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In-Vivo evaluation of anti-diarrhoeal activity of Ethanolic fruit and root extracts of *Carissa carandas* Linn. (Apocynaceae)

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Abstract:
Carissa carandas (L.) belonging to the Apocynaceae family and it is represented about 89 species in India. It is distributed throughout India in dry, sandy and rocky grounds. It naturally grows in the Himalayas at a height of 300 to 1800 meters in the Siwalik Hills from sea level and require fully exposure to sun and unfavorable to humidity. The plants having different chemical constituents include steroids, which may enhance intestinal absorption of Na^+ and water. In the first method, Castor oil induced diarrhoea due to its active metabolite, ricinoleic acid. At doses of EFCC 200 and 400mg/kg, the extracts significantly decreased (*p < 0.05) the total number of wet feces (1.1 ± 0.19 at 200mg/kg and 2.18 ± 0.14** at 400mg/kg) were ERCC (1.19 ± 0.08 at 200mg/kg and 2.42 ± 0.14** at 400mg/kg) when compared to the control (0.87 ± 0.03 at 5ml/kg, p.o). The highest dose of the EFCC and ERCC extracts was similar effects to that of the standard drug, Loperamide (2.88 ± 0.13** at 5mg/kg). During the second methods, propulsion of the charcoal meal through the gastrointestinal tract was decreased significantly (*p < 0.05) from EFCC 43.76 ± 0.40 at 200mg/kg and 54.9 ± 0.50* at 400mg/kg and from ERCC 45.11 ± 0.36 at 200mg/kg and 56.14 ± 0.30* at 400mg/kg, compared to control (36.02 ±0.27 at 5ml/kg, p.o.). Similarly, the highest dose of the EFCC and ERCC extracts was similar effect to that of the standard drug, Atropine sulphate (66.46 ± 0.43* at 5mg/kg). It concludes that the investigation revealed that the ethanolic fruit and root extracts of plants contained pharmacologically active substances which are responsible for anti-diarrhoeal activities.

Keywords: *Carissa carandas*, steroids, ricinoleic acid, anti-diarrhoeal

Full Length Original Research Paper

Introduction

The beneficial role of plant derived product in therapeutic treatment is an important breakthrough in the history of mankind. The Modern approach for the development of plant derived lead molecules involves isolation, Characterization and Pharmacological studies of using high tech instruments (Shailajan et al., 2012, 2013). Apocynaceae family is represented by about 89 species in India. Many of the plants in this family have milky sap and many species are poisonous if ingested and some plants of this family are used therapeutically (Singh et al., 2011). *Carissa carandas* (L.) (F. Apocynaceae) its botanical name has been changed to *Carissa congesta* Wight (syn. *Carissa carandas* Auct., formerly widely shown as *Carissa carandas* Linn.) (Anonymous, 1985; Itankar et al., 2011). Numerous branches, leaves are evergreen, opposite oval or elliptic; the white flowers in terminal corymbose cymes and ellipsoid are tubular with 5 hairy lobes. The Fruit cluster of 3 to 10 is oblong, broad-ovoid or round, ½ to 1 inch (2.5-7.5 cm) long, has fairly thin but tough skin, purplish- red turning dark-purple or nearly black and shiny when ripe, normally 8 seeds berries (Anonymous, 1995; Dipanjali et al., 2014). It grows naturally in the Himalayas at a height of 300 to 1800 meters in the Siwalik Hills from sea level and require fully exposure to sun and unfavorable to humidity (Fartyal et al., 2014). Flowering and fruiting season is on August and September (Sankaran et al., 2006). The plant is native and common throughout much of India, Sri Lanka, Java, Malaysia, Myanmar and Pakistan. The plants having different chemical constituents include steroids, terpenes, tannins, flavonoid, benzenoids, phenylpropanoid, lignans, sesquiterpenes and coumarins (Ya'u et al., 2008).

Diarrhoeal diseases are a major problem in most of the countries and are responsible for the death

of millions of people every year (Shoba et al., 2001). Diarrhoea is an alteration in normal bowel movement and is characterized by an increase in the water content, volume or frequency of stools (Guerrant et al., 2001; Meite et al., 2009). In developing countries, there are large numbers of epidemiological and experimental evidence pertaining to worldwide acute–diarrhoeal disease, which is one of the principal causes of death in the infants, particularly in malnourished and which is of critical importance in developing countries (Syder et al., 1982; Lutterodt et al., 1989). Diarrhoea is one of the leading causes of death among children under five years globally. More than one in 10 child deaths- about 800 000 each year is due to diarrhoea (Nuthakki et al., 2014). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Park et al., 2000; Hardman et al., 1992). Therefore, the search for safe and more effective agents has continued to be an important area of active research.

Figure 1: Photograph of *C. carandas* var. amara Linn. (F. Apocynaceae)

Preparation of extract

The powdered fruits and root of *C. carandas* (500 g) were extracted with ethanol using Soxlet apparatus. The resulting extracts was evaporated in vacuum or Rota vapor and stored in desiccators for future use. The crude extract was dissolved 2% Tween 80 prior to the experiment and used (Maiti et al., 2007).

Animals

Swiss albino rats (150 – 180 g) of either sex were selected for the experiments. Animals were allowed to be acclimatizing for a period of 3 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (6 animals per cage), maintained under standard laboratory conditions (i.e., 12:12 hour light and dark sequence; at an ambient temperature of 25±2°C; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum* (Mitjans et al., 2008; Maiti et al., 2007). The principles of Laboratory Animal Care (NIH, 1985) were followed and the study protocol was approved by Institutional animal ethical committee (CPCSEA Approval No.-1013/PO/c/06/CPCSEA) were maintained throughout the experiment.

Drugs and Chemicals

Atropine sulphate, 5mg/kg, i.p. and Loperamide, 5mg/kg, p.o. (Standard reference anti-diarrhoeal drugs), Castor oil, 1ml per animal, p.o. (as a laxative agent), normal saline solution (0.9% NaCl), charcoal meal (3% activated charcoal in 5% gum acacia) and vehicle (2%v/v Tween 80 in distilled water) were used. Atropine sulphate was procured from Samarth Pharma. Pvt. Ltd., Mumbai, India and Loperamide were purchased from Maiden Pharm. Pvt. Ltd., Delhi, India.

Phytochemical screening

The ethanolic fruit and root extracts was tested and observed different Phytochemical such as carbohydrates, alkaloids, glycosides, anthraquinone glycosides, saponins, terpenoids, tannins, flavonoids and steroids with standard

procedures (Ghani, 2003; Madakatti et al., 2011; Akter et al., 2013).

Preliminary acute toxicity test

It was observed that oral administration of the fruits and root extracts of C. *carandas* to the mice up to 2000 mg/kg neither showed mortality nor any apparent signs of weakness in the animals (OCED Guideline no. 424).

Castor oil-induced Diarrhoea

The rats fasted for 18 hr were randomly allocated to four groups of six animals each. The Group I (Saline 5 ml/kg, p.o.) served as control, Group II received Loperamide (5 mg/kg, p.o.) as Standard drug and Group III and IV were administered orally with test EFCC and ERCC each (200 and 400mg/kg) respectively. After 60 mints each animal was administered with 1 ml of Castor oil by gavages and place in a separate cage, the floor of which was lined by blotting paper. The floor lining was changed every hour. The consistency of the fecal matter and the number of both the wet and the dry diarrhoeal droppings were counted every hour for a period of 4 hours. Animals were placed beneath each cage and the characteristic diarrhoeal dropping was noted. During an observation period, the total number of feces which were excreted by the animals was noted (Rao et al., 2012; Sasidharan et al., 2007)

Table 1: Effect of ethanolic fruits and root extracts of C. *carandas* (EFCC & ERCC) on Castor oil-induced diarrhoea in rats

Treatment	Dose (mg/kg, p.o.)	Mean amount of Faeces/group (gm)
Control (Saline 5ml/kg, p.o.)	-	0.87 ± 0.03
Loperamide	5	2.88 ± 0.13**
EFCC	200	1.1 ± 0.19
EFCC	400	2.18 ± 0.14**
ERCC	200	1.19 ± 0.08
ERCC	400	2.42 ± 0.14**

Values are mean ± S.E.M (n=6); *P < 0.05 **p < 0.001 vs. control; Student's t-test.

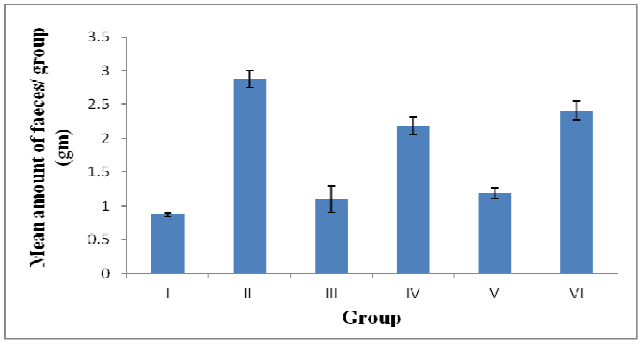

Figure 2: Effect of ethanolic fruits and root extracts of C. *carandas* (EFCC & ERCC) on Castor oil-induced diarrhoea in rats

Effect of Gastrointestinal Motility

The rats were divided into four groups of six animals each and fasted for 18 hr but water was freely provided. The Group I received Atropine sulphate (0.1mg/kg, i.p.) as standard drug, Group II received saline (5ml/kg, p.o.) as control. While, Group III and IV received orally, EFCC and ERCC (200 and 400mg/kg body weight, respectively). 30 mint later, each animal was given 1 ml/rat of charcoal meal (3% deactivated charcoal in 5% gum acacia) via the oral route. All animals were sacrificed 30 mint thereafter and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of distance moved (Meite et al., 2009; Pazhani et al., 2001).

Table 2: Effect of ethanolic fruits and root extracts of C. *carandas* (EFCC & ERCC) on gastrointestinal motility in rats

Treatment	Dose (mg/kg, p.o.)	Distance travelled by charcoal meal (%)
Control (Saline 5ml/kg, p.o.)	-	36.02 ± 0.27
Atropine sulphate	5	66.46 ± 0.43*
EFCC	200	43.76 ± 0.40
EFCC	400	54.9 ± 0.50*
ERCC	200	45.11 ± 0.36
ERCC	400	56.14 ± 0.30*

Values are mean ± S.E.M (n=6); *p < 0.05 vs. control; Student's t-test.

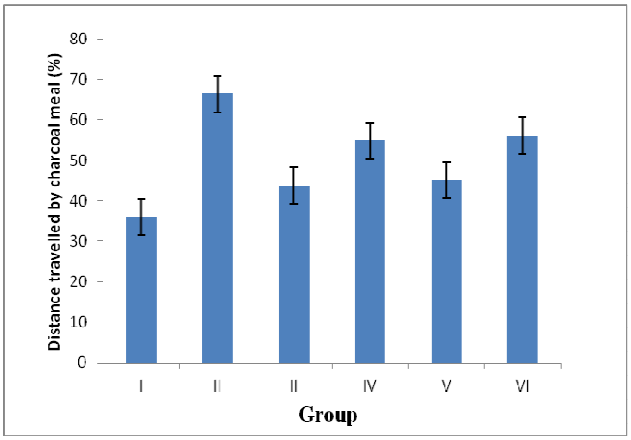

Figure 3: Effect of ethanolic fruits and root extracts of *C. carandas* (EFCC & ERCC) on gastrointestinal motility in rats

Result and Discussion

The Phytochemical screening reveals the presence of Phytoconstituents like; steroids, alkaloids, Flavonoids, glycosides, reducing sugar, terpenoid, Phenolic compounds and tannins are present in ethanolic fruit and root extracts of *C. carandas*. The Phytoconstituents Steroids are mostly useful for the treatment of diarrhoea and it may enhance intestinal absorption of Na^+ and water (Longanga et al., 2000; Goodman et al. 1996). Diarrhoea results from an imbalance between the absorptive and secretary mechanisms in the intestinal tract, which is accompanied by an excess loss of fluid in the feces (Ammon et al., 1974). Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the re-absorption of water.

In this study, the ethanolic fruit and root extracts of *C. carandas* exhibited a significant dose-dependent anti-diarrhoeal activity. In the castor oil-induced diarrhoea experiment, the plant extracts produced a marked anti-diarrhoeal effect in the rats, as shown in Table 1. At doses of EFCC 200 and 400mg/kg, the extract significantly decreased (*p < 0.05) the total number of wet faeces produced upon administration of castor oil (1.1 ± 0.19 at 200mg/kg and 2.18 ± 0.14** at 400mg/kg) where ERCC (1.19 ± 0.08 at 200mg/kg and 2.42 ± 0.14** at 400mg/kg) compared to the control group (0.87 ± 0.03 at 5ml/kg, p.o). The effect of the highest dose of the EFCC and ERCC extracts was similar to that of the standard drug (2.88 ± 0.13** at 5mg/kg). Therefore, it can be assumed that the anti-diarrhoeal action of the extracts was mediated by an anti-secretory mechanism. This was also evident from the inhibition of castor oil-induced fluid accumulation (enteropooling) by the extracts.

During the propulsion of the charcoal meal through the gastrointestinal tract was decreased significantly (*p < 0.05) from EFCC 43.76 ± 0.40 at 200mg/kg and 54.9 ± 0.50* at 400mg/kg and from ERCC 45.11 ± 0.36 at 200mg/kg and 56.14 ± 0.30* at 400mg/kg, compared to control group (36.02 ±0.27 at 5ml/kg, p.o.). Similarly, the effect of the highest dose of the EFCC and ERCC extracts was similar to that of the standard drug (66.46 ± 0.43* at 5mg/kg) results are shown in Table 2. Above observations suggest that the extracts in graded doses reduces diarrhoea by inhibiting peristalsis, gastrointestinal motility and castor oil-induced enteropooling. It is equally effective in prevention and curing of diarrhoea.

Conclusion

The results of this investigation revealed that the Ethanolic fruits and root extracts of *C. carandas* contained pharmacologically active substances with anti-diarrhoeal properties. Further research has to be carried out to fractionate and purify the extracts, in order to find out the bioactive molecule which is responsible for the anti-diarrhoeal activity which was observed.

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