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Research Article

EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF ETHANOLIC EXTRACT OF CELTIS TIMORENSIS LEAVES IN EXPERIMENTAL RATS.

PRASANTH KUMAR. M[1*], SUBA. V[2], RAMIREDDY. B[3], SRINIVAS BABU.P[4]

[1]Research scholar, Faculty of Pharmaceutical Sciences, JNTU, Hyderabad, Andhrapradesh. [2]Assistant professor, Pharmacology, National Institute of Siddha, Tambaram Sanatorium, Chennai. [3]Director-Formulations, NATCO Pharma, Hyderabad, Andhrapradesh. [4]Principal, Vignan Pharmacy College, Vadlamudi, Guntur, Andhrapradesh. Email: prasanth.pharmacology@gmail.com

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ABSTRACT

Objective: To evaluate the antidiarrhoeal activity of ethanolic extract of *celtis timorensis* leaves.

Materials and Methods: The antidiarrhoeal activity of ethanolic extract of *Celtis timorensis* leaves was investigated by using castor oil induced diarrhoea, castor oil induced enteropooling, charcoal meal test models and atropine was used as a standard reference drug in all the three models.

Results: The diarrhoeal severity was reduced significantly ($p<0.01$) by the extract by 56.85% at 200 mg/kg and 69.48% at 400 mg/kg, whereas 82.12% inhibition was found for the standard drug atropine at dose 3mg/kg. In castor oil induced enteropooling, the extract at doses 200 mg/kg and 400 mg/kg, standard drug atropine showed significant reduction in the volume of the intestinal fluid ($p<0.01$). In the charcoal meal test, the distance travelled by charcoal meal was significantly reduced by the extract at doses 200 mg/kg and 400 mg/kg ($P<0.01$) when compared to the castor oil control group. The percent inhibition of charcoal meal was found to be 9.94±1.63 for control group, 15.26±2.11 at 100 mg/kg, 46.81±2.32 at 200 mg/kg, 63.61±2.61 at 400 mg/kg and 75.76±1.72 for standard group.

Conclusion: The present findings concluded that the ethanolic extract of *celtis timorensis* leaves has a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

Keywords: Diarrhoea, *Celtis timorensis*, Motility, Castor oil, Enteropooling and Charcoal.

INTRODUCTION

Diarrhoea is one of the major fatal outcome causing diseases particularly in developing countries of the world and causes death in millions of population for every year. It is a major health threat to the population in developed countries also though there is an improvement in the health and wealth of the population [1]. It is estimated that diarrhoea accompanied with infectious disorders will persist as a major cause of global health concern [2]. In Nigeria, diarrhoea is the number one fatal outcome disease among children under five years age who are more susceptible for the occurrence of the disease [3]. According to the estimation of the world health organisation, diarrhoea accounts for 3-5 billion cases worldwide annually of which 1 billion cases and 5% of mortality occurring in children [4]. Various synthetic drugs are available in the market for the treatment of diarrhoea. These synthetic drugs have many undesirable side effects which included allergy, pyrexia, dryness of the mouth, constipation, skin rashes, abdominal pain, nausea, metallic taste, drowsiness, an increase in eosinophil count and exanthema [5]. As the herbal products possess few side effects when compared to the synthetic drugs, the WHO has supported various studies for the control of diarrhoea which involves the usage of herbal medicines based on the traditional medicinal practices [6]. Various traditional medicinal plants showed antidiarrhoeal activity by reducing the intestinal transit, suppressing the gut motility and stimulating the water adsorption or by reducing the electrolyte secretion [7].

Celtis timorensis belonging to cannabacea family is a flowering plant commonly known as stinking wood or stink wood. The plant has been recommended in ayurveda for the improvement of memory and in the treatment of nervous disorders. The plant extract has been reported for antidepressant, anticonvulsive and nervous disorders. The extract also enhanced learning and memory in humans [8]. It also helps in repairing of neurons which were damaged in specific brain regions. The plant extract also showed a neuroprotective effect against oxidative stress in the hippocampus of rat brain [9]. Traditionally the leaf extract of *celtis timorensis* is given during dysentery conditions [10]. So far, no scientific reports available on the antidiarrhoeal activity of *celtis timorensis*. Hence the present study has been undertaken to evaluate the antidiarrhoeal activity of ethanolic extract of *celtis timorensis* by using castor oil induced diarrhoea, castor oil induced enteropooling and charcoal meal test in rats.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of *celtis timorensis* were collected from Tirupathi, Andhrapradesh. The plant was identified and authenticated taxonomically by Assistant professor K.Madhava chetty of the Department of Botany, S.V. University, Tirupathi, AndhraPradesh, India. A voucher specimen of the collected sample was deposited in the herbarium of the institution for future reference.

Preparation of the extract

The leaves are shade dried and made into coarse powder and extracted with 70% ethanol by cold maceration method for 72 hours with intermittent shaking. The extract was filtered and concentrated at high vacuum. The extract was stored in the refrigerator till further use.

Animals

Albino Wistar rats (weighing between 150 gms-200 gms) of both sexes were selected for the experiment. They had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 28-30 degrees centigrade. Animals are allowed for a one week of acclimatization period prior to the study. The experimental protocol was approved by the IAEC (institutional animal ethical committee) and care of the experimental animals was taken according to the CPCSEA guidelines.

Drugs and chemicals

Castor oil and Atropine sulphate obtained from Himedia, Mumbai, India and all the other drugs and chemicals used for the experiment were of analytical grade**.**

Castor oil induced diarrhoea: This test is done by following the method of Shoba et al and Uddin et al [11, 12]. Rats selected for the experiment are fasted for 18 hours with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100 mg/kg of ethanolic extract p.o, group III received 200 mg/kg of ethanolic extract p.o, group IV received 400 mg/kg of ethanolic extract p.o and group V received standard drug atropine 3 mg/kg i.p. After 1 hour of treatment with the extract, standard drug and vehicle, 1 ml of castor oil was given orally to all the five groups of animals. The rats were then placed in metabolic cages and the floor of the cages was lined with adsorbent papers in order to collect the faeces. All the animals are to be observed for four hours for the presence of diarrhoea.

Antidiarrhoeal activity of the drug or extract was expressed in terms of percent inhibition of diarrhoea. The percent inhibition of defecation was calculated by using the formula:

% Inhibition of defecation= [(X-Y)/X] x100

X is the mean number of defecation caused by castor oil and Y is the mean number of defecation caused by drug or extract.

Castor oil induced enteropooling

The castor oil induced enteropooling was done according to the method of Robert et al [13]. Rats selected for the experiment are fasted for 18 hours with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100 mg/kg of ethanolic extract p.o, group III received 200 mg/kg of ethanolic extract p.o, group IV received 400 mg/kg of ethanolic extract p.o and group V received standard drug atropine 3 mg/kg i.p. After 1 hour of treatment with the extract, standard drug and vehicle, 1 ml of castor oil was given orally to all the five groups of animals. After 1 hour of treatment with castor oil, rats from all the groups were sacrificed and whole intestine from the pylorus to caecum was removed after tying the ends with thread and weighed. The contents of the intestine were collected by milking into a graduated tube. The intestine was reweighed and the difference between the full and empty intestine was measured in grams**.**

Charcoal meal test or gastrointestinal motility test

This test was performed according the method of Mascolo et al [14]. Rats selected for the experiment are fasted for 18 hours with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100 mg/kg of ethanolic extract p.o, group III received 200 mg/kg of ethanolic extract p.o, group IV received 400 mg/kg of ethanolic extract p.o and group V received standard drug atropine 3 mg/kg i.p. After 1 hour of treatment with the extract, standard drug and vehicle, 1 ml of castor oil was given orally to all the five groups of animals. After 1 hour of treatment with castor oil, 1 ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally to all the animals in all the five groups. The rats were sacrificed after one hour and the small intestine was removed surgically and the distance travelled by the charcoal meal from pylorus to caecum was measured after keeping the intestine on a clean transparent clean glass and the values are expressed as percentage with respect to the total length of the intestine from pylorus to caecum. The percentage of inhibition was also calculated by using the following formula [15].

Distance travelled (%) = Distance travelled by charcoal/ Total length x100

Inhibition (%) = Total length-Distance travelled by the charcoal/ Total length x100

Statistical analysis

Results are expressed as mean ±standard error mean (SEM). Data obtained was analyzed by using one way ANOVA followed by Dunnett's test and $p<0.05$ was considered as statistically significant.

RESULTS

Effect of ethanolic extract on Castor oil induced diarrhoea

In castor oil induced diarrhoea model, the ethanolic extract of *celtis timorensis* showed considerable antidiarrhoeal activity (Table: 1). The extract at all three doses showed significant decrease in the total number of faecal droppings when compared to the castor oil control group. At a dose of 100 mg/kg, there was no significant reduction in the number of diarrhoeal faeces when compared to the castor oil control group. However, at doses 200 mg/kg and 400 mg/kg there was a significant reduction in the total number of diarrhoeal faeces when compared to castor oil control group ($p<0.01$) and the effect is almost similar to that of standard drug. The results showed that 100 mg/kg dose of the extract showed 13.70% inhibition of diarrhoea whereas doses 200 mg/kg and 400 mg/kg showed 56.85% and 69.48% inhibition respectively. The standard drug atropine showed 82.12% inhibition of diarrhoea.

Table 1: Effect of ethanolic extract of *Celtis timorensis* on castor oil induced diarrhoea

Treatment Group	Total number of faeces	% inhibition of faeces	Total number of diarrhoeal faeces	% inhibition of diarrhoea
Vehicle plus castor oil 1 ml	26.66 ±1.14	-	15.83 ± 0.87	
EECT (100 mg/kg)	22.16 ±1.42*	16.87	13.66 ±1.05^{ns}	13.70
EECT (200 mg/kg)	16.66 ± 0.88**	37.50	6.83 ±0.94**	56.85
EECT (400 mg/kg)	10 ±0.96**	62.49	4.83 ± 0.47**	69.48
Atropine (3 mg/kg)	6.16 ± 0.60**	76.89	2.83 ±0.30**	82.12

EECT= Ethanolic extract of *celtis timorensis*. Values are expressed as mean±SEM. *P<0.05 and **P<0.01 when compared to castor oil control group.

Effect of ethanolic extract on Castor oil induced enteropooling

In castor oil induced enteropooling, the extract at a dose of 100 mg/kg did not show any significant reduction in the intestinal volume and weight of intestinal content when compared to castor oil control group. However, 200 mg/kg and 400 mg/kg, p.o. dose produced 36.56% and 44.02% inhibition of volume of intestinal content respectively with significance ($p<0.01$). The weight of the intestinal content was also significantly reduced at doses 200 mg/kg and 400 mg/kg.

Table 2: Effect of ethanolic extract of *Celtis timorensis* on castor oil induced enteropooling.

Treatment Group	Weight of the intestinal content (g)	Volume of intestinal fluid (ml)	%inhibition
Vehicle plus castor oil 1 ml	6.1 ±0.83	2.68 ±0.28	-
EECT(100 mg/kg)	5.85 ±0.12^{ns}	2.2 ±0.16^{ns}	17.91
EECT (200 mg/kg)	4.48 ±0.31*	1.7 ±0.18**	36.56
EECT (400 mg/kg)	2.45 ±0.28**	1.5 ±0.17**	44.02
Atropine (3 mg/kg)	1.55 ±0.14**	0.73 ±0.13**	72.76

EECT= Ethanolic extract of *celtis timorensis*. Values are expressed as mean±SEM. *P<0.05 and **P<0.01 when compared to castor oil control group.

Effect of ethanolic extract on Charcoal meal test

In charcoal meal test, the extract at 100 mg/kg dose did not show any significant reduction in the distance travelled by the charcoal

meal when compared to the castor oil control group. The gastrointestinal distance travelled by the charcoal meal in the rats significantly reduced by the extract at doses 200 mg/kg and 400 mg/kg (P<0.01). The percent inhibition of charcoal meal was found to be 9.94±1.63 in control group, 15.26±2.11 in 100 mg/kg dose group, 46.81±2.32 in 200 mg/kg dose group, 63.61±2.61 in 400 mg/kg dose group and 75.76±1.72 in standard group.

Table 3: Effect of ethanolic extract of *Celtis timorensis* on charcoal meal test

Treatment Group	Total length of the intestine (cm) Mean±SEM	Distance travelled by the charcoal meal (cm) Mean±SEM	Distance travelled by the charcoal meal (%) Mean±SEM	%Inhibition Mean±SEM
Vehicle plus castor oil 1 ml	68.83 ±1.60	62 ±1.93	90.04 ±1.63	9.94 ±1.63
EECT (100 mg/kg)	67.33 ±1.43	57 ±1.52^{ns}	84.72±2.11^{ns}	15.26 ±2.11^{ns}
EECT (200 mg/kg)	69.66 ±1.20	37 ±1.50**	53.17 ±2.32**	46.81 ±2.32**
EECT (400 mg/kg)	66.66 ± 2.86	24 ±1.15**	36.37 ±2.61**	63.61 ±2.61**
Atropine (3 mg/kg)	69.83 ±2.75	17 ±1.52**	24.22 ±1.72**	75.76 ±1.72**

EECT= Ethanolic extract of *celtis timorensis*. Values are expressed as mean±SEM. **P<0.01 when compared to castor oil control group

DISCUSSION

Diarrhoea is an important health problem among the developing countries and is a common cause of morbidity and mortality particularly in children and infants. Despite of the major development in modern medicine, many people still depends on traditional medicinal plants in the treatment of various ailments. In India, many medicinal plants are available for the treatment of diarrhoea and dysentery which are used in folklore medicine and by the local area people [16]. Various reports have shown that pre-treatment with some plant extracts had a protective effect on the intestinal tract [17]. Antidiarrhoeal activity of ethanolic extract of *celtis timorensis* was evaluated by using castor oil induced diarrhoea, castor oil induced enteropooling and charcoal meal test models. The seeds of *Ricinus communis* are the main source for castor oil extraction. The main active component of castor oil is ricinoleic acid which is mainly responsible for the induction of diarrhoea [18]. After the ingestion of castor oil, ricinoleic acid is liberated in the small intestine by lipases [19, 20]. Due to polar nature of ricinoleic acid, it was poorly absorbed and as a result of presence of the ricinoleic acid in the small intestine, there were changes in permeability of electrolytes of the intestinal mucosa and peristaltic activity in the intestine which in turn produces hypersecretion and fluid accumulation [21]. The presence of ricinoleic acid in the intestine causes mucosal irritation and increases inflammatory responses leading to secretion of prostaglandins which in turn stimulates the secretions in the intestine and hypermotility [22]. Prostaglandins which belong to E series are considered as potential diarrhoeal inducing agents in both humans and animals and diarrhoea induced by castor oil was found to be delayed by prostaglandin biosynthesis inhibitors [23]. The ricinoleic acid released from the castor oil readily reacts with sodium and potassium salts in the intestinal lumen to form ricinoleate salts. The anti-absorptive effect of the ricinoleate on the mucosa can be due to several mechanisms [24]. It inhibits the activity of intestinal sodium-potassium ATP ase enzyme and causes a cytotoxic response on isolated enterocytes. It may activate adenyl cyclase present in the epithelial cells, stimulation of prostaglandin secretion and platelet activating factor [25]. Most recent studies demonstrated the involvement of nitric oxide in the absorptive and secretory mechanisms. Nitric oxide could lead to secretions through the stimulation of cAMP and cGMP concentration [26].

In the present study, ethanolic extract of *celtis timorensis* significantly decreased the total number of faeces when compared to the castor oil group in castor oil induced diarrhoea model. The extract at doses 200 mg/kg and 400 mg/kg showed significant activity just like the standard drug atropine 3 mg/kg. The low dose 100 mg/kg is considered to be less effective in the reduction of the number of faeces when compared to 200 mg/kg and 400 mg/kg. In castor oil induced enteropooling, the extract at doses 200 mg/kg and 400 mg/kg significantly inhibited the castor-oil induced intestinal fluid accumulation (enteropooling) and weight of intestinal content. These results suggested that extract reduced diarrhoea by stimulating reabsorption of electrolytes and water or by inhibiting the fluid secretion into the intestine. In order to evaluate the mechanism of antidiarrhoeal activity, the study was extended to determine the intestinal motility. Previous studies showed that activated charcoal prevents the absorption of drugs and chemicals by adsorbing them actively on the surface of charcoal. Thus gastrointestinal motility test was carried out to determine the effect of the extract on the peristaltic movement. In charcoal meal test, the extract significantly reduced intestinal propulsive movement at doses 200 mg/kg and 400 mg/kg, this was almost similar to standard drug atropine. This activity is possible due to the extract's ability to inhibit the intestinal movement. The reduction in distance travelled can be used a tool in order to explain the intestinal smooth muscle relaxation. Several antidiarrhoeal medications are well known for reduction of intestinal contractions and thereby reducing the intestinal transit [27]. Atropine suppresses the movement of the charcoal meal in the study due to its anticholinergic effect [28]. From the present study, it was found that the ethanolic extract of *celtis timorensis* suppressed the movement of charcoal meal and thereby increasing the absorption of electrolytes like sodium, potassium and water.

Preliminary phytochemical analysis of the ethanolic extract showed the presence of flavonoids, tannins, glycosides, alkaloids and carbohydrates. Flavonoids, tannins, triterpenoids, alkaloids, saponins and tannic acid are the main chemical constituents of traditional medicinal plants which are responsible for antidiarrhoeal activity [29, 30, 31]. Tannins and tannic acid form protein tannate complex by protein denaturation and the complex formed makes the mucosa of the intestine more resistant by coating and thus reduce secretion [32]. Antidiarrhoeal activity, Anti-irritant activity, antibacterial activity and anti-phlogistic activity of the medicinal plants were observed due to the presence of tannins as main chemical constituents. There were changes in intestinal motility and hydroelectrolytic secretions during diarrhoea conditions and flavonoids are well known to reduce these secretions and motility [33]. Various experiments showed that the secretions induced by prostaglandins are inhibited by flavonoids. Flavonoids are well known for their antioxidant properties which are assumed to be responsible for the inhibition of various enzymes that are involved in the metabolism of arachidonic acid [34]. Thus the presence of phytochemicals such as alkaloids, flavonoids and tannins may be responsible for antidiarrhoeal activity.

CONCLUSION

The present study validates the use of *celtis timorensis* leaves as antidiarrhoeal agent in traditional medicine. Further investigation is necessary for isolation, identification and characterisation of various active constituents of the extract in order to elucidate the mechanism of action, responsible for these properties on different biological systems.

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REFERENCES

1. Casburn-Jones AC, Farthing MJG. Management of infectious diarrhoea. Gut 2004; 53: 296–305
2. Meyrowitsch DW, Bygbjerg IBC. Global burden of disease—a race against time. Danish Medical Bulletin 2007; 54: 32–34.
3. Ahmed AA, Zezi AU, Yaro AH. Antidiarrhoeal activity of the leaf extracts of Daniella oliveri, Hutch and Dalz (Fabaceae) and Ficus sycomorus, MIQ (Moraceae). Afr J Trad Compl & Alt Med 2007; 4: 524-528.
4. Awe EO, Kolawole SO, Wakeel KO, Abiodun OO. Antidiarrhoeal activity of Pyrenacantha staudtii Engl. (Iccacinaceae) aqueous leaf extract in rodents. Journal of Ethnopharmacology 2011; 137: 148-158.
5. Sharma HL, Sharma KK. Principles of Pharmacology, first ed. Hyderabad. Paras Medical Publisher; 2007. p.412–414.
6. Jebunnessa, Shaikh Bokhtear U, Mahabub-Uz-Zaman M, Rasheda A, Nazim Uddin A. Antidiarrhoeal activity of ethanolic bark extract of Mitragyna diversifolia. Bangladesh J Pharmacol 2009; 4: 144-146.
7. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytotherapy Research 2006; 20: 717–724.
8. Rajaneekar D, Sathyavathi D, Anusha K. Evaluation of Methanolic Extract of Celtis timorensis for its Antidepressant Activity. Indo American journal of Pharmaceutical research 2012; 1 (11): 1178-82.
9. Pullaiah T. Encyclopedia of world medicinal plants. 2006; 4: p.2019.
10. Singh HB, Singh RS and Sandhu JS Ed. Herbal medicine of Manipur, a colour encylopaedia. 2003; p. 67.
11. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. Journal of Ethnopharmacology 2001; 76: 73-76.
12. Uddin SJ, Shilpi JA, Alam SM, Alamgir M, Rahman MT, Sarker SD. Antidiarrhoeal activity of the methanol extract of the barks of Xylocarpus moluccensis in castor oil- and magnesium sulphate induced diarrhoea models in mice. Journal of Ethnopharmacology 2005; 101: 139-143.
13. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay, a test for diarrhoeal produced by prostaglandins. Prostaglandins 1976; 11: 809-828.
14. Mascolo N, Izzo AA, Avtore G, Barboto F, Capasso F. Nitric oxide and castor oil induced diarrhoeal. Journal of Pharmacology and Experimental therapeutics 1994; 268: 291-295.
15. Mujumdar AM. Antidiarrhoeal activity of Azadirachta indica leaf extract. Indian drugs 1998; 35 (7):417-420
16. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research (CSIR) Publication; 1956.p. 276–278.
17. Manonmani S, William S, Subramanian S, Govindasamy S. Biochemical studies on the antidiarrhoeal effects of cauvery-100 an ayurvedic formulation in rats. Biochemistry International 1991; 24: 701-708.
18. DiCarlo GD, Mascolo N, Izzo AA, Capasso F, Autore G. Effects of quercetin on the gastrointestinal tract in rats and mice. Phytotherapy Research 1994; 8: 42–45.
19. Meyer H. Ueber den wirksamen Bestandtheil des Ricinusöls. [On the active component of castor oil.] Arch Exp Path Pharmak 1890; 28: 145-152.
20. Watson WC, Gordon RS. Studies on the digestion, absorption and metabolism of castor oil. Biochem Pharmacol 1962; 11: 229-236.
21. Ammon HV, Thomas PJ, Phillips SF. Effect of oleic and ricinoleic acid on net jejunal water and electrolyte movement. Journal of Clinical Investigation 1974; 53: 374–379.
22. Pierce NF, Carpenter CCJ, Elliott HZ, Greenough WB. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology 1971; 60: 22–32.
23. Sorin T, Till FA, Rolf MN, Martin D, Stefan O. Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. Proc Natl Acad Sci USA 2012; 109: 9179-9184.
24. Venkat Rao N, Chandra Prakash K, Shanta Kumar SM. Pharmacological investigation of Cardiospermum halicacabum (Linn) in different animal models of diarrhoea. Indian Journal of Pharmacology 2006; 38: 346–349.
25. Munson PL, Muller RA, Breese GR. Principles of Pharmacology: Basic Concept and Clinical Applications. New York: Chapman and Hall (An International Thomson Publishing Company); 1995.
26. Liang YC, Liu HJ, Chen SH, Chen CC, Chou LS, Tsai LH. Effect of lipopolysaccharide on diarrhoea and gastrointestinal transit in mice: Roles of nitric oxide and prostaglandin E2. World Journal of Gastroenterology 2005; 11: 357-361.
27. Mathad VSB, Chandanam S, Setty SRT, Ramaiyan D, Veerama B, Lakshminarayanettry ABV. Antidiarrhoeal evaluation of Benincasa hispida (Thumb) Cogn. fruit extracts. Iranian J Pharmacol & Ther 2005; 4: 24-27.
28. Brown JH, Taylor P. Muscarinic receptor agonists and antagonist. In: Hardman JG, Limbird LE, editors. Goodman and Gilman, the pharmacological basis of therapeutics. 9th ed. New York: 1996. p.148-154.
29. Havagiray R, Ramesh C, Sadhna K. Study of antidiarrhoeal activity of Calotropis gigantea r.b.r. in experimental animals. J Pharm Pharm Sci 2004, 7: 70-75.
30. Brijesh S, Daswani P, Tetali P, Antia N, Birdi T: Studies on the antidiarrhoeal activity of Aegle marmelos unripe fruit:Validating its traditional usage. BMC Complement Altern Med 2009, 9(47):1–12.
31. Yadav AK, Tangpu V. Antidiarrhoeal activity of Lithocarpus dealbata and Urena lobata extracts: therapeutic implications. Pharm Biol 2007; 45(3):223–229.
32. Westendarp H. Effects of tannins in animal nutrition. Deutsche Tierarztliche Wochenschrift 2006; 113:264-268.
33. Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Kumar SGV, et al. Antidiarrhoeal potential of asparagus racemosus wild root extracts in laboratory animals. Journal of Pharmacy and Pharmaceutical Sciences 2005; 8: 39-45.
34. Bimlesh K, Kalyani D, Prashant T, Mino S, Diwakar G. Evaluation of anti diarrhoeall effect aqueous and ethanolic extracts of fruit pulp of *Terminalia belerica* in rats. International journal of drug development and research 2010; 2: 769-779.