ANTIDIARRHOEAL ACTIVITY OF Curcuma Neilgherrensis Wt.

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ABSTRACT

Turmeric is one of the wonderful product of Curcuma longa as major spice in Indian food used as antibiotic and other in medicinal formulations. And it is significant in using many traditional and auspicious occasions. The wild turmeric Curcuma neilgherrensis is also used by the local herbalists and ethnic group of seshachalam hill regions as spice ingredient in food preparations and used to cure many health disorders like skin diseases, wounds, ulcers, bone fractures, inflammations, arthritis, cold and cough, diarrhoea, worm infestations, chicken pox and small pox, menstrual disorders and diarrhoea. Rhizome and leaf were pharmacognostically evaluated for the presence of phytoconstituents, antibacterial and antifungal activities and anthelmintic studies, and proved its effective activity. Hence it is interested to evaluate against anti diarrhoeal activity of rhizome methanol and aqueous extracts and also its acute oral toxicity studies to prove as harmless drug on Albino wistar rats. Rhizome methanol extracts at 1000 mg/kg b.wt proved more effective to that of Atropine the standard drug with 70 % antidiarrhoeal activity and 60 % of fluid inhibition than the aqueous extracts may be due to the presence of tannins, terpinoids, glycosides, flavonoids and saponins. And it is proved as the safe drug upto 5000 mg/kg b.wt without any behavioural changes.

KEY WORDS: Albino rats, rhizome methanol extracts, toxicity studies, behavioural changes, safe drug, ethnic group.

INTRODUCTION

Diarrhoeal diseases are a major problem in third world countries and are responsible for the death of millions of people every year.[1] Medicinal plants are promising source of antidiarrhoeal drugs.[2] For this reason, international organizations including the World
Health Organization (WHO) have encouraged studies pertaining to the treatment and prevention of diarrhoeal diseases using traditional medical practices.\(^3-5\)

Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools.\(^6\) According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries affecting mainly the infants and children.\(^7\) It is often caused by enterotoxins which are produced by bacteria such as *Escherichia coli*, *Salmonella typhi*, *S. typhimurium*, *Clostridium difficile*, *C. freundii*, *Aeromonas hydrophila*, *Campylobacter jejuni* and *Vibrio cholera*.\(^8\) Drugs like Diphenoxylate, Loperamide, Diloxanide furoate for protozoal infections induced diarrhoea and dysentry, racecadotril, muscarinic receptor blockers like atropine sulfate etc; are available in the market for treating diarrhoea. But all of the existing drugs lead for adverse effects like the induction of bronchospasm, vomiting by racecadotril; intestinal obstruction and constipation by loperamide.\(^9\)

The herbal medicinal plant *C. neilgherrensis* (Zingiberaceae; Adavi pasupu) commonly has been used for different treatments by the sugalis, Yanadis, irula, yerukala and Nakkala tribes along the sesachalam hill ranges. Leaf and Rhizome paste was applied directly to cure skin diseases, cuts, boils and wounds; decoction of the leaf and rhizome was taken in case of mouth and stomach ulcers; leaf, rhizome and root paste along with white egg, black gram and onion is used to cure bone fractures, inflammations; rhizome along with the egg white, black gram, onion and cow ghee is made in to paste and bandaged around the infected area to cure arthritics (chronic inflammations). A piece of rhizome about 1gm is boiled along with the milk and used in case of cold and cough. Rhizome paste along with coconut/gingelly oil was used in case of burns and also as antiseptic. Tender leaf decoction was taken against worm infestation and diarrhoea. The paste of the rhizome is used in case of small pox and chicken pox. A pinch of rhizome powder is taken by the women with an empty stomach early in the morning to cure menstrual disorders.\(^10\)

*C. neilgherrensis* (Kattumanjal) rhizomes are used to heal foot cracks.\(^11\) Rhizomes are eaten as vegetables; leaf and rhizome used as demulcent, astringent, used against diarrhea and fever; juice from crushed rhizomes rubbed on the body swellings; rhizome paste bound with cloth in case of fractures; whole plant is used for rituals and ceremonials; flowers used in worshipping and also worn by the women. Rhizome used as in case of chronic hepatitis,
antiarthritic, antiseptic and menstrual disorders.\textsuperscript{[12]} Rhizomes used as a spice to flavor the cooked food; also used for the cardiae and abdominal disorders.\textsuperscript{[13]}

\textit{C.neilgherrensis} reported many important secondary metabolites like essential oils, phenols, flavonoids, terpinoids, steroids, tannins, saponins, glycosides, anthocyanidins and alkaloids.\textsuperscript{[10]} methanol and alcohol extracts of leaf and rhizome proved most effective antifungal herbal drug on \textit{Candida albicans} (31.3 mm) and \textit{Aspergillus niger} (29.5 mm) at 10mg/well than the standard drug \textit{Nystatin} with 10.2 and 12.1 mm zone of inhibition with 0.156 and 0.312 mg as minimum fungal inhibitory concentration \textsuperscript{[14]}. It also acts on \textit{Staphylococcus aureus} as the most susceptible with leaf and rhizome alcohol extracts (27.8 mm) followed by \textit{Pseudomonas aeruginosa} (23 mm), \textit{Bacillus subtilis} (22.7 mm) and \textit{E.coli} (22.9mm). MIC of bacteria ranges from 0.078 to 2.5mg.\textsuperscript{[15]}

Anthelmintic activity with alcohol and methanol rhizome extracts at 5 mg with 1:1 ratio of \textit{C.neilgherrensis} and \textit{Zingiber officinale} extracts on earthworms observed the shortest time for the paralysis by 6.9 and 6.4 sec and 15.6 and 16.6 sec for the death of worms compared with \textit{C.neilgherrensis} rhizome alcohol and methanol extracts with 10.2 and 10.3 sec for paralysis and 18.2 and 20.3 sec for death of worms; and also when compare with the standard drug \textit{Albendazole} at 5 mg as 90.9 sec for paralysis and 110.0 sec for death of worms supports its herbal usage against worm infestation and diarrhea.\textsuperscript{[16]} Pharmacognostic analysis of \textit{C.neilgherrensis} showed its close relationship in solubility and in ash content with \textit{C. amada}. Leaf bitter in taste, and presence of trichomes; Rhizome conical in shape and brownish, flavor shows resemblance to that of \textit{C.caesia} wild \textit{Curcuma} shows some with special characters in having fusiform long tuberous roots with secondary branching than other \textit{Curcuma} species. Presence of saponins and calcium oxalate crystals in rhizome also distinct character than other species and it is distributed in a varied habitat among the rocky substratum at an higher altitudinal ranges more than 1000 meters especially in association with an endemic gymnosperm \textit{Cycas beddomei}.\textsuperscript{[17]} \textit{C. neilgherrensis} starchy rhizomes and tender leaf decoctions are used as remedy for microbial infections, inflammations, diarrhoea, gastric and skin disorders by the local herbalists. Hence the crude extracts of the leaf and rhizome are subjected for the bioassaying studies in rats for scientific validation to prove the traditional usage against diarrhoea.
MATERIAL AND METHODS

Collection and identification of Plant material

Plant material *C. neilgherrensis* was collected from Tirumala and Talakona along the Seshachalam Hill Ranges during April – September, 2011, authenticated by Prof. N.Yasodamma and voucher specimens DC 921, DC 922 were prepared and preserved in the herbarium Department of Botany, S.V.University, Tirupati as per the standard method \[18\]. Leaves and rhizomes were collected, thoroughly washed, cut in to pieces and further dried under shade at 28 ± 2 ° C for about 10 days. The dried parts were ground well in to a fine powder in a mixer grinder and sieved to particle size of 50 – 150mm. The powders were stored in a polythene bags at room temperatures.

Extract preparation:

Shade dried leaves and rhizome powders were subjected to soxhlet extraction with Methanol. Simultaneously aqueous extracts also prepared. The above obtained semisolid extracts were preserved in air tight bottles at 4°C in a refrigerator until further use.

Animal selection

For acute toxicity studies and Antidiarrhoeal activity male Wistar albino rats weighing between 150 - 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25±2°C) and maintained on 12 hours light; 12 hours dark cycle. They were fed with *ad libitum*. The experiment was conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical CPCSEA/IAEC/SVU/NY-BK/dt: 19/11/2011).

Acute oral toxicity study: (AOT)

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD-425) guidelines. Wistar rats (n =6) were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 1000, 2000, 3000 and 5000 mg/kg body weight by intragastric tube and animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. Observations were also made on the changes of skin, fur, eyes, behaviour pattern, body weight, food consumption, fluid intake and psycho-motor activities were recorded daily. If mortality was observed in 2-3 animals, then
the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. Mortality was observed and then the LD_{50} was calculated. If no mortality of animals then it is considered to be the safe dose. All the observations were made as per the OECD Guidelines.

**Castor oil induced diarrhoea**

The Antidiarrhoeal activity of leaf, rhizome aqueous and methanol extracts were studied by castor oil induced diarrhoea method.\(^{[19]}\) The male Wistar albino rats weighing 150 - 200 g were fasted for 18 hrs before the test and divided into eleven groups of six rats each were treated as per the following regimen. Aqueous and methanol crude extracts of leaf and rhizome were mixed with vehicle - 1% Tween 80 and used for the study. Group I served as the negative control and received 2 ml of 1% Tween 80 in water / kg b.wt, group II received castor oil 10 ml/kg b.wt, and next nine groups received 10 ml of respective dosages 250, 500 and 1000 mg/kg b.wt. half an hour after castor oil administration. Group III served as the positive control and received the reference drug **Atropine** 3 mg/kg b.wt, group IV and V received methanol leaf extract 250, 500 and 1000 mg/kg b.wt, group VI and VII received aqueous leaf extract 250, 500 and 1000 mg/kg b.wt, group VIII and IX received methanol rhizome extract 250, 500 and 1000 mg/kg b.wt, group X and XI received aqueous rhizome extract 250, 500 and 1000 mg/kg b.wt. were given to the animals in the treatment groups and the animals were placed separately in cages with filter paper, which was changed every hour and the severity of diarrhoea was assessed each hour for 4 hrs. The frequency of defecation and number of diarrhoeal feces excreted in the recorded time were scored and compared with control group. The results were expressed in percentage of inhibition\(^{[20]}\).  

\[ \text{% of Inhibition} = \left( \frac{\text{Control} - \text{Test}}{\text{Control}} \right) \times 100. \]

**Castor oil- induced enteropooling**

Intra-luminal fluid accumulation was determined by the standard method.\(^{[21]}\) Overnight fasted rats were divided eleven groups of six animals each and were treated as per the following regimen. Aqueous and methanol crude extracts of leaf and rhizome were mixed with vehicle-1% Tween 80 and used for the study. Group I served as the negative control and received 2 ml of 1% Tween 80 in water /kg b.wt, group II received castor oil 10 ml/kg b.wt, and the III group served as the positive control and received the reference drug **Atropine** 3 mg/kg b.wt, group IV and V received methanol leaf extract 250, 500 and 1000 mg/kg b.wt, group VI and VII received aqueous leaf extract 250, 500 and 1000 mg/kg b.wt, group VIII and IX received
methanol rhizome extract 250, 500 and 1000 mg/kg b.wt, group X and XI received aqueous rhizome extract 250, 500 and 1000 mg/kg b.wt Castor oil was administered orally after 1 hr of drug administration. Two hours later the rats were sacrificed by cervical dislocation, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

\[
\% \text{ of Intestinal content} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100
\]

**Statistical analysis**

The results were analyzed for statistical significance using One way ANOVA followed by dunnet’s test. The \( p < 0.01 \) was considered significant.

**RESULTS**

**Acute Oral Toxicity**

There is no toxicity symptoms and any behavioral changes were observed up to the dose of 5000 mg/kg b.wt on wistar albino rats. Hence 1/10th dose was taken as effective and sub acute (Therapeutic dose) dose as the cutoff value of LD\(_{50}\) was 500 mg/kg b.wt and upto 1/5th dose 1000 mg/kg b.wt was selected for antidiarrhoeal activity.

**Castor oil induced diarrhoea and enteropooling: (Table: 1; Figure 1a, 1b)**

Leaf methanol extracts at 1000 mg/kg b.wt showed 6.1 dry and 2.1 wet defecations after 4 hours with 65% of diarrhoeal inhibition, where as rhizome methanol extracts 6.3 dry and 1.8 wet defecation with 70% of inhibition to that of the standard drug as 6.4 dry and 2.4 wet defecations with 60% of inhibition. Enteropooling activity also very effectively reduced with rhizome methanol extracts as 1.4 ml and 60% of inhibition and 1.7 ml with 51.4% leaf methanol extracts to that of the control drug *Atropine* with 1.6ml and 54.2% of intestinal fluid inhibition. Aqueous extracts antidiarrhoeal activity was comparatively less than methanol extracts.
Table 1: Effect of Leaf and Rhizome extracts on castor oil induced diarrhoea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) b.wt</th>
<th>Mean dry defecation 4 hours</th>
<th>Mean wet defecation 4 hours</th>
<th>% antidiarrhoeal activity</th>
<th>Fluid Volume (ml)</th>
<th>% Fluid Volume content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>4.24±0.08</td>
<td>0±0.0</td>
<td>-</td>
<td>1.5±0.00</td>
<td>-</td>
</tr>
<tr>
<td>Control (castor oil)</td>
<td>10ml/kg</td>
<td>8.2±0.09</td>
<td>6.0±0.09</td>
<td>-</td>
<td>3.5±0.008</td>
<td>-</td>
</tr>
<tr>
<td>Atropine</td>
<td>3mg</td>
<td>6.4±0.09**</td>
<td>2.4±0.09**</td>
<td>60.0</td>
<td>1.6±0.01**</td>
<td>54.2</td>
</tr>
<tr>
<td>Leaf Aqueous</td>
<td>250</td>
<td>8.8±0.01**</td>
<td>5.0±0.03**</td>
<td>16.6</td>
<td>3.3±0.03**</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.6±0.03**</td>
<td>3.8±0.01**</td>
<td>36.6</td>
<td>2.8±0.01**</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>6.7±0.04**</td>
<td>2.2±0.03**</td>
<td>63.3</td>
<td>2.0±0.03**</td>
<td>42.8</td>
</tr>
<tr>
<td>Leaf Methanol</td>
<td>250</td>
<td>9.0±0.03**</td>
<td>5.2±0.01**</td>
<td>13.3</td>
<td>3.4±0.01*</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8.3±0.03</td>
<td>3.9±0.01**</td>
<td>35.0</td>
<td>2.6±0.01**</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>6.1±0.03**</td>
<td>2.1±0.03**</td>
<td>65.0</td>
<td>1.7±0.03**</td>
<td>51.4</td>
</tr>
<tr>
<td>Rhizome Aqueous</td>
<td>250</td>
<td>9.0±0.01**</td>
<td>5.1±0.03**</td>
<td>15.0</td>
<td>3.2±0.03**</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8.2±0.01</td>
<td>3.8±0.03**</td>
<td>36.6</td>
<td>2.6±0.03**</td>
<td>25.7</td>
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<tr>
<td></td>
<td>1000</td>
<td>6.5±0.01**</td>
<td>2.0±0.03**</td>
<td>66.6</td>
<td>1.7±0.03**</td>
<td>51.4</td>
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<tr>
<td>Rhizome Methanol</td>
<td>250</td>
<td>9.1±0.03**</td>
<td>5.2±0.01**</td>
<td>13.3</td>
<td>3.4±0.01*</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8.2±0.01</td>
<td>3.5±0.01**</td>
<td>41.6</td>
<td>2.5±0.03**</td>
<td>28.5</td>
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<tr>
<td></td>
<td>1000</td>
<td>6.3±0.01**</td>
<td>1.8±0.03**</td>
<td>70.0</td>
<td>1.4±0.03**</td>
<td>60.0</td>
</tr>
</tbody>
</table>

All the data are expressed as mean±SEM: **indicate p<0.01 and* p<0.05 as compared to control group, n=6: (One–way ANOVA followed by Dunnett’s test)

a. Percentage Inhibition of Antidiarrhoeal activity
b. Percentage of Fluid Content

DISCUSSION AND CONCLUSION

Castor oil produces diarrhoea due to its most active metabolite ricinoleic acid by hypersecretory response, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa.[22-23] Castor oil also
stimulates the release of endogenous prostaglandins E and F which cause stomach cramp and diarrhoea due to the effect on the smooth muscle and secretion.\textsuperscript{24}

In the present study rhizome and leaf methanol extracts at 1000 mg/kg b.wt showed effective diarrhoeal activity may be due to the inhibition of electrolite permeability and prostaglandin release in the intestine causes suppression of intestinal fluid accumulation of Na\textsuperscript{+} and K\textsuperscript{+} secretion induced by the Castor oil. Antidiarrhoeal activity of the herbal drugs may be due to the presence of tannins, alkaloids, saponins, flavonoids, sterols and triterpenes.\textsuperscript{25} Antidiarrhoeal activity of \textit{C.neilgherrensis} rhizome and leaf extracts may be due to the presence of terpenoids, steroids, tannins, flavonoids and saponins.\textsuperscript{10} Presence of melilotic acid (rhizome) known for its choleretic and analgesic effects and also diuretic, anti ulcerogenic acts against gastro intestinal disorders, antimicrobial and anti spasmodic.\textsuperscript{26-27} Methanolic rhizome extracts of \textit{Alpinia conchigera} at 250 and 500 mg/kg reduced the induction time of diarrhoea and total weight of the faecal material in mice.\textsuperscript{28} The turmeric antidiarrhoeal activity also reported in \textit{C.longa} aqueous rhizome extract at 200 mg/kg b.wt on both gastro intestinal motility and experimentally induced diarrhoea in mice.\textsuperscript{29} Acetone rhizome extract of \textit{Kaempferia galanga} at 200 mg/kg b.wt with 80 % antidiarrhoeal activity and 77.36 % inhibition of defecation shown in mice.\textsuperscript{30}

CONCLUSION

Methanolic rhizome extracts of \textit{C.neilgherrensis} showed effective antidiarrhoeal activity due to the presence of a wide range of secondary metabolites to that of \textit{Alpinia conchigera}, \textit{C.longa}, \textit{Kaempferia galanga} (Zingiberaceae) and also proved its usage by the local herbalists and tribes. Hence further studies recommended to isolate the respective terpenoids and tannin compounds design the drugs against diarrhoeal activity.

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