

Evaluation of anti-diarrhoeal activity of *Quisqualis indica* L. leaves

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The aim of the present study is to evaluate the anti-diarrhoeal activity of the petroleum ether extract of leaves of *Quisqualis indica* L. against experimentally induced diarrhoea. Petroleum ether extract of the drug were evaluated for anti-diarrhoeal activity, using experimentally induced diarrhoea models: castor-oil induced diarrhoea, and charcoal induced gastrointestinal motility test in albino wistar rats of either sex. The earlier reported preliminary phytochemical screening of petroleum ether extracts revealed the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds. petroleum ether extract of *Quisqualis indica* L. leaves at the doses (100, 200 mg/kg, p.o) were administered through the oral route. The plant extracts exhibited dose dependent anti-diarrhoeal effects in the all treated groups and the results were compared with that of standard drugs (Loperamide (3 mg/kg, p.o) and Atropine sulphate (0.1 mg/kg intraperitoneal), respectively as reference standard drugs. Statistically processed results support the conclusion, that the petroleum ether extracts of *Quisqualis indica* L. leaves (100 mg/kg and 200 mg/kg) possesses dose dependent, significant ($P < 0.05$) antidiarrhoeal activity against experimentally induced diarrhoea.

Keywords: *Quisqualis indica*, Rangoon creeper, Anti-diarrhoeal, Atropine sulphate, Gastrointestinal motility.

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Introduction

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain¹. It is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the pediatric ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries².

To combat the problem of diarrhoea in developing countries, the World Health Organization (WHO) has constituted a diarrhoea disease control program (DDC) which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches³. The synthetic anti-diarrhoeal drugs have various side effects like rashes, fever, joint pain, nausea, vomiting, headache⁴, etc. Antibiotics used as anti-diarrhoeal drugs sometimes provoke adverse effects and

microorganisms tend to develop resistance toward them⁵. Therefore, the search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine.

Quisqualis indica L. (Family-Combretaceae) is a strong climber, ligneous vine that can reach from 2.5 m to up to 8 m. It is commonly known as Rangoon creeper.

It is indigenous in Africa, Indo Malaysian region and cultivated all over India. Flowers numerous, pendent, 7.5 cm long, 3.8 cm wide. At first they are white in color then they become deep red⁶. Pharmacognostic and phytochemical studies have reported the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds in petroleum ether extracts of leaves⁷.

The plant is also used as a cough cure. In Amboina the leaves are given in a compound decoction for flatulent distension of abdomen. In India also it is used for same purpose and seeds are given with honey as an electuary for the expulsion of entozoa in

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children. In Philippines the fruit and seeds are used as a vermifuge⁷ whereas in China, Thailand and Indo-China region seed are used as vermifuge and for rickets in children. In Bangladesh seeds are used for diarrhoea, fever, boils, ulcers and helminthiasis⁸. Leaves contain rutin, trigonelline, L-proline, laspargine and quisqualic acid whereas flower gum contains pelargonidin-3-glucoside. Seed oil contains linoleic, oleic, palmitic, stearic and arachidic acids. Ellagitannins, quisqualin A & B is present in fruits of this plant⁹ and flower contains linalool oxides (furanoid and pyranoid), 2,2,6-trimethyl-6-vinyl-3-oxo-tetrahydropyran, (E, E)- α -farnesene, (Z)-3-hexenyl benzoate and benzyl benzoate¹⁰. Four diphenyl propanoids were isolated from stem bark¹¹. Antimicrobial, antibacterial, antifungal and anticoccidial activities of *Q. indica* have been already reported¹²⁻¹⁴. Methanolic extract of leaves also shown significant antipyretic property¹⁵. No report was found on anti-diarrhoeal property of the leaves of this species, therefore, authors selected this topic for study.

Material and Methods

Plant material

The mature green leaves of *Q. indica* L. were collected in the morning locally from Jaipur District, Rajasthan, India, in the month of August 2009. The plant was identified and authenticated by the Botanist from the Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen (RUBL20663) is deposited in the Department of Botany, University of Rajasthan. After authentication fresh leaves were collected in bulk, dried under shade and pulverized in a grinder. The coarse powder was used for further studies.

Preparation of extract

About 500 g of dry powder was taken in a closed bottle and it was extracted with petroleum ether by using cold maceration process for 7-8 days with occasional shaking. The petroleum ether extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent^{16,17}. For *in-vivo* studies, the concentrated petroleum ether extract of *Quisqualis indica* (PEQI) was administered orally after suspending in 0.5% Tween 80 in distilled water¹⁸. The freshly prepared solution of PEQI was used in each experiment. 100 mg/kg and 200 mg/kg b.w. test doses were selected for the oral acute toxicity study in mice.

Acute toxicity studies

In accordance with the OECD 423 guidelines the acute toxicity test was carried out in mice. All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range up to 2000 mg/kg body weight. Two doses of 100 mg/kg and 200 mg/kg of body weight of petroleum ether extract were selected for further screenings¹⁹.

Animals

Wistar albino rats of either sex, weighing 150-200 g were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions and fed animals with standard diet (Hindustan Liver, Mumbai, India) with *ad libitum*. Pharmacological study was approved by Animal Ethical Committee of School of Pharmacy; Suresh Gyan Vihar University, with CPCSEA Reg no. 1234/a.08/CPCSEA.

Drugs and chemicals

All chemicals and solvents used in the study were of analytical grade. Loperamide was purchased from Ranbaxy laboratory, Gurgaon, Castor oil from Central drug house, New Delhi and Atropine sulphate from Ives Drugs (India) Pvt Ltd, Indore. Chemicals used in the study were procured from Central drug house, Delhi.

Evaluation of anti-diarrhoeal activity

Castor oil induced diarrhoea

The animals were divided randomly into four groups containing six animals each group. All groups received castor oil at a dose of 1 mL/animal orally, 30 min after castor oil administration, Group I received vehicle (0.5% Tween 80 in distilled water). The second group received the reference drug Loperamide (3 mg/kg) orally whereas the third and fourth group received the extract dose PEQI 100 and PEQI 200 mg/kg p.o. After this administration, the animals were placed separately in metabolic cages with filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 6 h. The total number of faeces and diarrhoea faeces excreted and the total weight of faeces were recorded within a period of 24 h and compared with the control group. The total number of diarrhoea faeces of the control group was considered 100%. The result was expressed as a percentage of inhibition of diarrhoea²⁰.

Gastro-intestinal motility test model

Albino rats of either sex weighing 150-200 g were used and they were divided into four groups of six animals each group. The first group (the control group) was orally administered the vehicle (0.5% Tween 80 in distilled water). The second group received the standard drug, atropine sulphate (0.1 mg/kg body weight intraperitoneal). The third and fourth groups received PEQI 100 mg/kg and PEQI 200 mg/kg body weight, respectively. 30 min after administration, each animal was given 1 mL of charcoal meal orally (10% activated charcoal in 5% gum acacia)²¹. Also, 30 min after this administration, each animal was sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of distance moved²².

Statistical analysis

All the values were expressed as Mean \pm SEM and analyzed for ANOVA followed by Dunnett's t-test. Differences between groups were considered significant at $P < 0.05$ levels. The statistical analysis was carried out using Graph Pad Prism 5 software.

Results and Discussion**Castor oil-induced diarrhoea**

In the castor oil-induced diarrhoea experiment, the rats that did not receive the plant extract, showed typical diarrhoea signs such as watery and frequent defecation. The petroleum ether extract of *Q. indica* produced a marked anti-diarrhoeal effect in the rats. Both doses of PEQI significantly ($P < 0.05$) decreased the total number of wet faeces produced by administration of castor oil (15.32 at the dose of 100 mg/kg and 12.45 at the dose of 200 mg/kg) as compared to the castor oil-treated control group (21.16). The percentage of inhibition of castor oil induced diarrhoea in PEQI treated rats was 27.59% and 32.23%, respectively at 100 and 200 mg/kg dose (Table 1 and Fig. 1). The average weight of faeces in the control group was 13.62 g. Treatment with 100 mg and 200 mg of PEQI significantly reduced ($P < 0.05$) the weight of faeces to 10.16 and 9.37 from 13.62 (Table 1 and Fig. 2).

Charcoal-induced gut in transit changes

The administration of PEQI also slowed down the propulsion of charcoal meal through the gastro-intestinal tract when compared to the castor

Table 1—Effect of petroleum ether extract of *Quisqualis indica* L. leaves at the doses (100, 200 mg/kg. p.o) in castor oil-induced diarrhoea in albino rats

Groups	Total no. of faeces	Total no. of Diarrhoeal faeces	% Inhibition	Total weight of faeces	% Inhibition
Group I	25.16 \pm 1.472	21.16 \pm 1.941	-	13.62 \pm 1.062	-
Group II	8.83 \pm 0.894	5.50 \pm 1.049***	74.01	1.45 \pm 0.087***	89.35
Group III	20.12 \pm 1.032	15.32 \pm 1.359**	27.59	10.16 \pm 0.412**	25.40
Group IV	17.05 \pm 0.62	12.45 \pm 0.983***	32.23	9.37 \pm 0.356***	31.21

Values are expressed as mean \pm S.E.M (n = 6) in each group. Statistical analysis ANOVA followed by Dunnett t-test.* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with Group I (control group).

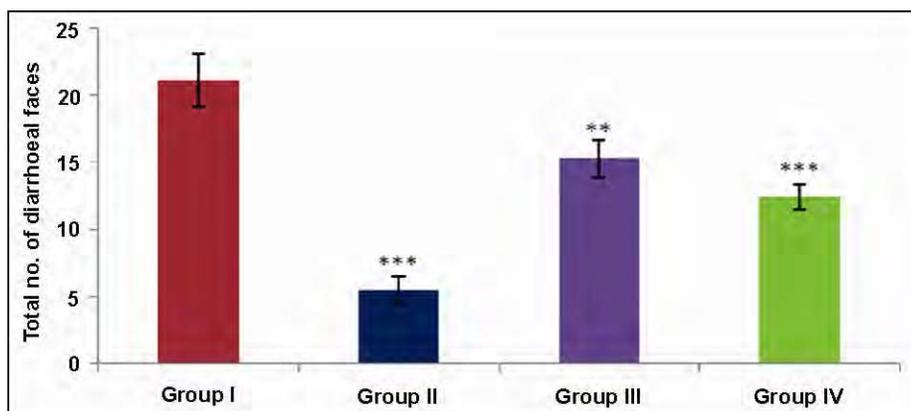


Fig. 1—Total number of diarrhoeal faeces in castor oil-induced diarrhoea in albino rats

oil-treated rats. The percentage of intestinal length travelled by charcoal meal in PEQI pre-treated (100 and 200 mg/kg) and castor oil treated rats was 68.12, 66.34 and 73.25%, respectively. Atropine on its part, produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal meal was 43.82 (Table 2 and Fig. 3).

Castor oil causes diarrhoea due to its active metabolite, ricinolic acid²³, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin²⁴.

In this study, the petroleum ether extract of *Q. indica* exhibited a significant anti-diarrhoeal

Table 2—Effect of petroleum ether extract of *Quisqualis indica* L. leaves at the doses (100, 200 mg/kg.p.o) in charcoal-induced gut in transit changes in albino rats

Groups	Mean length of small intestine	Percentage of distance travelled by charcoal meal	% Inhibition
Group I	101.65±0.98	73.25±1.12	-
Group II	101.60±1.36	43.82±1.13***	56.87
Group III	105.53±1.60	68.12±0.72**	35.44
Group IV	103.38±1.25	66.34±1.33***	35.82

Values are expressed as mean ± S.E.M (n=6) in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P<0.05, **P<0.01, ***P<0.001 as compared with Group I (control group).

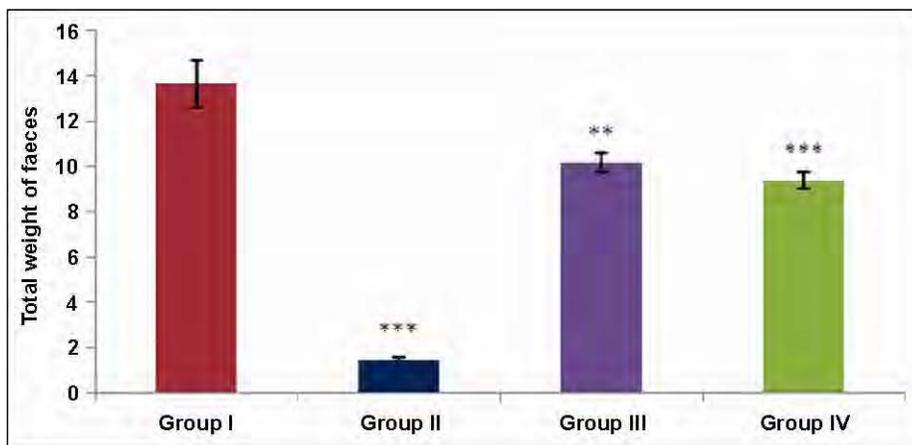


Fig. 2—Total weight of faeces in castor oil-induced diarrhoea in albino rats

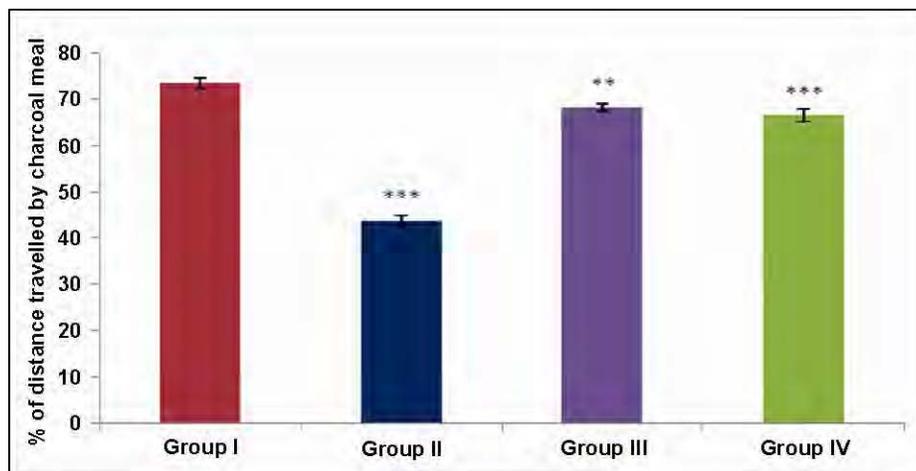


Fig. 3—Percentage of distance travelled by charcoal meal (cm) in albino rats

activity. Its effect depends on the dose. PEQI significantly reduced intestinal transit as observed by the decrease in intestinal motility of charcoal meal. The earlier reported preliminary phytochemical screening of petroleum ether extracts of leaves of *Q. indica* revealed the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds⁷. Also earlier studies have reported that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars^{25, 26}.

Hence, tannins, alkaloids and flavonoid present in this plant may be responsible for the mechanism anti-diarrhoeal activity. The anti-diarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydro electrolytic conditions²⁷. Flavonoids present in the plant extracts are reported to inhibit release of autocoids and prostaglandins, there by may inhibit motility and secretion induced by castor oil²⁸. Tannins and tannic acid present in anti-diarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion²⁹. The presence of these constituents may mediate the anti-diarrhoeal property of the extract. Loperamide, apart from regulating the gastrointestinal tract is also reported to slow down transit in the small intestine, reduce colon flow rate and consequently any effect on colonic motility³⁰. Atropine significantly reduced intestinal transit time. This is possible due to its anticholinergic effect³¹. Furthermore, a decrease in intestinal transit time with atropine could also be due to reduction in gastric emptying³².

Conclusion

The results of this investigation revealed that PEQI contains pharmacologically active substances with anti-diarrhoeal properties. These properties confirm the use of *Q. indica* as an anti-diarrhoeal drug used by traditional healers.

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