Comparative study of antidiarrheal effect of proposed new formulae from D.innoxia hydroalcoholic leave extract against E.coli O:157 induced diarrhea in goats

Aula Hasoon Obeid Duraid Abdul Hadi Abbas
College of Veterinary Medicine /Baghdad university
Email:drduraid cvm@yahoo.com

Abstract

New proposed formulation of 5% and 7.5% of hydroalcoholic leave extract of D.innoxia were tested as new drug in order to treat induced diarrheal infection by pathogenic E.coliO:157 strain isolate in 16 healthy local goats divided in to equal four groups according to following treatment groups T1 and T2 represented (25 and 37.5 mg/kg D.innoxia), ciprofloxacin (5mg/kg) and DMSO vehicle group 0.5 ml /kg as positive control. Dosing continue orally daily for 14 days after induction of diarrheal infection .The efficacy of different treatment were evaluated according to improvement in clinical symptoms in which the formulation showed dose dependent effect with superiority of higher dose in T2 over ciprofloxacin in reduction body temperature and respiration rate , improvement in body weight gain and dehydration signs with reduction of fecal bacterial count (E.coli ) to nearly normal with completely ceasing diarrhea at the end of treatment . This was due to the presence of multiphytochemical that have not only antibacterial effect but also astringent , anti-inflammatory , analgesic , antispasmodic, narcotic , antipyretic and antiallergic that help to over come the stress that accompanied diarrheal infection.

Key word :Datura, diarrhea, dehydration , formula,E.coliO:157
Introduction

Datura innoxia (Family: Solanaceae, locally known as Elsakran) is used for many medicinal purposes. Datura precise and natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions. It contains atropine alkaloids such as scopolamine, hyoscyamine, hyoscine norscopolamine, meteloidine (1) and (2). Traditional medicine uses flowers, leaves and seed of Datura. Innoxia (D.innoxia) medically treat for skin eruptions and infection, colds, nervous disorders, antipyritic, anti-inflammatory, analgesic, aspasmolytic, astringent and antidiarrheal effect.

Material and methods

D.innoxia preparation and extraction

D.innoxia leave were collected from college agriculture farms. The plant classification was done in Ministry of Agriculture/ State Board for Seeds Testing and Certification (S.B.S.T.C) in Abu Graib /Baghdad. The leaves dried in shade, blended to powder and extracted by using hydroalcoholic method in 30% ethanol according to (3) the yield of extract was calculated according to the dry plant powder.

Preparation of D.innoxia formulae

D.innoxia leave extract formula was prepared in two concentration 5% and 7.5% by dissolving the extract in 50% (dimethyle sulphoxide:DMSO) and after supported ingredients. Different trial were done to prepare the best formulation that its ingredient kept secret for how know purpose?. The formulae were prepared under sterilized condition under UV light and using 0.5 millipore filter paper to filtrate extract that filled in sterilized well sealed bottle of 25 ml to be given for treatment of diarrheal goats.

Detection of phytochemical components

Different phytochemical components were determined according to methods of detection mentioned by (3) for alkaloid, flavonoid, steroid, terpenoid, resin, phenol, saponin, tannins as well as glycosides.

Isolation and identification of pathogenic E.coli isolate

Pathogenic bacteria isolate were collected from severely infected diarrheal goats with hemorrhagic diarrhea. Analytical profilesIndex (API-20) as well different biochemical methods were used for identification of E.coli growth while the special growth color in chrome and sorbitol maccAonky agars were used for identification of E.coli O:157 strain.

In-Vivo study of antidiarrheal effect of D. innoxia formulations

Sixteen healthy local goats weighed (22-25) kg examined clinically for normal signs as well as for fecal bacterial growth that showed normal flora but without E. Coli O:157 strain growth. The goat divided equally into four treatment groups that kept in separated places in well closed table with special fodder and water given adlibitum. Treatment begins after 48 hrs. of inducing infection after appearance signs of diarrhea that treated according to the following groups:

Group (T1): infected and treated orally daily with 25 mg/kg b.w (5%) formulation for two weeks.
Group (T2): infected and treated orally daily with 37.5 mg/kg b.w (7.5%) formulation for two weeks
Group (cipro): infected and treated orally daily with 5 mg/kg b.w of ciprofloxacin at concentration 50 mg/ml. (safcoceieb/ Syria) Company
Group(DMSO): infected and treated with formulated vehcical (DMSO) with
out plant extract at dose 0.5 ml/kg b.w.

The following parameters have been studied (before, after infection and after treatment):

A- Physiological and clinical symptoms observation (body weight change, body temperature, respiratory rate)

B- Fecal bacterial count for E. coli bacteria.

**Inducing Infection (Diarrhea)**

After a pilot study, the challenge dose that was chosen to induce infection (acute diarrhea) was $3.5 \times 10^9$ cfu/ml of E. Coli suspension, the inoculum preparation-standardized according to viable counting method-pour plate technique. E. coli O157 bacteria was inoculated in 10 ml of brain heart infusion broth (1 ml) was given orally by modified warming gun method. Then animals were watched for development symptoms of diarrhea in goats in all treated groups after 24 hrs of infection (4)(5).

**Statistical analysis**

Data that subjected to statistical analysis were represented as mean ± standard error. Statistical analysis of data was performed by two-way ANOVA using Least significant difference (LSD) to compare means of collected data at level (P<0.05).

**Result**

1- Phytochemicals components in *D. innoxia* dry leaves Powder Extract

The plant leaves powder extract screening showed the presence of the following phytochemicals: alkaloids, phenol, saponins, flavonoids, tannins and glycosides. Alkaloid, phenols, saponins and tannins seemed to be found in a high level in dry extract. These results were in agreement with that study conducted by (6).

2- Identification of pathogenic *Escherichia coli* O:157 isolate:

The results of biochemical tests have shown that these isolates gave positive results for catalase, insole and methyl red, while giving negative results for oxidase and Citrate utilization, and the bacterial isolates expressed gram-negative stain, this biochemical characteristic microscopic feature and this characterized growth especially on chromagar that showed pink or mauve pink colour for E. coli O:157 strain that different from other strains of E. coli, because other strains of E. coli have blue color on chromagar(7) also growth on MacConky and sorbitol agar that showed specific straw to white or colourless colonies so these biochemical characteristics test indicating that the bacterial colonies belonged to *E. coli* O157. Fig.(1) This result was similar to that reported by (7) who used the same tests to reach such identification result conclusion of pathogenic strain of E. coli:O:157.
3. preparing infectious dose after counting colonies of pathogenic E.coli

The result of bacterial counting of the strain E.coli used in this study was 3.5 x10^9 cfu/ml that confirmed by serial dilution count. This dose was used to induce infection in goats orally by the aid of modified worming gun.

4. clinical observation signs

All the animals before infection were seem healthy with normal temperature and respiration fig (2-A). While after infection with E.coli O:157 isolate in all goats groups that exhibited clinical signs of enteritis, profuse diarrhea with mucous and blood, pasty or loose faeces, anorexia, emaciation, rough body coat, poor body weight gain and reduced elasticity of skin, loss of hair indicating dehydration fig (2-B).

Efficacy of different treatment against induced diarrheal infection are listed in table (4-1) according to treated groups. Group DMSO: untreated diarrhoeic animals showed symptoms of severe dehydration, profuse to hemorrhagic diarrhea, tachycardia, anorexia, reluctance movement and dyspnoea, that observed throughout the experimental period. Mortality in the treated positive infected control animal group by DMSO were found to be 100% after the end of treatment period.

The animals of Group T1 receiving D.innoxia 5% antidiarrhoeal formulation at dose of 25gm/kg for 14 days did not show complete recovery at the end of 14 days treatment since clinical signs were relatively mild. Group T2 animals treated with 37.5gm/kg D.innoxia extract formulae for 14 days exhibited faster recovery from day 7th treatment while complete recovery was attained on day 14th. Group cipro, given antibiotics ciprofloxacin at dose 5mg/kg for 14 days showed recovery after 8th day onward, as listed in table (1).

Table (1): Responsive of treatment of E.coli O:157 induced infection in goats according to improvement in clinical sings after treatment with D.innoxia extract formulae, ciprofloxacin and DMSO:

<table>
<thead>
<tr>
<th>Groups</th>
<th>After one week treatment</th>
<th>After two week treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 D.innoxia formulae at dose 25 mg/kg</td>
<td>Profuse watery diarrhoea with body weight loss, fever, and severe to moderate dehydration</td>
<td>mild diarrhoea with less weight loss, moderate dehydration, rough coat with loss of hair, decrease in body temperature and respiratory rate.</td>
</tr>
<tr>
<td>T2 D.innoxia formulae at does 37.5 mg/kg</td>
<td>moderate dehydration and watery diarrhea with foul odor, anorexia, dry mucous, mambaram of eyes, loss hair, anorexia, and rough body coat</td>
<td>Improvement in all clinical symptoms manifested by eating and drinking normally, increase in body weight gain normal, movement inside the field with decrease to normal body temperature and respiration rate, mild dehydration and stopped body weight loss with</td>
</tr>
</tbody>
</table>
### Ciprofloxacin
At dose 5 mg/kg

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>The behavior sign of depression, weight of loss, lethargy, moderate dehydration, soft to watery diarrhea, dry mucus mambarane of eyes, loss of hair, anorexia, rough body coat</td>
<td>Mild diarrhoea with e pale yellow colour mucous membrane, decrease in body temperature and respiratory rate, with mild dehydration, stopped diarrhea and body weight loss.</td>
</tr>
</tbody>
</table>

### DMSO vehicle
0.5 ml /kg

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green to blackish color, watery profuse diarrhea, severe dehydration, loss of weight, anorexia, representing inability to grazing in the field. As well as paleness of eye mucous membrane, it can not on eating and drinking, rough skin and recombancy.</td>
<td>Profuse watery to bloody diarrhoea with progressive weight loss and severe emaciation, signs manifested by recombancy, inability to eat, fever, and rapid to shallow breathing rate with increase mortality occur at the end of treatment. fig (2-D)</td>
</tr>
</tbody>
</table>

---

Fig(2)-(A)-healthy normal goats befor infection. (B) sever diarrhea in all groups during infection period. (C) stopped diarrhea after 2 week at 37.5 mg /kg D.inoxia formula treatment (d) dehydration and bloody diarrhea and mortality in DMSO treatment group goat.

**Body weight**
After infection and even after treatment periods there were significant decrease (P<0.05) in body weight after infection. While after treatment plant groups, showed some significant improvement (p<0.05) recorded in body weight gain in animal of T1 and T2 groups in comparison with ciprofloxacin group at the end of treatment or DMSO group that showed the signs of emaciation and sever dehydration during all course of treatment periods. Animals of DMSO group persisted in its significant decrease in body weight (P<0.05) during all treatment period in comparison with the other groups T1,T2 and cipro. (table 2)

**Table(2): Effect of different treatment with D.innoxia plant formulae, ciprofloxacin and DMSO on body weigh (Kg) of infected goats with E.coli O157 isolate**

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Pre infection</th>
<th>Infection without treatment</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>23.70 ± 0.32</td>
<td>20.30 ± 0.77</td>
<td>22.72 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC a</td>
<td>BC b</td>
<td>A ab</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>25.50 ± 0.37</td>
<td>22.22 ± 0.67</td>
<td>23.17 ± 0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A a</td>
<td>A b</td>
<td>A b</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>24.52 ± 0.43</td>
<td>21.17 ± 0.36</td>
<td>20.40 ± 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AB a</td>
<td>AB b</td>
<td>B b</td>
</tr>
<tr>
<td>D.M.S.O</td>
<td></td>
<td>22.70 ± 0.28</td>
<td>20.32 ± 0.60</td>
<td>18.30 ± 1.00</td>
</tr>
<tr>
<td>(vehicle)</td>
<td></td>
<td>C a</td>
<td>C b</td>
<td>C b</td>
</tr>
<tr>
<td>LSD value</td>
<td></td>
<td>1.106</td>
<td>1.922 *</td>
<td>1.996 *</td>
</tr>
</tbody>
</table>

T1 = group treated with *Datura innoxia* formulae at dose 25mg /kg bw  T2 = group treated with D.i. At dose 37.5 mg/kg bw  Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw  Values represent mean ±S. E.. Different small letters mean significant (P< 0.05) results between periods.Different capital letters mean significant (P< 0.05) results between groups

**Body temperature**

The result of body temprature change in goat groups dose with D.innoxia formula T1, T2 and ciprofloxacin. That showed signs of significantaly decrease in feverish temperature (P< 0.05) after treatment ranged between (38.30-38.75°C0) when compared with infected group with DMSO that showed high temp. of 40.52°C0. The reduction in body temperature in T1 and T2 groups animals were lesser than that treated with ciprofloxacin. (Table3)

**Table (3):Effect of different treatments with D.innoxia plant formulae, ciprofloxacin and DMSO on body temperature (C0) of infected goats with E.coli O157 isolate ;**

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Pre infection</th>
<th>Infection without treatment</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>38.42 ± 0.08</td>
<td>41.00 ± 0.22</td>
<td>38.32 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AB b</td>
<td>AB a</td>
<td>B b</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>38.30 ± 0.10</td>
<td>40.67 ± 0.32</td>
<td>38.30 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B b</td>
<td>B a</td>
<td>B b</td>
</tr>
</tbody>
</table>
Table 4: Effect of different treatment with D.innoxia plant formulae ,ciprofloxacin and DMSO on( No. respiration per minute) of infected goats with E.coli O157 isolate

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Pre infection</th>
<th>Infection without treatment</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>25.67 ± 2.01</td>
<td>33.32 ± 1.43</td>
<td>31.00 ± 0.71</td>
<td>B a</td>
</tr>
<tr>
<td></td>
<td>A b</td>
<td>B a</td>
<td>B a</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>24.67 ± 0.62</td>
<td>35.00 ± 1.08</td>
<td>29.32 ± 0.47</td>
<td>BC a</td>
</tr>
<tr>
<td></td>
<td>B b</td>
<td>A a</td>
<td>BC a</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24.00 ± 0.41</td>
<td>32.67 ± 0.23</td>
<td>29.00 ± 0.41</td>
<td>C a</td>
</tr>
<tr>
<td></td>
<td>AB b</td>
<td>AB a</td>
<td>C a</td>
<td></td>
</tr>
<tr>
<td>D.M.S.O (vehicle)</td>
<td>23.32 ± 0.94</td>
<td>34.32 ± 0.84</td>
<td>33.25 ± 0.85</td>
<td>A a</td>
</tr>
<tr>
<td></td>
<td>BA b</td>
<td>A a</td>
<td>A a</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>3.613</td>
<td>3.081 *</td>
<td>1.959 *</td>
<td></td>
</tr>
</tbody>
</table>

T1 =group treated with Datura innoxia formulae at dose 25mg /kg bw T2=group treated with D.i. At dose 37.5 mg/kg bw  Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw  Values represent mean ±S. E. Different small letters mean significant (P< 0.05) results between periods.Different capital letters mean significant (P< 0.05) results between groups

Rectal bacterial count

There were a significant decrease (P<0.05) in the rectal bacterial count of all treated groups in comparison to its count after inducing infection . Rectal bacterial count significantly reduced (P<0.05) after 14 days of treatment in comparison to its highly increasing count after inducing infection. Animal of T2 and ciprofloxacin groups possibly returned to normal bacterial count as before inducing infection after 14 days.
of treatment, indicating complete recovery while T1 showed signs of lesser antibacterial effect at the end of treatment recording \((6.07 \times 10^4)\) in comparison with \((4.20 \times 10^2\) and \(4.55 \times 10^2\) cfu/ml of fecal sample for T2 and ciprofloxacin groups respectively indicating not complete antibacterial effect than high formulae dose at 37.5 mg /ml or ciprofloxacin treatment. While group (DMSO) group showed no change in bacterial count, table (5).

**Table (5):** Effect of different treatment with D.innoxia plant formulae, ciprofloxacin and DMSO on Fecal bacterial count (cfu/1ml) of infected goats with E.coli O157 isolate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>After infection with out treatment</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1</strong></td>
<td>A</td>
<td>10.84 x10^6 ± 0.01</td>
<td>6.07 x10^4 ± 0.48</td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td>A</td>
<td>9.58 x10^7 ± 0.64</td>
<td>4.20 x10^2 ± 0.51</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>AB</td>
<td>10.34 x10^6 ± 0.29</td>
<td>4.55 x10^2 ± 0.49</td>
</tr>
<tr>
<td>D.M.S.O (vehicale)</td>
<td>AB</td>
<td>10.60 x10^6 ± 0.25</td>
<td>10.35 x10^3 ± 0.29</td>
</tr>
</tbody>
</table>

LSD value 1.151 * 1.401 *

T1 = group treated with *Datura innoxia* formulae at dose 25mg /kg bw T2 = group treated with D.i. At dose 37.5 mg/kg bw Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw Values represent mean ±S. E.. Different small letters mean significant (P< 0.05) results between periods. Different capital letters mean significant (P< 0.05) results between groups.

**Discussion**

**Clinical observation signs/Gram-negative bacterial infection by Escherichia coli:** O:157 occur frequently in farm animals. Infection by this organisms result in release of endotoxin from outer bacterial membrane into the host’s internal environment which is responsible for some of the most common diseases in farm animals such as enteritis, endometritis, dehydration. Animals treated with endotoxin exhibited different behavioral changes post-inoculation in comparison to the control animals as listed in table (1). There were clear dose dependent effect in response to diarrheal therapy manifested at the end of therapeutic period. Including complete recovery in T2 group even better than cipro. While T1 group showed better response but not complete recovery because of very virulent E.coli inducing diarrhea in all experimental groups that led to 100% mortality in all DMSO group goats after few days from the end of treatment periods for 14 days because of deterioration of health due to profuse diarrhea and dehydration. Ciprofloxacin is recommended antibiotic therapy in bacterial enteritis. Chosen because fluoroquinolones, produced 99% reduction in E.coli bacterial count, also prevented the emergence of resistant organisms. (8) The results of T1&T2 could be attributed to D.innoxia extract composition of secondary metabolites like; alkaloids, flavonid, phenols, tannins, saponines, steroids, and glycosides, since these secondary metabolites have reported to have antibacterial
activity with different mechanisms of 
action (9). This is interesting results 
since one of the favorable character of 
anti-diarrhoeal drug its analgesic and 
anti-inflammatory effects(10), as well 
as astringents and antispasmodic 
effects .(11).

Bodyweight/ Serial measurements of 
body weight, provide the best 
indication of hydration status, 
especially when fluid loss is acute, as 
in this study. This statement is similar 
to (12) that reported the percentage 
decrease in body weight was assumed 
to be equal to the degree of 
dehydration, because weight loss in 
this preruminant goat diarrhea model 
approximated to the decrease in 
extracellular fluid volume. Weight loss 
from under nutrition was considered 
minimal. Watery diarrhea lead to 
dehydration, and that cause weight loss 
initial body weight, within a 48-hour 
period. Goats had clinical evidence of 
severe dehydration at that time, as 
measured by extent of 
enophthalmos,and prolonged cervical 
and thoracic skin-tent duration. Most 
goats with severe dehydration and 
diarrhea have cold extremities 
because of poor peripheral perfusion.

Body temperature/ The results of 
treatment in T1 and T2 showed more 
decrease in fever when compared with 
ciprofloxacin group, this may be due to 
the D.innoxia have antipyretic effect 
in its active secondary metabolites of 
D. extract plant, like flavenoiod, 
saponine, tanine, alkaloids, and resin 
that in agreement with (13) who 
reported that secondary metabolites of 
plant like saponine and flavenoiod were 
present with antinflammatory and 
antipyretics effects as well they have 
algesic and antiallergic activities. 
The superiority of D.innoxia extract 
formulae versus ciprofloxacin 
treatment in reducing feverish body 
temperature after E.coli infection was 
due to the lack of ciprofloxacin of such 
antipyretic effect, of the multi active 
metabolites present in plant extract 
formulae. The presence of enough 
active ingredient in hydroalcoholic 
extract of D.innoxia leaves like 
flavnioids, phenols, Alkalioids and 
steroids that reported to have analgesic 
as well as anti-inflammatory effect. 
Probably other substances in relieving 
pain also have antipyretic effects. (15).

Respiration rate/ The high ratio of 
alkaloid components in leaves of 
Datura innoxia when it compared with 
other parts of the plant. In addition to 
that, in this study extracted leaves 
were taken from vegetative, young 
leaves of plant which according to 
(14) . Tropane alkaloids act as 
competitive blockers at muscarinic 
cholinergic receptors with different 
specificity for individual receptor 
subclasses. Symptoms associated with 
tropane alkaloids include dryness of 
the mucosa in the upper digestive and 
respiratory tract, alterations in the 
heart rate and central nervous effects 
such as irritability, ataxia, seizures and 
respiratory depression. So it is 
hydroalcoholic leaves extract contain 
some active ingredients with narcotic 
effect as well as other reported active 
ingredients with non-narcotic 
algesic effect like flavinoids, tannin, alkaloid, phenols and 
glycosides. (15).

Rectal bacterial count/ The results of 
bacterial counts at the end of treatment 
were indicative of virulence of E.coli 
isolated that cause deterioration of 
healthy status in all DMSO group 
goats ended with their death after 2 
week. Pharmacologically and 
therapeutically activity of datura 
extract plant have antimuscarinic
effect causing clearly antispasmodic and antidiarrheal (16). The body defense mechanism cannot overcome such virulent infection of E. coli O:157 isolate as reported with other bacteria. (7). The superiority of extract formulae over ciprofloxacin especially in high dose (37.5 mg/kg) were possibly due to antispasmodic, anti-inflammatory, antipyretic, as well as analgesic, astringent and antimicrobial effect of extract formulae of its active metabolite like flavonoids, phenols, tannins, saponines, alkaloids and other phytochemical compounds. that were useful to over come other symptoms that act as stress for bacterial infection cause diarrhea, colic, pains inflammation, GIT, irritation as well as the antibacterial effect that shared with ciprofloxacin effect. (15).

References


