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Evaluation of Antidiarrheal Efficacy of Traditionally Used Plants in Rats and Dairy Calves

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ABSTRACT

Plants have been used since time immemorial to treat a variety of diseases. The present study was conducted to comparatively evaluate the antidiarrheal efficacy of plants traditionally used to treat diarrhea viz. semi ripe fruit of *Aegle marmelos*, rhizome of *Curcuma longa*, leaves of *Dalbergia sissoo*, bark of *Mangifera indica*, leaves of *Psidium guajava* and fruit rind of *Punica granatum*. Methanolic plant extracts were tested for their antidiarrheal efficacy in castor oil induced diarrhoea in rats at three different doses (@ 100, 200 and 400 mg/Kg body weight). Of the six extracts tested, *Aegle marmelos* fruit extract was found to be the most effective, followed by *Dalbergia sissoo* leaf extract; both resulted in dose dependent inhibition of diarrhea in rats. *A. marmelos* fruit extract was further evaluated for its therapeutic potential in acute undifferentiated diarrhea of calves @ 400 mg/Kg/Day, orally in two divided doses. Efficacy of the methanolic extract of *Aegle marmelos* was found comparable to that of oral pefloxacin given to a group of calves @ 10 mg/Kg body weight, orally, once a day. In conclusion, *Aegle marmelos* can effectively be used as a non-specific antidiarrheal in acute diarrhea of cattle calves.

HIGHLIGHTS

- Comparative evaluation of antidiarrheal efficacy of methanolic extracts of traditionally used plants in in rats and neonatal dairy calves.
- *Aegle marmelos* can effectively be used as a non-specific antidiarrheal in acute undifferentiated diarrhea of cattle calves.

Keywords: *Aegle marmelos,* antidiarrheal, calf, dairy calves, *Dalbergia sissoo, diarrhea,* rats

Despite major advances in management and veterinary care of farm animals, neonatal diarrhea is still a major cause of concern for livestock owners and veterinarians (Srivastava *et al.,* 2016; Srivastava *et al.,* 2022). It causes significant economic loss to cattle industry (Radostits *et al.,* 2000). Neonatal diarrhea is a complex syndrome having a multifactorial etiology like infections and change or abnormality in diet associated with a number of stress factors. Several enteropathogens are responsible for causing diarrhea in livesctock which are mainly enterotoxigenic *E.coli* (Sharma *et al.,* 2022), rotavirus, corona virus, *Cryptosporidium* and *Salmonella* spp. industry (Radostits *et al.,* 2000). Mostly, more than one of these enteropathogens interact with each other to produce the clinical syndrome and it is very difficult to differentiate between the common causes of diarrhea on the basis of clinical signs alone. The disease therefore, is termed as 'acute undifferentiated diarrhoea of newborn calves' (Radostits *et al.,* 2000).

Despite the evidences of multifactorial etiology of calf diarrhea and increasing pressure from the medical

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fraternity in terms of drug resistance, various antimicrobial agents are currently being used for treatment of diarrhea. Deleterious effects of various orally fed antimicrobial agents on structure and function of small intestine of calves has been reported (Constable, 2004). A wide variety of Indian plants have been used traditionally by different indigenous communities as a nonspecific treatment of diarrhea. A few clinical trials have also been conducted to evaluate the safety and tolerability of traditional herbal antidiarrheal preparations which indicated minimal side effects (Palombo, 2006).

Considering all these facts, antidiarrheal efficacy of six plants *(A. marmelos*, *D. sissoo*, *C. longa*, *P. granatum*, *P. guajava* and *M. indica)*, commonly used as nonspecific antidiarrheal, were comparatively evaluated in castor oil induced diarrhea in rats followed by the assessment of clinical efficacy of the most effective plant extract in clinical cases of acute undifferentiated diarrhea in neonatal calves.

MATERIALS AND METHODS

Identification of common plant materials effective against diarrhea

Plant materials, which are traditionally used by the local people and which were claimed to be effective in treating diarrhea were selected for the present study. On these bases, six different plants materials were selected for the study viz. semi ripe fruit of *A. marmelos*, leaves of *D. sissoo*, rhizome of *C. longa*, fruit rind of *P. granatum*, leaves of *P. guajava* and bark of *M. indica*. Plant materials for the present study were collected from the Bareilly district of North Central India. A specimen each of these plant materials was deposited in the Botany department of Bareilly College, India for proper identification and cataloguing. The methanolic extracts of the plants were prepared as per the standard method (Paech and Tracy, 1956) and percentage yield for each plant material was calculated (Table 1).

Evaluation of antidiarrhoeal activity of extracts of identified plant materials in castor oil induced diarrhea in rats

A total of 100 Albino-wistar rats [sourced from Laboratory Animal Resource Section, IVRI] divided into 20 groups of 05 each of either sex weighing 150-200 grams, were used for the study during 2005-2007. The trial was pre-approved by the institutional animal ethics committee (IAEC) of IVRI. The trial was conducted as per the standard method (Awouters *et al.,* 1978). The rats were fasted overnight before commencement of the trial. Clean drinking water was provided to rats *ad libitum* throughout the trial. For each plant extract, three different doses of the extract (100, 200 and 400 mg/Kg in 5% Tween-80 solution) were administered to three different groups of rats (n=05) orally by gavage as a suspension. Lomotil®, Pfizer India Ltd. (Diphenoxylate hydrochloride @ 2.5 mg + Atropine sulphate @ 0.025 mg/ tablet) was used as the standard antidiarrheal drug for comparison. Rats belonging to untreated control group (n=05) were given 05 mL of 5% tween- 80 solution, orally only whereas rats belonging to standard control group (n=05) were given Lomotil® @ 05 mg/Kg body weight, PO. One hour after treatment each animal received 1 ml castor oil orally by gavage and was then observed for defecation.

Table 1: Methanolic extract yield (g %) of plant materials by Soxhlet apparatus

Sl. No.	Plant species	Part used	Extract yield (g%)
1	Aegle marmelos	Semi ripe fruit	32.0
2	*Curcuma longa*	Rhizome	15.0
3	*Dalbergia sissoo*	Leaves	12.0
4	*Mangifera indica*	Stem bark	13.5
5	*Psidium guaja*va	Leaves	16.0
6	*Punica granatum*	Fruit rind	16.5

Total number of fecal droppings was recorded for each group over the 4 hr period after castor oil administration and average number of droppings per animal was calculated. Percentage reduction in the fecal output was calculated for each group in comparison with the untreated control group considering 100% establishment of diarrhea in this group. All the test groups exhibiting more than 50 % reduction in fecal output were considered to be efficacious against castor oil induced diarrhea in rats.

After completion of the trial (4 hrs after castor oil challenge), rats were anaesthetized with diethyl ether and 1.0 – 2.0 ml of blood was collected from each rat by puncturing the orbital venous plexus through the inner eye canthus using heparinised microcapillary tube

for estimation of hematocrit, serum sodium and serum potassium. After completion of the trial, all the rats were provided feed and water *ad libitum*.

Therapeutic evaluation of the effective plant extract in clinical cases of calf diarrhoea

Dairy calves (under one month of age) of an organized dairy herd (dairy farm of the Indian Veterinary Research Institute) suffering from acute undifferentiated diarrhea were selected for the trial. Diarrheic calves were divided randomly into 03 groups of 05 each for undertaking the therapeutic trial study of the most effective plant extract based on the results of rat trial described above. First group was treated with antibiotic (Pefloxacin, Tab Pelox®, Wokhardt India Ltd. @ 10 mg/Kg B.Wt., PO, OD for 03 days) whereas the test group was treated for 03 days with the estimated dose of the plant extract exhibiting highest antidiarrheal activity in the rat trial (as described above). A group of 05 healthy calves served as healthy control.

Fecal consistency, depression and dehydration score in respect of calves were recorded on day 0 (the day of clinical diagnosis of diarrhoea) and day 03 (post therapy) as per the standard method (Walker *et al.*, 1998) (Table 2).

05 ml blood was collected from the calves aseptically through jugular venepuncture on day 0 (the day of clinical diagnosis of diarrhea) and day 03. Two fractions were collected, one with anticoagulant (Na_2-EDTA + sodium fluoride) for estimation of hematocrit and plasma glucose and another without any anticoagulant for separation of serum to estimate total serum protein (Doumas *et al.*, 1971), serum sodium and serum potassium (Oser, 1965).

STATISTICAL ANALYSIS

Statistical analysis of the data obtained was done using SPSS statistical software. Data obtained during *in vivo* trial (in rats) was analyzed by one way analysis of variance (ANOVA) at 95 % level of significance. Data obtained during therapeutic evaluation study (in calves) was analyzed by paired t-test at 95 % level of significance.

RESULTS AND DISCUSSION

Characteristic semi solid diarrhoeic droppings were evident in overnight fasted rats of the untreated control group after 1 hr of castor oil administration. An average of 6 defecations per animal was recorded in the untreated (castor oil induced) control group whereas an average of 2.2 was recorded in the treated control (Lomotil®) group, which was 63.37 % less than the untreated control group. Similarly, average number of droppings was recorded for each of the 18 test groups (Table 3). *A. marmelos* fruit extract @ 400 mg/kg was found to be most effective which resulted in 73.33 % reduction in faecal output followed by *D. sissoo* leaf extract @ 400 mg/kg (60 % reduction), *A. marmelos* fruit extract @ 200 mg/kg (56.67% reduction) and *D. sissoo* leaf extract @ 200 mg/kg (53.32 % reduction) (Table 3). No significant antidiarrheal activity was observed with other extracts.

It was also observed that fecal pellets passed by the rats were very well formed in *A. marmelos* and Lomotil® treated group but the same was not observed in *D. sissoo* treated group.

Table 2: Clinical scoring of diarrheic calves

Score	Faecal consistency score	Depression score	Dehydration score
0	Normal, well-formed faeces	Normal	Normal, eyes are bright
1	Pasty faeces	Mild depression, calf suckles but not vigorously	Mild dehydration, skin tents < 3 seconds, eye balls don't recede into the orbit
2	Semi-liquid faeces still with a solid component	Moderate depression; calf is able to stand, suckling is weak or disorganized	Moderate dehydration, skin tents for 3 -10 seconds, eye balls slightly recessed into the orbit
3	Watery faeces	Severe depression, calf is unable to stand	Severe dehydration, skin tents > 10 seconds, eye balls markedly recessed into the orbit

No definite pattern was observed in the alteration of values of hemato-biochemical parameters (hematocrit, serum sodium and serum potassium) of all the groups during the experiment except the group treated with *A. marmelos* @ 400 mg/Kg which provided statistically significant protection from hemoconcentration [only hematocrit of the group treated with *A. marmelos* fruit extract @ 400 mg/kg (38.00 ± 0.707%) varied significantly from that hematocrit of untreated (castor oil) control group (42.00 ± 0.633%)]. However, there were no significant differences among the hematocrit of all the other groups. It is interesting to observe that the mean hematocrit of treated (Lomotil®) control (39.00 ± 1.140%) itself was not significantly ($P < 0.05$) different from the untreated control and was even higher than many of the test groups. Results are summarized in the Table 3.

Therapeutic evaluation of *A. marmelos* fruit extract in clinical cases of calf diarrhea

During screening of plant extracts for their antidiarrheal property in rats, *Aegle marmelos* extract was found to be most effective with a dose rate of 400 mg/kg. This extract was therefore further tried in the diarrheic calves at a dose rate of 400 mg/kg in two divided doses for three days.

Statistically significant improvement (between pretreatment and post treatment values) was observed in all the clinical parameters observed (Fecal consistency, depression and dehydration score) as well as in hematocrit, serum protein and plasma glucose. Statistically insignificant improvement was observed in the values of serum sodium and serum potassium in the test (*A. marmelos* extract) group whereas significant improvement

Table 3: Effect of plant extracts on castor oil induced diarrhoea in rats (Mean ± SEM for PCV, Serum sodium and Serum potassium)

Sl. No.	Group (N= 05)	Average no. of fecal droppings / animal in 4 hrs.	% reduction in droppings compared to untreated control	PCV (%)	Serum Sodium (mEq/L)	Serum Potassium (mEq/L)
1	Untreated Control	6.0	00.00	42.00 ± 0.633^{A}	132.444±2.437	6.60 ±0.509
2	Treated Control (Lomotil® @ 5 mg/Kg)	2.2	63.37*	39.00 ± 1.140	137.358 ±1.461	5.80 ± 0.489
3	*A. marmelos* @ 100 mg/Kg	4.0	33.32	39.60 ± 1.208	132.678±3.541	7.00 ± 0.547
4	*A. marmelos* @ 200 mg/Kg	2.6	56.67*	39.00 ± 1.140	132.578±2.426	6.80 ± 0.735
5	*A. marmelos* @ 400 mg/Kg	1.6	73.33*	38.00 ± 0.707^{B}	140.166±2.653	5.40 ± 0.510
6	*D. sissoo* @ 100 mg/Kg	5.2	13.32	39.80 ± 1.241	138.762±1.364	6.60 ± 0.600
7	*D. sissoo* @ 200 mg/Kg	2.8	53.32*	39.00 ± 1.871	138.294±1.007	6.40 ± 0.600
8	*D. sissoo* @ 400 mg/Kg	2.4	60.00*	38.60 ± 1.166	134.316±3.148	5.20 ± 0.374
9	*C. longa* @ 100 mg/Kg	5.0	16.65	39.20 ± 1.356	137.826±2.875	6.80 ± 0.663
10	*C. longa* @ 200 mg/Kg	4.0	32.32	39.20 ± 0.663	139.230±2.189	6.00 ± 0.316
11	*C. longa* @ 400 mg/Kg	3.8	36.65	40.80 ± 1.562	138.996±3.170	6.80 ± 0.583
12	*P. granatum* @ 100 mg/Kg	6.0	00.00	38.80 ± 0.800	136.186±4.902	6.40 ± 0.872
13	*P. granatum* @ 200 mg/Kg	4.6	23.32	39.20 ± 1.068	133.848±3.674	6.40 ± 0.678
14	*P. granatum* @ 400 mg/Kg	3.0	50.00	39.80 ± 1.241	133.848±2.299	6.80 ±0.583
15	*P. guajava* @ 100 mg/Kg	5.6	06.65	38.40 ± 0.812	137.124±3.255	5.80 ± 0.735
16	*P. guajava* @ 200 mg/Kg	5.0	16.65	38.40 ± 0.510	135.954±3.905	6.00 ± 0.548
17	*P. guajava* @ 400 mg/Kg	5.4	9.98	38.80 ± 0.860	132.678±5.013	6.00 ± 0.548
18	*M. indica* @ 100 mg/Kg	6.0	00.00	39.00 ±1.000	133.614±2.779	6.40 ± 0.510
19	*M. indica* @ 200 mg/Kg	5.8	3.31	40.00 ± 0.837	131.508±3.961	7.00 ± 0.707
20	*M. indica* @ 400 mg/Kg	5.4	9.98	40.60 ± 0.510	135.252±1.908	6.80 ± 0.663

* Significant (more than 50 %) reduction in fecal output compared to that in untreated control group; Values with different letters as superscript (across the column) vary significantly ($P < 0.05$).

was observed in all the parameters studied in the standard (Pefloxacin) group after three days of treatment (Table 4).

Diarrhea is usually considered a consequence of altered motility and fluid accumulation in intestinal tract. Castor oil after being ingested produces an active metabolite - ricinoleic acid which increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water. All these changes result in hypersecretory response and diarrhoea (Gaginella *et al.,* 1975). Diphenoxylate is an opioid which stimulates contraction of circular smooth muscles of intestine resulting in increased segmentation and increase in transit time of ingesta through the alimentary tract. Atropine sulphate, although present in a very insignificant amount in the Lomotil®, by virtue of its anticholinergic activity inhibits both motility and secretions of alimentary canal.

In this study, extracts of *A. marmelos* followed by that of *D. sissoo* exhibited significant reduction in faecal output and *A. marmelos* extract was also able to provide effective protection from hemoconcentration. These effects may be due to capability of *A. marmelos* fruit extract to protect the mucosa, reduce the gut motility and ability to prevent excessive fluid and electrolyte losses in the gut lumen. Absence of any remarkable change in hemato-biochemical profile of the rats may be due to lack of sufficient time for the clinical changes to be evident hemato-biochemically.

C. longa, M. indica, P. granatum, and *P. guajava* extracts did not result in significant reduction in faecal output in this study. However, *C. longa* (Duke *et al.,* 2002)*, P. granatum* (Das *et al.,* 1999), and *P. guajava* (Ojewole *et al.,* 2008) have been reported to possess anti-diarrheal property via different mechanisms.

Plant extracts which were found to have highest efficacy during *in vivo* trial for non-infectious diarrhoea in rats i.e. *A. marmelos* was validated further in clinical cases of calf diarrhoea. For this purpose, calves suffering from 'acute undifferentiated diarrhoea' were selected without ascertaining the exact etiology of the diarrhea. In field cases, it is neither possible nor imperative to look for the exact etiological agent responsible for diarrhea in calves, and most of the times all that requires is to treat the clinical signs. There are several reports about the direct bactericidal

Table 4: Clinico-hematobiochemical profile of different therapeutic regimens in clinical cases of acute undifferentiated calf diarrhoea

Parameter	Day	Group I**	Group II***	Group III****
Faecal consistency score (0-3)	0	0.00±0.000^{A}	2.750±0.250^{A}	2.600±0.244^{A}
	3	0.00±0.000^{A}	0.000±0.000^{B}	0.600±0.400^{B}
Depression score (0-3)	0	0.00±0.000^{A}	1.500±0.288^{A}	1.600±0.244^{A}
	3	0.00±0.000^{A}	0.000±0.000^{B}	0.200±0.200^{B}
Dehydration score (0-3)	0	0.00±0.000^{A}	1.750±0.250^{A}	1.800±0.200^{A}
	3	0.20±0.123^{A}	0.000±0.000^{B}	0.400±0.24 5^{B}
PCV (%)	0	32.400±3.140^{A}	33.740±3.301^{A}	30.400±2.713^{A}
	3	32.800±3.056^{A}	31.000±3.189^{B}	28.600±2.638^{B}
Plasma glucose (mg/dL)	0	62.299±3.487^{A}	62.080±2.588^{A}	62.584±4.320^{A}
	3	62.010±3.705^{A}	68.182±2.125^{B}	65.646±4.144^{B}
Total serum protein (g/dL)	0	5.804±0.667^{A}	6.600±0.507^{A}	6.330±0.505^{A}
	3	5.864±0.652^{A}	5.768±0.380^{B}	5.622±0.613^{B}
Serum sodium (mEq/L)	0	137.400±3.906^{A}	131.800±5.324^{A}	132.800±4.587^{A}
	3	137.400±4.082^{A}	137.400±4.545^{B}	136.200±4.862^{A}
Serum potassium (mEq/L)	0	5.000±0.447^{A}	6.000±0.707^{A}	5.800±0.800^{A}
	3	4.800±0.374^{A}	5.000±0.447^{B}	5.200±0.735^{A}

*Values (Mean ± SEM) with different letters across the column (for each parameter) as superscript vary significantly ($P < 0.05$); **Group I – Healthy control; ***Group II – Antibiotic (Pefloxacin @ 10 mg/Kg, orally, once in day) control; ****Group III – Treated with *A. marmelos* fruit extract @ 400 mg/kg/day, orally in 2 divided doses.

activity of *A. marmelos* fruit or leaf extract against different pathogenic bacteria including *E. coli* (Sharma *et al.,* 2022; Srivastava *et al.,* 2016; Rajasekaran *et al.,* 2008). Several biologically active phytochemicals, such as aurapten, luvangetin, marmelide marmelosin, psoralen, and tannin have been isolated from fruit of *A. marmelos*. Of these, marmelosin is reported to have antibacterial whereas psoralen and tannin have antispasmodic and astringent property, respectively (Maity *et al.,* 2009). Tannins and flavonoids have consistently been proposed to be responsible for antidiarrheal activity of different phytodrugs (Palombo, 2006). *P. granatum* is reported to have high hydrolysable tannin content (Wang *et al.,* 2010) which has antidiarrheal activity but it didn't result into its superior antidiarrheal efficacy over and above to that of *A. marmelos* and *D. sissoo*. This contradicts the observations of the present study. Therefore, in the present scenario it can be assumed that antidiarrheal activity of methanolic extract of *A. marmelos* and *D. sissoo* cannot be effectively attributed to any single chemical constituent. Safety of *A. marmelos* fruit extract has already been established in the previous studies (Kamalakkannan *et al.,* 2003).

Results of the present study indicate that both *Aegle marmelos* fruit extract and *Dalbergia sissoo* leaf extract possess significant antimotility and antidiarrheal activity. Phytodrugs containing *Aegle marmelos* or *Dalbergia sissoo* or both can therefore be effectively used as standalone or supportive therapy in clinical cases of acute undifferentiated diarrhea of neonatal calves.

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