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Research Article

STUDY ON ANTIDIARRHOEAL POTENTIALS OF MANGIFERA INDICA TENDER FRUITS SEED KERNEL (MITFSK)

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A B S T R A C T

Objective: *Mangifera indica* Tender Fruits Seed Kernel (MITFSK) is traditionally used for the treatment of gastrointestinal disorders like diarrhoea and dysentery. Traditionally peoples of Tamil Nadu and Andhra Pradesh uses whole young fruit in the name of avakai used in pickle preparation. To understand the traditional claim of this fruits young seed kernel, the present study was undertaken to screen antidiarrhoeal activity using animal models. **Materials and Methods:** Castrol oil induced diarrhoeal model was considered to study dirrhoeal faecal output, gastro enteropooling, gastrointestinal motility, haematological parameters and small intestinal NA^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and Nitric oxide activity. **Results:** Results revealed that all the methods clearly evidenced the role of MITFSK on diarrhoeal control. This plant extracts not only control diarrhoea but also restore all the haematological parameters. This also scavenges nitric oxide in small intestinal tissues. **Conclusion**: This study concluded that MITFSK is one of the best alternative therapies for the treatment of diarrhoea without any side effects.

INTRODUCTION

Diarrhoea is one of the most important leading causes of morbidity and mortality among children (Shalini and Rajan, 2015). Incidence of this disease is due to unhygienic livelihood condition of the third world counties. Microbial agents and non microbial agents are responsible for the cause of diarrhea. Bacteria, virus, helminthes and parasites are the main cause of diarrhoea. Infectious agents cause change in bowl movement, irritation of intestinal epithelium, inflammation of bowl cells and leads to diarrhoea (Poongothai *et al.,* 2012). It also increases fluid secretion in intestinal region. Non microbial agents like $MgSo_4$, Castrol oil also able to cause diarrhoea by inducing adenylate cyclase, prostaglandins etc.,. Water, food and unhygienic environment also induce diarrhoea. Diarrhoea is treated with symptomatic as well as antibiotic therapy. Though various strategies are adopted by government and private agencies to culminate diarrhoea, still there are about 3.5% morbidity and mortality are due to diarrhea. Traditionally medicinal plants and plant preparations are take part in curing diarrhoeal diseases, scientific developments in the field of medicine reduces the use of plants, semi synthetic and synthetic preparations overcome the race, cures all kinds of diseases with lots of side effects including organ failure, cancer etc.,.

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To overcome these troubles, people turned towards traditional system of medicine, which also encouraged by WHO and other international agencies worldwide. Today about 25% of commercial drugs are made from medicinal plants in the name of phytotherapy. *Mangifera indica* Tender Fruits Seed Kernel (MITFSK) was selected in this study to reduce the consequences of diarrhoea. This plants young fruit along with seed kernel (Avaakai) used for pickle preparations (Bharathi and Rajan, 2018). Seed kernel of this plant is used to cure chronic diarrhoea. It has the ability to expel tapeworms (Antihelminthic) and other worms in ulcers (Prabhu and Rajan, 2015). It also possesses antioxidant, antidiarrhoeal, anti ulcer and antimicrobial properties. The kernel powder is used as astringent in bleeding piles. MITFSK is reported in traditional medicine as a cure for vomiting, dysentery and burning (Prabhu and Rajan, 2014). Many studies are available on mature fruits seed kernel of this plant where as no studies were indicated on young fruits seed kernel of this plant. Hence this study was undertaken to screen anti diarrhoeal activities of MITFSK using animal models.

MATERIALS & METHODS

Plant Material: *Mangifera indica* Tender Fruits Seed Kernel (MITFSK) was collected from Perambalur, Tamilnadu, India during summer months of 2016. The plant material was identified by Dr. John Britto, Professor, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and specimen was deposited in PG and Research department of

Microbiology, M. R. Government Arts College, Mannargudi, Tamilnadu, India.

***Preparation of Extracts**:* The powdered plant material (150gm) was mixed with water and alcohol (1500mL). The MITFSK powder was mixed with water for 3 days and filtered with a muselin cloth and it was condensed in hot air oven at 50˚C. Coarsely powdered MITFSK material was soaked in ethanol for 3 days, filtered and allowed to condense at 50˚C. The aqueous and alcoholic extract were stored in a container and refrigerated for future use (Jonathan, 2009).

Castor Oil-Induced Diarrhoea in Rats: The animals were starved for 18 h prior to the experiments, but allowed free access to water. Animals were randomly distributed into nine groups of 10 (five males and five females) animals per study dose. Group I was treated with 0.2ml of normal saline, which served as control; Group II received only castor oil, considered as disease control .Group III received standard drug (Loperamide 3mg/kg). Rats of the groups IV, V, VI groups were administered orally by gavage 100, 200 and 400 mg/kg body weight of the ethanolic extract, respectively. Groups VII, VIII and IX received orally by gavage 100, 200 and 400 mg/kg body weight of the aqueous extract, respectively. Sixty minutes after drug treatment, each animal was administered castor oil orally (1 ml/100 g body weight). The latent period (the time between castor oil administration and appearance of first diarrheic drop) was recorded. Observation for defecation continued up to 6 h on filter paper placed beneath the individual perforated rat cages. This paper was replaced every hour after noting its weight (M1). Finally, the filter paper was exposed in the laboratory for drying over a period of 14 h and it was reweighed (M2). The fecal water content was calculated as (M2 - M1) g. The percentage of rats that responded to diarrhoea, the latent period, the mean stool frequency, frequency of diarrheic drops and water content were recorded (Murugesan *et al.,* 2000), The purging indices (Mohd *et al*., 2004) the percentage inhibition of defecation and diarrheic drops (Ezekwesili *et al*., 2004) were evaluated.

$$\text{Purging index} = \frac{\%\ \text{respondants x average number of stool}}{\text{Average latent period}}$$

$$\text{Inhibition of defecation}\,(\%) = \frac{\text{Mc} - \text{Md}}{\text{Mc}} \text{x} 100$$

$$\text{Inhibition of diarrhoeic drops} = \frac{\text{Mo} - \text{Me}}{\text{Mo}} \text{x} 100$$

Effects of Plant Extracts on Castor Oil-Induced Enteropooling: Enteropooling (intraluminal fluid accumulation) was determined as described by Murugesan *et al.,* (2009). Animals were randomly distributed into nine groups of 10 (five males and five females) animals per study dose. Group I was treated with 0.2ml of normal saline, which served as control; Group II received only castor oil, considered as disease control .Group III received standard drug (Loperamide 3mg/kg). Rats of the groups IV, V, VI groups were administered orally by gavage 100, 200 and 400 mg/kg body weight of the ethanolic extract, respectively. Groups VII, VIII and IX received orally by gavage 100, 200 and 400 mg/kg body weight of the aqueous extract, respectively.Thirty minutes after drug treatment, castor oil was administered to all the rats (1ml/100 g body weight). Thirty minutes after castor oil administration, each rat was sacrificed and the ends of the small intestine were tied (at both the pylorus and the caecum). This section was dissected out and its length was measured. The intestinal content was collected by milking into preweighed (m0) graduated tubes and the new weight (m 1) was measured. The volume (ml) of the intestinal content was read directly from the graduation while the mass was obtained as (m1 -m0) g.

Effects of Plant Extracts on Gastrointestinal Motility: The method described by Akah *et al*., (1998) was used. Animals were randomly distributed into nine groups of 10 (five males and five females) animals per study dose. Group I was treated with 0.2ml of normal saline, which served as control; Group II received only castor oil, considered as disease control .Group III received standard drug (Loperamide 3mg/kg). Rats of the groups IV, V, VI groups were administered orally by gavage 100, 200 and 400 mg/kg body weight of the ethanolic extract, respectively. Groups VII, VIII and IX received orally by gavage 100, 200 and 400 mg/kg body weight of the aqueous extract, respectively. After 50 min, all the animals were given 1 ml of vegetable charcoal meal as a food tracer prepared at 10% in normal saline (0.9% sodium chloride). After an observation period of 40 min, each rat was sacrificed and dissected. The small intestine was removed and its total length was measured (cm). The movement of charcoal from the pylorus was equally measured (cm). The intestinal charcoal transit was expressed as a percentage of the distance moved by charcoal to the total length between the pylorus and the caecum.

Hematology: After the in vivo experiment, haematological study of blood parameters of the treated rats was carried out. Blood sample (2ml) was collected from their jugular vein with a disposable syringe and needle and immediately transferred into sterile Ethylene Diamine Tetra-acetic Acid (EDTA) embedded vials for haematological study (Freeman and Brain, 1996) of total erythrocyte (RBC), leukocyte (WBC) counts, Hemoglobin (Hb) content.

Small intestinal NA+K+ATPase, Ca2+ATPase, Mg2+ATPase: The animals were sacrificed by decapitation and exsanguinated. Brain and kidneys were immediately removed and placed in 20 volumes of ice-cold 0.32 M sucrose containing 1 mM EDTA. Each crude synaptosomal preparation was obtained by homogenation of a pool of 6 animal brains or kidneys in the sucrose solution. The homogenates were centrifuged at 1000 × g at 4 ◦C for 10 min. The supernatants were removed and centrifuged at 12,000 × g at 4 ◦C for 20 min (Gordon-Weeks, 1987; Maier and Costa, 1990). The pellets were resuspended in ice-cold 0.32 M sucrose containing 1 mM EDTA and used to determine ATPases activity according to Maier and Costa (1990). Total ATPase activity was determined spectrometricaly quantifying inorganic phosphorus produced by ATP hydrolysis and Mg2+-ATPase activity was measured following a similar method in a K+-free medium containing ouabain 1 mM. The difference of the two activities is taken as Na+,K+-ATPase activity. Inorganic phosphorus (Pi) was determined by a colorimetric assay of Taussky and Shorr (1953) and protein content was determined by the method of Lowry *et al*. (1951). Inhibition curves of Na+,K+-ATPase activity using ouabain ranged from 0.1 × 10−3 to 0.1 mM were prepared and compared with the curves obtained using strictosamide ranged from 0.25 to 2 mg/mL, in order to determine the in vitro effects of this compound on Na+,K+-

ATPase and Mg2+-ATPase activities. For the in vivo experiments, strictosamide was dissolved in distilled water (vehicle) and i.p. administered in single doses of 50, 100 or 200 mg/kg body weight to each group of six mice. The control group received i.p. vehicle. All the animals were sacrificed at 3 h of assay.

Determination of Nitric Oxide Concentration: The procedure described by Wo *et al*., (2013) was used to determine the concentration of nitric oxide in the small intestine supernatants of the animals. A known volume (0.5 mL) of the supernatant was added to 2 mL of 75 mmol/L ZnSO4 solution and 2.5 mL of 55 mmol/L NaOH. The solution was mixed thoroughly, adjusted to a pH of 7.3, incubated for 10 minutes, and centrifuged at 504 ×g for 10 minutes. The blank was constituted in a similar manner like the test except that 0.5 mL of supernatant was replaced by 0.5 mL of distilled water. Furthermore, 1 mL of glycine-NaOH buffer each was added to the test sample and blank. A known volume (2 mL) of deproteinized solution was added to the test and blank and the volume adjusted to 4.0 mL with deionized distilled water. The reaction was initiated by the addition of freshly activated cadmium granules and, after 60 minutes, 2.0 mL each of test and blank was added to tubes containing 2.5 mL of ethylenediaminetetraacetic acid solution, 3.0 mL of 1.0 mol/L HCl, and 0.3 mL of 1.0 g/L fuchsin acid solution, mixed thoroughly and incubated for 2 minutes. Next, 0.2 mL of 0.05 mol/L resorcinol and 3.0 mL of 1.0 mol/L NH_4OH were added. The absorbance of the test solution was read against the blank at 436 nm. The concentration of serum nitrite was extrapolated from the calibration curve of nitrite.

RESULT

Ethanolic extract of MITFSK at 400µg/ml (Gp VI) showed higher latency period (178.00±8.10). Purging indices of aqueous and ethanolic extracts at 400µg/ml are less than 2.90 (Gp VI and Gp IX). Both extracts of MITFSK are effectively controlled the effect of castor oil, which is evidenced in table 1. Aqueous extract provides effective control in faecal output upto 73.74±3.39% at 400µg/ml concentration (Gp VI), which is greater than drug control (Gp III). Similarly ethanolic extract produced 70.44±2.10% inhibition of faecal output.

Volume of intestinal fluid output was decreased in dose dependent manner. Increased concentration of extract dose decreases intestinal fluid output. Ethanolic extract at 400µg/ml concentration inhibited 57.22±2.00% of defacation and 53.57±5.12% diarrhoea, where as lopramide inhibited only 36.67±1.92% of defecation and 12.07±7.12% diarrhoeal output (Gp III). Aqueous extract also inhibited defacation and diarrhoea higher than lopramide (Gp IX). This clearly illustrated the effect of MITFSK as an ideal antidiarrhoeal agent (Table 2). Aqueous extracts showed greater effect on intestinal fluid output upto 60.56±3.15 (Gp IX).

Table 1 Effect of Ethanol and Aqueous extract of Selected Plant on fecal characteristics in castor induced diarrheal rats

Groups	Percentage of respondents	Latency period (m)	Total number of feces	Number of diarrheal feces	Purging Indices	Mean drops of fecal drops	% inhibition of diarrhea
I	100	55.50±1.23	5.67±0.33	0.00±0.00	0.00	0.00±0.00	0.00±0.00
II	100	69.00±1.15	20.33±1.36	17.50±0.62	25.36	12.17±0.60	0.00±0.00
III	100	85.33±0.95	17.17±0.60	13.50±0.76	15.82	9.83±0.48	22.12±5.87
IV	100	92.33±8.53	14.00±0.58	11.33±0.67	12.27	8.33±0.42	34.61±5.24
V	100	129.67±2.39	11.50±0.43	7.17±0.60	5.53	6.50±0.43	58.62±4.17
VI	100	178.00±8.10	10.50±0.76	5.17±0.40	2.90	5.17±0.48	70.44±2.10
VII	100	103.67±3.00	13.67±0.49	9.50±0.67	9.16	7.67±0.49	45.34±4.51
VIII	100	145.83±8.80	11.33±0.42	6.67±0.33	4.57	6.17±0.31	61.87±1.43
IX	100	267.17±15.77	9.50±0.43	4.50±0.43	1.68	4.67±0.33	73.74±3.39

Table 2 Effect of Ethanol and Aqueous extract of Selected Plant on fecal characteristics and intestinal fluid in castor induced diarrheal rats

Groups	Fecal Moisture Content (g)	Weight of stools (g)	Mean volume of intestinal fluid (ml)	% inhibition of diarrhea	% inhibition of defecation
I	1.55±0.10	0.16±0.01	0.00±0.00	0.00±0.00	0.00±0.00
II	3.28±0.19	1.12±0.02	12.33±0.56	0.00±0.00	30.00±3.55
III	2.91±0.09	0.63±0.02	10.67±0.49	12.07±7.12	36.67±1.92
IV	2.46±0.11	0.53±0.04	9.17±0.48	25.30±3.88	41.67±1.43
V	1.94±0.07	0.35±0.01	7.83±0.54	35.98±4.67	48.89±2.94
VI	1.70±0.04	0.22±0.02	5.67±0.61	53.57±5.12	57.22±2.00
VII	2.04±0.06	0.45±0.01	8.67±0.33	29.46±2.03	45.00±1.88
VIII	1.83±0.05	0.30±0.01	6.50±0.43	46.45±5.00	51.67±2.82
IX	1.67±0.11	0.19±0.01	4.83±0.31	60.87±1.36	60.56±3.15

Table 3 Effect of Ethanol and Aqueous extract of Selected Plant on intestinal charcoal transit in castor induced diarrheal rats

Groups	Total length of intestine (cm)	Distance travelled by marker (cm)	% intestinal transit	% of inhibition
I	86.99±1.93	26.90±1.68	30.78±1.30	0.00±0.00
II	84.63±0.69	67.47±0.75	79.72±0.46	0.00±0.00
III	85.16±1.04	58.82±0.65	69.08±0.25	13.33±0.61
IV	86.62±1.14	52.67±0.94	60.79±0.41	23.74±0.68
V	82.59±1.12	41.70±0.95	50.47±0.63	36.70±0.50
VI	86.13±1.17	35.02±0.93	40.62±0.54	49.04±0.73
VII	85.02±0.99	49.71±0.88	58.46±0.54	26.66±0.83
VIII	85.93±1.07	39.79±0.94	46.28±0.62	41.96±0.59
IX	85.91±1.30	28.76±0.98	33.43±0.68	58.04±0.99

Study on intestinal transit also indicated the effect of MITFSK extracts at 400µg/ml concentration. Ethanolic extract produced about 49.04±0.73% of intestinal transit inhibition (Gp VI). Whereas lopramide inhibited intestinal transit up to only 13.33±0.61% (Table 3). Aqueous extract inhibited intestinal transit up to 58.04±0.99%

Haematological parameter abnormalities were restored when animals were treated with aqueous and ethanolic extracts of MITFSK (Gp IV, V, VI, VII, VIII and IX). WBC levels were restored effectively when compared to standard drug lopramide (Gp VI). Haemoglobin content also increased with increased concentration of plant extract. Ethanolic extract effectively restored haematological parameters when compared to aqueous extract (Table 4).

is one of the most important ingredients in siddha and ayurvedha preparations. Castor oil is used to study antidiarrhoeal activity of MITFSK. Ricinoleic acid present in castor oil initiates diarrhoea via several mechanisms such as causing irritation and inflammation of the intestinal mucosa (Mbagwu and Adeyemi, 2008); affecting electrolyte transports and smooth muscle contractility in the intestine (Palombo, 2006); by preventing the reabsorption of water; interfering with oxidative and being cytotoxic to intestinal epithelial cells (Mascolo *et al*., 1993). These sequences of events may be related to the release of eicosanoids, prostaglandins, nitric oxide, platelet activating factor, cAMP and tachykinins by the intestinal mucosa.

Table 4 Table 5 Effect of Ethanol and Aqueous extract of Selected Plant on hematological parameters in castor induced diarrheal rats

Groups	RBC (10^6 Cells/mm^3)	WBC (10^3 Cells /mm^3)	Haemoglobin (g%)	Hematocrit
I	9.97±0.47	7.18±0.48	21.64±0.06	36.17±1.45
II	7.02±0.43	13.69±0.50	15.45±0.03	21.33±0.49
III	7.29±0.46	12.15±0.43	15.88±0.07	25.00±1.06
IV	7.55±0.59	11.19±0.49	16.70±0.06	27.50±0.76
V	8.46±0.50	9.10±0.46	17.86±0.10	30.83±0.60
VI	8.95±0.49	7.46±0.43	18.73±0.11	34.17±0.79
VII	8.22±0.50	9.46±0.44	17.32±0.02	29.50±0.76
VIII	8.54±0.48	8.29±0.46	18.31±0.01	32.50±0.76
IX	9.63±0.49	7.20±0.56	20.30±0.06	35.33±1.31

Values are expressed as Mean±SE (n=6); Non Significant differences for each group vs control values.

Table 5 Effect of Ethanol and Aqueous extract of Selected Plant on small intestinal NA^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and NO in castor induced diarrheal rats

Groups	Small Intestine NA^+K^+ATPase (µg of pi liberated/ g tissue/min)	Small Intestine Ca^{2+}ATPase (µg of pi liberated/ g tissue/min)	Small Intestine Mg^{2+}ATPase (µg of pi liberated/ g tissue/min)	Small intestine nitric oxide Concentration (µmol/L)
I	38.53±0.52	96.91±0.45	77.69±0.51	79.33±1.02
II	24.52±0.77	53.76±0.62	55.74±1.26	267.17±6.56
III	24.89±0.57	68.07±0.80	61.19±0.57	140.67±3.94
IV	27.68±0.63	73.08±0.53	64.74±0.92	122.50±4.79
V	31.64±0.58	79.89±0.55	71.50±0.65	105.00±3.65
VI	34.84±0.49	92.08±0.50	74.88±1.07	87.50±2.05
VII	28.55±0.53	76.28±1.61	65.90±0.46	116.67±4.77
VIII	32.53±0.51	83.02±0.74	69.57±0.61	96.67±1.84
IX	36.68±0.47	95.04±0.61	76.02±0.73	82.17±1.51

Levels of intestinal tissue NA^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were increased when the diarrhoeal animals were treated with lopramide (Gp III), aqueous extract (Gp VII, VIII and IX) and ethanolic extract (Gp IV, V and VI). Group IX animals showed restored NA^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase activity as like group I animals. This indicated that aqueous extract of MITFSK proved to be an antidiarrhoeal agent when compared to etahanolic extract and lopramide. Nitric oxide concentration also restored as like normal at 400µg/ml concentration of MITFSK aqueous aswell as ethanolic extract (table 5).

DISCUSSION

Traditional healers in India use plants and plant products as trademark medicine in villages. They used plant medicine without disturbing natural metabolism of an individual. Tender fruit of *Mangifera indica* seed kernel (Aavakai) is used to prepare pickle in India. Masuad Parvez (2016), Bharathi and Rajan, (2018) and Rajan *et al*., (2011) indicated the uses of MITSK in diarrhoea, dysentery, haemorrhages, haemorrhoids, diabetes, heat burn etc.,. *Mangifera indica* seed kernel (MISK)

Hence in this study appropriate standard methods were adopted to analyze volume of intestinal content, intestinal transit and release of wet stool.

Results revealed that MITFSK reduces diarrhoeal diseases in experimental animals, which is evidenced through reduction in defecation rate, % of intestinal transit and volume of intestinal content. The clinical effect of the MITFSK aqueous and ethanolic extract as antidiarrhoeal agent was demonstrated at 100 and 400 mg/kg body weight. The extract might have exerted its antidiarrhoeal activity via secretary mechanism as evident from reduction in total number of wet faeces. Furthermore, this antidiarrhoeal activity could have resulted from the inhibitory activity of aqueous and ethanolic MITFSK extracts on prostanglandins synthesis, nitric oxide and platelet activating factors production, as inhibitors of prostaglandins and nitric oxide syntheses are known to delay diarrhoea induced by castor oil (Adzu *et al*., 2003; Tangpu and Yadav, 2004). Similar effects were reported in several studies by Qnais *et al*., (2005), Akindele and Adeyemi (2006) and Appidi *et al*., (2010). Seed kernel of matured *Mangifera indica* also exhibited similar kind of effect. The increase in the activity of

Na+-K+ ATPase (Table 5) as well as decrease in the concentration of nitric oxide (table V - Gp VI and IX) in the small intestine of extract treated animals may be one of the mechanisms by which the extract exhibits its antidiarrhoeal effect. This anti-enteropooling effect of MITFSK could be due to the presence of tannins and flavonoids in the extract, as the phytochemical have been reported to inhibit intestinal motility and hydroelectrolytic secretion (Bharathi and Rajan, 2018). Lopramide is known to produce an antidiarrhoeal effect on intestinal transit. The extract appears to have acted on all parts of the intestine producing inhibitory effect on both the gastrointestinal propulsion and fluid secretion. The findings in this study are similar to the report by Maridass (2011). Previous studies have implicated a wide array of phytochemicals with antidiarrhoeal activity. These include tannins, alkaloids, saponins, flavonoids, sterols, terpenoids and reducing sugars (Prabhu and Rajan, 2014; Shoba, 2001; Venkatesan *et al*., 2005). Flavonoids and saponins are known to inhibit the release of autocoids and prostaglandins thereby reducing the motility and secretion induced by castor oil (Sairam, 2003; Perez *et al*., 2005). Tannins also exerts antidiarrhoeal effect by precipitating surface proteins, there by prevents the release of $Ca^{2+,}$ Mg^{2+}, Na^{+}, K^{+} ions (Rajan *et al*., 2011)

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