Studies on the effect of garlic preparation on *E. coli* O157:H7 causing enteritis in lambs.

By
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**SUMMARY**

In this study, a total of 20 faecal samples as well as tissue specimens of the internal organs of 16 dead lambs were suffered from haemorrhagic diarrhea were collected. These samples were subjected to bacteriological examination. *E. coli* O157:H7 isolated in incidence of 60% from faecal samples of diarrheic lambs and from dead lambs in an incidence of 16.5%, 56.25%, 75%, 93.75%, 87.5%, 93.75% and 16.5% from brain, lung, liver, kidneys, spleen, intestine and testis; respectively. The histopathological examination revealed that *E. coli* O157:H7 had a drastic severe pathological alteration (systemic haemorrhagic syndrome) represented by neuropathy, haemorrhagic pneumonia, haemorrhage in liver and necrosis of hepatocytes, hemolytic-uremic syndrome, haemorrhagic colitis as well as necrosis of spermatogenesis series cells which may lead to sterility. In vitro, garlic powder induced inhibitor results on the growth of *E. coli* O157:H7 with MIC value 10000 mg/liter. In order to study the effect of treatment or prophylactive role of garlic powder in vivo, Fifty male Wister rats of 10-12 weeks old and weighing approximately 100 gm, the rats were divided into 5 equal groups (infected-non treated [I], infected-treated [II], prophylactic-infected [III], treated-non infected [IV] and Control [V]). The mortality rate were 70% in group (I) as well as (0%) on rats of other groups. The results revealed that the highest antibody response measured by ELISA was in group (II) and group (III). Re-isolation of *E. coli* O157:H7 from different groups of dead rats allover the experimental period and sacrificed rats at the end of the experiment were 10%, 40%, 70%, 80%, 100% and 20% from brain, lung, liver, kidneys, spleen, intestine and testis; respectively. While *E. coli* O157:H7 couldn't be isolated from any internal organs from rats of other groups. The histopathological examination of rats of group (I) which dead during the experiment showed nearly the same lesions as naturally infected lambs. While the rats of either the infected-treated group or the prophylactic-infected group showed mild pathological changes such as mild necrosis and mild infiltration of mononuclear cells and their cells tend to be in normal state. It is concluded that garlic has
the ability to fighting EHEC, either by its detoxified effect against the produced toxin or by its immunostimulant effect. Garlic is a cheapest and most available drug can be used as either a treatment or a prophylactic to the suspected infected animal with E. coli O157:H7.

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**INTRODUCTION**

Diarrhoea is still the most common and costly disease affecting neonatal small ruminants. Diarrhoea in lambs and goats is a complex multi-factorial disease involving the animal, the environment, nutrition and infectious agents. The four major causes of diarrhoea in lambs and kids during the first month of life are *E. coli*, retrovirus, *Cryptosporidium sp.* and *Salmonella sp.* E. coli scour is most common. *Enterohaemorrhagic Escherichia coli* (EHEC) has emerged in developed countries over the past 20 years as an important cause of human intestinal disease (Schoenian, 2008).

*E. coli* strains of serotype O157:H7 belong to a family of pathogenic *E. coli* called *Enterohaemorrhagic E. coli* (EHEC) strains are characterized by the production of cytotoxins called shiga toxins (STX1 and STX2) or verotoxins (Nystrom, 1997).

*E. coli* O157:H7 an important zoonotic pathogen (Moxley, 2004) that causes both outbreaks and sporadic cases of disease, haemorrhagic colitis and hemolytic uremic syndrome (Baker *et al.*, 2007).

Garlic (*Allium sativum L*) with its active ingredient allicin was reported to have antibacterial, antiprotozoal and antifungal properties. So, it was employed today in flock medicine in all parts of the world for both prophylaxis and for treatment of a variety of diseases (Mandour and Hedaya, 1999).

Besides allacin, the other antimicrobial principle of garlic is methylmethane thiosulfinate (MMTSO) which is converted to S-methyl-L cysteine sulfoxide. The antimicrobial effect of garlic is due to interaction between the thiosulfinates present, (Kirubaharan *et al.*, 1999).

Gupta and Ravishankars (2005) found that the fresh garlic pasts showed strongest antimicrobial activity with complete inacti-
vation of *E. coli* O157:H7 at 3 days and 4°C in vitro.

Dried garlic powder and tablets still contain the unreacted alliin and the alliinase enzyme. The powder is then activated by adding water, the enzymatic reaction occurs and allicin results so dried product is closes in its composition to fresh garlic (*Cronin, 2001*).

**MATERIALS AND METHODS**

* Field samples:
   Faecal samples were collected from 20 diarrheic lambs and internal organ samples (Brain, lung, liver, spleen, intestine and testis) were collected from 16 dead lambs which had a history of haemorrhagic colitis with bloody diarrhoea from governmental farm in El-Dakhalia governorate. The samples were collected in a period from June to September 2007. Each collected tissue samples were divided into 5 parts, the first, second and third parts were transferred rapidly on ice box to the laboratory for bacteriological, virological and anaerobical examinations. The fourth part were kept in 10% buffered neutral formalin for histopathological studies. The fifth part was examined for any virological and parasitological pathogen.

* Isolation and identification of causative bacteria:
   It was carried out on the basis of *Koneman et al. (1983)*. Ten grams from each tissue samples were inoculated into nutrient broth and incubated aerobically at 37°C for 24 h. Then a loopful from the inoculated broth were streaked onto plates of MacConkey's bile salt agar, sorbitol MacConkey agar and blood agar. Suspected purified isolates were identified according to *Quinn et al. (2002)*.

* Garlic preparation:
   It is commercially obtained as "Tomex plus" tablets, the product of ATOS Pharma, Cairo-Egypt. The tablets (300 mg/day/rat) were used after grinding and resuspension in water and administrated to rats via gastric tube (*Koch and Lawson, 1996*).

* Antibacterial effect of garlic powder on *E. coli* O157:H7:
   Susceptibility of *E. coli* O157:H7 for garlic was determined by agar dilution method (*Yirsaw, 2003*) using tryptic soya agar (TSA) containing garlic powder in concentration of 0, 1000, 2000, 5000, 10000, 12500, 15000 and 17500 mg/liter. Agar plates (3 plates for each concentration) were inoculated with 0.01 ml of *E. coli* 1x10⁹ and incubated 24-48h. Then the plate were investigated for *E. coli* growth and detect MIC values.

* Experimental Design:
   Fifty male Wister rats of 10-12 weeks old and weighing ap-
approximately 100 gm, were divided into 5 equal groups. The rats were kept for 2 weeks before the beginning of the experiment for adaptation and also for make sure that they free from any pathogen.

Group (I): Ten rats were challenged with *E. coli* O157:H7 at a dose $1 \times 10^9$ CFU/ml intraperitoneal injection (Infected-non treated group).

Group (II): Ten rats were challenged with $1 \times 10^9$ CFU/ml *E. coli* O157:H7 I/P and simultaneously administrated garlic extract (Infected-treated group).

Group (III): Ten rats were firstly administrated the garlic extract for 3 weeks before challenge with $1 \times 10^9$ CFU/ml *E. coli* O157:H7 I/P (prophylactic-infected group).

Group (IV): Ten rats were administrated the garlic extract only.

Group (V): Ten rats were kept as control group.

All animals were given standard chow diet and drinking water ad-libitum.

* Mortality rates:

Mortality rates calculated as numbers of dead rats allover the experimental period in relation to all inoculated rats in each group.

- Samples collected from experimental animals:
  - Blood samples were collected from all rats of each group 1, 2, 3 and 4 weeks post-challenge of *E. coli*. The serum was separated by centrifugation and stored at -20°C until used for ELISA testing.
  - The post mortem examination was performed either on the dead rats allover the experiment or to the sacrificed rats after the end of the experiment (4 weeks post challenge). Tissues specimens were collected from all animals (brain, lung, liver, spleen, intestine and testis) and divided into 2 portions, first part subjected to bacteriological examination for isolation and identification of *E. coli* O157:H, while, the second one were fixed in 10% buffered neutral formalin for histopathological studies. Parts from different places of large intestine of infected-non treated group (Group 1) were preserved in formalin with glutaraldehyde at 1:1 mixture to be processed for electron microscopy examination.

* ELISA test:

Serum immunoglobulin G (IgG) antibodies titres were determined by an enzyme-linked immunosorbent assays by previously described techniques by Snyder and Marquard (1989). 100 µl LPS prepared as described by Westphal and Luderit (1964). Diluted 2.5 µg/ml in carbonate bicarbonate buffer as coating antigen which kept over night in refrigerator. Plates were washed three times
with washing solution (PBS containing 0.1% Tween 20). Blocking solution 0.3 ml/well was added (1% BSA in PBS) plates were incubated at 37 °C for 60 minutes. Then washed three times with washing solution. Diluted 100 µl of each serum samples 1:100 in 1% BSA was distributed into appreciate well, positive and negative control sera were added and then incubated at 37 °C for 60 minutes. Washing the plates three times, 100 µl of rabbit antirate IgG peroxidase conjugate diluted 1:1000 in BPS containing 1% BSA were added to each well and plates were incubated at 37 °C for 60 minutes. Washing 3 times again and then add 100 ml of diluted OPD (1 tablet plus 75 ml bidistilled water and immediately H₂O₂ 30% for each diluted OPD) incubated 20 minutes in dark place at room temperature. Absorbance values at 490 mmA were measured using ELISA reader.

* Bacteriological examination of experimental tissues:

Brain, lung, liver, spleen, intestine and testis of experimental animals were collected under aseptic condition. Re-isolation of E. coli O157:H7 was done as previously mentioned according to method of Kudve et al. (1997).

* Histopathological studies:

Tissue specimens collected from both lambs and rats were pre-

served in 10% neutral buffered formalin, and then processed to obtain five micron thick paraffin sections to be stained with haematoxylin and eosin according to Bancroft et al. (1996) for histopathological examination.

* Ultrastructure studies:

The tissues which previously preserved in formalin with glutaraldehyde were processed according to Weakly (1981) for Electron microscopy examination.

**RESULTS**

The clinical symptoms appeared on the lamb were repre-

sented by abdominal cramps, bloody diarrhoea, nausea with low grade of fever.

The virological, anaerobic and parasitological examinations revealed negative results. The bacteriological examination revealed that E. coli O157:H7 was isolated from 12 diarrheic lambs out of 20 examined lambs in percentage of 60% (Table, 1). Regarding to dead lambs (16), E. coli O157:H7 isolated from brain, lung, liver, kidneys, spleen, intestine and testis in a percentage of 2 (16.5 %), 9 (56.25 %), 12 (75 %), 15 (93.75 %), 14 (87.5 %), 15 (93.75% and 2 (2.5 %); respectively. All E. coli O157:H7 isolates didn't ferment sorbitol.

The use of garlic powder
(Table, 2) induced inhibitory results on the growth of *E. coli* O157:H7. Garlic powder had MIC 10,000 µg/ml.

Antibody response measured by ELISA test (Table, 3) was illustrated in Table (3) which showed that an increase in the antibody titers from 1st week post infection in both infected- treated and prophylactic-infected groups (II & III) which more than that recorded in infected-non treated group (I). This increase in antibody titre increase gradually till reach peak at 3 weeks post infection.

Mortality rate (Table, 4) in infected non treated group was 70%, while mortality rates were 0% among rats of other groups of the experiment.

Reisolation of *E. coli* O157:H7 from different organs of dead rats all over experimental period and sacrificed rats at the end of the experiment gave variable results. In infected-non treated group (I) *E. coli* O157:H7 isolated in a percent of 10%, 40%, 70%, 70%, 80%, 100% and 20% from brain, lungs, liver, kidneys, spleen, intestine and testis, respectively. While, in rats of infected-treated and prophylactic-infected groups, *E. coli* O157:H7 couldn't be isolated from any internal organ (Table, 5).

The post-mortem examination of infected lamb showed congested brain, congested and enlarged lungs, liver, kidneys, spleen and intestine with haemorrhagic diarrhoea in its lumen.

The histopathological examination of lambs showed necrosis of some neurons accompanied with neurophagia, axonal swelling (degeneration) as well as perineural and perivascular oedema with brain malasia (Fig. 1). Also, mild gliosis with thrombus formation in the blood vessels were seen (Fig. 2). Lungs showed haemorrhage filled the alveoli accompanied with lymphocytic infiltration (Haemorrhagic pneumonia) as well as destruction and sloughing of the epithelial cells lining the bronchi-oles, complete destruction of the endothelial cells lining the blood vessels with thrombus formation (Fig. 3). Liver showed haemorrhage, infiltration of mononuclear inflammatory cells, fibrin deposition specially around the portal area with necrosis of some hepatocytes (Fig. 4). Kidneys of lambs showed haemorrhage, necrosis of epithelial cells lining some renal tubules, infiltration of mononuclear inflammatory cell as well as vacuolation and necrosis of the endothelial cells lining the blood vessels of the gromeruli accompanied with infiltration of mononuclear inflammatory cells inside the bow- man's capsule (Fig. 5). Spleen was suffered from severe depletion of the lymphocytes of the white bulb.
as well as accumulation of hemosidrin pigments scattered in the red bulb (indication of haemorrhage) (Fig. 6). Intestine specially the right colon was the most severely affected part which showed severe haemorrhage, oedema, atrophied villi, fibrinous to fibrinohaemorrhagic exudates were fill the intestinal lumen (Fibrinohaemorrhagic colitis), necrosis of the epithelial cells lining the intestinal glands with sever infiltration of mononuclear inflammatory cells (Fig. 7). Testes showed necrosis of some spermatogenesis series cells, the seminefrous tubules were devoid of sperms with oedema in between the seminefrous tubules as well as deformity of their shape (Fig. 8).

While the infected-non treated rats were suffered from abdominal pain, nausea and bloody diarrhoea which ended by death. Some rats can survived till the end of the experiment, had the same symptoms with non bloody diarrhoea. Rats of other groups (prophylactic-infected and infected-treated as well as non infected-treated) act as normal control group and hadn't any abnormal clinical symptoms.

The dead rats of the infected-non treated group showed the nearly the same lesions in all organs as the naturally infected lambs. While, the survived rats of this group which sacrificed at the end of the experiment, showed necrosis of some neurons with neurophagia and axonal swelling of the brain. Lungs showed thrombus of small blood vessel, infiltration of fibrous connective tissues around the bronchioles and the blood vessel, hyperproliferation of the cells lining the bronchioles as well as thickening of the wall of the blood vessel accompanied with infiltration of mononuclear inflammatory cells (Fig. 9). Liver was suffered from severe oedema with focal necrosis of hepatocytes and infiltration of inflammatory cells (Fig. 10). Kidneys showed vacuolation of glomerular tuft, necrosis of epithelial cells lining most of renal tubules with infiltration of mononuclear inflammatory cells (Fig. 11). Spleen was suffered from depletion of the lymphocytes of the white bulb (Fig. 12). Some animals showed increase in the goblet cells of the intestinal villi with oedema.
and infiltration of inflammatory cells in-between the intestinal glands with fibrinotic exudates tinged with inflammatory cells in its lumen (Fig. 13). Some villus appeared eroded with sloughed of its epithelial cells and infiltration of mononuclear inflammatory cells. (Fig. 14). Other villi were fused together with complete necrosis of their lining epithelial cells (Fig. 15). Complete destruction of the villi and also the intestinal gland in the payer's patch part of the intestine which engorged with lymphocytes (Fig. 16).

The ultrastructure examination revealed bacteria adherent to the epithelial cells of large intestine with loss of the cellular web (lost their brush border) leading to what it called attaching and effacing (A/E) lesions. The distribution of bacteria was patchy in ileum and diffuse in colon as well as some bacteria were seen intracellular in case of heavy colonization (Fig. 17). Moreover, cupping of the plasma membrane around the individual bacteria were seen (Fig. 18).

The histopathological examination of rats of infected-treated (group II) as well as prophylactic-infected (group III) were represented by mild vacuolar degeneration in brain (Fig. 19). Liver showed mild oedema, few necrotic hepatocytes and very mild infiltration of mononuclear inflammatory cells (Fig. 20). Kidneys showed normal glomeruli with few lymphocytes cell infiltration (Fig. 21). Spleen showed very mild depletion of lymphocytic cells of white bulb (Fig. 22). Intestine showed few inflammatory cells in the lumen of the villi (Fig. 23). Testis showed normal spermatogenesis series cells with production of sperm cells (Fig. 24).

Rats of non-infected-treated rats didn't show any pathological alterations except mild vacuolar degenerative changes in liver.
Table (1): Isolation of sorbitol and non-sorbitol fermenting *E. coli* O157:H7 from diarrheic and dead lambs.

<table>
<thead>
<tr>
<th>Examined lambs</th>
<th>Types of samples</th>
<th>No. of examined samples</th>
<th>No. (%) of <em>E. coli</em> O157:H7 isolation *</th>
<th>Sorbitol fermentation</th>
<th>Non sorbitol fermenting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrheic lambs (20)</td>
<td>Fecal</td>
<td>20</td>
<td>12(60%)</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>16</td>
<td>2 (16.5%)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lungs</td>
<td>16</td>
<td>9 (56.25 %)</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>16</td>
<td>12 (75 %)</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Dead lambs (16)</td>
<td>Kidneys</td>
<td>16</td>
<td>15 (93.75 %)</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>16</td>
<td>14 (87.5 %)</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Intestine</td>
<td>16</td>
<td>15 (93.75 %)</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Testis</td>
<td>16</td>
<td>2 (16.5 %)</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Table (2): Antibacterial effect of garlic on *E. coli* O157:H7 in vivo.

<table>
<thead>
<tr>
<th>Concentration of garlic (mg/1 liter)</th>
<th>Growth of <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>++++ a</td>
</tr>
<tr>
<td>1000</td>
<td>+++</td>
</tr>
<tr>
<td>2000</td>
<td>++ b</td>
</tr>
<tr>
<td>5000</td>
<td>+ c</td>
</tr>
<tr>
<td>10000</td>
<td>- d</td>
</tr>
<tr>
<td>12000</td>
<td>- d</td>
</tr>
<tr>
<td>15000</td>
<td>- d</td>
</tr>
<tr>
<td>17500</td>
<td>- d</td>
</tr>
</tbody>
</table>

a = heavy growth.,
b = average No. colonies (30 to 50 colonies.),
c = Average No. of colonies less than 10 colonies.
d = negative.
Table (3): Overall mean of ELISA optical density among experimental animal groups.

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Weeks post infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>0.280 ±0.006</td>
</tr>
<tr>
<td>II</td>
<td>0.295 ±0.009</td>
</tr>
<tr>
<td>III</td>
<td>0.313 ±0.31</td>
</tr>
<tr>
<td>IV</td>
<td>0.121 ±0.003</td>
</tr>
<tr>
<td>V</td>
<td>0.142 ±0.001</td>
</tr>
</tbody>
</table>

Table (4): Protective immunity induced by garlic powder in rats challenged by *E. coli* O157:H7.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>No. of dead rats/week post challenging</th>
<th>Dead/Total</th>
<th>Mortality rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table (5): Re-isolation of *E. coli* O157:H7 from different organs of dead as well as sacrificed rats during and at the end of the experiment.

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Brain</th>
<th>Lungs</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Intestine</th>
<th>testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1/10 (10%)</td>
<td>4/10 (40%)</td>
<td>7/10 (70%)</td>
<td>7/10 (70%)</td>
<td>8/10 (80%)</td>
<td>10/10 (100%)</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>II</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>III</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>IV</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>V</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
</tbody>
</table>
Fig. (1): Brain of infected lamb with *E. coli O157:H7* showing necrosis of some neurons accompanied with neuropagia, axonal swelling (degeneration) as well as perineural and perivascular edema with brain malasia (H & E, X 400).

Fig. (2): Brain of infected lamb with *E. coli O157:H7* showing mild gliosis with thrombus formation in the blood vessels (H & E, X 200).

Fig. (3): Lung of infected lamb with *E. coli O157:H7* showing haemorrhagic pneumonia) and complete destruction of the endothelial cells lining the blood vessels with thrombus formation (H & E, X 200).

Fig. (4): Liver of infected lamb with *E. coli O157:H7* showing haemorrhage, infiltration of mononuclear inflammatory cells, fibrinotic deposition specially around the portal area with necrosis of some hepatocytes (H & E, X 400).
Fig. (5): Kidney of infected lamb with *E. coli* O157:H7 showing haemorrhage, necrosis of epithelial cells lining some renal tubules, infiltration of mononuclear inflammatory cell as well as vacuolation and necrosis of the endothelial cells lining the blood vessels of the glomeruli (H & E, X 200).

Fig. (6): Spleen of infected lamb with *E. coli* O157:H7 showing severe depletion of the lymphocytes of the white bulb as well as accumulation of hemosidrin pigments scattered in the red bulb (H & E, X 200).

Fig. (7): Intestine of infected lamb with *E. coli* O157:H7 showing fibrinohæmorrhagic colitis and necrosis of the epithelial cells lining the intestinal glands with sever infiltration of mononuclear inflammatory cells (H & E, X 100).

Fig. (8): Testis of infected lamb with *E. coli* O157:H7 showing necrosis of some spermatogenesis series cells, the seminefrous tubules were devoid of sperms with edema in between the seminefrous tubules as well as deformity of their shape. (H & E, X 200).
Fig. (9): Lung of infected rats with *E. coli O157:H7* showing thrombus of small blood vessel, fibrous connective tissues around the bronchioles and the blood vessel, hyperproliferation of the cells lining the bronchioles, thickening of wall of the blood vessel accompanied with infiltration of mononuclear inflammatory cells (H & E, X 200).

Fig. (10): Liver of infected rats with *E. coli O157:H7* showing severe edema with focal necrosis of hepatocytes and infiltration of inflammatory cells (H & E, X 200).

Fig. (11): Kidney of infected rats with *E. coli O157:H7* showing vacuolation of glomerular tuft, necrosis of epithelial cells lining most of renal tubules with mononuclear inflammatory cells (H & E, X 200).

Fig. (12): Spleen of infected rats with *E. coli O157:H7* showing depletion of the lymphocytes of the white bulb (H & E, X 200).
Fig. (13): Intestine of infected rats with *E. coli* O157:H7 showing increase in the goblet cells of the intestinal villi with edema and infiltration of inflammatory cells in-between the intestinal glands with fibrinotic exudates tinged with inflammatory cells in its lumen (H & E, X 200).

Fig. (14): Intestine of infected rats with *E. coli* O157:H7 showing some villi appeared eroded with sloughed of its epithelial cells with infiltration of mononuclear inflammatory cells (H & E, X 200).

Fig. (15): Intestine of infected rats with *E. coli* O157:H7 showing villi were fused together with complete necrosis of their lining epithelial cells (H & E, X 200).

Fig. (16): Intestine of infected rats with *E. coli* O157:H7 showing complete destruction of the villi and also the intestinal gland in the pyre's patch part of the intestine which engorged with lymphocytes (H & E, X 200).
Fig. (17): Ultrastructure of colon of infected rats with *E. coli O157:H7* showing loss of bruch border of the epithelial cells as well as adherent of bacteria on the cell membrane or sometimes bacteria showed intercellular. (Electron Microscopy, x 10000).

Fig. (18): Ultrastructure of colon of infected rats with *E. coli O157:H7* showing cupping of the plasma membrane around the individual bacteria (Electron Microscopy, x 30000).

Fig. (19): Brain of infected –treated group by garlic powder showing mild vacuolar degeneration in brain (H & E, X 400).

Fig. (20): Liver of infected –treated group by garlic powder showing mild edema, few necrotic hepatocytes and very mild infiltration of mononuclear inflammatory cells (H & E, X 200).
Fig. (21): Kidney of infected –treated group by garlic powder showing showed normal glomeruli with few lymphocytes cell infiltration (H & E, X 100).

Fig. (22): Spleen of infected –treated group by garlic powder showing very mild depletion of lymphocytic cells of white bulb (H & E, X 100).

Fig. (23): Intestine of infected –treated group by garlic powder showing few inflammatory cells in the lumen of the villi (H & E, X 200).

Fig. (24): Testis of infected –treated group by garlic powder showing normal spermatogenesis series cells with production of sperm cells (H & E, X 200).
DISCUSSION

Diarrhoea in lambs is a common problem affecting animals in their few weeks of life. The disease causes loss of animal health and death may occur due to enterotoxicty of causative agents.

Sheep, the second commonly reared species of ruminant food animals, appears to have a role of similar to that of cattle as natural reservoir of E. coli O157:H7. Lambs were infected by E. coli O157:H7 in Jun-September (Summer season). These results were coincided with Boyee et al. (1995) who stated that the infection by this microorganism are more common in warmer months than in colder months with a peak incidence from June through September. The mortality rate of infected lambs reached 60%, while Schoenian (2008) recorded 46% of lambs mortality in a study at USA Experimental Station. The highest in the mortality may be due to the virulence of the microorganism and the immunosuppression status of our national lambs due to hotness.

The clinical symptoms appeared either on lambs or on infected-non treated rats which died during the experiment which were also observed by Robinson et al. (2006) and Bonardi et al. (2007). Also, the rats which suffered only from non-bloody diarrhoea was noticed by Nystrom (1997) and Karch et al. (2005) who reported that the infection by E. coli O157:H7 cause non bloody diarrhoea in some cases of infected calves.

Non sorbitol fermenting E. coli O157:H7 could be isolated either from lambs suffered from diarrhoea or rats of infected-non treated group (I). Also, E coli O157:H7 was isolated from the faecal samples of the diarrheic lambs in an incidence of 60% of diseased animals. The results observed come in parallel with Bonardi et al. (1999) who recovered E. coli O157:H7 at 33.33% of examined animals where two community outbreak the year before and disagree with Euvelink et al. (1998) who recorded that E. coli O157:H7 was isolated from faecal samples of sheep in a percentage of 4%, this may be attributed to the difference in age of animals used in their experiments and virulence of the microorganism.

The ability to compare published prevalence data is limited because of the use of large variety screening methods. Several recent studies demonstrated that selective enrichment significantly improved the sensitivity of direct plating faecal samples from cattle (Sanderson et al., 1995) As regards to D-Sorbitol fermentation, all E. coli O157:H7 isolates were non sorbi-
tol fermenting. This observation could be confirmed by March and Ratman (1989) who reported that SMAC medium is a useful rapid and reliable screening and for the detection of E. coli O157:H7.

The post mortem examination which represented by congested enlarged of all internal organs with haemorrhagic diarrhea in the intestinal lumen were similarly recorded by Robinson et al. (2006) and Bonardi et al. (2007).

The histopathological examination of either infected lamb or infected non treated rats which dead during the experiment revealed encephalopathy, severe haemorrhage, oedema, destruction of the endothelial cells lining the blood vessels with thrombus formation and infiltration of mononuclear inflammatory cells, i.e systemic haemorrhagic syndrome (haemolytic uremic syndrome and fibrohaemorrhagic colitis) which lead to the death of the infected animals. These results were coincided with those noticed by Nystrom et al. (2003); Gracia et al. (2006) and Baker et al. (2007) who attributed these lesions to the infection with E. coli O157:H7. Also, the most affected part of intestine were the right colon, which indicated that this was the predilection seat of E. coli O157:H7. These result were agree with Naylor et al. (2003) who recorded that payer's patches was the target seat of this microorganism. All these lesions may be attributed to the fact that the isolates of E. coli O157:H7 produced several factors which contribute to their virulence; Shiga like toxin (SLTs) 1, 2 and several proteins encoded in locus of enterocyte effacement pathogenicity mainly endothelial cells lining the blood vessels leading to vascular damage and haemorrhage (Baker et al., 2007 and Zotta et al., 2008). In addition, Nystrom (1997) mentioned that following bacterial colonization of the intestine, the toxins are thought to enter the systemic circulation SLTs were then transferred to endothelial cells and cause damage with fibrin deposition in, also SLTs appeared to be capable of causing direct damage to tissues other than the endothelial cells. While, Boyee et al. (1995) added that the vascular damage by SLTs may allow to lipopolysacharride and other inflammatory mediators to gain access of circulation and initiating the hemolytic-uremic syndrome. Gracia et al. (2006) and Baker et al. (2007) mentioned that lesions which appeared in naturally infected calves and lamb were similar to those found in experimentally infected animals with E. coli O157:H7, such as piglet and rats.

Rats of infected-non treated group which can survival and slaughtered at the end of experi-
ment may be attributed to individ-
ual host immune status (Girard et
al., 2005). So, they showed necro-
sis of epithelial cells of all exam-
ined organs with oedema and infil-
tration of lymphocytes which re-
fect as depletion of the lympho-
cytes of white bulb of spleen. While, intestine showed all stages
of sloughed, eroded and complete
ecrosis of the villi as well as the
epithelial cells lining the intestinal
glands. Moreover, the increase in
the goblet cells as well as the dif-
fusion of some villi together, act as
a defense trial from the body
against the microorganism inva-
sion and its toxin production.
These results were come in agree-
ment with Sandhu and Gyles
(2002) and Karch et al. (2005). It
was suggested that a cooperation
between SLTs and tumor necrosis
factor (TNF) may be important in
producing the pathologic changes.
i.e. TNF and SLTs exhibited syn-
nergistic cytotoxic activity towards
endothelial and epithelial cells of
these organs (Isogai et al., 1998).

The ultrastructure examina-
tion revealed attaching and effac-
ing (A/E) lesions as well as the en-
terocyte bacteria and the cupping
of the plasma membrane around
the bacterial cells. These results
were similar to previously men-
tioned by Sandhu and Gyles
(2002) and Wales et al. (2005). In
fact, bacteria couldn’t be seen ad-
hering to the mucosal cell unless
the brush border was absent i.e.
bacteria were in close contact only
with epithelial cells that had lost
their brush border (Takeuchi et
al., 1978). Moreover, the H7 fla-
gellum induces production of
chemokines such as interleukins 8
and leucocytic infiltration of intes-
tinal mucosa which in turn may
enhance SLTs up take across the
intestinal epithelium, the toxin
bind to intestinal crypt cells and
submucosal lymphocytes, it may
be suppress mucosal immunity yet
enhance other effects that promote
intestinal colonization (Moxley,
2004). Also, SLTs enhance the ca-
pacity of EHEC to adhere to
epithelial cells and to colonization
(Robinson et al., 2006).

Many authors ruled out the
use of antibiotics and favored the
use of antioxidant. Phytochemicals
as garlic that exhibited antimicro-
biabial activity against wide range
of Gram positive and Gram –ve
bacteria without mutagenicity
(Adeleye and Opiah, 2003). The
bacteriological studies revealed
that garlic powder had inhibitory
effect on E. coli O157:H7 (MIC
10.000 mg/liter).

A growing interest in using
herbs and other material therapies
in animal production has been
made just a complementary medi-
cine. These results showed that
daily administration of garlic pow-
der enhance antibody levels in
both infected-treated and prophylactic-infected groups (II & III). These findings explained by Geong and Lee (1998) who stated that pretreatment with garlic extract or its components diallyle sulfide restored suppression of antibody induced by naturally occurring immune toxic agents, thus garlic stimulate humeral immunity. Also Arzomastev (1993) found that garlic extract could induce two fold increase in titer of haemagglutinins as well as antibody forming cells in the spleen of mice. From the results illustrated in table 4 and 5, it is clear that mortality rates and reisolation of E. coli O157:H7 from internal organs in rats of infected-treated and prophylactic-treated groups (II & III) were 0% each. This may be due to the antioxidant power of the garlic extract (Borek, 2001).

While, rats of either the infected-treated group or the prophylactic-infected group showed mild pathological changes such as mild necrosis and mild infiltration of mononuclear cells and their cells tend to be in normal state. These results may be referred to the action of garlic powder as antibacterial against enteric bacteria as previously mentioned by Ross et al. (2001). Also, Garlic seems to have antibacterial and immunostimulating properties enhance fibrinolytic activity and exert favorable effects on platelet aggregation and adhesion (Painter, 1995). Moreover, Allicin was found to exhibit antimicrobial activity against a wide range of Gram –ve and Gram +ve bacteria including multiderugs-resistant enterotoxigenic strain of E. coli (Ankris and Mirelman, 1999). Allicin has the ability to block the enzymes by reacting with one of their important components known as sulphydryl (SH) groups of the microorganisms. Unlike most bacteria, animal tissues cells contain detoxifying molecules (glutathione) which helps maintain appropriate sulfhydryl levels which needed in enzymes involved in some of the body's vital processes (ASM, 1997 and Youssef, 2000). Garlic extract increase some detoxifying cytochromes P-450 (e.g CYP2B1 and CYP2B2) (Dwivedi et al., 1998). Fulder (1991) reported that garlic, even at low doses, has the ability not only to kill invaders but simply stop their multiplication, has antitoxified effect against the poisoning produced by the invaders, affording the body an opportunity to marshal its own defense. These theory explain the protective effect of garlic powder against EHEC itself and its toxin produced (SLTs) not only as immune stimulating before infection (Prophylactive dose) but also by treating dose even in the incubation period of infection and before the appearance of the symptoms. Moreover,
Dehydrated garlic powder was effective in recovery of spermatogenesis and stimulated acetaldehyde detoxification (Kasuga et al., 2001).

Otherwise there wasn’t any pathological alteration were seen among the organs of non-infected treated rats except mild vacuolar degeneration in hepatocytes. The current results were in consistence with Al naqeeb et al. (1996) and Soliman (2000) who reported the presence of mild vacuolation in liver cells after treating with 50 mg/Kg/day of raw garlic extract for 3 weeks.

From this study, it is concluded that EHEC O157:H7 is a virulence microorganism caused severe drastic pathological lesions can lead to the death of animals either by naturally or experimentally infection. Rats is represented a good model of EHEC infection. Garlic is the only herbal extract has the ability to fitting EHEC, a detoxified effect against their toxins and give the chance to the body to protect himself via its immunostimulant effect. Garlic is a cheapest and most available herbal drug can used as a prophylactive dose to protected animals suspected infection by EHEC.

REFERENCES


in weaned pigs." Infect. & Immun., 73(9): 5514-5523.


Kudva, I. T.; Halffield, P. G. and


دراسات عن تأثير مستحضرات الثوم على الإشريشيا كولي المسببة لالتهاب الأمعاء في الحملان

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الملخص العربي

في هذه الدراسة تم فحص 20 عينة براز والأعضاء الداخلية من 16 حمل نافق مصاب باسحمل مدمم. بالفحص البكتريولوجي وجد أن نسبة الإصابة بميكروب الإشريشيا كولي (E. coli O157:H7) بنسبة 100 % من عينات براز الحيوانات التي تعاني من الإسهام المدمم و بنسبة 16.6 % و 11 % من كل من المخ، الرئة، الكبد، الكلي، الطعام، الأمعاء و الخصية على التوالي من الحيوانات الناقفة والتي كانت تعاني من نفس الأعراض.

وقد أظهر الفحص الهستوباثولوجي أن هذا الميكروب له أعراض بالباثولوجية شديدة تتخلص التهاب بخلايا المخ، التهاب رئوي نزيف، نزيف وانكسرس بخلايا الكبد، تتكسر
بالخلايا المبطنة للأوعية الدموية بحافظة يومان بالكلى مع وجود نزيف وتتركس بالخلايا المبطنة للأوعية البولية وأيضا الخلايا المبطنة للأوعية المنوية بالخصبة.

وقد منع مستخلص الثوم الجاف نمو الميكروب على الأطباقي بالعمل.

وفي إطار دراسة التأثير الواقع والعلاجي للثور المجفف في حيوانات التجربة، استخدم 90 فأر ذكر عمر 12-16 أسبوع يزن 100 جم تقريبا وقد قسمت بالتساوي كالتالي: المجموعة الأولى: المصاب بال mikrobi]

المصابة والمعالجة بالثور المجفف، المجموعة الثالثة: أعطي الثور المجفف أولا لمدة 3 أيام ثم تم إصابتها بالميكروب، والمجموعة الرابعة أعطيت الثور المجفف فقط، المجموعة الخامسة استخدمت كمجموعة ضابطة) واستمرت التجربة لمدة 4 أسابيع بعد الإصابة بالميكروب. وقد أظهرت النتائج أن نسبة الوفيات كانت 20% في المجموعة الأولى ولم تسجل أي وفيات في باقي المجموعات. وأيضاً ارتفاع نسبة الأجسام المناعية التي تم قياسها بواسطة اختبار الإليزا في كل من المجموعتين الثانية والثالثة. وتم عزل الميكروب من الأعضاء الداخلية للذئاب التي فقفت خلال التجربة والذي تم ذبحها بعد انتهاء التجربة من المجموعة الأولى بنسبة 10% 40% 60% 80% 100% و20% من المخ، الرئة، الكبد، الكلى، الطحال، الأمعاء، والخصبة على التوالي. بينما لم يتم عزل الميكروب من أي عضو من فئران المجموعات الأخرى.

وقد أظهرت نتائج الفحص البصري والإثاثولوجي للفئران المصاببة تقريبا نفس الصورة التي ظهرت عند فحص الأعضاء الداخلية للحولان المصاببة طبيعاً بهذا الميكروب. وقد ظهرت بعض التغيرات البيولوجية الطفيفة عند فحص كل من الكبد والكلى والأمعاء لكل من المجموعتين الثانية والثالثة مقارنة بالمجموعة الضابطة.

وقد أوضحت جميع النتائج التأثير الواقع والعلاجي للثور المجفف على الحيوانات E. coli O157:H7 .

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