Research Article

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ANTIDIARRHOEAL ACTIVITY OF ROOT EXTRACTS OF *RUBIA CORDIFOLIA LINN*

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ABSTRACT

The aim of the present study was is to prepare and analyze various extracts for phyto-constituents and to study the anti-diarrhoeal activity in alcohol and aqueous extracts of Rubia cordifolia (Linn) The whole plant was extracted successively with different solvents (petroleum ether, chloroform, alcohol and distilled water) by soxhlet apparatus and the extracts were analyzed by standard procedures for the phyto-constituents present with them. In acute toxicity studies in mice no mortality was seen even at a dose of 2000 mg/kg so 1/5th, 1/10th and 1/^{20th} of doses was selected for the entire studies. The preliminary phytochemical investigation revealed the presence of different phytochemical constituents like sterols, carbohydrates, tannins, flavonoids and triterpines in alcoholic and aqueous extracts. The two extracts used i.e. alcohol and aqueous extracts have exhibited significant anti-diarrhoeal activity in experimental animal models. Aqueous extract has exhibited better anti-diarrhoeal activity than the alcoholic extract in castor oil and magnesium sulphate induced diarrhoea. In charcoal meal test alcoholic extract is more potent than aqueous extract.

KEY WORDS: Rubia Cordifolia, Antidiarrhoeal, Sterols, Carbohydrates, Tannins, Flavonoids, Triterpines

INTRODUCTION:

Rubia cordifolia (Linn) is a less explored plant for its varying activities hence an effort has been made here to investigate the potential uses of this plant. Tannins[1], flavonoids[2], reducing sugars, sterols and triterpines[3] are reported for their anti-diarrhoeal activity. Diarrhoea caused by intestinal pathogens is a global health concern and one of the primary causes of infant mortality especially in developing countries[4]. According to the World Health Report[5], diarrhoea is the cause of 3.3% of all deaths. In young children, it can lead to death due to dehydration and in survivors impaired growth and malnutrition. In adults, while the impact is less severe, it nevertheless can lead to nutritional deficiencies especially in the case of persistent diarrhoea.

A majority of diarrhoeal cases are due to bacterial enteropathogens, diarrhoeagenic Escherichia coli being the most common cause in developing countries[6]. The two main bacterial groups causing traveller's diarrhoea are diarrhoeagenic E. coli, mainly enterotoxigenic and enteroaggregative[7] and invasive bacterial pathogens like Shigella, Campylobacter and Salmonella[8]. Amongst the viral agents, rotavirus is the most common[9]. Oral rehydration therapy (ORT) has been the key strategy for effective case management and has been instrumental in reducing diarrhea-related deaths[10]. However, patients often express their dissatisfaction with ORT since it does not decrease the frequency of stools. Moreover, there is an increasing threat of drug resistance to antibiotics[11]. Thus an important niche exists for development of cost-effective alternative approaches for the treatment of diarrhoea which can possibly be filled by the use of tested and well standardized medicinal plants.

The virulent features of diarrhoeal organisms have been studied in great detail and the pathogenesis of infectious diarrhoea is largely well understood[12–14]. However, most of the studies reporting antidiarrhoeal activity of medicinal plants overlook the pathogenesis of infectious diarrhoea and evaluate their efficacy on the basis of antimicrobial action alone. Targeting the virulence parameters as an alternative approach to define the divergent mechanism(s) of antidiarrhoeal activity of medicinal plants, especially in the absence of antimicrobial activity, has been previously demonstrated[15–18].

METHODS:

Plant materials:

The roots were purchased from Yucca enterprises, Mumbai, India and authenticated Pharmacognostically by Dr. K.Madhava Chetty, Dept. of Botany, SV University, Tirupathi, A.P.

Extract preparation:

Roots of RC were shade dried, ground to fine powder and subjected to successive extraction by using different solvents in the increasing order of polarity (pet ether, chloroform, alcohol and water) in soxhlet apparatus, until the eluent became colorless and then macerated with chloroform water. The extracts were dried under reduced pressure. From this extract, on evaporation of water a brick red substance was obtained which was kept in air tight container until use[19].

Preliminary phytochemical investigation:

All the extracts were subjected to phytochemical test. All the extracts were subjected to preliminary phytochemical tests. The entire test reveal that the plant posses steroids, glycosides, triterpenoids, tannins. Mucilage and flavonoids.

Since alcoholic and aqueous extracts has shown better yield and chemical constituents, this extracts were selected for further study.

Animals:

Adult Albino rats (180-220 g) and albino mice (18-22 g) were used in this study. They were housed in well-ventilated rooms under standard conditions (23 ± 2^{o} C, humidity 65-70 %, 12 h light / dark cycle), fed with a synthetic standard diet from Amrut laboratories & Pranav Agro industries Ltd. Sangli, and with tap water *ad libitum*. Permission was obtained from institutional ethical committee for the use of animals in experiments.

Acute toxicity studies:

The acute toxicity of alcoholic and aqueous extracts of roots of Rubia cordifolia Linn was determined in albino mice of either sex weighing between 18-22 gms those maintained under standard husbandry conditions.

The animals were fasted 3 hrs prior to the experiment according to up and down procedure (OECD guideline no. 425) method of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (short term) toxicity. Based on the drug short-term profile the dose of the next animals were determined as per as OECD guideline 425. All the animals were

observed for long term toxicity (7 days) and then $1/5^{th}$, $1/10^{th}$, $1/25^{th}$, $1/50^{th}$ of the lethal dose was taken as effective dose ED_{50}[20, 21].

Castor oil induced diarrhea: [22]

The method described by Awouters F. et.al, was followed here with some modifications. In he present study albino rats of the either sex weighing 160-190 gms were divided into eight groups of each comprising of six animals. They were fasted overnight prior to the test with free access to water all the time.

After 30 minutes each rat received 1 ml of castor oil orally. Each rat was then housed separately in the metabolic cages with special provision to separate urine and faeces. Then the diarrhoeal episode was observed for a period of 4 hours. During this period number and weight of diarrhoeal dropping were noted. Percentage of diarrhoea and percentage of inhibition was calculated by making use of mean weight of the stools. Anti-diarrhoeal activity was determined in terms of percentage of protection. The percentage protection of diarrhoea was calculated by following formula:

Percentage of protection (1%) =A-B/A

Where, **'A'** is the total weight of stools of control animals.

'B' is the total weight of stools of extracts treated animals.

Gastro-intestinal motility test[23]**:**

The method was described by Pazhani G.P.et.al. was used in this study. In the present study albino rats of the either sex weighing 160-200 gms were divided into eight groups of each comprising of six animals. They were fasted for 24 hours prior to the test with free access to water all the time. After 30 minutes, 1ml of charcoal meal (3% deactivated charcoal in 10% normal saline) was administered by oral route to all the animals. 30 minutes after this treatment, all rats were sacrificed and distance traveled by the charcoal meal in each animal's intestine from pylorus to caecum was noted. The distance travelled by the charcoal meal in control and extracts treated groups was compared with that of standard group.

Percentage travelled and percentage of inhibition was calculated by the following formulae,

% traveled= (A/B)*100.

% of inhibition= {(B-A)/B}*100.

Where **'A'** is the distance traveled by the charcoal meal,

'B' is the total length of small intestine.

Magnesiumsulphate induced diarrhea:[24]

Method: The method described by Mujumdar A.M. et.al. was followed here with some modifications. In he present study albino mice of the either sex weighing 20-25 gms were divided into eight groups of each comprising of six animals. They were fasted overnight prior to the test with free access to water all the time.

After 30 minutes each rat received magnesium sulphate orally. Each mice was then housed separately in the metabolic cages with special provision to separate urine and faeces. Then the diarrhoeal episode was observed for a period of 4 hours. During this period number and weight of diarrhoeal dropping were noted. Percentage of diarrhoea and percentage of inhibition was calculated by making use of mean weight of the stools. Anti-diarrhoeal activity was determined in terms of percentage of protection. The percentage protection of diarrhoea was calculated by following formula:

Percentage of protection (1%) = A-B/A

Where **A** is the total weight of stools of control animals.

B is the total weight of stools of extracts treated animals.

RESULTS AND DISCUSSION:

Castor oil induced diarrhoea in rats:

The percentage inhibition of standard drug Loperamide was 94.98% and the percentage inhibition in weight of the stool with alcohol (low, medium, high) and aqueous(low medium, high) extracts were 27.19%, 45.31%, 79.45% and 24.16%, 45.61%, 84.89% respectively compared with control (received castor oil with vehicle only). ANOVA studies have shown that there is a significant difference among the groups. Dunnet "t" test indicates a significant reduction of diarrhoea (stool) in drug treated animals when compared with stool in control animals. The potency of the anti-diarrhoeal activity was in the order Loperamide > aqueous > alcoholic.

Effect on gastro intestinal motility:

The percentage reduction of gastro intestinal motility with alcoholic (low, medium, high) and aqueous (low, medium, high) extracts were 21.94%, 43.70%, 54.07% and 19.51%, 39.75%, 53.58% respectively and the standard, atropine sulphate has shown 66.21% reduction in gastro intestinal motility, compared with motility of control animals. According to the percentage inhibition of charcoal movement in intestine

the order of the potency of anti-diarrhoeal activity was atropine sulphate > alcoholic > aqueous. ANOVA studies have shown that there is a significant difference in movement of charcoal among the drug/extracts treated groups. Dunnet "t" test indicates a significant reduction with intestinal propulsion drug treated animals compared with stool in control animals.

Magnesium sulphate induced diarrhoea in mice:

The percentage inhibition of standard drug Loperamide was 85.83% and the percentage inhibition in weight of the stool with alcohol (low, medium, high) and aqueous(low medium, high) extracts were 24.46%, 58.79%, 69.95% and 33.04%, 63.51%, 75.96% respectively compared with control (received magnesium sulphate with vehicle only). ANOVA studies have shown that there is a significant difference among the groups. Dunnet "t" test indicates a significant reduction of diarrhoea (stool) in drug treated animals when compared with stool in control animals. The potency of the anti-diarrhoeal activity was in the order Loperamide > aqueous > alcoholic. Inhibition of experimental diarrhoea and reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential anti-diarrhoeal agent. Reduction of gastrointestinal motility and secretions are the mechanism by which many anti-diarrhoeal agents can act. It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and induces peristaltic changes in the mucosal fluid and electrolyte transport that result in a hypersecretory response and diarrhoea[25, 26]. The experimental studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil. Ricinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused an increase of the net secretion of the water and electrolytes into the small intestine[27].

The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulates motility and secretion[28]. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea. It has been show that E type of prostaglandins cause diarrhoea in experimental animals as well as in human beings.

There mechanism has been associated with dual effect on gastrointestinal motility as well as on water and electrolyte transport. PGE_2 also inhibits the absorption of glucose,

a major stimulus to intestinal absorption of water and electrolytes[29].These mechanisms of action may be suggested for the two extracts since these have showed marked reduction of the peristaltic movement of the gut, reduction in the fecal output and intestinal secretion. Results support the traditional use of Rubia cordifolia Linn in controlling diarrhoea in animals and humans.

In the anti-diarrhoeal studies, there has been a significant anti-diarrhoeal activity by both the extracts against the incident and severity of diarrhoea produced in experimental animals by three models (i.e. Castor oil induced diarrhoea, Charcoal meal test, and magnesium sulphate induced diarrhoea) which was comparable to the standard drugs. The activity may be due to the presence of tannins, steroids, flavonoids, triterpines and anthracene glycosides. Tannins can evoke an anti-diarrhoeal effect. Since these substances may precipitate proteins of the electrolytes, reduce peristaltic movement and intestinal secretion.

Tannins, reducing sugars, sterols, flavonoids[30], and triterpines[31] are reported for there anti-diarrhoeal activity. The anti-diarrhoeal activity of flavonoids has been described to there ability to inhibit intestinal motility and hydro-electric secretion, which are known to be altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by prostaglandin E_2.

In addition, flavonoids posses antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism. The both extracts (i.e. alcoholic and aqueous) of Rubia cordifolia Linn contains tannins, flavonoids, sterols, and triterpines, these may have contributed for the anti-diarrhoeal activity exhibited.

Fig no. 1: Intestinal charcoal coal movement

Table-1: Anti-diarrhoeal activity of root extracts of Rubia cordifolia Linn on castor oil induced.

Group	Treatment	No.of Animals	Dose (mg/kg)	Mean weight of Stools ± SEM after 4 hours (gms)	Percentage Inhibition
1	Control	6	-	3.316±0.127	-
2	Standard (Loperamide)	6	3.0	0.166±0.166	94.98%
3	RC ALC (Low dose)	6	100	2.416±0.104	27.19%
4	RC ALC (Medium dose)	6	200	1.816±0.110	45.31%
5	RC ALC (High dose)	6	400	0.683±0.168	79.45%
6	RC AQ (Low dose)	6	100	2.516±0.217	24.16%
7	RC AQ (Medium dose)	6	200	1.8±0.115	45.61%
8	RC AQ (High dose)	6	400	0.5±0.134	84.89%

Table No-2: Spasmogenic effect-Charcoal meal test

G	Treatment	Dose (mg/kg)	Mean total length ±SEM(cms)	Mean distance traveled ±SEM(cms)	Mean percentage ±SEM(cms)	Percentage Inhibition (%)
1	Control	-	91.83±0.74	81.16±0.65	88.41±1.05	-
2	Standard(Atropine)	0.1	92.33±0.91	31.16±0.54	34.17±0.77	66.21
3	RCALC(Low dose)	100	92.33±0.918	72±1.26	78.04±1.91	21.94
4	RCALC(Medium dose)	200	91.83±0.749	51.83±0.47	56.44±0.67	43.70
5	RCALC(High dose)	400	91.83±0.749	42.16±0.70	45.91±0.70	54.07
6	RC AQ (Low dose)	100	92.83±1.01	74.66±0.88	80.47±1.32	19.51
7	RCAQ(Medium dose)	200	91.33±0.42	55±1.22	60.23±1.40	39.75
8	RC AQ (High dose)	400	92.66±0.80	43±0.966	46.40±1.01	53.58

Table No-3: Anti-diarrhoeal activity by Magnesium sulphate induced diarrhoea in mice

Group	Treatment	No.of Animals	Dose (mg/kg	Mean weight of Stools ± SEM after 4 hours (gms)	Percentage Inhibition (%)
1	Control	6	-	2.33±0.13	-
2	Standard (Loperamide)	6	3.0	0.33±0.21	85.83
3	RC ALC (Low dose)	6	100	1.76±0.06	24.46
4	RC ALC (Medium dose)	6	200	0.96±0.15	58.79
5	RC ALC (High dose)	6	400	0.7±0.11	69.95
6	RC AQ (Low dose)	6	100	1.56±0.09	33.04
7	RC AQ (Medium dose)	6	200	0.85±0.18	63.51
8	RC AQ (High dose)	6	400	0.56±0.14	75.96

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