Evaluating the Potential of *Annona muricata* L. Plant Compounds as Reducing Agents for Toxicity of Ethylene Glycol

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Abstract

*Annona muricata* L. is a tropical plant, which is considered as an attractive species due to its medical uses and bioactivity. This study evaluated the potential of *Annona* plant for decreasing the toxic effect of ethylene glycol. Twenty five male rabbits were divided into five groups, i.e., control group (without any additives), group fed with ethylene glycol and the rest of groups were fed with *Annona muricata* L. compounds (flavonoid, glycoside and alkaloids) to monitor its impact on liver activity. Results revealed that ethylene glycol increased the levels of aspartate transaminase, alanine transaminase, gamma-glutamyltransferase and alkaline phosphatase, whereas Annona compounds decreased the toxicity of ethylene glycol. A significant difference (P˂ 0.05) in total protein, albumin, globulin values as well as A/G ratio was observed in the control group in comparison to ethylene glycol group and ethylene glycol groups with *Annona muricata* L. plant compounds. Lipid peroxidation resulted in high level of malondialdehyde in the ethylene glycol group in comparison to the control group, however, Annona compounds reduced the level of ethylene glycol, which in turn decreased the oxidative damage. Glutathione, catalase as well as glutathione peroxidase levels increased among the groups fed with Annona compounds in comparison to that fed with ethylene glycol only. Superoxide dismutase recorded approximately the same levels in all tested groups. There was a significant difference (P˂ 0.05) in MDA and CAT values in all groups, while no significant difference (P> 0.05) was observed in other parameters, i.e., GSH, GPx as well as SOD levels.

Key word: Annona, ethylene glycol, Lipid peroxidation, liver function, Antioxidant


Introduction

The liver is an essential organ in the living organisms, i.e., humans and animals, which is considered as a center of metabolic activity and waste excretion (1). In particular, the main function of the liver is refining the absorbed substances from the digestive system prior distributing to blood circulation or bile (1), i.e., proteins, carbohydrates, amino acids, lipids, cholesterol as well as vitamins. Moreover, the liver has 15 types of cells with hepatocytes being the most abundant type that comprising 60% of total cells (2). Plenty of chemicals are metabolized in liver. Consequently, hepatocytes are exposed to considerable concentrations of these substances (3), such as ethylene glycol (EG).
Ethylene glycol is an organic compound, which is utilized in various industries (plastics, chemicals as well as energies). In particular, it has different applications such as a coolant and anti-freeze substance in automobile (4). A wide range of studies had been dedicated for EG applications, for instance, evaluating the pathology and toxicity of EG on body organs in addition to diagnosis and treatment (4), as ingesting small amounts will metabolized in liver to form oxalic acid that precipitates in kidney creating crystals of calcium oxalate (5).

In order to evaluate the function of liver, liver enzymes should be monitored, i.e., alanine amino transferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) to detect the damage of hepatocytes, which is called serum enzyme tests (6). In hepatocytes, AST and ALT are essential indicators for liver injury. Thus, the normal range for AST is 40 U/L and that for ALT is 55 U/L (7). Nevertheless, high levels of ALP results in cholestatic disorders. In general, the deterioration of liver, leads to fail of releasing ALP enzyme (6). γ Glutamyl transpeptidase (GGT) enzyme is also a serious indicator of cholestasis when elevated in liver and can be stimulated by drugs (8).

Moreover, liver lipid peroxidation is also important to assess, as it is a biomarker for enhancing cell survival or stimulating cell death (9).

Material and Methods

Plants material: Annona muricata L. plant was used for running the experiment. The plant samples were collected from the market then identified and approved by the herbariums at the College of Education in University of Mosul, Iraq.

Preparation of plant compounds: Preparation of plant compounds, i.e., glycoside, flavonoid as well as alkaloids were conducted as described by (10).

Animals: Twenty five male rabbits were used for running the experiment. Their weights were between 750-850g. Male rabbits were divided into five groups and each group had 5 rabbits, i.e., control group (fed with regular diet), a group fed with water containing 0.75% of ethylene glycol (EG) to stimulate the production of renal calculi till day 30. The third, fourth and fifth groups were fed with Annona plant compounds, i.e., glycoside, flavonoid and alkaloids (dose of 100mg/kg of body weight) from day 15 to 30, as the male rabbits were fed twice a day.

Liver function tests: Blood samples were taken from male rabbits after 60 days of feeding with Annona plant compounds. The serum was extracted from each blood sample to determine aspartate transaminase (AST), alanine transaminase (ALT) (11), gamma-glutamyltransferase (GGT) (12) and alkaline phosphatase (ALP) (13), which are liver enzymes. Total protein and albumin was also determined by (14) using spectrophotometer. The globulin values and Albumin/globulin ratios were also calculated.

Determining lipid peroxidation and antioxidant enzymes.

Tissue samples were taken after 60 days of feeding. The malondialdehyde (MDA) levels were determined by using Thiobarbituric Acid Reactions (TBA). Glutathion (GSH) levels were determined as described by (15). Antioxidant enzyme activities (catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) were determined by using (16, 17 and 18) methods respectively.
Results

A number of parameters (tests), i.e., AST (U/L), ALT (U/L), GGT (U/L), ALP (U/L), Total protein (g/dL), Albumin (g/dL), Globulin (g/dL) as well as albumin/globulin ratio (A/G ratio) were set to evaluate its potential on the function of liver of male rabbits that had been fed with ethylene glycol (EG) and Annona plant compounds such as flavonoid, glycoside and alkaloids.

Results have shown that AST, ALT, GGT, ALP and total protein values in the control group of male rabbits were lower (30.13±0.41, 35.66±1.3, 100±4.3, 31.81±0.78 and 10.72±0.03) respectively than that of EG group, i.e., 61.33±0.32, 52.19±2.02, 210±11.5, 67.11±1.13 and 12.46±0.11 respectively, however, albumin value in the control group was slightly higher (6.35±0.03) than that of EG group (5.79±0.55).

Globulin value was slightly lower (3.57±0.05) in the control group than that of EG group, i.e., 4.44±0.23. Finally, A/G ratio was nearly the same in both control group and EG group, i.e., 1.78±0.04 and 1.31±0.23 respectively (Table 1).

The comparison between the control group and EG groups with Annona muricata L. plant compounds such as, flavonoid, glycoside and alkaloids revealed that ALT, GGT and ALP values in the control group were lower (35.66±1.2, 100±4.3 and 31.81±0.78) respectively than that in the EG group with flavonoid (43.22±1.76, 154±3.9 and 35.73±1.91) respectively, EG group with glycoside (43.32±1.6, 147±4.8 and 45.73±1.19) respectively and EG group with alkaloids (49.62±1.16, 109±.6 and 39.13±1.00) respectively. However, AST value in the control group, i.e., 30.13±.41 was lower than that in the EG group with flavonoid (35.95±1.57); the control group value was higher than that in the EG groups with glycoside and alkaloids (27.95±1.37 and 28.25±1.47) respectively (Table 1).

Total protein value in the control group, i.e., 10.72±0.03 was slightly lower than that of EG groups with flavonoid and glycoside (11.89±0.11 and 12.11±0.10) respectively, while that of EG group with alkaloids (10.79±0.11) was almost as same as the control group, i.e., 10.72±0.03. Albumin value in the control group (6.35±0.03) was nearly the same as that in the EG groups with flavonoid, glycoside and alkaloids, i.e., 7.17±0.61, 7.77±0.42 and 6.47±.42 respectively. Globulin value in the control group, i.e., 3.57±0.05 was almost the same as that in the EG groups with flavonoid, glycoside and alkaloids (4.54±0.12, 4.44±0.12 and 4.21±0.11) respectively. Finally, A/G ratio in the control group (1.78±0.04) revealed nearly the same values as that in the EG groups with flavonoid, glycoside and alkaloids, i.e., 1.57±0.20, 1.75±0.12 and 1.53±0.12 respectively (Table 1).

The comparison between EG group and EG groups with Annona muricata L. plant compounds (flavonoid, glycoside and alkaloids) revealed that AST, ALT, GGT and ALP values in the EG group were higher than that in the EG groups with plant compounds, whereas total protein value in the EG group (12.46±0.11) was nearly the same as that of EG groups with plant compounds (flavonoid, glycoside and alkaloids), i.e., 11.89±0.11, 12.11±0.10 and 10.79±0.11 respectively (Table 1).
Table 1 Effect of ethylene glycol and *Annona muricata L.* plant compounds on different parameters in the liver of male rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>EG group</th>
<th>EG group with flavonoid</th>
<th>EG group with glycoside</th>
<th>EG group with alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>30.13±0.41</td>
<td>61.33±0.32</td>
<td>35.95±1.57</td>
<td>27.95±1.37</td>
<td>28.25±1.47</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>35.66±1.2</td>
<td>52.19±2.02</td>
<td>43.22±1.76</td>
<td>43.32±1.56</td>
<td>49.62±1.16</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>100±4.3</td>
<td>210±11.5</td>
<td>154±3.9</td>
<td>147±4.8</td>
<td>109±5.6</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>31.81±0.78</td>
<td>67.11±1.13</td>
<td>35.73±1.91</td>
<td>45.73±1.19</td>
<td>39.13±1.00</td>
</tr>
<tr>
<td>Total Protein (g/dL)</td>
<td>10.72±0.03</td>
<td>12.46±0.11</td>
<td>11.89±0.11</td>
<td>12.11±0.10</td>
<td>10.79±0.11</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>6.35±0.03</td>
<td>5.79±0.55</td>
<td>7.17±0.61</td>
<td>7.77±0.42</td>
<td>6.47±0.42</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>3.57±0.05</td>
<td>4.44±0.23</td>
<td>4.54±0.12</td>
<td>4.44±0.12</td>
<td>4.21±0.11</td>
</tr>
<tr>
<td>A/G Ratio</td>
<td>1.78±0.04</td>
<td>1.31±0.23</td>
<td>1.57±0.20</td>
<td>1.75±0.12</td>
<td>1.53±0.12</td>
</tr>
</tbody>
</table>

Analysis of Variance (ANOVA) was used to analyze data. Results revealed that there was a significant difference (P< 0.05) in total protein, albumin, globulin values as well as A/G ratio in the control group in comparison to EG group and EG groups with *Annona muricata L.* plant compounds that showed no significant difference (P> 0.05) (Table 2).

Other parameters were also monitored to evaluate its impact on the function of liver in male rabbits, i.e., MDA (lipid peroxidation), GSH, CAT, GPx as well as SOD that are antioxidants. Results revealed that MDA value in the control group, i.e., 0.85±0.02 was lower than that in EG group, EG group with glycoside and EG group with alkaloids (1.99±0.02, 1.57±0.03 and 1.44±0.02) respectively, while the control group was as same as EG group with flavonoid, i.e., 0.91±0.03. GSH value in the control group was higher (75±3.11) than that in EG group, EG group with glycoside as well as EG group with alkaloids (57±2.8, 59±3.3 and 64±2.9) respectively, however, GSH value in the control group was lower than that in EG group with flavonoid (78±3.3)(Table 2).

Another parameter, i.e., CAT showed a similar value of 0.17±0.01 in the control group with that for EG group and EG groups with plant compounds (flavonoid, glycoside and alkaloids), i.e., 0.09±0.01, 0.12±0.02, 0.14±0.02 and 0.14±0.02 respectively. GPx values revealed a small variation between the control group (6.80±0.51) and EG groups with plant compounds, i.e., flavonoid and glycoside (5.55±0.42 and 5.44±0.49) respectively, whereas EG group and EG group with alkaloids were lower (3.43±0.32 and 4.09±0.69) respectively than that of the control group. Finally, SOD values were similar in the control group (2.30±0.2), EG group (2.43±0.2) and EG group with flavonoid (2.17±0.12), however, the control group value was slightly higher than that of EG groups with glycoside and alkaloids (1.92±0.12 and 1.96±0.12) respectively (Table 2).
Table 2 Changes in liver lipid peroxidation and antioxidant enzymes in male rabbits fed with ethylene glycol and Annona plant compounds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>EG group</th>
<th>EG group with flavonoid</th>
<th>EG group with glycoside</th>
<th>EG group with alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (mM/100 g tissue)</td>
<td>0.85±0.02</td>
<td>1.99±0.02</td>
<td>0.91±0.03</td>
<td>1.57±0.03</td>
<td>1.44±0.02</td>
</tr>
<tr>
<td>GSH (mg/100mg tissue)</td>
<td>75±3.11</td>
<td>57±2.8</td>
<td>78±3.3</td>
<td>59±3.3</td>
<td>64±2.9</td>
</tr>
<tr>
<td>CAT (mmol/l)</td>
<td>0.17±0.01</td>
<td>0.09±0.01</td>
<td>0.12±0.02</td>
<td>0.14±0.02</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>6.80±0.51</td>
<td>3.43±0.32</td>
<td>5.55±0.42</td>
<td>5.44±0.49</td>
<td>4.09±0.69</td>
</tr>
<tr>
<td>SOD</td>
<td>2.30±0.2</td>
<td>2.43±0.2</td>
<td>2.17±0.12</td>
<td>1.92±0.12</td>
<td>1.96±0.12</td>
</tr>
</tbody>
</table>

Data were analyzed using ANOVA to determine if there is a significant difference in parameters values, i.e., AST, ALT, GGT, ALP, total protein, albumin, globulin as well as A/G ratio among the animal groups in the current study. Results revealed that there was a significant difference (P< 0.05) in MDA and CAT values in all groups, whereas no significant difference (P> 0.05) was observed in other parameters, i.e., GSH, GPx as well as SOD levels.

Discussion

Effect of ethylene glycol and Annona muricata L. plant compounds on liver enzymes

The current study had investigated the effect of Annona muricata L. plant compounds, i.e., flavonoid, glycoside and alkaloids on the function of liver enzymes and its impact on EG. Results have shown that there was a considerable increase in AST, ALT, GGT as well as ALP values in the liver of male rabbits group that were fed with only EG in comparison to that of the control group, which was in line with that reported by (19). Both AST and ALT enzymes are found in the cytoplasm of hepatocytes in animals and an important signs of hepatocellular injury (20). In general, they are important parameters for monitoring the cytolysis of liver, as their existence refers to dysfunction of the organ (21). Moreover, the increase in GGT value leads to increase mortality, as it is related to liver injury (8). The high value of ALP after administration could be attributed to an increase in liver activity as reported by (21). This increase may consider as a threat for the cells and results in cytolysis (21). Both GGT and ALP enzymes are common markers for cholestasis in animals. Moreover, ALP is more active in bile membrane, while GGT is allocated in the biliary epithelial cell and hepatocytes (22). These enzymes are considered as indicators for epithelial deterioration, hepatobiliary injury as well as stasis of the bile duct when their levels increased (23). In contrast, male rabbits group that was fed with both EG and Annona muricata L. plant compounds exhibited a decrease in enzymes level in comparison with the group that was fed with EG only, which indicates that Annona muricata L. compounds had reduced the fatal effect of EG on liver. This was in line with that obtained by (19), when Annona muricata L. plant compounds reduced the enzymes level in the
kidney of rabbits. Moreover, total protein value in EG group was slightly higher than that of the control group and groups fed with *Annona muricata L.* plant compounds except EG group with glycoside, which showed a similar value as that of the EG group.

**Effect of ethylene glycol and *Annona muricata L.* plant compounds on liver lipid peroxidation and antioxidant enzymes**

The current study had also evaluated the effect of EG and *Annona muricata L.* plant compounds on the lipid peroxidation and the antioxidant enzymes in the liver of male rabbits. Results have shown that MDA value in the EG group was slightly higher than that of both the control group and EG group with flavonoid, however, MDA value in the EG group was as same as that of both EG groups with glycoside and alkaloids. GSH value in the control group was higher than that of the EG group, EG groups with glycoside and alkaloids, whereas that of EG group with flavonoids was higher than that of the control group. Results were online with that obtained by (19), as lipid peroxidation damage was expressed by high levels of MDA and low levels of antioxidant enzymes in the serum such as, GSH, GPx. The production of MDA may alter the properties of the molecule and leads to pathological conditions, as reported by (9) Nevertheless, *Annona muricata L.* plant compounds reduced MDA level and increased the activity of antioxidant GSH, CAT, GPx as well as SOD, which indicates its protection role against oxidative stress.

**Conclusion**

*Annona muricata L.* plant compounds could be efficient in reducing the toxic effect of ethylene glycol.

**References**


