Clinicopathological Studies on Neem and Ginger Effects as Feed Additives in Normal and
E. coli Infected Weaned Rabbits

Nariman M. M. Edrees,1 Ibtisam M. Gamal El Dien,2 Salwa A. M. Eid2*
1Clinical Pathology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt
2Animal Health Research Institute, Zagazig, Egypt

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Abstract

The present study was performed to investigate the clinicopathological effects of neem and ginger as feed additives. One hundred and twenty weaned White New Zealand rabbits were divided into 6 equal groups. Group (1) kept as control. Groups (2 and 5) received ration contained neem leaves daily (5% of diet). Groups (3 and 6) received ration contained ginger powder daily (2% of diet). Groups (4, 5 and 6) were experimentally infected by E. coli (O103 once orally with a dose of 3 ml of suspension containing 3x10⁷ CFU at the end of the 4th week of experiment. The results revealed normal parameters in none infected as well as neem and ginger treated groups (1, 2 and 3). However a significant decrease in the serum total protein, albumin, globulin and catalase levels (CAT) on the 1st, 3rd and 15th day PI was observed in infected non treated animals (Group 4). On the other hand a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde level (MDA), phagocytic percentage and phagocytic index were observed in the same group. In Groups 5 and 6; animals showed a significant increase in total protein, albumin, globulin, CAT and a significant decrease in the other parameters comparing with the infected group. It could be concluded that both neem and ginger can be used as feed additives in rabbit ration to enhance hepatic, renal and antioxidant activities beside cell mediated immunity .Moreover, Neem was better than ginger in amelioration of the harmful effects of E. coli infection.

Keywords: Antibacterial, Immunostimulant, Neem, Ginger, Antioxidant

Introduction

There is a worldwide trend towards the use of natural additives in food as spices and herbal medicine Neem and ginger are important members of herbal medicine [1,2]. Both of them have compounds of many biological activities as hepatoprotective and renoprotective effects in addition to anti-oxidant and immunostimulant properties [2,3].

The effect of dietary neem leaves meal on serum biochemical parameters was investigated by Obikaonu et al. [4]. The AST, ALT and ALP levels were decreasing by increasing neem leaves percent in diet comparing with the control. Mukherjee et al. [5] found that the administration of oral mixture of leaves and seed extract at 10% concentration for 6 weeks in rodent caused specific activation of T-lymphocytes and phagocytic cells. Biswas et al. [1] found an increase in catalase activity (CAT) while malondialdehyde level (MDA) was decreased. Lebda et al. [6] studied the effect of different ginger treatments on hepatic oxidative stressed male New Zealand rabbits. The results revealed that AST, ALT, ALP and urea were decreased with increased creatinine level comparing with control. Onu and Aja [7] recorded a significantly decreased MDA level with increased CAT activity using ginger (0.25% of diet) on weaned rabbits as supplement for 10 weeks. Oral ginger administration as feed additives stimulated phagocytic activity [8].

Escherichia coli is one of the most important etiological agents of enteritis and losses in rabbit industry [9] The most pathogenic
serotypes inducing high mortalities are (O109:H2), with neonatal diarrhea, (O103:H2) weanling diarrhea, O123 and O132 [10, 11].

This work aimed to study hepatic and renal protective effects as well as antioxidants and immunological effects of neem leaves and ginger rhizomes on normal and *E. coli* experimentally infected rabbits.

**Material and Methods**

**Experimental Design**

One hundred and twenty weaned white New Zealand rabbits apparently healthy were obtained from a private farm near Menia Elkameh, Sharkia Governorate. The basal and balanced growing ration was given to animals. The rabbits were divided into 6 equal groups. Group 1 was kept as control. Groups (2 and 5) received neem leaves powder as 5% of diet [12]. Groups (3 and 6) received ginger powder 2% of diet daily for 6 weeks [6]. Groups (4, 5 and 6) were experimentally infected rabbits by *E. coli* with of O103 strain once orally with a dose of 3 ml of suspension containing $3 \times 10^7$ CFU [13] at the end of the 4th week of experiment. The animals were observed for clinical examination daily along the experiment.

**Feed additives**

Neem, fresh matured neem leaves were harvested from garden in El-Asher Men Ramadan City. The samples were identified in Botany Department, Faculty of Science, Zagazig University. The leaves were cleaned, dried in shaded place and powdered. The powder was added to ration as 5% of diet [12].

Ginger rhizomes were collected from local markets as dry rhizomes then were ground well till became fine powder and added to ration as 2% of diet [6].

Pathogenic *E. coli* O103 was obtained from Animal Health Research Institute, Dokki, Giza, Egypt and was used for infection.

**Blood samples**

Blood samples were collected from ear vein on the 1st, 3rd and 15th day PI. The first sample was collected on heparin for phagocytic activity determination [13] and the second one was collected for serum biochemical analysis AST, ALT [14] ALP [15] total protein [16] albumin [17] globulin was calculated, urea [18] and creatinine [19].

**Antioxidant activities**

Catalase activities and MDA levels were calorimetrically determined using Kits of Bio Diagnostics [20].

**Phagocytic activity**

The Phagocytic percent and index were evaluated according to Wilkinson [21]. The phagocytic percent is the total number of phagocytic cells at any stage of phagocytosis in 100 mononuclear phagocytic cells. Phagocytic index (PI) is the number of *C. Albicans* ingested by 100 phagocytic cells.

**Statistical analysis**

The obtained data were statistically analyzed by F-test [22] using MSTAT-C computer program.

**Results and Discussion**

Neem and ginger are important members of herbal medicine [1, 2]. Both of them have biological activities as hepato and renal protective in addition to anti-oxidant and immunostimulant properties [2, 3].

The use of neem or ginger as feed additives in rabbit ration with a percent of 5% and 2% respectively were investigated for their beneficial impact on rabbit biochemical and immunological properties as well as their antibacterial efficacy.

Our results revealed that Groups (2 and 3) treated non infected animals showed no significant changes in AST, ALT and ALP hepatic enzymes all over the experimental periods compared to control group (Tables 1, 2). This may be due to that neem leaves meal or ginger are safe and has no deleterious effect on the liver and these results confirmed histopathologically where there is no pathological lesions in the hepatic tissues. These results are in agreement with Haque *et al.* [23] and Ogbuewu *et al.* [12] who stated that AST and ALT were non significantly changed in neem and ginger treated groups comparing with the control.
Table (1): Effect of Neem and Ginger on hepatic enzymes and proteinogram in infected and normal rabbits through 15 days PI (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hepatic enzymes</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Proteinogram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>AST (U/L)</td>
<td>ALT (U/L)</td>
<td>ALP (U/L)</td>
<td>Total protein (g/dL)</td>
<td>Albumin (g/dL)</td>
<td>Globulins (g/dL)</td>
</tr>
<tr>
<td>Group</td>
<td>1st day</td>
<td>3rd day</td>
<td>15th day</td>
<td>1st day</td>
<td>3rd day</td>
<td>15th day</td>
<td>1st day</td>
</tr>
<tr>
<td>GP (1)</td>
<td>Control</td>
<td>40.20</td>
<td>40.40</td>
<td>41.00</td>
<td>40.60</td>
<td>42.00</td>
<td>44.00</td>
</tr>
<tr>
<td>GP (2) Neem</td>
<td>39.60</td>
<td>39.40</td>
<td>38.00</td>
<td>41.60</td>
<td>41.00</td>
<td>40.00</td>
<td>33.20</td>
</tr>
<tr>
<td>GP (3)</td>
<td>40.40</td>
<td>39.20</td>
<td>39.40</td>
<td>38.20</td>
<td>40.40</td>
<td>41.20</td>
<td>35.40</td>
</tr>
<tr>
<td>GP (4) E. coli</td>
<td>67.40</td>
<td>90.60</td>
<td>60.60</td>
<td>58.60</td>
<td>80.00</td>
<td>60.20</td>
<td>57.20</td>
</tr>
<tr>
<td>GP (5) Neem</td>
<td>50.60</td>
<td>68.20</td>
<td>44.20</td>
<td>49.00</td>
<td>64.40</td>
<td>48.50</td>
<td>48.20</td>
</tr>
<tr>
<td>+ E. coli</td>
<td>55.60</td>
<td>74.80</td>
<td>50.20</td>
<td>45.80</td>
<td>60.20</td>
<td>50.10</td>
<td>52.4</td>
</tr>
<tr>
<td>F-test</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<td>*</td>
</tr>
</tbody>
</table>

N.S.: non significant  *: Significant at 0.05 probability  PI: post infection

However, The increase in serum ALT, AST and ALP activities was observed in group (4) which may be attributed to the damaging effect of E. coli (lipopolysaccharide) on the liver and increased cell membrane permeability [24-25]. This confirmed pathologically (Figure 1A and B) which showed severe congestion of the hepatic blood vessels, hydropic degeneration and coagulative necrosis of the hepatocytes with pyknotic and karyolytic nuclei. The significant decrease in the hepatic enzymes in groups (5 and 6) comparing with group (4) may be due to administration of neem and ginger which contain hepatoprotective and antibacterial active principals [26,27]. This was confirmed pathologically (Figure 1C) and was manifested by as reduced degenerative changes in hepatocytes. Ginger has hepatoprotective effect and repair the damaged hepatic tissue [28,29]. Decreasing in proteinogram in group (4) may be due to E coli effects on liver and kidney [9]. Serum total protein, albumin and globulin levels were increased in groups (5
and 6) on the (3rd and 15th) day post infection comparing with infected group [29,30].

A significant increase in urea and creatinine (Table 2) was found in group (4). *E. coli* infection (lipopolysaccharide) caused damage in renal tissue [9,25,31]. This was confirmed pathologically (Figure 1D) with multiple cystic dilatations of renal tubules with hyaline and cellular casts. Renal protective and antibacterial effects of neem and ginger ameliorated the elevation in urea and creatinine levels in groups 5 and 6. Similar results were obtained previously by lebdet al. [6] and Ezz-Din et al. [32]. Also this may be due to polyphenols and flavonoids present in ginger that remove waste products from plasma [6].

In respect to antioxidant activity, Groups (2 and 3) showed a significant increase in CAT and a significant decrease in MDA on the 1st, 3rd and 15th day PI (Table 3). It could be explained by the antioxidant effects of neem that may increase the synthesis of antioxidant molecules as mentioned previously by Dkhill et al. [33]. Also, this may be due to ginger contains antioxidants that act as a free radical scavenger. A significant decrease in CAT and increase MDA was recorded in group (4) which may be attributed to lipopolysaccharide [34,35]. Groups (5 and 6) showed a significant increase in CAT and decrease in MDA level in comparison to infected group. This may be attributed to scavenging effects of neem [33, 36] and ginger [37] among free radicals and so relieving oxidative stress of *E. coli* infection.

Groups (2, 3, 5 and 6) showed a significant increase in phagocytic percent and index all over the experimental period (Table 4). This may be due to neem increase nonspecific immune response [36,38]. Ginger stimulated phagocytic activity as described by Ajith et al., and Mallikarjuna et al. [39,40]. Phagocytic percent and index were significantly increased in group (4). This may be referred to immune response against *E. coli* as explained by Eisa and Alam [41, 42]. Leukocytosis, neutrophilia and monocytosis were found in groups 4, 5, and 6 on 1st day PI. Neutrophilia and monocytosis were still present on 3rd day. Lymphopenia was found in the same groups on the 1st and 3rd day PI. These changes may be due to the acute defense mechanism against bacterial infection as recorded by Edrees et al., and El-Boushy et al. [9,25]. A significant decrease in TLC was found in group 4 on the 3rd and 15th day PI. The changes in leukogram may be due to immunodepressant effect of *E. coli* infection as stated by many authors [31,41,43,44].

**Conclusion**

It could be concluded that neem (5% of diet) and ginger (2% of diet) enhanced hepatic, renal and antioxidant activities of rabbits. Also, they acted as immune stimulant and ameliorated the harmful effects of *E. coli* infection. Moreover, neem effects were stronger than ginger effects.
Table (2): Effect of Neem and Ginger on Serum urea and creatinine in infected and normal rabbits through 15 days PI (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum urea (mg/dL)</th>
<th>Serum creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day PI</td>
<td>3rd day PI</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP (1) Control</td>
<td>17.30 ± 0.104 b</td>
<td>17.17 ± 0.100 d</td>
</tr>
<tr>
<td>GP (2) Neem</td>
<td>17.16 ± 0.094 b</td>
<td>17.68 ± 0.554 d</td>
</tr>
<tr>
<td>GP (3) Ginger</td>
<td>17.36 ± 0.066 b</td>
<td>17.30 ± 0.074 d</td>
</tr>
<tr>
<td>GP (4) E. coli</td>
<td>19.0 ± 0.447 a</td>
<td>26.80 ± 0.622 a</td>
</tr>
<tr>
<td>GP (5) Neem + E. coli</td>
<td>17.39 ± 0.191 b</td>
<td></td>
</tr>
<tr>
<td>GP (6) Ginger + E. coli</td>
<td>17.88 ± 0.333 b</td>
<td></td>
</tr>
<tr>
<td>F-test</td>
<td>*</td>
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</tr>
</tbody>
</table>

*=significant at 0.05 probability.  PI: post infection

Table (3): Effect of Neem and Ginger on antioxidant activities of liver tissues of infected and normal rabbits Through 15 days PI (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Catalase (Mu/mg)</th>
<th>MDA (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day PI</td>
<td>3rd day PI</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP (1) Control</td>
<td>1.36 ± 0.058 b</td>
<td>1.36 ± 0.066 b</td>
</tr>
<tr>
<td>GP (2) Neem</td>
<td>1.53 ± 0.056 a</td>
<td>1.60 ± 0.046 a</td>
</tr>
<tr>
<td>GP (3) Ginger</td>
<td>1.49 ± 0.032 a</td>
<td>1.50 ± 0.023 a</td>
</tr>
<tr>
<td>GP (4) E. coli</td>
<td>0.78 ± 0.048 a</td>
<td>0.71 ± 0.031 a</td>
</tr>
<tr>
<td>GP (5) Neem + E. coli</td>
<td>1.13 ± 0.050 c</td>
<td></td>
</tr>
<tr>
<td>GP (6) Ginger + E. coli</td>
<td>1.10 ± 0.007 c</td>
<td></td>
</tr>
<tr>
<td>F-test</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*=significant at 0.05 probability.  PI: post infection
Table (4): Effect of Neem and Ginger on phagocytic % and phagocytic index in infected and normal rabbits through 15 days PI (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day PI</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day PI</th>
<th>15&lt;sup&gt;th&lt;/sup&gt; day PI</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day PI</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day PI</th>
<th>15&lt;sup&gt;th&lt;/sup&gt; day PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) Control</td>
<td>63.04 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.12 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.18 ± 0.177&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.38± 0.037&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46± 0.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46± 0.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Group (2) Neem</td>
<td>70.44± 0.15&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>70.26± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.24± 0.172&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.06± 0.092&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.09± 0.060&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.55± 0.063&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group (3) Ginger</td>
<td>65.92± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.94± 0.21&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>71.18± .139&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56± 0.050&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.66± 0.092&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.74± 0.067&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group (4) E. coli</td>
<td>80.60± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.34± 0.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>62.70± 0.234&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.62± 0.073&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82± 0.058&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02± 0.058&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group (5) N + E. coli</td>
<td>86.66± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.10± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.10± 0.234&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.34± 0.050&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.58± 0.037&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.69± 0.124&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group (6) G + E. coli</td>
<td>83.64± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.44±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.14± 0.163&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.58± 0.037&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.69± 0.124&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

C: Control, N: Neem, G: Ginger

Figure 1: A) Liver (Group 4), 3 days PI showing severe congestion of the hepatic blood vessels (H&E x130); B) Liver (Group 4), 3 days PI showing severe hydropic degeneration and coagulative necrosis of the hepatocytes with pyknotic and karyolytic nuclei (H&E x520); C) Liver (Group 5), on the 3<sup>rd</sup> day PI, showing congestion of the hepatic blood vessels together with mononuclear leukocytic cells infiltration (H&E x520); D) Kidney (Group 4), 3 days PI showing many cystic dilatations of some renal tubules with hyaline and cellular casts inside it (H&E x520); E) Kidney (Group 5), 3 days PI showing mild congestion of renal blood vessels in the renal cortex together with mononuclear leukocytic cell infiltration (H&E x300); F) Kidney (Group 6), 3 days PI showing mononuclear leukocytic cells infiltrating the renal cortex (H&E x 520).

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgment

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References


