Escherichia coli Isolated from Horses and Study the Effect of the Peganum harmaline Extract In Vitro and In Vivo and Antibiofilm Effect In Vitro

Aseel Mohammed Hamzah

ABSTRACT

Out of a hundred horses, fecal samples Escherichia coli was isolated from 37 samples of different ages. The isolated samples had been used to examine the effect of peganum harmaline on isolated E.coli in vitro and in vivo.

The goal of this research was to determine the antimicrobial activity of peganum harmaline extract by means of (ethanol: methanol 1:1) in opposition to Escherichia coli at various 40,20, 10, 5, 2.5, 1.25 and 0.625 mg/ml concentrations in each plastic and glass tube. The values of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) for the extract against E.coli were equal to 0.625 mg/ml for MIC and (10 mg/ml) for MBC on bacteria which cultured on glass tube while the MIC value was 40 mg/ml and MBC was10 mg/ml on plastic tube. The effect of Peganum harmaline on the formation of E.coli biofilm was investigated and the biofilm inhibitory concentrations were 40-6.25mg / ml in vitro. In vivo, a group of laboratory mice used, the LD50 of peganum harmaline extract was tested orally by up and down method and found that the sample transport to the bacteriology laboratory of zoonotic diseases unit/college of veterinary medicine/Baghdad university.

INTRODUCTION

Escherichia coli infection is one of the most important diseases that affected many animal species that can cause diarrhea, dysentery or urinary tract infection depend on pathotype of E.coli [1, 2]. There was an extensive study of the virulence factors and mechanisms by which E.coli strains can cause diarrhea in humans, pigs, and calves [3].

In recent years the bacteria become resist many kinds of drug [4] so that we found other sources as antibacterial. There are many natural plants in nature, have antibacterial activity depend on their chemical composition [5]. Peganum harmaline contain alkaloid that is making the plant antimicrobial effectiveness [6].

There are many studies around the world on P.harmaline effect in many diseases Parkinson’s disease [7], anti leishmanial [8].

This study aimed to isolate the E.coli bacteria from horses and used of P.harmaline as an antibacterial and antibiofilm drug in vitro and in vivo.

MATERIAL AND METHOD

1. Samples

Fecal samples had been accumulated from horses at different ages in a septic disposable plastic container after that the sample transport to the bacteriology laboratory of zoonotic diseases unit/college of veterinary medicine/Baghdad university.

2. Bacterial isolation

Each one gram of samples diluted with three ml of sterile phosphate buffered saline, then one loopful cultured on MacConkey’s agar after 24hr of incubation at 37°C the pink colony cultured on eosin methylene blue agar then confirm the isolate by API 20E [9].

3. Plant extraction

The seed was percushed from the local market, then grinding and the extract obtained as describe in [10].

4. Antibiotic susceptibility testing

4.1. Agar well diffusion for P.harmaline

A density of 10⁶ cells/ml of 0.5 McFarland standard was prepared for bacterial broth cultivation. The liquor was distributed evenly over the Muller Hinton agar By swab of sterile cotton, Equal distance holes with a disinfectid 5 mm in diameter, cork borer, 2 mm from the edge of the plate, were made on each plate as describe by [11]. Various concentrations were prepared from crude P. harmaline, 150,100,50,20,10 and Five micro liters of each plant extract (500 mg / ml) were inserted aseptically into the respective well of the agar, after that incubated the agar plate overnight at 37°C, then read the zone of inhibition in diameter.
4.2. Detection of Minimum inhibitory concentration of P. harmaline (MIC)
In a sequence of eight test tubes, the extracts of P. harmaline was diluted double fold (2:2) with Muller Hinton broth. Each tube as well as control tube were inoculated with an aliquot of 1ml of the bacterial suspension (1x10⁸ cfu/ml). With the same quantity of Muller Hinton broth, except the last tube use as control injected with 1 ml of 10⁶ CFU/ml bacterial culture plus 1 ml of Muller Hinton broth. All the tubes have been incubated for 24hrs at 37°C. The determination of the MIC assay by bacterial growth visualization by using the salt (P-iodonitrotetrazolium violet) (INT) (0.04mg / ml) was added to each tube with as 0.5 ml and all tubes were incubated at room temperature for 6 hours. The tubes have been checked for change of color and the first transparent tube showed the MIC, which did not change to a red color compared with control tubes [12].

4.4. Detection of Minimal bactericidal concentration of P. harmaline (MBC)
MBC has been performed by subculturing a loopfull of each MIC exhibit no growth (clearance). MBC was taken as the largest dilution (smaller concentration) that did not produce a single colony of bacteria.

4.5. P. harmaline extract’s effect on the production of bacterial biofilms
The development of biofilms in plastic and glass sterile test tubes has been measured with two-fold dilution of plant extract with muller hinton broth started from (20) ended with 0.312 mg/ml concentrations, clinical isolate of E. coli cultured in broth and Standardizing as 0.5 McFarland turbidity put 1ml in each tube and incubated the tube for 4-5 hr at 37°C. Allowing a bacteria to adhere therefor apply 1ml of each concentration to each tube furthermore incubated the tube 24 hours at 37°C, control tube contain bacteria with muller hinton only.

4.6. Bacteria’s capacity to bind in tubes
By utilizing of 1 % crystal violet staining to measure the adhered biomass of the cells each tube wash three times with phosphate buffer as described by [12].

4.7. LD50 dose of P. harmaline in laboratory mice
Oral LD50 was established as an initial attempt to assess the toxicity of alkaloids using the up-and-down approach [13]. Based on the toxicity level chart, the alkaloids tested were considered to be marginally toxic to mice (1070 mg / kg body weight [14], Sub-lethal doses (500 and 1000 mg / kg body weight) were survived mice, exhibit lethargy and prostration, lack of appetite, trembling, difficulty walking and sometimes loss of consciousness until recovery or death. P. harmaline extract LD50 was (1030 mg / kg B.W) and the dose treated was 103 mg / kg B.W orally.

4.8. Infective dose of E.coli in laboratory mice
Isolated bacteria cultivate in the tryptic soya broth overnight at 37°C, bacterial count done by M.iles and M.isra approach to get 1x10⁶ CFU/ml [15,16]. A group of laboratory mice 3-4 month of age divided into four groups all mice administrated with 0.2 ml of 1x10⁶ CFU/ml E.coli suspension except the control group administrated normal saline as control negative. First group: Animals were administrated daily for ten days orally 0.2 ml of P. harmaline extract, then the animals were sacrificed and collect the internal organ for histopathology, second group treated for twenty days then sacrificed else third group treated for along thirty days the animals were sacrificed.

RESULTS

1-Isolation
E.coli was isolated from 37 out of hundred horses fecal samples obtained in Baghad's Al-Furusia group, which are listed in Table1.

<table>
<thead>
<tr>
<th>Horses fecal samples</th>
<th>Number of samples</th>
<th>Number of positive</th>
<th>Percente of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>37</td>
<td>37%</td>
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</tbody>
</table>

2-P. harmaline susceptibility testing in agar well diffusion test
Crude extracts of P. harmaline demonstrate inhibition of bacterial growth of 150 percent, 100 percent, 50 percent, 20 percent, and 10 percent against E.coli, with no inhibition of 5 percent (table 2). The plant has inhibited bacterial growth from 44 mm to 7 mm in diameter.

<table>
<thead>
<tr>
<th>P. harmaline concentrate extract</th>
<th>Zone of inhibition /mm</th>
</tr>
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<tbody>
<tr>
<td>150</td>
<td>44mm</td>
</tr>
<tr>
<td>100</td>
<td>41mm</td>
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<tr>
<td>50</td>
<td>35mm</td>
</tr>
<tr>
<td>20</td>
<td>33mm</td>
</tr>
<tr>
<td>10</td>
<td>7mm</td>
</tr>
<tr>
<td>5</td>
<td>No zone</td>
</tr>
</tbody>
</table>
3-Minimum inhibitory concentration and Minimum bactericidal concentration of P.harmaline (MIC and MBC)

The result of the MIC of P.harmaline extract was comparable to that obtained in the agar-well diffusion technique, all plant extract concentrated (started with 20 to 0.625 mg/ml) has MIC activity against E.coli except 0.312 mg/ml in glasses tubes, whereas plant extract concentrated from 20 to 1.25 mg/ml the MIC activity against E.coli in plastic tube.

Whereas the findings have been reported for minimum bactericidal concentration (MBC) was 10mg/ml in glasses tube and 20 mg/ml in plastic tube.

4- Antibiofilm

Anti-adhesion tests were performed using crystal violet experiment in order to evaluate plant extract inhibition ability against E.coli attachments at concentration MIC value in glass and plastic tube (figure 1 and 3). Results of alcoholic extract of P.harmaline in vitro antibiofilm activity could inhibit bacteria cell attachment Biofilm formation inhibition induced on E.coli up to 95% show in figure 6 and 8 of glass and plastic tube respectively.

**Figure 1:** Antibiofilm of plant extract concentration A:20; B:10; C:5; D:2.5; E:1.25; F:0.625; G:0.312 mg/ml and H: control positive in glass tube

**Figure 2:** Antibiofilm of plant extract concentration curve revealed the higher inhibition at 20mg/ml of P.harmaline at 590 nm on glass tube
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Figure 3: Antibiofilm of plant extract concentration curve revealed the higher inhibition at 40mg/ml of P.harmaline at 590 nm on plastic tube.

5. Histopathological study of P.harmaline against E.coli infection

The intestine was shown to invade few mononuclear cells in the intestinal mucosal lamina properia. after ten days of treatment with plant extract, likewise aggregation of mononuclear cell in the sub-mucosa and mucosal glands after twenty days of treated animals as shown in, furthermore the intestine of animal at day 30 of treatment shows marked mononuclear cells infiltration between mucosal glands(figure 4) whereas the control group showed focal enteritis (figure 5).

Figure 4: Section in the intestine animal at day 30 shows marked mononuclear cells infiltration between mucosal glands (H&E stain 400X)

Figure 5: section of small intestine shows; focal enteritis (En). H&E stain.40x in control positive group. Liver sections showed pathological changes in different treated group compare with control group that appear sever changes without treatment (Figure 6 and 7).

Figure 6: Section in the liver animal at day 30 shows few mononuclear cells infiltration in portal area (H&E stain 400X)
Antileishmanial activity of Peganum harmala extracts with antibacterial effects, have elected plant extracts, particularly for calves and are beneficial in the role of plant extract for treated E.coli [27].

CONCLUSIONS

P.harmaline have antimicrobial and antibiofilm activity towards E.coli infection with lowest MIC values furthermore Oral dosing of P.harmaline was stopped pathogenic E. coli infection in mice and increased induction of macrophage.

CONFLICT OF INTEREST

None

REFERENCES


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