Curative role of green banana and red apple on aspirin induced gastric ulcer in rats

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Abstract
The biochemical effects of ulcer induced by acetyl salicylic acid on rats and the anti-ulcer activity of unripe banana and red apple supplementation was evaluated. One hundred male adult albino rats were divided into five groups (n=8). One group fed balanced diet, while other groups fed balanced diet supplemented with unripe banana and red apple at two tested doses (10 and 20%). After one month, ten rats from each group were starved 48 hours and were given aspirin at a dose level of 200mg/kg/body weight and sacrificed after six hours and noted as groups (6, 7, 8, 9, and 10). Aspirin administration caused a significant increase in serum malondialdehyde, serum alkaline phosphatase activity and nitric oxide level. Reduced glutathione level, erythrocyte Superoxide dismutase and plasma catalase activities were significantly decreased compared to healthy control group. Total titrable acidity, ulcer index, ulceration percent and ulcer score were significantly increased in ulcer control group while mucosal thickness and relative weight of stomach were significantly decreased in ulcer control group compared to healthy control group. Aspirin administration significantly decreased the level of hemoglobin, red blood cells and platelets counts but increased the level of white blood cells, neutrophils and lymphocytes counts. Consumption of green banana and red apple at the tested doses by healthy and ulcerated rats resulted in a significant decrease in oxidative biomarkers and significant improvement in antioxidants levels; complete blood picture, total titrable acidity, ulcer index, ulceration percent and ulcer score as well as mucosal thickness and relative weight of stomach as compared with ulcerated rats.

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1. Introduction

Gastric ulcer is a complex polycausal disease and is known to develop due to imbalance between aggressive and protective factors (Glavin and Szabo, 1992). The aspirin injures the gastrointestinal mucosa and because oxygen-derived free radicals mediate injury of this mucosa, oxy-radicals may play a pathogenetic role in the evolution of aspirin – induced erosive gastritis (Adams, 1996). Reactive oxygen species (ROS) may interact with cellular proteins, lipids and DNA, causing alterations in cell function. Cells can either repair the damage or directly reduce the pro-oxidative state via enzymatic and non-enzymatic antioxidants. Non-enzymatic (vitamins E and C, flavonoids, etc.) and enzymatic as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) antioxidants have been shown to scavenge free radicals and ROS (Uzun et al., 2010).

Recently, much attention has been paid to naturally derived products from fruits or vegetables which may beneficially affect a number of pathologic conditions of the gastrointestinal tract. In particular, it has been demonstrated that dietary anti-oxidants play a crucial role in maintaining gastrointestinal homeostasis by counteracting the gastrointestinal mucosa damaging effects of ROS (D’Argenio et al., 2012). The preventive and protective role of these foods is due to significant
presence of phytochemicals which, on one hand prevent oxidative damage brought about by ROS inside the gastro intestinal tract, and the other hand stimulate the cell to “self-defence” stimulating intracellular mechanisms that counteract oxidative stress and inflammation (Ricciardiello et al., 2011; D’Argenio et al., 2012).

Phenolic compounds represent an important part of phytochemicals in the diet and, in particular, the phenolic components of apple represent 22% of total polyphenols in the diet of United States consumers (D’Argenio et al., 2012).

Flavonoids and other polyphenols have a gastroprotective property which is attributed to their free radical scavenging antioxidant activity (Alanko et al., 1999). In addition, they also alter GSH metabolism, scavenge free radicals and inhibit Ca^{2+} influx (Ishige et al., 2001). The protective capacity of green bananas cannot be confined to only one active component. Pectin phosphatidylcholine and polyphenolic flavonoid (leucocyanidin) may protect gastric mucosa (Dunji et al., 1993). Apple prevents damage to human gastric epithelial cells due to its antioxidant activity. A diet rich in apple antioxidants have a beneficial effect in the prevention of gastric diseases related to generation of ROS (Woods et al., 2003; Bouayed and Bohn, 2010).

2. Materials and Methods

Materials: Aspirin, acetyl salicylic acid was obtained from EL-Gomhouria Company for Trading Chemicals, Cairo, Egypt.

Animals: One hundred adult male albino rats of Sprague-Dawley strains weighing (150±5g) were supplied by the Faculty of Medicine Research Center, Ain Shams University, Egypt. Animals were maintained on a natural light/dark cycle and given food and tap water ad libitum.

Diet: - The experimental diet used was the standard diet containing unripe bananas and red apples at a dose level of 10% or 20% of the diet weight that prepared according to AIN-1993 and adjusted by (Reeves et al., 1993).

Experimental Design: - Unripe bananas and red apple were purchased from local market, bananas were peeled, sliced while apples sliced and freshly added. Animals randomly enrolled into five groups of twenty animals each and treated as following: Group I: rats fed on balanced diet; Group II: rats fed on balanced diet + 10% banana; Group III: rats fed on balanced diet + 20% Banana; Group IV: rats fed on balanced diet + 10% apple; Group V: rats fed on balanced diet + 20% apple. At the end of the experimental period (one month) for ulceration induction, ten rats from each group were starved 48 hours and were given a single dose of acetyl salicylic acid (aspirin) orally by stomach tube at a dose level of 200mg/kg of body weight according to Kannappan et al. (2008), and noted as groups (6, 7, 8, 9, 10) and after 6 hours rats were sacrificed under diethyl ether anesthesia while the other ten rats were sacrificed after an overnight fasting. Blood samples were collected from the hepatic portal vein into three dry clean centrifuge tubes for analysis. Abdomen opened at greater curvature, stomachs ligated from esophageal were removed, gastric juice collected and centrifuged.

Biochemical analysis:- Whole blood was analyzed for the immediate determination of complete blood picture (CBC) according to Dacie and Lewis (1984) and non enzymatic antioxidant GSH according to Beutler et al. (1963) and the separation of RBC’S for the assay of antioxidant enzymes SOD activity according to Nishikimi et al. (1972). Serum was analyzed for ALP activity according to Belfield and Goldberg (1971), (MDA) according to Draper and Hadley (1990) and NO concentration according to Montgomery et al. (1961). Plasma was analyzed for CAT activity according to Aebi (1984). Gastric juice was analyzed for determination of titrable acidity according to Abdelaziz et al. (2006).

Stomach examination:- Stomachs were incised along the greater curvature and ulceration was scored according to Raju (2009). Evaluation of degree of ulceration was expressed in terms of ulcer score which is calculated by dividing the total number of ulcers in each group by number of rats in that group according to Robert et al. (1968). The degree of ulceration was also expressed as ulcer index and calculated by multiplying ulcer score ×100 according to Radwan et al. (2003). Percent of ulceration=

\[
\text{Percent of ulceration} = \frac{\text{Number of ulcerated rat} - \text{Number of non ulcerated rat}}{\text{Number of rat in each group}} \times 100
\]

Stomachs were dissected out and fixed instantaneously in 10% formalin solution for 24 hours. Paraffin blocks were prepared and 5µm thick sections were subjected to Morphometric Study using Leica Qwin 500 LTD computer assisted image analysis system in the assessment of the thickness of gastric epithelium and microscopic examination according to Kiernan (2001).
Statistical Analysis: The data were statistically analyzed using SPSS software (version 11.5). The data are expressed as means ± SD. The difference among groups means were tested using the least significant differences (LSD). P-values ≤ 0.05 were considered statistically significant according to Steel and Torrie (1980).

3. Results

3.1 Effect of banana and apple fruits administration on change in body weight, food intake, feed efficiency ratio (FER), relative weight of stomach, total titrable acidity, and mucosal thickness in control and ulcerated rats

Table (1) showed that values of feed intake of control rats exceeded that of ulcerated rats, also the values of change in body weight affected on FER showed significant decrease in ulcerated rats.

While the treatment of rats with banana and apple caused a significant decrease. Also, as shown in table (1) aspirin caused increased gastric acid production and secretion which is reflected in the significant increase in the value of total titrable acidity (mEq/L) by 46.08 % in aspirin ulcerated control rats in comparison with healthy control rats, meanwhile there was a significant reduction in the value of total titrable acidity in banana and apple treated groups and also in banana and apple ulcerated treated groups.

Also, table (1) showed that aspirin caused a significant decrease in mucosal thickness (µm) by 97.45 % and relative weight of the stomach (%) by 14.13 % while the treatment with banana and apple showed a significant increase in these values to reach the levels of healthy rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>change in body weight (g)</th>
<th>food intake (g/day)</th>
<th>Feed efficiency Ratio (%)</th>
<th>Relative weigt of stomach (%)</th>
<th>Total titrable acidity (mEq/L)</th>
<th>Mucosal thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.9±0.72</td>
<td>9.78±0.24</td>
<td>0.19±0.28</td>
<td>0.92±0.069</td>
<td>43.00±2.27</td>
<td>614.6±27.55</td>
</tr>
<tr>
<td>10% banana</td>
<td>27.8±0.64</td>
<td>9.37±0.51</td>
<td>0.09±0.0056</td>
<td>0.96±0.037</td>
<td>4.03±0.78</td>
<td>615.8±26.54</td>
</tr>
<tr>
<td>20% banana</td>
<td>25.7±0.83</td>
<td>8.71±0.69</td>
<td>0.10±0.0069</td>
<td>1.02±0.037</td>
<td>38.85±0.62</td>
<td>630.6±37.11</td>
</tr>
<tr>
<td>10% apple</td>
<td>26.00±1.03</td>
<td>9.18±0.84</td>
<td>0.09±0.0056</td>
<td>1.28±0.07</td>
<td>36.52±0.33</td>
<td>640.7±29.47</td>
</tr>
<tr>
<td>20% apple</td>
<td>24.5±1.22</td>
<td>8.81±0.59</td>
<td>0.09±0.0056</td>
<td>1.32±0.05</td>
<td>33.17±0.23</td>
<td>660.6±45.63</td>
</tr>
<tr>
<td>Ulcer</td>
<td>29.75±0.65</td>
<td>9.78±0.24</td>
<td>0.10±0.001</td>
<td>0.79±0.05</td>
<td>79.75±2.10</td>
<td>153.6±17.88</td>
</tr>
<tr>
<td>Ulcer+10% banana</td>
<td>27.65±0.61</td>
<td>9.34±0.83</td>
<td>0.10±0.002</td>
<td>0.83±0.05</td>
<td>70.3±0.47</td>
<td>225.5±19.21</td>
</tr>
<tr>
<td>Ulcer+20% banana</td>
<td>25.62±1.09</td>
<td>8.65±0.71</td>
<td>0.09±0.003</td>
<td>0.82±0.14</td>
<td>64.25±0.50</td>
<td>277.39±29.78</td>
</tr>
<tr>
<td>Ulcer+10% apple</td>
<td>26.28±0.99</td>
<td>9.28±0.92</td>
<td>0.09±0.005</td>
<td>0.93±0.03</td>
<td>59.25±0.50</td>
<td>506.9±76.91</td>
</tr>
<tr>
<td>Ulcer+20% apple</td>
<td>24.50±1.13</td>
<td>9.00±0.65</td>
<td>0.08±0.008</td>
<td>0.98±0.03</td>
<td>45.50±0.40</td>
<td>577.18±31.01</td>
</tr>
<tr>
<td>L.S.D (p≤0.05)</td>
<td>0.90</td>
<td>0.65</td>
<td>0.08</td>
<td>0.06</td>
<td>1.09</td>
<td>26.39</td>
</tr>
</tbody>
</table>

3.2 Effect of banana and apple fruits administration on serum malondialdehyde (MDA), nitric oxide levels, plasma catalase activity, reduced glutathione level, erythrocyte superoxide dismutase and alkaline phosphatase (ALP) activities in control and ulcerated rats

Table (2) showed that aspirin induced oxidative stress which is reflected in the significant increase in the values of MDA by 181.77 % and NO by 99.68 % in aspirin ulcerated control rats compared to healthy control rats, the reduction percentage were found in banana and apple treated groups and also in banana and apple ulcerated treated groups, beside oxidative stress aspirin induced tissue inflammation which is reflected in the significant increase in the activity of ALP by 111.25 % in aspirin ulcerated control rats in comparison with healthy control rats, the treatment with green banana and red apple at the tested doses (10 % and 20 %) significantly reduced ALP activity by 20.80 %, 23.78 %, 36.35 % and 46.39 % in banana and apple treated ulcerated groups. There were also a significant decrease in antioxidant enzyme activities of SOD and CAT as well as GSH level, the reduction percentages were 47.35 %, 47.10 %, and 35.85 % respectively in aspirin ulcerated control rats in comparison with healthy control rats.
Treatment with banana and apple significantly increased these percentages and improved anti-oxidative enzymes activities as well as non-enzymatic such as GSH level.

**Table 2:** The effect of administration of banana and apple fruits on serum malondialdehyde (MDA), nitric oxide levels, plasma catalase activity, reduced glutathione level, erythrocyte superoxide dismutase and alkaline phosphatase (ALP) activities in control and aspirin ulcerated rats  
- Values are expressed as means ±S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts in the same column.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (µmol/L)</th>
<th>Nitric oxide (µmol/L)</th>
<th>Catalase (U/L)</th>
<th>Reduced glutathione (mg/dL)</th>
<th>Superoxide dismutase (U/mL)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.14±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.12±0.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>99.25±2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.35±1.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>307.02±5.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.00±0.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% banana</td>
<td></td>
<td>1.88±0.14&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16.60±0.90&lt;sup&gt;f&lt;/sup&gt;</td>
<td>103.87±1.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>81.12±0.90&lt;sup&gt;d&lt;/sup&gt;</td>
<td>314.83±2.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.15±0.49&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>20% banana</td>
<td></td>
<td>1.79±0.19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.65±1.12&lt;sup&gt;h&lt;/sup&gt;</td>
<td>104.12±1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.88±0.61&lt;sup&gt;e&lt;/sup&gt;</td>
<td>324.92±2.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.43±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% apple</td>
<td></td>
<td>1.59±0.16&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.41±1.67&lt;sup&gt;h&lt;/sup&gt;</td>
<td>106.25±1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.93±0.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>340.55±2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.00±0.73&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>20% apple</td>
<td></td>
<td>1.20±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.78±0.66&lt;sup&gt;f&lt;/sup&gt;</td>
<td>108.25±1070&lt;sup&gt;e&lt;/sup&gt;</td>
<td>86.51±0.67&lt;sup&gt;i&lt;/sup&gt;</td>
<td>355.88±2.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.55±1.00&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td>6.03±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.18±0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.25±1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.26±1017&lt;sup&gt;g&lt;/sup&gt;</td>
<td>162.39±3.53&lt;sup&gt;i&lt;/sup&gt;</td>
<td>50.70±1.14&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer+10% banana</td>
<td></td>
<td>4.80±0.40&lt;sup&gt;e&lt;/sup&gt;</td>
<td>30.27±0.61&lt;sup&gt;e&lt;/sup&gt;</td>
<td>64.75±2.50&lt;sup&gt;e&lt;/sup&gt;</td>
<td>55.43±0.62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>194.93±4.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.15±0.73&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer+20% banana</td>
<td></td>
<td>4.19±0.64&lt;sup&gt;e&lt;/sup&gt;</td>
<td>26.21±0.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.37±1.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>58.20±0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210.85±16.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38.64±0.74&lt;sup&gt;ci&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer+10% apple</td>
<td></td>
<td>3.81±0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.93±0.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>77.87±1.31&lt;sup&gt;f&lt;/sup&gt;</td>
<td>60.73±0.40&lt;sup&gt;f&lt;/sup&gt;</td>
<td>42.57±1.76&lt;sup&gt;g&lt;/sup&gt;</td>
<td>32.27±3.61&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer+20% apple</td>
<td></td>
<td>3.16±0.12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19.78±0.76&lt;sup&gt;e&lt;/sup&gt;</td>
<td>82.62±1.79&lt;sup&gt;e&lt;/sup&gt;</td>
<td>66.54±0.80&lt;sup&gt;f&lt;/sup&gt;</td>
<td>281.90±5.41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>27.18±0.71&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>L.S.D (p&lt;0.05)</td>
<td></td>
<td>0.37</td>
<td>0.95</td>
<td>1.76</td>
<td>0.79</td>
<td>5.83</td>
<td>1.32</td>
</tr>
</tbody>
</table>

3.3 Effect of banana and apple fruits administration on Hb level, RBCs, PLT, WBCs, NEUT and LYM counts in control and ulcerated control rats 
Table (3) showed the hematological evaluation for RBCS, WBCS, PLTs, and RBCS counts as well as Hb level. There were reduction in RBCS and PLTs counts as well as Hb level and raise in WBCS count in aspirin ulcerated control rats compared to healthy control rats, while the groups that were treated with banana and apple showed improvement in these values.

**Table 3:** The effect of administration of banana and apple fruits on Hb level, RB’s, PLT, WBCs, NEUT and LYM counts in control and aspirin ulcerated control rats  
- Values are expressed as means ±S.D, n=10.
- There was no significant difference between means have the same alphabetical superscripts in the same column.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Hemoglobin (g/dL)</th>
<th>RBCs (10³/µL)</th>
<th>Platelets (10³/µL)</th>
<th>WBCs (10³/µL)</th>
<th>NEUT (10³/µL)</th>
<th>LYM (10³/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>15.13±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.35±0.41&lt;sup&gt;e&lt;/sup&gt;</td>
<td>553.50±23.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.61±0.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.76±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% banana</td>
<td></td>
<td>15.15±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.65±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>553.25±28.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.54±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.60±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.26±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20% banana</td>
<td></td>
<td>15.27±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12±0.45&lt;sup&gt;f&lt;/sup&gt;</td>
<td>572.00±80.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.41±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.17±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.92±0.66&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>10% apple</td>
<td></td>
<td>15.20±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.90±0.55&lt;sup&gt;e&lt;/sup&gt;</td>
<td>563.50±15.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.45±0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.58±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.08±0.59&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20% apple</td>
<td></td>
<td>15.29±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.07±1.09&lt;sup&gt;e&lt;/sup&gt;</td>
<td>582.50±40.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.20±0.38&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.01±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.91±0.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td>8.60±0.60&lt;sup)b&lt;/sup&gt;</td>
<td>3.35±0.31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>397.00±28.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.90±0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.20±0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.70±0.91&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>L.S.D (p&lt;0.05)</td>
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<td>0.59</td>
<td>0.78</td>
<td>32.11</td>
<td>0.87</td>
<td>0.86</td>
<td>0.66</td>
</tr>
</tbody>
</table>

3.4 Effect of banana and apple fruits administration on ulcer score, ulcer index, and percent of ulceration (%) in ulcerated rats 
In table (4), stomach sections of aspirin ulcerate rats showed increased the values of ulcer index, percent of ulceration and ulcer score in comparison with healthy control rats while the treatment with banana and apple showed fewer degeneration and...
stomach appear more or less like control as well as reduced the value of ulcer index, percent of ulceration and ulcer score.

Table 4: The effect of administration of banana and apple fruits on ulcer score, ulcer index, and percent of ulceration (%) in aspirin ulcerated rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>ulcer score</th>
<th>ulcer index</th>
<th>percent of ulceration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer</td>
<td>9.5</td>
<td>950</td>
<td>100</td>
</tr>
<tr>
<td>Ulcer+10% banana</td>
<td>8</td>
<td>800</td>
<td>85.7</td>
</tr>
<tr>
<td>Ulcer+20% banana</td>
<td>7.5</td>
<td>750</td>
<td>71.4</td>
</tr>
<tr>
<td>Ulcer+10% apple</td>
<td>6</td>
<td>600</td>
<td>43</td>
</tr>
<tr>
<td>Ulcer+20% apple</td>
<td>4.5</td>
<td>450</td>
<td>14.28</td>
</tr>
<tr>
<td>L.S.D (p≤0.05)</td>
<td>0.43</td>
<td>46.38</td>
<td>14.00</td>
</tr>
</tbody>
</table>

* Values are expressed as means ±S.D, n =10.
* There was no significant difference between means have the same alphabetical superscripts in the same column.

3.5 Effect of banana and apple fruits administration on microscopical examination of the stomach

The results of microscopical examination of stomach illustrated in figures (1-15) showed the degenerative changes caused by aspirin and the curative role of green banana and red apple on ulcerated gastric mucosa.

Figure 1: Photomicrograph of a section in the rat stomach of healthy control rats showing the mucosa which consists of tubular straight gastric glands, extend to the level of muscularis mucosa. (H. &E. x200)

Figure 2: Photomicrograph of a section in the rat stomach of healthy control rats showing gastric glands with acidophilic parietal cells are distributed along the length of the glands and basophilic peptic cells (P) mainly at the base of the glands. Note minimal lamina propria in-between the glands. (H. & E. x400)

Figure 3: Photomicrograph of a section in the rat stomach of ulcerated control rats showing multiple swollen cells with vacuolated cytoplasm of the gastric glands, some ballooned cells with vacuolated cytoplasm and the loss of the architecture of some glandular cells, degradation of the superficial cells with decreased thickness of the mucosa and dilatation of the blood vessels of the lamina propria and submucosa, superficial ulceration of the gastric mucosa, ulceration includes all thickness of the gastric mucosa as well as wide separation of gastric glands in the lamina propria as shown in figures (3-10).

Figure 4: Photomicrograph of a section in the rat stomach of ulcerated control rats showing multiple dark nuclei in the cells of the gastric glands. (H. & E. x400)

Figure 5: Photomicrograph of a section in the rat stomach of ulcerated control rats showing some ballooned cells with vacuolated cytoplasm. Note loss of the architecture of some glandular cells (A). (H. & E. x400)
Ulcerated rats treated with banana revealed a significant improvement in microscopical examination of the stomach as shown in figures (11-13) a decreased thickness of gastric glands with superficial loss of cells. Also, some glands appeared with loss of architecture, some swollen cells with vacuolated cytoplasm of the gastric glands with moderate dilatation of blood vessels only in 10% banana treated ulcerated groups. While 20 % banana treatment caused restore thickness of the gastric mucosa with moderate dilatation of the blood vessels in comparison with ulcerated control group.
Figure 11: Photomicrograph of a section in the rat stomach of 10% banana treated ulcerated rats showing decreased thickness of gastric glands with superficial loss of cells. Also, some glands appeared with loss of architecture (H. & E. x200).

Figure 12: Photomicrograph of a section in the rat stomach of 10% banana treated ulcerated rats showing swollen cells with vacuolated cytoplasm of the gastric glands. Note moderate dilatation of blood vessels (V) (H. & E. x400).

Figure 13: Photomicrograph of a section in the rat stomach of 20% banana treated ulcerated rats showing restore thickness of the gastric mucosa with moderate dilatation of the blood vessels (V). (H. & E. x200)

On the other hand ulcerated rats treated with apple at doseses (10% and 20%) caused a significant improvement in microscopical examination of stomach as shown in figures (14 and 15) the gastric mucosa restored thickness of the gastric mucosa with localized loss of cells with dilatation of the blood vessels in 10% apple treated ulcerated group, while, 20% apple treatment caused restored thickness of the gastric mucosa with minimal dilatation of the blood vessels in comparison with ulcerated control group.

Figure 14: Photomicrograph of a section in the rat stomach of 10% apple treated ulcerated rats showing restore thickness of the gastric mucosa with localized loss of cells (L). Note dilatation of the blood vessels (V). (H. & E. x200)

Figure 15: Photomicrograph of a section in the rat stomach of 20% apple treated ulcerated rats showing restore thickness of the gastric mucosa with minimal dilatation of the blood vessels (V). (H. & E. x200)

Our results demonstrated that in ulcerated rats which fed on 20% apple supplemented diet caused the most improvement in the microscopical examination of the stomach, followed by 20% banana treated ulcerated group, followed by 10% apple treatment and finally 10% banana treated ulcerated group.

4. Discussion

Oxidative stress is caused by an imbalance between the production of ROS or oxygen free radicals (OFRs) and a biological system’s ability to readily detoxify the reactive intermediates or easily repair the resulting damage leading to important health implications (Rainjbar et al., 2005). Aspirin is a commonly used non-steroidal anti-inflammatory drug (NSAIDs) and a potent cyclooxygenase
inhibitor which increases acid secretion and produce microvasculature damage by generation of free radicals (Sen et al., 2009). Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and block diffusion of H+ (Mabrouk et al., 2009). Aspirin blockade of cyclooxygenase-1 (COX-1) and (COX-II) results in reduction of prostaglandin synthesis. The interruption of prostaglandin synthesis results in impairment of mucosal damage repair, thus facilitating mucosal injury (Mabrouk et al., 2009).

The antiulcer effects of banana may be due to its high content of 5-hydroxytryptamine (5HT) that would produce peristaltic reflexes inhibiting gastric secretion (Orhan, 2001). In addition to phenolics, pectin in apple fruit may function as an antiulcerative factor. (Nergard et al., 2005). In toxicological studies, change in body weight is an important criterion for evaluation of toxicity. It is considered as one of the most sensitive indicators of the animal’s condition (Gad and Weil, 1994). Results of this study showed that, rats consumed experimental diets with apples and banana have got less body weight gain than control groups. These results were in agreement with (De Oliveira et al., 2003 and Sembries et al., 2004).

De Oliveira et al., (2003) reported the hypothesis of weight loss with increased intake of fruits which is based on three premises: the low-energy density of most fruits, their higher fiber composition, and a less striking variation of diets high in fruit. Also, Sembries et al., (2004) reported that apple dietary fiber in experimental diets led to lower weight gain. Consequently, the net body weight gain of the animals intoxicated with aspirin was less as compared to the normal controls, suggesting that, the poor body weight gain may be attributed to the overall increased degeneration of lipids and proteins as a result of the direct effect of the aspirin intake. While the intake of green banana and red apple caused a normal decrease in body weight as a result of their fiber content resulting in fullness feeling causing decreased feed intake. There is substantial evidence to support the claim that ROS are involved in gastric injury caused by aspirin exposure. OFRs play a key role in the mechanism of aspirin induced acute gastric mucosal Lesions (McCord, 2000).

Results of the current study showed significant increase in NO level in aspirin administered rats when compared to healthy controls which are similar to the results of (Odabasoglu et al., 2006 and Wang et al., 2011) who attributed this to neutrophil infiltration into gastric mucosal tissues that have led to increase and over expression in inducible NO synthase activity (iNOS).

Flavonoids has anti-inflammatory activity leading to decreased neutrophil infiltration into gastric mucosal tissues also have antioxidative properties that scavenge OFRs so banana and apple can reduce the NO level and protect the gastric mucosa from its detrimental effect.

Results of the present study showed significant increase in alkaline phosphatase (ALP) activity in aspirin ulcerated rats when compared to healthy controls, these results were found to be in accordance with (Naryan et al., 2004 and Ologundudu et al., 2008).

Naryan et al. (2004) attributed this to the damage of tissues. The release of this enzyme has been suggested to have a role in tissue necrosis associated with various models of gastrointestinal ulceration. Similarly, Ologundudu et al., A (2008) attributed this to tissue necrosis and damage associated with tissue ulceration.

Meanwhile, our results showed a significant decrease in ALP among rats given diet supplemented with banana as well as apple; this was in agreement with Juskiewicz et al. (2002) who connected this with metabolism of flavonoids absorbed from the digestive tract, and due to green banana and red apple content of different flavonoids. Their intake leads to decreased inflammation and correction of ALP (Abeysekera et al., 1999).

In this study, CAT and SOD activities as well as GSH levels were significantly decreased in aspirin ulcerated rats. These results were found to be in
Jainu and Devi, (2004) reported that there were a significant decrease in SOD, CAT and GSH in aspirin induced rats when compared with those of control rats as a result of oxidative stress associated with aspirin ulceration. Meanwhile, Ologundudu et al. (2008) stated that there was a significant decrease in the activity of the antioxidant enzyme, CAT in the aspirin treated rats in comparison with normal control rats due to oxidative stress associated with aspirin ulceration. Angelo et al. (2010) confirmed that, a significant decrease of gastric GSH level in aspirin ulcerated rats as a result of tissue oxidative stress. Also, Thamotharan et al. (2010) stated that, aspirin produced depletion of CAT and SOD, also decreased the level of GSH which is a non enzymatic antioxidant due to consumption of enzymatic antioxidant and non enzymatic antioxidant in fighting oxidants formed after aspirin ulceration. Oxygen handling cells have antioxidant enzymes such as CAT, SOD, and GSH which are the first line of cellular defense against oxidative injury (Millan et al., 1998).

Flavonoids and antioxidative properties of banana and apple are potent antioxidants and are known to modulate the activities of different enzymes due to their interactions with various biomolecules. The antioxidant properties of flavonoids are due to their ability to directly scavenge some radical species. Catechin has iron chelating and antioxidant properties. Quercetin also has a cytoprotective role which may be due to its ability to interact with and penetrate the lipid bilayer. Antioxidants have been shown to inhibit free radical formation (Durak et al., 2010).

Results of the current study showed significant decrease in RBC’s count and Hb levels in aspirin ulcerated rats after ulceration, on the other hand there was significant increase in RBC’s count and Hb levels among groups treated with green banana and red apple these results were found to be in accordance with many studies. Izzettin et al., (2010) who reported that, Post-treatment with NSAIDs Hb level was lower than those measured before the treatment; this could be due to the NSAID-induced blood loss in the animals. Also, Wang et al., (2011) attributed this to hemorrhagic gastric ulcers covered with coagulated blood in the aspirin administered group. Meanwhile, Pari et al., (1999) on their study on banana, oral administration of extract of the banana flowers resulted in a significant increase in Hb level.

Kanter et al., (2009) reported that, RBC membrane is rich in poly unsaturated fatty acids which are very susceptible to free radical mediated peroxidation which cause hemolysis.

Inflammation and neutrophil infiltration are also important in the pathogenesis of the gastric damage induced by NSAIDs (Souza et al., 2004 and Odashima et al., 2006). Flavonoids have been reported to act in the gastrointestinal tract as antiulcer, antispasmodic or antisecretory (Rao et al., 2003). In the present study, Aspirin caused decrease in RBC and, Hb which is due to bleeding associated with ulcer formation process. PLT also affected by ulceration process as a result of clot formation while leucocytosis observed indicates the response to inflammation as well as secondary infection. This study demonstrated that aspirin treatment to animals resulted in significant abrogation of the hematological indices while the treatment with banana and apple normalized these indices and has protective effects which may be due to the antioxidants properties of flavonoids they contain.

Results of this study showed that, aspirin caused increased gastric acid production and secretion which is reflected in the significant increase in the value of total titrable acidity (Meq/L) in aspirin ulcerated rats in comparison with healthy control rats, meanwhile there was a significant reduction in banana and apple treated groups. Our results agree with the results of (Angelo et al., 2010 ; Thamotharan et al., 2010; Sivaraman and Muralidharan, 2010 and Mradul et al., 2011) they attributed it to decreased prostaglandin level following aspirin administration leading to increased acid production.

Antioxidants have been shown to inhibit free radical formation (Durak et al., 2010). The antioxidant constituents in banana and apple scavenge radical species leading to decreased total acidity output in ulcer treated groups in comparison with ulcer control group.

Lewis et al. (1999) stated that bananas have long been recognized for their antacid effects that protect against stomach ulcers and ulcer damage. A flavonoid in the banana, leucocyanidin has been found to significantly increase the thickness of the mucous membrane layer of the stomach.

Results of this study showed that aspirin caused a significant decrease in mucosal thickness and relative weight of the stomach while the treatment with banana and apple showed a significant increase in mucosal thickness and relative weight of stomach to reach the levels of healthy rats. These results were found to be in accordance with many studies. Nair et al.(2006); Fesharaki et al.(2006) and Angelo et al.(2010) they reported
that, aspirin reduces endogenous prostaglandin biosynthesis as a result of cyclooxygenase inhibition resulting in decreased mucin level and reduced protein concentration as a result of accumulation of toxic free radicals in the mucosal cells resulting in decreased both mucosal thickness and relative weight of stomach.

Meanwhile, Best et al. (1984) suggested that, banana and apple provided protection against the action of aspirin by increase in protein and mucin content of the gastric mucosal tissue and also due to banana’s ability to stimulate growth of gastric mucosa which is responsible for the rapid healing of ulcers in rats treated with banana after aspirin administration. Also, Hamauzu et al. (2007) stated that, Procyanidins oligomers phenolic in apple act as a protective coating of mucosal tissue having a radical scavenging activity. Current study displayed that stomach sections of aspirin ulcerated rats showed increased the value of ulcer index, percent of ulceration and ulcer score in comparison with healthy controls while the treatment with banana and apple showed fewer degeneration and stomach appear more or less like control as well as reduced the value of ulcer index, percent of ulceration and ulcer score.

Our results were supported by Jainu and Devi, (2004); Burke et al. (2006); Ologundudu et al. (2008); Mabrouk et al. (2009) and Thamotharan et al. (2004); Mabrouk et al. (2009) and Thamotharan et al. (2008); Mabrouk et al. (2009) and Thamotharan et al. (2008) they attributing this to decreased prostaglandin synthesis by inhibition of cyclooxygenase enzyme resulting in stomach tissue damage and apoptosis. Also, sen et al. (2009) explained this due to increased acid secretion and production of microvasculature damage by generation of free radicals. Supporting this, others explained it due to direct and systemic effects causing imbalance between the aggressive and the defensive factors leading to failure in gastro protective and repair mechanisms ending in disrupted mucosal barrier and mucosal lesions (Sivaraman and Muralidharan,2010 and Mradul et al., 2011) . On the other hand, the antioxidant and anti-ulcerative action of phenolics in apple might be due to the high concentration of procyanidins and their binding ability to the surface mucosa, which is thought to produce a protective mucosal coating and reduced lesion formation (Saito et al., 1998 and Nergard et al., 2005).

Also, pectin in apple fruit may function as an anti-ulcerative factor (Yamada, 1994 and Galati et al., 2002). Also, Pectin, phosphatidylycholine, and polyphenolic flavonoid (leucocyanidin) from green banana (Dunji et al., 1993) may stimulate growth of gastric mucosa and protect it and decrease gastric secreations (Best et al., 1984) that leads to reduced gastric lesion.

Results of this study showed that aspirin caused an observed change on the mucosal thickness of the stomach as a result of ulceration and perforation of the gastric mucosa of ulcerated rats while green banana and red apple caused a significant improvement in ulcerated tissues with increased mucosal thickness and decreased the level of ulceration. Our results agree with Wang et al. (2011) who reported that, the macroscopic findings of the opened stomach showed hemorrhagic gastric ulcers covered with coagulated blood that were more apparent in the aspirin administered group than control group. Apple and banana antioxidants play a role in preventing oxidative stress and play a curative role on gastric ulcer tissues. Procyanidins oligomers phenolic in apple demonstrate greater ability to bind to the surface of mucosal tissue and act as a protective coating having a radical scavenging activity (Hamauzu et al., 2007).

Conclusion: Both green banana and red apple at the tested doses (10% and 20% of normal diet) had antiulcerative and anti-inflammatory effects which were probably mediated by strong antioxidants (leucocyanidin in green banana and quercetin, catechin, phloridzin and chlorogenic acid in red apple).

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