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Evaluation of Anti-Diarrhoeal Activity of *Lippia nodiflorai Leaves* Extracts in Experimental Rats

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ABSTRACT

Diarrhoea is one of the principal causes of death in infants, particularly in developing countries. An array of medicinal plants with anti-diarrhoeal properties has been widely used by the traditional practitioners; however, the efficacy of many of these anti-diarrhoeal conventional medicines has not been scientifically evaluated. The present study was undertaken to explore anti-diarrhoeal activity of *Lippia nodiflora* leaves. Male Albino rats of 150 to 200g were used for study and anti-diarrhoeal activity was evaluated by castor oil-induced Diarrhoea model. The extract showed significant ($p < 0.001$) protection against castor oil-induced diarrhoea and castor oil-induced enteropooling at (100 mg/kg). The investigated may be exploited for wide range of diarrhoeal stages and further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *Lippia nodiflora* leaves.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems and leading cause of mortality and morbidity in the developing countries (Rajamanickam et al 2010) and produces more illness and causes death of more infants and children below 5 years old than all other diseases combined. (Dalal *et al* .,2011) Diarrhoea is a symptom marked by rapid and frequent passage of semisolid or liquid faecal material through the gastrointestinal tract and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the absorption of fluid and thus a loss of electrolytes particularly Na+ and water. It is also called loose motions. Diarrhoea is not itself a disease, but can be a symptom of several diseases. It is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside the digestive system. (Rang *et al*., 2003)

According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea (Saralaya *et al* .,2010) and the cause of 3.3% of all deaths (Daswani *et al.*, 2010). Around 88% of diarrheal related deaths are caused due to inadequate sanitation and poor hygiene (Kose *et al* .,2003). Diarrhoea can be either acute (short term) or chronic (long term). Acute diarrhoea is a common problem that lasts for 1 or 2 days and chronic diarrhoea is that which lasts for at least 4 weeks. Chronic diarrhoea symptoms are continual or they may come and go. Diarrhoea lasting for more than 2 days is a sign of a more serious problem. It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used antidiarrhoeal drugs, which are not completely free from adverse effects.

Lippia nodiflora Mich. (Verbenaceae) is a creeping much-branched herb with small white flowers, a weed of wet ground and grassy pastures. (Chopra *et al* .,1958) The herb is known as Poduthalai in Tamil, Kattuttippali in Malayalam, Bhuiokra in Hindi and Vashira in Sanskrit. The plant is distributed throughout India, Ceylon, Baluchistan and Africa. The plant is used for the treatment of diuretic, aphrodisiac, diseases of heart, ulcers, bronchitis, fever and cold. (Gamble.,1957) *L. nodiflora* is reported to possess acrid, cooling, aphrodisiac, astringent, anthelmintic, alexiteric, emmenagogue, bactericide, diuretic, antiseptic, antitussive, antipyretic and anti-inflammatory properties and is effective against bronchitis, respiratory diseases, arthritis, fever, dyspepsia, hookworm, gonorrhea, ulcers, stomachic, wounds, burning sensation,

asthma, thirst, loss of consciousness, diaper rash, erysipelas, neuralgia, sores, spasms and vertigo. (Shukla *et al* .,2009; Pascual *et al* ., 2001) So in the present study the aqueous leaf extract of *Lippia nodiflora* were evaluated for their antidiarrhoeal activity by castor oil induced diarrhoea and study on Castor oil-induced enteropooling method.

MATERIALS AND METHODS

Plant

Fresh leaves of *Lippia nodiflora* were collected from the Thanjavur. The collected leaves were identified and authenticated by a botanist, Prof. Dr. M. Jegadesan, Department of Herbal and Environmental science, Tamil University, Thanjavur, Tamil Nadu. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction.

Preparation of crude extract

The leaves were air-dried and ground into powder. A total of 200g of the powdered leaves were macerated in 500 ml of distilled water for 8 h and filtered through cheese cloth, glass wool and Whatman No. 1 filter paper. The filtrate was evaporated to dryness by heating in a water bath at a temperature of 100°C. The dry aqueous extract was stored in the refrigerator and used as the crude extract.

Animal

Healthy male albino rats (*Rattus norvegicus*) of weight 150 to 200g, were obtained from the Animal House of the Tamil University. All the animals were housed in a clean and well-ventilated house conditions [temperature 23 ± 1°C; photoperiod (the interval in a 24-h period during which an organism is exposed to light)]: 12 h natural light and 12 h dark: humidity: 45 to 50%]. They were also allowed free access to Balanced Trusty Chunks (Sai Durga Foods Ltd., Banglore) and tap water. The cleaning of the cages was done daily.

Castor oil induced diarrhea

Animals were divided into three groups (n = 6). The first group of animals, which served as control was administered with castor oil (1 ml/rats). The second group castor oil (1 ml/rats) + crude powder (100mg/100g body weight). Third group received standard drug, loperamide (3 mg/kg) orally as suspension.

Phytochemical screening

The aqueous crude leaves extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids, flavonoids, saponin and glycosides using standard procedures (Trease and Evans, 1996).

Castor oil-induced diarrhoea in rats

A total of 18 albino rats (*R. norvegicus*) were divided into three groups of six animals each. All rats were fasted for 18 h and received castor oil at a dose of 1 ml/animal orally (p.o.) using or gastric cannula for induction of diarrhea (Doherty, 1981). Thirty minutes after castor oil administration, rats of group I (control) received 1.0ml/100g of 0.9% NaCl in distilled water (normal saline) and rats of groups II, received 100mg/kg of *Lippia nodiflora* leaves crude powder extract p.o. and group III received standard drug, loperamide (3mg/kg p.o.), respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h. The total number of diarrhoea feces of the control group was considered 100%.

$$\% \text{ Inhibition} = (\text{Control} - \text{Test}) \times 100/\text{Control}$$

Castor oil-induced enteropooling

Castor oil-induced enteropooling was determined by the method of Robert et al. (1976). The adult rats (*R. norvegicus*) selected without sex discrimination were fasted for 18 h and divided into three groups of six animals each. Castor oil (1 ml) was administered orally to these animals. One hour later, Group I received 1ml/100g of normal saline solution and rats of groups II received 100mg/kg *Lippia nodiflora* leaves crude powder p.o and group III received standard drug, loperamide (3mg/kg orally), respectively. After 2 h of treatment, the rats were sacrificed by

ether anesthesia. The edges of the intestine from pylorus to caecum were tied with thread and the intestine was removed and weighed. Intestinal content was collected by measuring cylinder, and volume measured.

Statistical analysis

Results are presented as means ± standard deviation (SD) and simple percentages. The student 't' test' was used to determine the significant difference between two groups ($p < 0.001$).

RESULTS

Phytochemical analysis

The phytochemical results confirm the presence of alkaloids, flavonoids, tannins, saponins and glycosides in extracts (Table 1). These are the phytochemicals which are essential in many medicinal plants responsible for the antidiarrhoeal (Patricia et al., 2005). The reported medicinal property of the plant might be due to the presence of these bioactive components in *Lippia nodiflora*.

In the castor oil-induced diarrhoea experiment, aqueous extract of *Lippia nodiflora* produced a markedly antidiarrhoeal effect in the rats, as shown in Table 2 (Figure 1). At dose of 100mg/kg, the extract significantly decreased ($p < 0.01$) the total number of watery stool produced upon administration of castor oil (2.10±0.3 at 100mg/kg) compared to the control group (22.00±0.90). The effect of the dose of the extract was similar to that of the standard drug, loperamide (3mg/kg).

Lippia nodiflora leaves crude extract significantly ($p < 0.001$) inhibited castor oil-induced enteropooling in rats at oral dose 100mg/kg (Table 3 and Figure 2). The intestinal fluid in control animals was 3.29±0.06ml. The inhibition of intestinal accumulation was 76% ($p < 0.001$) at dose 100mg/kg of the drug. The standard drug, loperamide (3mg/kg) also significantly inhibited ($p < 0.001$) intestinal fluid accumulation (87%).

DISSCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by rush resulting in an excess loss of fluid in the faeces. In some

diarrhea, the secretory component predominates while other diarrhoea is characterized by hypermotility (Chitme *et al*., 2004). Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (Ammon *et al*., 1974; Watson and Gordon, 1962) which induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in a hyper-secretory response (decreasing Na+ and K+ absorption), stimulating peristaltic activity and diarrhoea (Zavala et al., 1998). Inhibitors of prostaglandin synthesis are known to delay diarrhoea induced with castor oil (Sunil *et al*., 2001). Its action also stimulates the release of endogenous prostaglandin (Galvez *et al*., 1993). Castor oil is reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the re-absorption of water. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (Pierce *et al* .,1971).

The results of this study revealed that the aqueous leaves extract of *Lippia nodiflora* produced statistically significant protection against diarrhoea and was found to be comparable to loperamide; is a butyramide derivative and an opiate agonist which improves the diarrhoea due to many causes (Karim and Aidakan 1977). The antidiarrhoeal effect of loperamide was attributed to these actions on motility (Nakayama *et al*., 1977) against diarrhea disorders which effectively antagonizes diarrhoea induced by castor oil, prostaglandin and cholera toxin (Niemegeers *et al*., 1974; Facack et al., 1981).

The antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Havagiray et al., 2004)., Most plant species that have anti-diarrheal potential confirm tannins as one of the major constituents (Yadav *et a*l .,2007). Tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray *et al*., 2004). While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion. (Venkatesan *et al*., 2005) Therefore, the antidiarrhoeal activity of *Lippia nodiflora* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids, saponins and glycosides in the crude extract.

CONCLUSION

Antidiarrhoeal activity of *Lippia nodiflora* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the crude extract. The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study are indications of antidiarrhoeal potential of *Lippia nodiflora* leaf crude extract. Further studies are however needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects.

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Table 1. Preliminary phytochemical analysis of *Lippia nodiflora Leaves* extracts

Phytoconstituents	Tannin	Alkaloids	Flavonoids	Saponin	Glycosides
Aqueous Extract	+	+	+	+	+

Table 2: Effect of *Lippia nodiflora Leaves* extract on castor oil induced diarrhea

Subject	Dose (mg/kg)	No. of Watery Diarrhea	% Inhibition
Control (Castor Oil)	1ml	22.0±0.90	--
Lippia nodiflora Leaves	100	2.5±0.22***	87
Loperamide	3	2.0±0.26***	90

Values are expressed as mean ± S.D. n = 6, *** P< 0.001 compared with Control

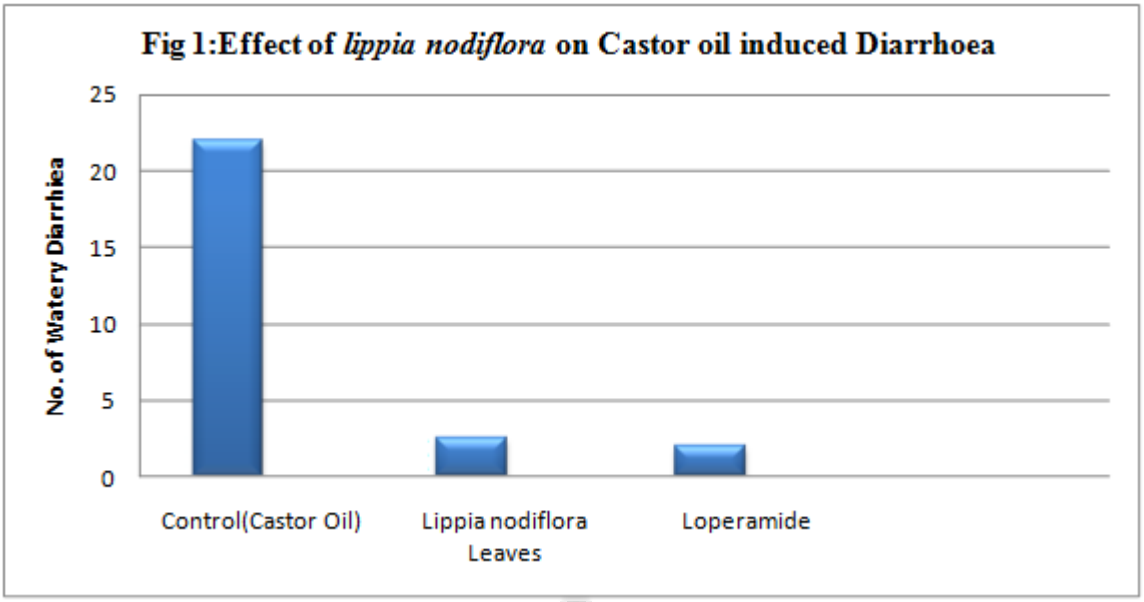
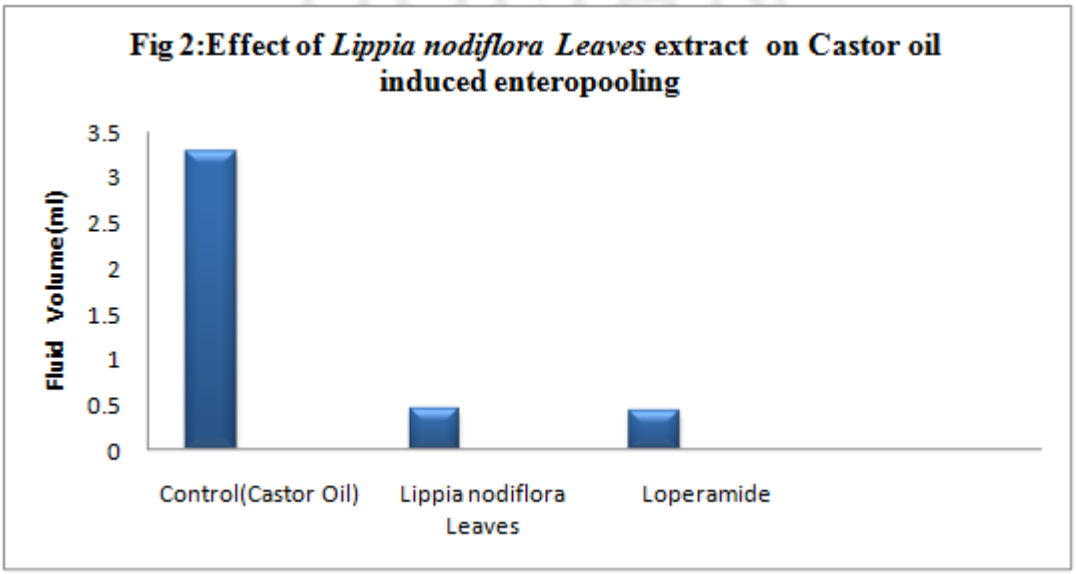


Table 3: Effect of *Lippia nodiflora Leaves* on Castor oil-induced enteropooling

Subject	Dose (mg/kg)	Fluid Volume (ml)	% Inhibition
Control (Castor Oil)	1ml	3.29±0.06	--
Lippia nodiflora Leaves	100	0.46±0.01***	86
Loperamide	3	0.43±0.01***	87

Values are expressed as mean ± S.D. n = 6, *** P< 0.001 compared with Control



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