STUDY OF THE ACTION OF CYPERUS ROTUNDUS ROOT DECOCTION ON THE ADHERENCE AND ENTEROTOXIN PRODUCTION OF DIARRHOEAGENIC ESCHERICHIA COLI

Diarrhoeal diseases are a major global public health problem. Although diarrhoea may be the outcome of several reasons, episodes of infectious diarrhoea due to bacteria, protozoa or viruses are common and widespread. The diarrhoeagenic organisms are versatile in their pathogenesis and well equipped with phenotypic and genotypic virulent features leading to the different forms of diarrhoea. The versatility of diarrhoeal illness is exemplified in the classification of the genus *Escherichia coli* into five categories of diarrhoeagenic *E.coli* (for a detailed review see Nataro and Kaper1) viz. enteropathogenic (EPEC), enteroadherant (EAEC), enteroinvasive (EIEC), enterotoxigenic (ETEC) and enterohaemorrhagic (EHEC). We have studied the action of a decoction of *Cyperus rotundus* (Linn.) (Cyperaceae), Musta known to have antidiarhoeal activity2 on adherence and enterotoxin production of 2 groups of *E.coli* viz. EPEC and ETEC.

Root bulbs of *C. rotundus* were collected by Megha herbal products from Dhamtari district of Madhya Pradesh, India in April 1996 and authenticated by Vaidya D.S Antarkar. These were air dried and stored at room temperature.

For each experiment, a decoction was prepared by boiling 1gm of plant material in 16 ml distilled water till the volume was reduced to 4 ml. The decoction was then centrifuged at 2,500 rpm for 10 minutes and filtered through a membrane of 0.22 u pore size before use.

A 1:2, 1:10, 1:100 and 1:1000 (v/v) of the decoction in appropriate media were tested in each assay.

The four strains of *E. coli* used were:

a) EPEC B-170 (0111:NH) and
b) EPEC E-2348 (0127:H6) - exhibiting localised adherence on HEp-2 cell line.
c) ETEC TX1 (078:H12) - producing heat stable toxin.
d) ETEC B831-2 (unknown) - producing heat labile toxin.

**HEp-2 adherence assay** for EPEC strains B-170 and E-2348: A log phase culture of *E.coli* (5 x 10<sup>8</sup>/ml) was incubated on the HEp-2 cell line on glass coverslips in presence of 1:2, 1:10, 1:100 and 1:1000 of the decoction in Dulbecco’s Modified Eagle Medium (DMEM-Gibco) and in DMEM alone (control) for 3 hours at 37°C. Following fixation and staining, two hundred healthy cells per coverslip were quantitated microscopically for adherence (having >5 *E.coli*). Percentage HEp-2 cells showing adherence and the average number of *E.coli* adhering/ cell was quantitated microscopically for adherence (having >5 *E.coli*).

**Table 1.** Effect of *Cyperus rotundus* (C.r) on the enterotoxin production of ETEC. Incubation of *E.coli* B831-2 (for labile toxin-GM1 ELISA) and TX1 (for stable toxin-suckling mouse assay) was carried out in Casein hydrolysate yeast extract broth (CAYE) alone and in presence of different dilutions of C.r in CAYE.

<table>
<thead>
<tr>
<th>Incubation of <em>E.coli</em> in CAYE (control)</th>
<th>Labile toxin (O.D at 492 nm)</th>
<th>Stable toxin (gut wt/carcass wt)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>24 hours</td>
<td>72 hours</td>
</tr>
<tr>
<td>CAYE (control)</td>
<td>1.09 ± 0.37</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>CAYE + 1:2 C.r</td>
<td>0.43 ± 0.12*</td>
<td>0.49 ± 0.1*</td>
</tr>
<tr>
<td>CAYE + 1:10 C.r</td>
<td>0.76 ± 0.32</td>
<td>0.93 ± 0.17</td>
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<tr>
<td>CAYE + 1:100 C.r</td>
<td>0.32 ± 0.44</td>
<td>0.82 ± 0.12*</td>
</tr>
<tr>
<td>CAYE + 1:1000 C.r</td>
<td>1.18 ± 0.23</td>
<td>1.09 ± 0.09</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD of three individual experiments.

*P <0.05 when compared to respective control.
determined. In addition to this the formation of characteristic EPEC micro-colonies on HEp-2 in the absence and presence of the decoction was also noted.

**Ganglioside monosialic acid enzyme linked immunosorbsent assay (GM-1 ELISA)** for labile toxin: *E. coli* B831-2 was incubated in casein hydrolysate yeast extract broth (CAYE, Himedia) alone (control) and in the presence of 1:2, 1:10, 1:100 and 1:1000 of the decoction in CAYE. Following a 24 and 72 hour incubation at 37°C, the bacterial sonicate (50 ng/ ml protein) was added to wells of an ELISA plate precoated with GM-1 (1.5 μg/ ml). A 1:200 and a 1:300 dilution of the primary antibody (anti-choler toxin) and the secondary antibody (peroxidase labelled swine anti-rabbit Ig) respectively were used. The reaction was developed using o-phenylenediamine (OPD) and the intensity of the colour developed read at 492 nm in a ELISA reader.

**Suckling mouse assay** for the stable toxin: The culture supernatants of *E. coli* TX1 grown in the presence of 1:2, 1:10, 1:100 and 1:1000 of the decoction in CAYE and in CAYE alone (control) for 24 hours were injected intragastrically for 3 hours at room temperature in 2-3 days old Swiss white suckling mice. Thereafter the pups were sacrificed and the ratio of gut weight to the remaining carcass weight calculated.

The results were analysed using the two tailed ‘t’ test. P <0.05 was considered significant.

*C. rotundus* decoction did not affect adherence (data not shown).

A significant inhibition in labile toxin production was noted at 24 hours at a 1:2 dilution and at 72 hours at 1:2 and 1:100 dilution (Table 1). Stable toxin was inhibited at 1:10, 1:100 and 1:1000 dilutions, maximum inhibition seen at 1:1000. Interestingly, an inverse correlation was observed between the stable toxin production and the concentration of the decoction (Table 1).

Most of the studies with antidiarrhoeal plants have concentrated on antimicrobial profile and the effect on the gastrointestinal motility. Here divergent bioassays targeting the important stages in the pathogenesis of bacterial diarrhoea have been used. *C. rotundus* selectively affected bacterial toxin production without affecting adherence or killing (data not shown). Hence its effectiveness would be most visible against ETEC strains.

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**REFERENCES**


