various doses (400 and 600 mg/kg b.w.) of the extract caused a dose dependent significant decrease in the white blood cell count of the treated groups (3 and 4) as compared to the untreated group 2 rats. These results suggest that the extract was able to restore normalcy in the rat after treatment and this could be as a result of the actions of some active phytoconstituents of the plant extract in stimulating the immune system to fight the disease state. This result also indicates that the extract has an immune-stimulatory effect on the component of the immune cells. Thus, it could be deduced that aqueous extract of *H. sabdariffa* has immuno-modulatory potentials. Thus, this result of Ademola et al. (2015) where the protective effect of pretreatment of rats with Calyx extract of *H. sabdariffa* against carbon tetrachloride-induced hemato-toxicity.

Furthermore, from Figure 4 and Table 5 administration of acetaminophen alone to groups 2 rats significantly increased (p<0.05) the neutrophil count when compared
Table 4. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on red blood cell and white blood cell count of acetaminophen-induced liver damage in Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (mm$^3$)</th>
<th>RBC (x10$^6$mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>4716.67 ± 538*</td>
<td>423.67 ± 35*</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>6450.00 ± 832</td>
<td>320.00 ± 56</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>4766.67 ± 445*</td>
<td>396.00 ± 12*</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>4966.67 ± 438*</td>
<td>366.67 ± 35*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

Eosinophil is a white blood cell containing granules and an eosinophil count typically helps to confirm a diagnosis, it have two distinct functions in the immune; they destroy invading germs like viruses, bacteria or parasites and they create inflammatory response especially when an allergy is involved. Thus, drug induced liver injury is not associated with eosinophil as can be seen in Figure 5 and Table 6 that induction of acetaminophen did not cause any significant increase or decrease (p>0.05) since it is mostly concern with allergy responses.

The results shown in Figure 6 and Table 4 shows that after induction of acetaminophen, there was a significant decrease (p<0.05) in the red blood cell count of the untreated group as compared to the normal control group 1. This suggests that the high level of free radical actions could lead to increase in the breakdown of the red cell or with the normal group 1 rats, however, there was no significant increase (p>0.05) in the lymphocyte counts of the treated groups when compared with the untreated group. Neutrophils are the primary white blood cells that respond to infection or any form of cell toxicity or cell inflammation while lymphocytes are also a part of the white blood cells but specific to acute viral infections such as viral hepatitis, cytomegalovirus and others such as protozoal infections. Thus, treatment with various doses (400 and 600 mg/kg b.w.) of aqueous extract of *H. sabdariffa* caused a significant decrease (p<0.05) in the neutrophil count of the treatment groups (3 and 4) as compared to the untreated group 2 rats. The results of this research are in line with the works of olatunji et al. (2005) where the hematological effect of *H. sabdariffa* petals on rats was determined.
Table 5. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on neutrophil count and lymphocytes count against acetaminophen-induced liver damage in Wistar Albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NEU. CNT (%)</th>
<th>LYM. CNT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>67.33 ± 7.76*</td>
<td>31.67 ± 2.18</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>85.67 ± 6.35</td>
<td>29.33 ± 5.81</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>68.67 ± 4.80*</td>
<td>30.00 ± 5.29</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>70.67 ± 5.76*</td>
<td>28.00 ± 4.60</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.
other unknown actions of the toxic metabolite in the body. However, after treatment with the extract at various doses (400 and 600 mg/kg b.w), there was a significant increase (p<0.05) in the red blood cell count of the treatment groups (3 and 4) when compared with the untreated group 2 rats. This suggests that the extract could contain some compounds that are potent in boosting the production of the red cells by the bone marrow. However, this result is in concordance with the works of Ademola et al. (2015) where the protective effect of pretreatment of rats with calyx extract of H. sabdariffa against carbon tetrachloride-induced hematoxicity induction of acetaminophen significantly decreases (p<0.05) the packed cell volume (PCV) count and the hemoglobin estimation of group 2 rats compare with normal control (group 1). As shown in Table 3 and Figure 7. This is probably due to the significant reduction in the weight of thymus and spleen, and this decrease could also be attributed to the low levels of hemoglobin which is a clinically condition characterized by low levels of PCV. The implication of this is suppression of the innate immune responses. This result is consistent with Fakeye et al. (2008), who observed a decrease in the above parameter after acetaminophen induction. However, administration of H. sabdariffa flower aqueous extract after acetaminophen administration significantly increased (p<0.05) PCV and hemoglobin estimation of the treated rats of group 3 and 4 as compared to the untreated rats of group 2. Thus, this suggests that the aqueous extract of H. sabdariffa flower could contain

### Table 6

Effect of aqueous leaf extract of *H. sabdariffa* on eosinophil count against acetaminophen-induced liver damage in Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Eosinophil CNT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Normal (Control)</td>
<td>0.6667 ± 0.06</td>
</tr>
<tr>
<td>Group 2 Positive Control (untreated rats)</td>
<td>0.6876 ± 0.06</td>
</tr>
<tr>
<td>Group 3 Acetaminophen + 400 mg/kg b.w. of extract</td>
<td>0.6766 ± 0.06</td>
</tr>
<tr>
<td>Group 4 Acetaminophen + 600 mg/kg b.w. of extract</td>
<td>0.6576 ± 0.06</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.
some active compounds that will be effective against anemia and other blood related disorders. However, the results of this research is in line with the works of Ahmed et al. (2013) where the effect of aqueous extract of H. sabdariffa seed on hematological parameters against anemic rats.

**Conclusion**

Conclusively, the results of this study suggest that flowers of H. sabdariffa contains some compounds that are effective against drug induced liver toxicity and more so has some phyto-constituents that could help to boost hematological parameters in an anemic condition.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


