Changes in Creatinine, Urea, Glutathione-S-Transferase and Uric Acid Levels in Acetaminophen Extra Overdosed Rabbits Treated with Watermelon Juice (Citrullus lanatus)

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Abstract:

**Background:** Watermelon juice that contains antioxidants in addition to other vitamins and minerals is being used as beverage to alleviate thirst in Nigeria. Acetaminophen extra is most available to people and could be irrationally used to relieve pain. **Aim and Objective:** This work was designed to evaluate the effect of watermelon juice in rabbits overdosed with acetaminophen extra using Creatinine, Urea, Glutathione-S-transferase, and Uric acid.

**Materials and Methods:** Thirty rabbits classified into six experimental groups labelled A-F (and sub groups as C1, C2, E1 and E2) with A as control were investigated. An overdose of acetaminophen extra of 1500mg/kg was administered into some of the rabbits for 5 days to induce toxicity. Fifteen millilitre of watermelon juice was used to protect and treat acetaminophen extra toxicity. Plasma Creatinine

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Urea, Glutathione-S-transferase, and Uric acid were analysed biochemically by spectrophotometry.

**Result:** The result showed a significantly increase plasma values of urea in rabbits simultaneously administered with 15ml/kg of watermelon juice and 1500mg/kg of acetaminophen extra than the normal control (<0.05). There was a significant increase in plasma value of GST in rabbits given watermelon juice only without acetaminophen extra and a significantly decrease difference in rabbits given 1500mg/kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/kg of watermelon juice and 1500mg/kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water (p<0.05). There was a significantly decrease urea level and a significantly increase plasma uric acid level in rabbits given 1500mg/kg of acetaminophen extra without watermelon juice than the normal control rabbits (p<0.05).

**Conclusion:** This study revealed a significant decrease and increase in plasma level of urea and uric acid respectively in the rabbits after the administration of 1500mg/kg acetaminophen extra. There was also a significant decrease in plasma Glutathione-S-transferase, in experimental rabbits following the administration of 1500mg/kg of acetaminophen extra for 5 days and also after the administration of 15ml/kg of watermelon juice compared with normal control rabbits. Further study should be carried out on the effect of watermelon juice on drug toxicity using another chemical substance.

**Key words:** Creatinine, Urea, Glutathione-S-Transferase, Uric Acid, Acetaminophen, Overdosed, Rabbits, Watermelon Juice.

**INTRODUCTION**

*Citrullus lanatus* (water melon) is made up of about 93% water, hence the name “water” melon (1). Naturally, it is a rich source of lycopene (in the red/pink succulent part), a carotenoid that has a great antioxidant capacity and potential health benefits (2). It also contains amino-acid citrulline, cucurbitacin,
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triterpenes, sterols and alkaloids \(^{(3)}\) \(^{(4)}\) \(^{(5)}\). Every part of watermelon fruit is highly nutritive including the back and the seeds \(^{(6)}\).

The fruit has some non-scientific traditional health benefits applied to treat some ailments in Nigeria, Ayurveda and Indian traditional system of medicine. In addition it contains phytochemicals like flavonoids, alkaloids, saponins, glycoside, tannins and phenols \(^{(7)}\) \(^{(8)}\). The root can be used as purgative and emetic \(^{(8)}\) while the seed is demulcent, pectoral and tonic \(^{(9)}\). It is sometimes been used to treat urinary tract infections \(^{(8)}\) as well as bed wetting. The fruit is also diuretic and very effective in the treatment of dropsy and renal stones \(^{(10)}\). It has antihypertensive effects and the fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms \(^{(11)}\) \(^{(12)}\). The rind of the fruit has been recommended for alcohol poisoning and diabetes \(^{(9)}\). \textit{Citrullus lanatus} in Northern Sudan is used for burns, swellings, rheumatism, gout and as laxative \(^{(13)}\) \(^{(14)}\). Lycopene is characterized by its distinctive red color in fruits and vegetable \(^{(15)}\). The fruit is used as a purgative in Senegal; also used to treat diarrhoea and gonorrhoea in Nigeria \(^{(16)}\) \(^{(17)}\). Plant medicine (phytomedicine) has been used in healthcare delivery in many parts of Africa and the rest of the world \(^{(17)}\) \(^{(18)}\). Fruits and vegetables are natural sources of various bioactive compounds \(^{(19)}\) which could be attributed to their phytochemical constituents especially flavonoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber, and carotenoids present in fruits and vegetables \(^{(20)}\). An example of these medicinal plants is \textit{Citrullus lanatus}. Although several of its uses in traditional medicine have been documented, many of these claims are yet to be validated by scientific researchers. Consequently, This work was designed to evaluate the effect of watermelon juice in rabbits overdosed with acetaminophen
extra using Creatinine, Urea, Glutathione-S-transferase, and Uric acid.

MATERIALS AND METHODS

Study area
This study was carried out at Achievers University animal house, Owo Local Government area of Ondo state in Nigeria.

Study population
Thirty rabbits were purchased in Owo through the Department of Biological Sciences, Achievers University, Owo – Nigeria and were divided into six groups.

Experimental Groups
Group A: 5 rabbits were fed with normal meal and water for 7 days (normal control)
Group B: Consist of 5 rabbits fed with normal meal and 15ml/Kg body weight of watermelon juice daily for 7 days
Group C: 5 rabbits were given 1500mg/kg of acetaminophen extra per oral for 5 days (C1) and thereafter were fed with normal meal and 15ml/Kg of watermelon juice daily for 7 days after 5 days of post acetaminophen extra administration(C2)
Group D: 5 rabbits given 1500mg/kg of acetaminophen extra per oral and fed with normal meal and water for 7 days
Group E: 5 rabbits fed with normal meal and 15ml/Kg of watermelon juice daily for 7 days after which each rabbit were given 1500mg/kg of acetaminophen extra per oral for 5 days with normal meal and water.
Group F: 5 rabbits given 1500mg/kg of acetaminophen extra per oral and were simultaneously fed with normal meal and 15ml/Kg of watermelon juice daily for 5 days.
Biological sample
Blood samples were collected from the vein lining the ears of each of the rabbits as control and from the experimental rabbits before and after the administration of 1,500mg/Kg BW and 15ml/Kg BW.

Preparation of watermelon juice
Water melon was purchased from Owo markets and was presented to the Department of biological sciences, Achievers University, Owo for confirmation and certification. The succulent red part was removed and kept in a sterile bowl. The seeds was aseptically removed. The remaining succulent red part of the watermelon was blended using electric blender and thereafter was filtered undiluted. Fifteen milliliter of the filtrate was administered to the rabbits as watermelon juice.

Preparation of acetaminophen extra powder
Acetaminophen extra tablets of GlaxoSmithKline was purchased and grinded into powder using Laboratory pestle and mortal.

Blood sample preparation
Whole blood samples collected from each of the rabbits was collected into Lithium heparinized tubes. The blood samples were spun using bench/macro centrifuge for the extraction of the plasma.

ESTIMATION OF UREA, URIC ACID, CREATININE AND GST
The UREA,URIC ACID, CREATININE were measured by using reagent kits purchased from Randox Laboratories Limited, Antrium (United Kingdom) according to the method described by Fawcett and Scott,(1960) and GST assay was
carried out using reagent from Sigma Aldrich (America) with biosino biochemical (China) according to the manufacturer’s instruction.

ANALYTICAL METHODS

CREATININE
Method: Jaffe-slot alkaline picrate creatinine method
Principle: Creatinine reacts with picric acid in an alkaline medium. The absorbance of yellow-red colour produced is measured in a colorimeter using a blue-green filter 490nm (Ilford No.603) or in a spectrophotometer at 490nm wavelength (21).

UREA
Method: Berthelot reaction
Principle: Urea is split into ammonia and carbon dioxide by the action of urease, an enzyme obtained from Jack Bean meal. The ammonia then reacts with alkaline hypochlorite and phenol in the presence of a catalyst-sodium nitroprusside. The resulting product is indophenol (a blue color) and the concentration of ammonia is directly proportional to the absorbance of indophenol (22).

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{CO}_2
\]
\[
\text{NH}_3 + \text{hypochlorite} + \text{phenol} \rightarrow \text{indophenol (blue compound)}
\]

GLUTATHIONE –S TRANSFERASE
Method: Enzymatic reaction
Principle: The principle is based on the fact that known glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4,-dinitrobenzene as the second substrate, consequently, the conventional assay for glutathione-S-transferase activity utilizes 1-chloro2,4,- dinitrobenzene as
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substrate. The absorption increase at the new wavelength of 340nm provides a direct measurement of enzymatic reaction \(^{(23)}\).

**URIC ACID**

*Method:* Colorimetric method

*Principle:* Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound \(^{(24)}\).

\[
\text{Uric acid} + \text{O}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{uricase}} \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 3,5\text{-dichloro-2-hydroxybenzenesulfonic} \quad \text{acid} + 4\text{-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzo-quinoneimine}
\]

**STATISTICAL ANALYSIS OF DATA**

A statistical package for social sciences (SPSS) 20.0 was used for the analysis of the data appropriately. Continuous variables were displayed as means and standard deviation (SD) and categorical variables were displayed as percentage. The level of significance was taken at 95% confidence interval and \(P \leq 0.05\) was considered significant.

**Results**

The results obtained showed no significant difference in the plasma value of creatinine in normal control rabbits compared with: rabbits given 15ml/Kg of watermelon juice without acetaminophen extra; rabbits given 1500mg/Kg of acetaminophen extra for five days without water melon juice; rabbits simultaneously administered with 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra for 5 days (\(p>0.05\)). No significant increase was obtained in the plasma value of creatinine in rabbits given 1500mg/Kg
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acetaminophen extra for 5 days before and after they were given 15ml/Kg of watermelon juice for seven days before and after the inducement of toxicity by giving 1500mg/Kg of acetaminophen extra for 5 days (p>0.05). There was no significant increase difference in the plasma value of urea and no significant increase difference in the plasma uric acid in normal control rabbits compared with rabbits given 15ml/Kg of watermelon juice without acetaminophen extra (p>0.05). No significant decrease difference was obtained in the plasma value of urea and no significant increase difference in plasma uric acid in rabbits given 1500mg/Kg acetaminophen extra before and after they were given 15ml/Kg of watermelon juice and also in rabbits given 15ml/Kg of watermelon juice for seven days before and after the inducement of toxicity by giving 1500mg/Kg of acetaminophen extra for 5 days (p>0.05). There was a significantly higher difference in plasma value of urea in rabbits simultaneously administered with 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra for 5 days than the normal control (p<0.05). There was a significant increase in plasma value of GST in rabbits given watermelon juice only without acetaminophen extra and a significantly decrease level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water (p<0.05). There was a significantly lower urea level and a significantly higher plasma uric acid level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice than the normal control rabbits (p<0.05).
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Table 1: The mean and standard deviation of CREATININE, UREA, GST and GST obtained in the rabbits

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Cr</th>
<th>Urea</th>
<th>GST</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.3±0.1</td>
<td>5.8±0.9</td>
<td>9.8±5.2</td>
<td>3.6±1.8</td>
</tr>
<tr>
<td>B</td>
<td>0.1±0.1</td>
<td>4.8±1.0</td>
<td>14±1.9</td>
<td>5.0±2.9</td>
</tr>
<tr>
<td>C1</td>
<td>0.2±0.1</td>
<td>4.3±1.9</td>
<td>5.5±2.5</td>
<td>5.3±1.3</td>
</tr>
<tr>
<td>C2</td>
<td>0.2±0.1</td>
<td>3.2±0.7</td>
<td>5.9±1.7</td>
<td>6.0±1.3</td>
</tr>
<tr>
<td>D</td>
<td>0.1±0.1</td>
<td>4.1±0.9</td>
<td>1.8±0.5</td>
<td>8.7±4.1</td>
</tr>
<tr>
<td>E1</td>
<td>0.1±0.1</td>
<td>4.8±1.0</td>
<td>14.1±1.9</td>
<td>5.3±1.3</td>
</tr>
<tr>
<td>E2</td>
<td>0.3±0.1</td>
<td>4.2±0.9</td>
<td>3.7±0.6</td>
<td>5.4±2.8</td>
</tr>
</tbody>
</table>

Table 2: The comparative analysis of mean and standard deviation of CREATININE, UREA, GST and URIC ACID obtained in the rabbits

<table>
<thead>
<tr>
<th></th>
<th>A Vs B</th>
<th>A Vs D</th>
<th>C1 Vs C2</th>
<th>E1 Vs E2</th>
<th>A Vs F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>2.12</td>
<td>0.10</td>
<td>0.45</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Urea (Mmol/L)</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>1.88</td>
<td>0.10</td>
<td>1.23</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>GST(Mmol/dl)</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>-4.88</td>
<td>0.00</td>
<td>-0.10</td>
<td>0.47</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>p&gt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>-0.52</td>
<td>0.31</td>
<td>-2.01</td>
<td>0.50</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.05</td>
<td>p&lt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

Figure 1: Showing creatinine mean result
DISCUSSION

The result showed a significantly increase plasma value of glutathione S-transferases in rabbits given watermelon juice only without acetaminophen extra; decrease GST in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water this could be as a result of over utilization of GST in detoxifying possible toxicity caused by the administration of 1500mg/Kg of acetaminophen extra because glutathione S-transferases are enzymes with many functions including a key role in cellular detoxification (25). It protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their
electrophilic sites, and renders the products more water-soluble (25). The glutathione conjugates are metabolized further to mercapturic acid and then excreted. GSTs are implicated in a variety of diseases by virtue of their involvement with GSH (25). There was a significantly decrease urea level and a significantly increase plasma uric acid level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice than the normal control rabbits.

In this work decrease plasma urea level obtained in the rabbits after the administration of 1500mg/Kg acetaminophen extra than in the control rabbits is consistent with the report of Waring et al. (26) that low serum urea concentration is not an independent risk factor for hepatotoxicity after paracetamol overdose. This could also be attributed to the fact that glutathione depletion increases the incidence of toxicity after paracetamol overdose. Risk factors for toxicity, including chronic ethanol excess and malnutrition, are associated with low serum urea concentrations. Therefore, they hypothesized that low serum urea concentration might itself be predictive of hepatotoxicity in patients that present to hospital after paracetamol overdose (26).

Raised uric acid found in the rabbits in this study after the administration of 1500mg/Kg of acetaminophen extra than in normal control rabbits agrees with the report of Wilding amd Heath (27) who reported on the effect of paracetamol on uric acid determination that apparently raised values of serum uric acid may be reported in patients receiving therapeutic levels of paracetamol and levels of serum uric acid were determined over a five-hour period in three patients who had received therapeutic doses of paracetamol.
CONCLUSION

This study revealed a significant decrease in plasma level of urea and a significant increase in plasma uric acid in the rabbits after the administration of 1500mg/Kg acetaminophen extra. There was also a significant decrease in plasma GST in experimental rabbits following the administration of 1500mg/Kg of acetaminophen extra and a significant increase after the administration of 15ml/Kg of watermelon juice compared with normal control rabbits.

RECOMMENDATION

Further study should be carried out on the effect of watermelon juice on drug toxicity using another chemical substance.

REFERENCES

consumption of Citrullus vulgaris (watermelon). Berichte der deutschen chemischen Gesellschaft; 28 (4): 467–472


