The antidiarrhoeal activity of the ethanolic extract of *Cissampelos pareira* (*Menispermaceae*) roots was assessed on experimental animals. The hydroethanolic extract (25–100 mg dry extract kg⁻¹ body mass, p.o.) exhibited a dose dependent decrease in the total number of faecal droppings (control 65, reduced to 26–46) and 29.2–60.0% inhibition in castor oil-induced diarrhoea. Further, *C. pareira* produced a significant (*p < 0.01*) and dose dependent reduction in intestinal fluids accumulation (26.0–59.0%). The extract showed a greater inhibitory effect on the concentration of Na⁺ (20.0 and 34.5%) than on the concentration of K⁺ (6.7 and 9.4%). The extract also reduced dose dependently the gastrointestinal transit from 46.4 and 38.7%, equivalent to 53.6 and 61.3%. However, *C. pareira* significantly reduced the lipid peroxidation and inhibited the decrease in antioxidant enzyme levels (superoxide dismutase and catalase) on prior administration to castor oil-induced fluid accumulation. The extract of *C. pareira* had no effect on normal defecation at 25 mg kg⁻¹ in mice. However, 50 and 100 mg kg⁻¹ inhibited defecation by 100% in the initial 2 h and the activity was reduced to 40.0 and 73.0%, respectively, in the third hour. 

**Keywords:** antidiarrhoeal, *Cissampelos pareira* extract, catalase, superoxide dismutase
The roots possess adstringent, mildly tonic, diuretic, stomachic, antilithic, analgesic, antipyretic activities and are prescribed for treating cough, dyspepsia, dropsy, urino-genital troubles such as prolapsus uteri, cystitis, haemorrhage, menorrhagia and calcular nephritis (3). The root and leaves contain several alkaloids and essential oil (0.2%) (2). The methiodide and methchloride derivatives of alkaloid hayatine were reported to be potent neuromuscular blocking agents that lower blood pressure (2). In ethnomedicine, the plant is also mentioned for its antidiarrhoeal properties (4), but to our knowledge there is no scientific report on the antidiarrhoeal activity. The aim of the present paper is to justify the traditional claims by studying the antidiarrhoeal activity with the help of various validated models.

EXPERIMENTAL METHODS

Plant material

The roots of *C. pareira* (*Menispermaceae*) were collected in the botanical garden of the National Botanical Research Institute, Lucknow, India, in September 2002. The plant material was identified and authenticated taxonomically at the National Botanical Research Institute, Lucknow, India. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Extract preparation

Fresh roots of *C. pareira* were washed with distilled water to remove dirt and soil, and were shade dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted thrice with ethanol (50%, V/V). The extracts were filtered, pooled and concentrated at reduced temperature (–5 °C) on a rotary evaporator (Büchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) under high vacuum (1.75 × 10^4 Pa) and at a temperature of –40 ± 2 °C (yield 3.4%, m/m). The drug extract was suspended in doubly distilled water containing carboxymethyl cellulose (CMC 1%, m/V). A standard orogastric cannula was used for oral drug administration.

Animals

Sprague-Dawley rats (140–160 g) and albino mice (18–24 g) of either sex were purchased from the animal house of the Central Drug Research Institute (Lucknow, India). They were kept in the departmental animal house in a well cross-ventilated room at 27 ± 2 °C, and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. Animals were provided with the standard rodent pellet diet (Amrut, India) and the food was withdrawn 24 h before the experiment but water was allowed *ad libitum*. All the experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (5).
Evaluation of the effect on normal defecation

Five groups of six mice each were placed individually in separate cages with filter papers at the bottom. The doses of extracts (25, 50 and 100 mg dry extract kg\(^{-1}\) body mass) were administered orally to different groups. The nonspecific antidiarrhoeal reference drug diphenoxylate HCl (5.0 mg kg\(^{-1}\), p.o.) and 1% CMC (10 mg kg\(^{-1}\), p.o.) were administered to two groups and they later served as controls (6). The total number of faecal droppings in each group was assessed every hour for the next 4 h. Percent reduction in the total number of faeces in the treated groups was obtained by comparison with control animals.

Castor oil-induced diarrhoea

The method of Awouters et al. (7), modified by Nwodo and Alumanah (8), was used. Briefly, rats fasted for 24 h were randomly allocated to five groups of six animals each. Group I received 1% CMC (10 mL kg\(^{-1}\), p.o.), groups II, III and IV received orally the drug extract (25, 50 and 100 mg kg\(^{-1}\)), respectively. Group V was given diphenoxylate HCl (5.0 mg kg\(^{-1}\), p.o.) in suspension. After 60 min, each animal was given 2 mL of castor oil with an orogastric cannula, and was placed in a separate cage and observed for 4 h. Transparent plastic dishes were placed beneath each cage and the characteristic diarrhoeal droppings were recorded.

Castor oil-induced fluid accumulation and Na\(^+\) and K\(^+\) secretion

This was determined according to the method of Robert et al. (9), modified by Di Carlo et al. (10). The rats fasted for 24 h but with free access to water were randomized and allocated to four groups of six rats each. Group I (control) was administered 1% CMC (10 mL kg\(^{-1}\), p.o.), group II was administered castor oil only (2 mL), groups III and IV were administered 50 and 100 mg kg\(^{-1}\) of C. pareira extract, respectively, 1 h prior to castor oil administration. After 30 min, the rats were killed by cervical dislocation and exsanguinated; the small intestine was ligated both at pyloric sphincter and at the ileo-caecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents was recorded. The fluid samples were analyzed for Na\(^+\) and K\(^+\) concentrations using flame photometer (Elico® CL361, India).

Small intestinal transit

Animals were divided into four groups of six rats each and each animal was given orally 1 mL of charcoal meal (5% activated charcoal suspended in 1% CMC) 60 min after an oral dose of drugs or vehicle. Group I was administered with 1% CMC (10 mL kg\(^{-1}\)) and animals in groups II and III received extracted drug (50 and 100 mg kg\(^{-1}\)). Group IV received atropine sulfate (0.1 mg kg\(^{-1}\), i.p.) as standard drug. After 30 min, animals were killed by cervical dislocation and the intestine was removed without stretching and placed lengthwise on moist filter paper. The length of the intestine (pyloric sphincter to caecum) and the distance travelled by the charcoal as a percentage of that length were eval-
uated for each animal, and group means were compared and expressed as percentage inhibition (11).

**Estimation of free radical generation**

The mucosal scraping of the small intestine of the rat was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 seconds. The homogenate was centrifuged at 800xg for 10 min, followed by centrifugation of the supernatant at 12,000xg for 15 min to get the mitochondrial fraction used for the following estimations (12). The levels of lipid peroxidase (LPO) (13) along with the activities of enzymes such as superoxide dismutase (SOD) (14) and catalase (CAT) (15) were estimated.

**General gross behaviour and acute toxicity studies**

Different doses (25–2000 mg kg⁻¹, p.o.) of the C. pareira extract were administered to groups of 10 mice for each dose, while one group of the same number of mice served as control. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behaviour changes, including the general motor activity, wri-thing, convulsions, response to tail pinching, gnawing, piloerection, pupil size, faecal output and feeding behaviour, and further up to 72 h for mortality. Acute LD₅₀ (50% lethal dose) values in mice were calculated by the method of Lorke (16).

**Statistical analysis**

All the data were presented as the mean ± standard error of the mean and analyzed by the Wilcoxon Sum Rank Test (17) followed by unpaired Student’s t-test for the possible significant interrelation between the various groups.

**RESULTS AND DISCUSSION**

The extract of C. pareira roots of 25 mg kg⁻¹ had no effect, 50 and 100 mg kg⁻¹ doses inhibited defecation by 100% in the initial 2 h compared to normal defecation in mice. The activity was reduced to 40.0% and 73.0%, respectively, at the higher doses in the third hour. In the present investigation, the ethanolic extract of C. pareira roots showed dose dependent antidiarrhoeal activity in various validated models in rats. Castor oil produced characteristic semisolid diarrhoea droppings in all animals of the control group. The effect of the C. pareira root extract at the dose of 25–100 mg kg⁻¹ caused a dose dependent decrease in the total faecal matter (29.2 and 60.0%). Diphenoxylate HCl, a standard antidiarrhoeal drug, inhibited the diarrhoea by 70.8% (Table I). The action of castor oil as diarrhoea inductors has been largely studied and it is known that its most active component is the ricinoleic acid, which produces an irritating activity in the small intestine (7, 8). Prostaglandins contribute to the patho-physiological functions of the gastrointestinal tract and act on the local electrical and mechanical activities of the ileal circular muscles (18). Treatment with the C. pareira extract (50 and 100 mg kg⁻¹) produced a significant and dose-dependent reduction in the intestinal fluid accumulation by 26.0%
to 59.0%. Na⁺ and K⁺ concentrations in the intestinal fluids after castor oil-induced fluid accumulation in rats revealed that C. pareira had a greater inhibitory effect on Na⁺ levels than on K⁺ concentrations (Table II). The physiological K⁺ concentration in the intestinal fluid of 5.1 ± 0.4 mmol L⁻¹ was not significantly different from the value of 6.4 ± 0.7 mmol L⁻¹ obtained after the castor oil administration. On the other hand, Na⁺ concentration in the castor oil-induced intestinal fluid was 158.7 mmol L⁻¹ and the value was reduced significantly (p < 0.05) and dose dependently (50 and 100 mg kg⁻¹); the percentage inhibition was 20.0 and 34.5% in Na⁺ and 6.3 and 9.4% in K⁺, respectively (Table II).

Castor oil increases the peristaltic activity and produces changes in the intestinal mucosal membrane permeability to electrolytes and water (19). Membrane bound enzyme Na⁺- and K⁺-ATPase has been related to sodium and potassium transport in the intestine (20). When a decrease in Na⁺ and K⁺-ATPase in diarrhoeal conditions relates to an interruption in the normal water and electrolyte absorption, diarrhoea results. Therefore, the decrease of water together with Na⁺ accumulation might affect the activity of Na⁺ and K⁺-ATPase (21). The stimulated fluid, Na⁺ and K⁺ secretion induced by the castor oil were inhibited by the C. pareira extract in a dose related manner. The effect of C. pareira on castor oil stimulated gastrointestinal transit was also significant and dose dependent. The reduction in the gastrointestinal transit of 53.6 to 61.3% was comparable to the standard antimuscarinic drug atropine sulphate (68.3%) (Table III). The highest inhi-

### Table I. Effect of C. pareira extract on 2 mL castor oil-induced diarrhoea in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Total no. of faecal droppings</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1%, 10 mL kg⁻¹ CMC)</td>
<td>–</td>
<td>65</td>
<td>–</td>
</tr>
<tr>
<td>C. pareira</td>
<td>25</td>
<td>46</td>
<td>29.2</td>
</tr>
<tr>
<td>C. pareira</td>
<td>50</td>
<td>38</td>
<td>41.5</td>
</tr>
<tr>
<td>C. pareira</td>
<td>100</td>
<td>26</td>
<td>60.0</td>
</tr>
<tr>
<td>Diphenoxylate HCl</td>
<td>5.0</td>
<td>19</td>
<td>70.8</td>
</tr>
</tbody>
</table>

* Values are presented as mean values of six rats in each group.

### Table II. Effect of C. pareira extract on castor oil-induced fluid accumulation in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Intestinal fluid (mL)</th>
<th>Na⁺ (mmol L⁻¹)</th>
<th>K⁺ (mmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1%, 10 mL kg⁻¹ CMC)</td>
<td>–</td>
<td>1.0 ± 0.04</td>
<td>120.5 ± 11.9</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Castor oil (2 mL)</td>
<td>–</td>
<td>3.9 ± 0.3b</td>
<td>158.7 ± 9.5c</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>C. pareira + castor oil</td>
<td>50</td>
<td>2.9 ± 0.2d</td>
<td>127.0 ± 9.7d</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>C. pareira + castor oil</td>
<td>100</td>
<td>1.6 ± 0.05e</td>
<td>103.9 ± 7.7e</td>
<td>5.8 ± 0.3</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM for six rats. Statistically significant difference in respect to the control: b p < 0.001, c p < 0.05 and to the castor oil group: d p < 0.05, e p < 0.001, f p < 0.01.
bition of gut motility was however obtained with the antimuscarinic drug atropine sulphate (7). These observations demonstrate the inhibitory effect of the *C. pareira* root extract on the castor oil-induced diarrhoea, intraluminal fluid accumulation and peristaltic activity in small intestine.

In support of this conclusion, several reports from different laboratories have demonstrated that reactive oxygen species (ROS) are involved in the pathogenesis of stress induced mucosal injury in rats (22, 23). Superoxide (O$_2^-$), hydrogen peroxidation (H$_2$O$_2$) and hydroxyl radical (OH-) are important ROS, which cause mucosal tissue damage, and the lipid peroxidation product malondialdehyde is an indicator of ROS generation in the tissue (22). A summary of Table IV indicates that the lipid peroxidase (LPO) was significantly increased in castor oil-induced diarrhoea in rats. Pretreatment with *C. pareira* (50 and 100 mg kg$^{-1}$) reduced the LPO significantly. These radicals appear to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing of cellular proteins (23). A detailed study was made to see the effect of *C.*

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**Table III. Effect of *C. pareira* extract on charcoal meal-stimulated gastrointestinal transit in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg$^{-1}$)</th>
<th>Mean intestinal length (cm)$^b$</th>
<th>Mean distance travelled by charcoal (cm)$^b$</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1%, 10 mL kg$^{-1}$ CMC) + charcoal meal</td>
<td>–</td>
<td>84.6 ± 4.3</td>
<td>62.5 ± 5.1</td>
<td>26.1</td>
</tr>
<tr>
<td><em>C. pareira</em> + charcoal meal</td>
<td>50</td>
<td>89.3 ± 3.8</td>
<td>41.4 ± 3.8$^b$</td>
<td>53.6</td>
</tr>
<tr>
<td><em>C. pareira</em> + charcoal meal</td>
<td>100</td>
<td>81.9 ± 4.2</td>
<td>31.7 ± 4.1$^c$</td>
<td>61.3</td>
</tr>
<tr>
<td>Atropine sulphate + charcoal meal</td>
<td>0.1</td>
<td>83.7 ± 5.2</td>
<td>26.5 ± 2.6$^c$</td>
<td>68.3</td>
</tr>
</tbody>
</table>

$a$ Values are mean ± SEM for six rats.
$b$ Statistically significant difference in respect to the control: $^b p < 0.01$, $^c p < 0.001$.

detailed study was made to see the effect of *C.*

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**Table IV. Effect of *C. pareira* on mucosal lipid peroxidase (LPO), superoxide dismutase (SOD) and catalase (CAT) levels in castor oil-induced fluid accumulation in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg$^{-1}$)</th>
<th>LPO (nmol mg$^{-1}$ protein)</th>
<th>SOD (units mg$^{-1}$ protein)</th>
<th>CAT (units mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1%, 10 mL kg$^{-1}$ CMC)</td>
<td>–</td>
<td>0.28 ± 0.01</td>
<td>134.5 ± 10.5</td>
<td>50.5 ± 3.8</td>
</tr>
<tr>
<td>Castor oil (2 mL)</td>
<td>–</td>
<td>0.54 ± 0.02$^b$</td>
<td>206.4 ± 11.8$^c$</td>
<td>28.9 ± 2.6$^b$</td>
</tr>
<tr>
<td><em>C. pareira</em> + castor oil (2 mL)</td>
<td>50</td>
<td>0.46 ± 0.02$^d$</td>
<td>181.0 ± 10.1</td>
<td>38.4 ± 3.1$^d$</td>
</tr>
<tr>
<td><em>C. pareira</em> + castor oil (2 mL)</td>
<td>100</td>
<td>0.37 ± 0.02$^e$</td>
<td>157.1 ± 9.6$^d$</td>
<td>45.5 ± 3.5$^f$</td>
</tr>
</tbody>
</table>

$a$ Values are mean ± SEM for six rats.
Statistically significant difference compound to the control: $^b p < 0.001$, $^c p < 0.01$
and castor oil group: $^d p < 0.05$, $^e p < 0.001$, $^f p < 0.01$. 

In support of this conclusion, several reports from different laboratories have demonstrated that reactive oxygen species (ROS) are involved in the pathogenesis of stress induced mucosal injury in rats (22, 23). Superoxide (O$_2^-$), hydrogen peroxidation (H$_2$O$_2$) and hydroxyl radical (OH-) are important ROS, which cause mucosal tissue damage, and the lipid peroxidation product malondialdehyde is an indicator of ROS generation in the tissue (22). A summary of Table IV indicates that the lipid peroxidase (LPO) was significantly increased in castor oil-induced diarrhoea in rats. Pretreatment with *C. pareira* (50 and 100 mg kg$^{-1}$) reduced the LPO significantly. These radicals appear to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing of cellular proteins (23). A detailed study was made to see the effect of *C.*
pareira on the enzyme antioxidant (superoxide dismutase and catalase) levels in castor oil-induced fluid accumulation in rats. Our studies have accredited an increase in the level of mitochondrial superoxide dismutase (SOD) activity, which correlated well with the increase in castor oil-induced fluid accumulation in rats. The increased SOD level indicates increased production of O$_2^-$ within the tissue since an elevated O$_2^-$ level is thought to increase the concentration of cellular SOD (24, 25). C. pareira significantly and dose dependently reduced the LPO and SOD levels and increased the catalase activity compound to the castor oil-induced groups.

Up to 2 g kg$^{-1}$ body mass of C. pareira extract given orally showed no gross evidence of any abnormalities or mortality in mice up to the end of the observation period. The C. pareira drug extract caused no abnormality or death during the course of the treatment.

CONCLUSIONS

The results of the present study justify the traditional claims of C. pareira extract being an antidiarrhoeal drug. Moreover, the active constituents responsible for the antidiarrhoeal activity remain to be identified. However, a detailed study is needed of the treatment of bacterial and viral diarrhoea.

Acknowledgements. – The authors thank Dr. M. P. Dubey, Retired Scientist and Head, Department of Pharmacology, Central Drug Research Institute, Lucknow, India, for his advice and critical approach during the study. The authors are also thankful to Mr. Mithilesh Kumar Singh for technical help and Dr. R. L. S. Sikarwar and Dr. Vivek, NBRI, Lucknow, for taxonomical identification of the plant materials.

REFERENCES

U radu je ispitivano antidijaroičko djelovanje etanolnog ekstrakta korijena biljke Cissampelos pareira (Menispermaceae) na štakorima i miševima. Peroralna primjena ekstrakta u dozi 25–100 mg kg⁻¹ izazivala je o dozi ovisno smanjenje broja fekalija (26 i 46 u odnosu na 65 u kontrolnoj skupini) i 29,2–60,0% inhibicije dijareje uzrokovane ricinusovim uljem. Nadalje, Cissampelos pareira je urokovala značajnu (p < 0,01) i o dozi ovisnu inhibiciju nakupljanja intestinalne tekućine (26,0–59,0%). Inhibitorni učinak ekstrakta na koncentraciju Na⁺ (20,0 i 34,5%) bio je veći nego na koncentraciju K⁺ (6,7 i 9,4%). Osim toga ekstrakt je reducirao gastrointestinalni tranzit od 46,4 i 38,7%, što je ekvivalentno s 53,6, odnosno 61,3%. Međutim, Cissampelos pareira značajno je smanjila peroksidaciju lipida i inhibirala je smanjenje koncentracije antioksidativnih enzima (superoksid dismutaze i katalaze) ako je primijenjena prije ricinusovog ulja. Ekstrakt biljke Cissampelos pareira nije imao učinak na normalnu defekaciju ako je primijenjen na miševima u dozi 25 mg kg⁻¹. Međutim doza od 50, odnosno 100 mg kg⁻¹ inhibirala je defekaciju 100% u početna dva sata, dok je treći sat smanjila defekaciju za 40,0, odnosno 73,0%.

Ključne riječi: antidijaroič, ekstrakt biljke Cissampelos pareira (Menispermaceae), katalaza, superoksid dismutaza