ORIGINAL ARTICLE

Anti-diarrheal effects of Methanol extract of *Curcuma Longa*

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ABSTRACT:

Objective: To evaluate the anti-diarrheal effect of Methanol extract of *Curcuma Longa,* and to compare it with Loperamide in albino rats.

Methodology: This experimental animal study was performed in the Department of Pharmacology and Therapeutics, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi, in collaboration with BMSI Animal house, from May 2013 to December 2014. Dried rhizomes of *Curcuma longa* were purchased from local market of Karachi and soaked in 100% methanol, which was later evaporated to yield a semisolid extract. Seventy-two albino rats were used, which were randomly assigned into 3 groups of 24 rats. Each group was further divided into 4 subgroups. In each group, one subgroup was control, one was standard (loperamide group) and two subgroups were given 100mg/kg and 200mg/kg of methanol extract of *Curcuma Longa* orally. Anti-diarrheal effect was assessed by counting total number of feces after castor oil-induced diarrhea, castor oil-induced enteropooling and gastrointestinal motility test.

Results: The methanol extract of *Curcuma Longa* showed significant anti-diarrheal activity evidenced by the reduction in defecation, decreased intestinal transit of charcoal meal and decreased enteropooling after castor oil-induced diarrhea.

Conclusion: *Curcuma Longa* had potent anti-diarrheal affect and can replace synthetic drugs like loperamide. These effects were related to phytochemicals present in it.

Keywords: *Curcuma Longa*, Enteropooling, Gastrointestinal motility, Phytochemicals

INTRODUCTION:

Natural products from plants are being used in pharmaceutical preparations either as pure compounds or as extracts. One example is *Curcuma Longa*[1].

Curcuma Longa is a herbaceous perennial plant of Zingiberaceae family, cultivated extensively in South East Asia, China, Indonesia and Malaysia, commonly known as "Haldi" in Urdu, and turmeric in English language. It has a large oval rhizome with orange colour inside. It has elliptical leaves, which can reach up to 1.2 meters in length and yellow flowers. It grows in temperature between 20°C to 30°C[2]. It has been in continuous use for its flavoring, and as a spice in both vegetarian and non-vegetarian food[3,4].

Curcuma longa produces different pharmacological effects and is a known anti-inflammatory agent. It has significant medicinal potential with minimal toxicity[5]. The most active component in turmeric is flavonoid curcumin, which makes up 2 to 5% of the total spice in turmeric. Curcumin is the principal curcuminoid. It is a diferuloylmethane and constitutes up to 75% of total curcuminoids. The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin which make up 24% and 8% respectively[6].

Diarrhea is one of the common health problems affecting people, especially in developing countries. The burden of diarrheal disease disproportionately affects children under five years where it is a primary cause of mortality, specifically in low- and middle-income population groups who have higher incidence rates due to inadequate water and sanitation, and nutritional risk factors, such as sub-optimal breastfeeding, zinc and vitamin A deficiency etc[7]. Worldwide, there are about two billion cases of diarrheal disease every year. Diarrhea can last several days, and can leave the body without water and salts necessary for survival. Cause of death is generally severe dehydration and fluid loss. World Health Organization (WHO) has estimated that 2.5 billion episodes of diarrhea in children younger than five years of age are reported every year in developing countries, which is responsible for 1.5 million deaths every year[8]. Despite decline in mortality due to diarrhea in last decade, it remains one of the principal causes of morbidity in children[9]. There are certain opioid and non-opioid derivatives which are currently used as anti-diarrheal agents but these are not devoid of toxicity and have contraindications, like loperamide should be avoided in bloody or suspected inflammatory diarrhea, or when associated with significant abdominal pain[10]. So there is need for natural remedy which must be non-toxic and integral component of our home kitchen[11].

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METHODOLOGY:

This animal study was conducted in the Department

of Pharmacology and Therapeutics, BMSI, JPMC, Karachi, after approval from JPMC ethical committee, from May 2013 to December 2014.

A total of 72 albino rats of either sex, 2-3 months old and weighing from 150–250 gram were selected for the study. The rats were randomly assigned into 3 groups of 24. Each group was further divided into 4 subgroups, which comprised of 6 albino rats. Animals were kept in a standard natural environmental condition with the provision of standard laboratory diet.

Preparation of extract:

C*urcuma Longa* was purchased from local market of Karachi and verified from Department of Pharmacognosy, Karachi University with taxonomy no109. It was coarsely ground into very fine particles and then soaked in 99% Methanol for two weeks. This mixture was filtered via filter paper. The filtrate was passed through rotatory evaporator and thick semisolid residue was isolated. This extracted material was kept in the open bottle for about two weeks so that remaining methanol was evaporated. After fourteen days, the bottle was kept closed so that extract was ready for use.

Group A animals (n=24) were given Castor oil to induce diarrhea[12]. Rats were divided into 4 subgroups (n=6) and fasted for 24 hours before giving drugs, i.e., normal saline 3ml to 1st group (control), loperamide 5mg/kg body weight to 2nd group (standard) and *Curcuma longa* 100mg/kg and 200mg/kg to 3rd and 4th group respectively[12,13]. After 30 minutes of drug administration, 1 ml of castor oil was given to all the animals. The albino rats were then kept in cages which were lined by white blotting paper, which were changed every hour for the next 4 hours. The total number of feces excreted was counted for the next 4 hours and weight of animals was also noted before and after the procedure. The total number of diarrheal feces of the control group was considered to be 100%, and % inhibition of diarrhea was calculated by following formula:

$$\text{Percentage inhibition} = \frac{\text{Mean diarrheal count (control group-treated group)} \times 100}{\text{Mean diarrheal count of control group}}$$

In Group B, Castor oil-induced enteropooling was carried out. The rats were divided into 4 subgroups (n=6) and kept on fasting for 24 hours before giving them drugs, normal saline 3ml to 1st group (control), loperamide 5mg/kg to 2nd group (standard) and *Curcuma longa* 100mg/kg and 200mg/kg to 3rd and 4th group respectively. After 30 minutes of drug administration, 1 ml of castor oil was given to them for diarrhea induction. After one hour the rats were sacrificed by ether overdose. Abdomen was dissected and small intestine was traced and isolated from pyloric end to ilieocecal junction. The intestinal contents of each rat were collected by milking into a graduated tube and the volume as well as the weight was measured[14].

In Group C animals, gastrointestinal motility test was done. The method for testing gastrointestinal activity was modified from Gunakkunru et al[15]. The rats were divided into 4 subgroups (n=6) as in other groups, and fasted for 24 hours before the drugs were given to them in the same doses as in groups A and B. After 30 minutes of drug administration, 1 ml of castor oil was given to each. After 1 hour, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum tragacanth) orally. The animals were sacrificed after 30 minutes by ether overdose, abdomen was dissected and small intestine was isolated. The distance traveled by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of the total distance of the intestine.

The data analysis was done on SPSS version 19. The results were expressed as mean ±SD for quantitative variables (stool weight, stool number, volume of intestinal contents and small intestinal transit). Statistical comparison between groups was done by analysis of variance (ANOVA). The student t test was performed in all quantitative variables. In all statistical analysis P-value <0.05 was considered significant.

RESULTS:

The effect of *curcuma longa* on castor oil-induced diarrhea was observed. The mean diarrheal stool count was significantly reduced in 100mg/kg (78%) and 200mg/kg (100%) doses in a dose-related manner which was comparable to loperamide group (100%) and the difference was highly significant when compared to control group. There was significantly less decrease in weight in both *curcuma* groups as compared to control group (table-1).

The affect of *curcuma longa* on intestinal transit showed significant reduction in intestinal transit of charcoal meal in dose-related manner i.e. 31% in 100mg/kg and 37% in 200mg/kg *curcuma* group as compared to control group. Delay in intestinal transit was highest in loperamide group i.e. 42% (table-2).

Effect of *curcuma longa* on enteropooling i.e. weight (gm) and volume (ml) of intestinal contents showed that both subgroups of *Curcuma longa* had significant inhibitory effect on enteropooling evidenced by decrease in both volume and weight of intestinal contents as compared to control. Loperamide group showed maximum decrease in intestinal contents (table-3).

Table: 1

Anti diarrheal effect of Methanol extract of *Curcuma Longa* on castor oil-induced dry diarrheal count in albino rats

Group-A Treatment	**No of Animals**	**No of stool count At 1st hour** mean ± SD	**No of stool count At 4th hour** mean ± SD	**Percentage Change**	**Mean weight after diarrhea** in gram
Normal saline+ Castor oil 3ml	6	2.00±0.63	1.66±0.51	-17%	160.00±18.97
Loperamide 5mg/kg + Castor oil 3ml	6	1.33±0.51	0.00±0.00*	-100%	186.66±23.16
C. longa 100mg/kg + Castor oil 3ml	6	1.50±0.54	0.33±0.51*	-78%	184.16±11.14*
C. longa 200mg/kg+ Castor oil 3ml	6	1.33±0.51	0.00±0.00*	-100%	186.66±15.05*

* P-value Significant at <0.05 level as compared to control

Table: 2

Effect of methanol extract of *Curcuma Longa* on castor oil-induced enteropooling in albino rats

Group B Treatment	**Volume of intestinal content** (ml)	**Weight of intestinal content** (gm)	**(%) Inhibition**
Normal saline (3 ml/kg) + Castor oil 3ml	8.15±0.56	3.91±0.53	--------
Loperamide (5 mg/kg)+ Castor oil 3ml	1.68±0.78*	1.08±0.58*	72.34%
C. longa Extract (100mg/kg)+ Castor oil 3ml	5.66±0.43*	2.63±0.38*	32.77%
C. longa Extract (200mg/kg) + Castor oil 3ml	2.00±1.04*	1.25±0.52*	68.09%

*P-value was highly significant as compared to control

Table: 3

Effect of methanol extract of *Curcuma Longa* on small intestinal transit in albino rats (cm)

Group C Treatment	No of Animals	Total Length mean ± SD	Transit Length mean ± SD	Percentage Change
Charcoal meal + normal saline + Castor oil 3ml	6	97.71±2.00	84.85±4.25	-19%
Charcoal meal + Loperamide + Castor oil 3ml	6	98.05±3.10	65.26±9.21*	-42%
Charcoal meal + *C. longa 100mg/kg* + Castor oil 3ml	6	97.31±3.12	55.76±14.69*	-31%
Charcoal meal+ *C. longa 200mg/kg* + Castor oil 3ml	6	99.65±2.57	63.08±6.42*	-37%

*P-value highly significant as compared to control

DISCUSSION:

Diarrhea is one of the main causes of infant mortality in developing countries, causing about nearly one in five child deaths, a loss of about 1.5 million lives each year[8]. Medicinal plants have been used traditionally as anti-diarrheal without any scientific basis[16]. To evaluate the efficacy of these herbal drugs, castor oil-induced diarrhea models in rats are useful to observe measurable changes in the number and consistency of stools. The suggested underlying mechanism is liberation of lipase enzymes from ricinoleic acid present in castor oil[17], which irritates intestinal mucosa causing inflammation and release of prostaglandins and nitric oxide[18], leading to increased epithelial permeability, gastrointestinal secretion and motility[19].

The results of present study showed that both 100 and 200 mg/kg of methanol extract of *C. longa* have significant inhibitory effects on intestinal motility which was apparent by significant decrease in dry and wet diarrheal stool counts, significant decrease in enteropooling and finally decrease in intestinal transit. Gilani et al[20] observed that crude extract of turmeric relaxed potassium-induced contractions in isolated rabbit jejunum; relaxation was mediated through blockade of Ca^{++} influx, as another study[21] described curcumin as a Ca^{++} antagonist.

The gastrointestinal model using activated charcoal as marker has been used for more than six decades as a simple and effective means to assess effects of laxatives. This method indicates maximum distance traveled by the marker in the small intestine in a given time interval after its administration[22]. According to the model, *C. longa* extracts at different concentrations significantly reduced intestinal motility, with a dose-dependent pattern when compared to the control group.

Gnanasekar and Perianayagam[23] demonstrated that sodium salt of curcumin significantly inhibited castor oil-induced diarrhea by inhibition of prostaglandin synthesis. Two different studies[24,25] showed that Curcuma increased intestinal transit time in albino rats in disorders of altered intestinal motility. Another study[26] evaluated myo-relaxant effect of *Curcuma longa* on ileum and colon in a Mouse experimental colitis. In another study, Curcumin reduced mucosal injury in mice in experimentally-induced colitis. Ten days prior to induction of colitis with 1,4,6-trinitrobenzene sulphonic acid, administration of 50 mg/kg curcumin resulted in a significant reduction of diarrhea, neutrophil infiltration and lipid peroxidation in colonic tissue[1]. Husssain et al[27] and Balekar et al[3], in two different studies evaluated anti-diarrheal activity of methanol extract of two herbal plants, *Musa sapientum* (banana) and *Malvastrum tricuspidatum* (kharenti) in which 100 and 200 mg/kg body weight methanol extract of *Musa sapientum* reduced frequency and severity of diarrhea in test animals and significantly delayed intestinal transit of charcoal meal as compared to control group. Methanol extract of *Malvastrum tricuspidatum* at the doses of 100, 250 and 500mg/kg also significantly inhibited diarrhea by 71%, 80% and 88% as compared to control. The anti-diarrheal effects of these plants was due to the presence of flavonoids and alkaloids in them. Flavonoids control diarrhea by their ability to inhibit peristaltic activity and hydroelectrolyte secretion responsible for diarrhea. A previous study[3] showed similar results and established that flavonoids were able to inhibit intestinal secretory response. As *curcuma longa* is rich in flavonoids, these flavonoids were most likely responsible for its anti-diarrheal effect.

CONCLUSION:

The study demonstrated significant antidiarrheal effect of *Curcuma longa* and justified its use in diarrhea. It can be a safe alternative to synthetic antimotility agents.

RECOMMENDATIONS:
Studies are recommended to explore the underlying detailed mechanism by which *C. longa* and its components exert antidiarrheal effects.

REFERENCES:

1. Nasri H, Sahinfard N, Rafieian M, Rafieian S, Shirzad M, Rafieian-kopaei M. Turmeric: A spice with multifunctional medicinal properties. J Herb Med Plarmacol. 2014; 3(1): 5-8
2. Labban L. Medical and pharmacological properties of Turmeric (Curcuma longa): A review. Int J Pharm Biomed Sci 2014; 5:17-23
3. Balekar N, Parihar G, Jain DK, Gupta S. Antidiarrheal potential of ethanolic leaf extract of Malvastrum tricuspidatum in albino rats. J Adv Pharm Edu Res 2014; 4:233-40
4. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "curecumin": from kitchen to clinic. Biochem Pharmacol 2008; 75:787-809
5. Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med. 2006; 6:10
6. Kita T, Imai S, Sawada H, Kumagai H, Seto H. The biosynthetic pathway of curcuminoid in turmeric (Curcuma longa) as revealed by 13C-labeled precursors. Biosci Biotechnol Biochem. 2008; 72: 1789-98
7. Walker CLF, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC Public Health 2012; 12: 220. http://doi.org/10.1186/1471-2458-12-220
8. WHO and UNICEF (World Health Organization and United Nations Children's Fund). 2009
9. Rice AL, Sacco L, Hyder A, Black RE. Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. Bull World Health Organ 2000; 78: 1207–21
10. Baker DE. Loperamide: a pharmacological review. Rev Gastroenterol Disord 2007; 7(3): S11-S18
11. Awouters F, Nimegeers CJE, Lenaerts FM, Janssen PAJ. Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J. Pharm. Pharmacol, 1978, 30: 41-5
12. Tannaz B, Poonam D, Brijesh S, Pundarikakshudu T, Arvind N, Noshir A. Newer insights into the mechanism of action of *Psidium guajava* L. leaves in infectious diarrhoea. BMC Complement Altern Med. 2010; 10: 33
13. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol. 2001; 76(1):73–6
14. Qnais EY, Elokda AS, Abu Ghalyun YY, Abdulla FA . Anti-diarrheal activity of the aqueous extract of Punica granatum (Pomegranate) peels. Pharm. Biol. 2007; 45(9): 715-20
15. Gunakkunru A, Padmanaban K, Thirumal P, Pritila J, Parimala G, Vengatesan N, et al. Antidiarrhoel activity of Butea monosperma in experimental animals. J of Ethnopharmacology. 2005; 98: 24 - 4
16. Konaté K, Yomalan K, Sytar O, Brestic M. Antidiarrheal and antimicrobial profile extracts of the leaves from Trichilia emetica Vahl. (Meliaceae). Asian Pac. J. Trop. Biomed. 2015; 5: 242–8
17. Chitme HR, Chandra R, Kaushik S. Studies on anti–diarrheal activity of Calotropis gigantean in experimental animals. J Pharmacol Pharm Sci 2004;7: 70 -5
18. Wang S, Zhao Y, Zhang J, Huang X, Wang Y, Xu X, et al. Antidiarrheal effect of Alpinia oxyphylla Miq. (Zingiberaceae) in experimental mice and its possible mechanism of action. J Ethnopharmacol. 2015; 168: 182–90
19. Dash PR, Nasrin M, Ibn Morshed MT, Ali MS. Study of Antinociceptive Activity of *Kaempferia galanga* from Bangladesh in Swiss albino Mice. American Journal of Food and Nutrition 2015; 3(3):64-8
20. Gilani AH, Shah AJ, Ghayur MN, Majeed K. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. Life Sci 2005; 76: 3089 –105
21. Dyer JL, Khan SZ, Bilmen JG, Hawtin SR, Wheatley M, Javed MU et al. Curcumin a new cell permeant inhibitor of inositol 1,4,5-triphosphate receptor. Cell Calcium 2002; 31: 45–52
22. Silva PCB, Neto JC, Silva ADS, Silva KM, Silva TMS, Agra MF, et al. Antidiarrheal activity of Solanum asterophorum in mice. Rev. Bras.Farmacogn. 2012; 22: 131-6
23. Gnanasekar N, Perianayagam JB. Influence of sodium curcuminate on castor oil induced diarrhoea in rats. Indian J Pharmacol. 2004; 36 (3): 177-8
24. Kumar A, Purwar B, Shrivastava A, Pandey S. Effects of curcumin on the intestinal motility of albino rats. Indian J Physiol Pharmacol 2012; 54:284–8
25. Itthipanichpong C, Ruangrungsi N, Kemsri W, Sawasdipanich A. Antispasmodic effects of curcuminoids on isolated guinea-pig ileum and rat uterus. J Med Assoc Thai 2006; 86:2S 2999-309
26. Aldini R, Budriesi R, Roda G, Micucci M, Loan P, D' ErricoGrigioni A, et al. Curcuma longa extract exerts a myorelaxant effect on the ileum and colon in a mouse experimental colitis model, independent of the anti-inflammatory effect. PLoS ONE 2012; 7:e44650
27. Hussain MS, Alam MB, Asadujjaman M, Zahan R, Islam MM, Mazumder MEH et al. Antidiarrheal, antioxidant and antimicrobial activities of the Musa sapientum seed. Avicenna J Med Biotechnol 2011; 3:95-105