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Therapeutic efficacy of polyherbal anti-diarrhoeal preparation in diarrhoeic goats

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Abstract

Diarrhoea has been recognized as one of the most important health problem and leading cause of mortality and morbidity in goats. The *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* possesses antidiarrheal activity, which acts by reducing the gastrointestinal motility and gastric secretion and possess lesser side effects than the conventional drugs. The present research work was undertaken to study the therapeutic efficacy of Polyherbal antidiarrhoeal tablet containing hydro-ethanolic extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* in diarrhoeic goats and to study the haematological profile in diarrhoeic goats. Total 25 diarrhoeic goats were selected and treated with polyherbal antidiarrhoeal tablet (600 mg) @ 1 tablet orally twice a day for 5 day along with fluid and supportive treatment depending on severity of case. The efficacy of Polyherbal antidiarrhoeal tablet was judged on the basis of restoration of faecal consistency to normal and improvement in haematological parameters in diarrhoeic goats after treatment. The mean rank of faecal consistency score was normal in diarrhoeic goats after treatment with Polyherbal antidiarrhoeal tablet. The haematological profile study showed significant ($P < 0.01$) improvement in altered PCV (%), TLC, TEC, neutrophils (%), lymphocytes (%) and eosinophils (%) on 5th day post treatment as compare to their corresponding values on '0' day (before treatment). The study concluded that the polyherbal antidiarrhoeal tablet containing *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* found effective and safe remedy in inducing recovery in diarrhoeic goats with improvement in haematological parameters.

Keywords: Polyherbal, antidiarrhoeal, goats, *Aegle marmelos*, *Dalbergia sissoo*, *Holarrhena antidysenterica*, haematology

Introduction

Among the diseases affecting goats, gastrointestinal diseases such as enteritis is one of the most important and common health problem. The etiological agent causing diarrhoea in goats are bacterial, viral, protozoa, parasitic, nutritional, management and stress etc. It is one of most common clinical signs of GIT disease and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and decrease in the absorption of fluid and thus, a loss of electrolytes and water results in severe dehydration and death [1]. Bacterial enteritis is one of the important cause of diarrhoea in goats. Indiscriminate usage of antibiotics in animals for the treatment of various bacterial infections has lead to emergence of resistant strains. Various treatments such as Diphenoxylate, Loperamide, racecadotril, bismuth subsalicylate, muscarinic receptor blockers like atropine sulfate etc; are available in the market for treatment of diarrhoea. However, these synthetic drugs possess various side effects [2]. Therefore, the World Health Organization (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices. Medicinal plants are of greater significance for treatment of diarrhoea in ruminants because of their complex stomach wherein herbs are better assimilated and good for rumen microbes and almost devoid of side effects. For this reason, in recent year the interest in study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. Therefore, situation requires a urgent need for development and promoting cheaper, affordable, easily available and eco-friendly, effective, safe alternative antibacterial and antidiarrhoeal agent to control diarrhoea in goats. The ancient Indian system of medicine, the 'Ayurveda' describes medicinal values of many herbs and their curative properties against a variety of diseases in man and animals [3]. There are many herbal plants such as *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* which possess antidiarrheal

activity by reducing the gastrointestinal motility and gastric secretion and exhibit lesser side effects than the conventional drugs and thus are safer to use [1].

Aegle marmelos is one of the most important medicinal plant of India. The half ripe *Aegle marmelos* fruit (Bael) has astringent property that reduces irritation in the digestive tract and is excellent treatment for diarrhoea and dysentery [4]. The unripe dried fruit is used as an astringent, digestive, stomachic in diarrhoea and dysentery. Decoction of unripe fruit is astringent, useful in diarrhoea and chronic dysentery [5]. The effectiveness of *A. marmelos* fruit in diarrhoea and dysentery has resulted in its entry into the British Pharmacopoeia [6]. *Dalbergia sissoo* is among the most important Indian timber tree. Various part of *D. sissoo* plant were being used for various therapeutic purposes. The bark of *Dalbergia sissoo* has astringent property and traditionally used for treating the diarrhea. Many workers reported significant anti-diarrhoeal activity of *D. sissoo* leaves extract in castor oil induced diarrhoea in mice [7, 8]. Some researchers reported very good anti-diarrhoeal activity of decoction of leaves of *Dalbergia sissoo* in diarrhoeic goats [9]. *Holarrhena antidysenterica* plant is used traditionally for a variety of health disorders, including colic, diarrhoea, dysentery, constipation and flatulence. The bark and seeds of *Holarrhena antidysenterica* have been used in the treatment of amoebic dysentery, diarrhoea, asthma, bronchopneumonia, piles, eczema and fever. The ethanolic extract of *H. antidysenterica* seeds showed strong antibacterial activity against *E. coli in vitro* and their anti-diarrhoeal activity on castor oil-induced diarrhoea in rats, *in vitro* [10].

All these plants possess anti-diarrhoeal, antibacterial, astringent, anthelmintic and anti-inflammatory activities as reported in literature. In earlier investigation, phytochemical analysis (qualitative and quantitative) and pharmacological screening (anti-diarrhoeal, antibacterial and acute toxicity study) of hydroethanolic extract of unripe fruits of *Aegle marmelos*, bark of *D. sissoo* and seeds of *Holarrhena antidysenterica* were undertaken [2]. Anti-diarrhoeal activity of different combinations of these plant extracts were evaluated in laboratory animals and antibacterial activity *in vitro*. Based on the results of phyto-pharmacological study conducted [2], the polyherbal anti-diarrhoeal formulation was prepared in the form of tablet containing hydroethanolic extracts of unripe fruits of *Aegle marmelos*, bark of *D. sissoo* and seeds of *Holarrhena antidysenterica* and its therapeutic efficacy along with haematological parameters in diarrhoeic goats was evaluated in the present study.

Material and method

The present study was carried out in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Teaching Veterinary Clinical Complex, Department of Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola.

Collection and Processing of Plant Material

The unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* were collected in the month of January and February from the Nagarjun Vanaushadhi Udhyan, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. P.D.K.V.), Akola, local market and campus of Post Graduate Institute of Veterinary and Animal Sciences, Akola. All the plants were identified and

authenticated from expert botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S). The shade dried unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* were processed to get fine powder with the help of pulverizing machine. Freshly prepared powder was subjected to cold hydro-ethanolic extraction. The extract thus obtained was used for further studies.

Preparation of cold Extract

The freshly prepared powder of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* (25 g) were immersed in hydro-ethanolic solution (40% distilled water + 60% ethanol) in a flask stoppered tightly with cotton plug and were kept on orbital shaker at room temperature for 48 hours at 150 rpm. The contents of the flask were filtered through Whatman No. 1 filter paper. Final filtrate, so obtained were evaporated and per cent extractability were determined. The extracts were stored in airtight screw cap vials and kept in the desiccator until further use in this study.

Formulation of dose of Polyherbal anti-diarrhoeal

The dose of polyherbal anti-diarrhoeal formulation containing extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* was formulated based on earlier phyto-pharmacological study [2] undertaken in the Department of Veterinary Clinical Medicine, PGIVAS, Akola as per the method of conversion of dose [11]. In present investigation same dose i.e 28 mg/Kg body weight was selected for therapeutic study in diarrhoeic goats.

Preparation of Polyherbal anti-diarrhoeal Tablets

The polyherbal anti-diarrhoeal tablets (600 mg) containing combination of extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* were prepared by defined steps viz; Pulverization and mixing, Granulation, Compression and Coating. The tablets were manufactured at Geetadevi Khandelwal Institute of Pharmacy Akola, Maharashtra as per the standard procedure. Each 600 mg tablet contains equal concentration (140 mg) of extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica*.

Therapeutic Study

a) Selection of animals

Total 29 adult goats of either sex (2-5 years of age) suffering from diarrhoea presented to Teaching Veterinary Clinical Complex, PGIVAS, Akola, District Veterinary Polyclinic, Akola and in and around Akola were selected and subjected for clinical and haematological parameters.

b) Treatment groups

Out of 29 diarrhoeic goats, 25 goats were selected and treated with polyherbal anti-diarrhoeal tablet (600 mg) containing extract of fruit of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* @ 1 tablet orally twice a day for 5 days along with fluid and supportive treatment depending on severity of case. One additional group of 10 normal healthy goats was kept without any treatment as control group. The faecal consistency score was recorded before treatment and daily for 5 days after treatment.

c) Haematological parameters

Blood samples were collected on '0' day (before treatment) and on 5th day after treatment. Blood samples were collected aseptically from jugular vein in sterile vial containing EDTA from all the animals under study. The haematological parameters like haemoglobin (g/dl), PCV (%), TLC ($\times 10^3/\text{cu.mm}$), TEC ($\times 10^6/\text{cu mm}$) and DLC (%) were estimated as per the standard methods [12].

Statistical analysis

Kruskal-Wallis Test, a one way ANOVA for non-parametric data was applied to compare faecal consistency scores. The significance of haematological parameters between both the groups means at before treatment as well as 5th day after treatment were studied using two mean 't' test. All statistical analyses were carried out using software SPSS version 21.0 and WASP version 2.0.

Results and Discussions

The efficacy of polyherbal antidiarrhoeal tablet was judged on the basis of restoration of faecal consistency to normal in diarrhoeic goats and restoration of haematological parameters to normalcy after treatment. In normal control group (T₁) all the goats were alert and healthy throughout the study period while diarrhoeic goats (T₂) showed signs of dullness, emaciation, inappetance, soiled perineum with faeces, loss of body weight etc. The symptoms like dullness, emaciation, soiled perineum, inappetance might be due to loss of fluid and electrolyte from the body as a result of diarrhoea. After treatment with polyherbal antidiarrhoeal most of the diarrhoeic goats showed improvement in general condition on 5th day post treatment.

The mean rank of faecal consistency score in normal control (T₁) and treatment group (T₂) on day '0' (before treatment) and at different intervals after treatment is presented in Table 1. In normal control group (T₁), the consistency of faeces was normal. The mean rank of faecal consistency was 30.50 from day '0' to 5 (Table 1). The statistical analysis revealed non-significant variation in mean rank of faecal consistency in normal control group (T₁), indicated that the faecal consistency was constant during different intervals in healthy goats. The faecal consistency score in group of diarrhoeic goats (T₂) was ranging from pasty to watery before initiation of treatment ('0' day). The statistical analysis showed significant variation in mean rank of faecal consistency score between different intervals in group T₂. The mean rank was higher (110.54) on day '0' and reduced gradually on subsequent days after treatment. The mean rank of faecal consistency score was 31.10 on day 5 after treatment, which was very close to mean rank of normal control group (T₁), indicated that faecal consistency was normal after treatment with polyherbal antidiarrhoeal in diarrhoeic goats (Table 1). In the present study, polyherbal antidiarrhoeal tablet showed remarkable improvement in faecal consistency on 5th day as consistency of faeces restored to normal indicating recovery in diarrhoeic goats after treatment with polyherbal antidiarrhoeal.

The result indicated that the polyherbal antidiarrhoeal preparation of extracts of unripe fruits of *Aegle marmelos*, bark of *D. sissoo* and seeds of *Holarrhena antidysenterica* was found effective and proved a potent antidiarrhoeal in diarrhoeic goats. The finding of present study is in agreement with the findings of the research worker [2], who also reported potent antidiarrhoeal activity of polyherbal formulation

containing extract of *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* in diarrhoeic goats.

The mechanism of action of polyherbal antidiarrhoeal might be because of antidiarrhoeal, antibacterial, astringent, antihelminthic and anti-inflammatory activities reported in the earlier studies. The *Aegle marmelos* fruit (Bael) has astringent property that reduces irritation in the digestive tract as reported by many workers [6, 5, 4]. Several researchers reported antidiarrhoeal activity and astringent property of *D. sissoo* [7, 8, 9]. The ethanolic extract of *H. antidysenterica* seeds showed strong antibacterial activity against *E. coli in vitro* and their antidiarrhoeal activity on castor oil-induced diarrhoea in rats, *in vitro* [10]. The improvement in diarrhoeic goats brought by polyherbal antidiarrhoeal could be due to active phyto-constituent such as tannins, flavonoids, alkaloids, saponins, sterols and terpenes present in these plants. These phyto-constituents have the ability to inhibit intestinal motility and hydroelectrolytic secretions which are responsible to alter in this intestinal condition. It has been shown that flavonoids are able to inhibit prostaglandin E-2. The ability of flavonoids to inhibit intestinal motility and block prostaglandin induced secretory process has been established the presence of the active principles like flavonoids and tannins in abundance in the polyherbal formulation is postulated to act as a contributing factor responsible for its antidiarrhoeal activity [13]. In the present study, the polyherbal antidiarrhoeal might have reduced the inflammation and irritation or biosynthesis of prostaglandins effectively and anti-secretory mechanism and anti-electrolyte permeability action of these phyto constituents resulted in recovery in diarrhoeic goats [13].

The mean values of haemoglobin (gm/dl), PCV (%), TEC, TLC and DLC in normal control (T₁) and treatment group (T₂) before ('0' day) and after treatment is presented in Table 2. The mean haemoglobin concentration in normal control group (T₁) was within the normal range throughout the study period (11.3 ± 0.11). The statistical analysis revealed significantly ($P < 0.01$) low haemoglobin (8.64 ± 0.28) in diarrhoeic goats (T₂) as compared to normal control group (T₁), indicated anaemia in diarrhoeic goats. The similar findings were also reported by many researchers [14, 15]. However, other workers reported increase in haemoglobin in diarrhoeic animals [16-20]. In present study, low haemoglobin concentration in diarrhoeic goats might be due to inappetance or anorexia and nutritional deficiency leading to poor body condition [21]. Many studies reported higher haemoglobin concentration in diarrhoeic goats, which might be due to haemoconcentration as a result of water loss from body due to dehydration [22, 18, 23, 20, 4]. The statistical analysis revealed non-significant variation in haemoglobin between '0' day (before treatment) and 5th day after treatment, indicated no effect of treatment in improving altered haemoglobin in diarrhoeic goats within 5 days period.

In present investigation, the mean PCV was significantly ($P < 0.01$) low (25.84 ± 0.99) in group of diarrhoeic goats (T₂) on '0' day (before treatment) as compare to normal (32.30 ± 0.61) control group (T₁). The findings of present study are contradictory with the findings reported in earlier studies [24, 25, 22, 18, 23, 14, 15], who reported significant increase in PCV in diarrhoeic animals. The decrease in PCV in diarrhoeic animals indicated anaemia in goats. The statistical analysis revealed significant ($P < 0.01$) increase in PCV (%) on 5th day after treatment as compare to PCV (%) on '0' day (before treatment), indicated effect of treatment in improving PCV (%) in diarrhoeic goats after treatment.

The mean TLC was significantly ($P<0.01$) higher (12.96 ± 0.50) in group of diarrhoeic goats (T_2) on '0' day (before treatment) as compare to normal (10.97 ± 0.43) control group (T_1). Similar observations were also reported by many workers [26, 27, 28, 14, 15]. The higher TLC in diarrhoeic goats might be due to normal reaction of body defense mechanism against infection and also due to dehydration and haemoconcentration [29, 15]. The mean TLC was significantly ($P<0.01$) improved on 5th day post treatment in group (T_2) treated with polyherbal antidiarrhoeal, indicated effectiveness of treatment in improving TLC in diarrhoeic goats. Simillar improvement in TLC in diarrhoeic goats after treatment with polyherbal antidiarrhoeal preparations containing extracts of *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* also reported in earlier studies [30].

The mean TEC was significantly low ($P<0.05$) in group (7.83 ± 0.24) of diarrhoeic goats (T_2) on '0' day (before treatment) as compare to normal control group (T_1), indicated anaemia in diarrhoeic goats. The findings of the present study are in accordance with the findings of previous studies [14, 30]. However, other workers reported increase in TEC in diarrhoeic animals [23, 20, 4]. The statistical analysis revealed significant improvement in TEC on 5th day post treatment in diarrhoeic goats as compared to '0' day, indicated effectiveness of treatment in improving TEC in diarrhoeic goats. The findings of present study supports the findings of earlier research work [30], who also reported improvement in TEC in diarrhoeic goats after treatment with polyherbal antidiarrhoeal preparations containing extracts of *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* [30].

The statistical analysis revealed non-significant variation in neutrophils (%), lymphocytes (%), monocytes (%) and basophils (%) between normal control group (T_1) and group of diarrhoeic goats (T_2) on '0' day (before treatment). The mean eosinophils (%) was significantly ($P<0.05$) higher in

group of diarrhoeic goats (T_2) on '0' day (before treatment) as compared to normal control group (T_1), indicating eosinophilia in diarrhoeic goats. These findings supports the findings of previous studies [29, 31, 27, 32, 15, 21, 30]. Some workers reported marked neutrophilia response with lymphopenia and attributed to acute bacterial enteritis [21].

The polyherbal antidiarrhoeal (T_2) showed significant ($P<0.01$) improvement in altered PCV (%), TLC, TEC, neutrophils (%), lymphocytes (%) and eosinophils (%) on 5th day post treatment as compare to their corresponding values on '0' day (before treatment). No significant variation was observed in monocytes (%) and basophils (%) on 5th day post treatment as compare to their corresponding values on '0' day (before treatment). Similar observations were also reported in earlier study [15, 21].

The overall study concluded that the polyherbal antidiarrhoeal tablet containing *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* @ 1 tablet twice a day along with fluid and supportive therapy for five days found effective and safe remedy in inducing recovery in diarrhoeic goats with improvement in haematological parameters.

Table 1: The mean rank of faecal consistency score in normal control group T_1 and polyherbal treatment group T_2 at different intervals (Kruskal-Wallis Test)

Day	Group T_1 (N=10)	Group T_2 (N=25)
	Mean rank	Mean rank
0	30.50	110.54
1	30.50	107.64
2	30.50	85.86
3	30.50	74.66
4	30.50	43.20
5	30.50	31.10
Chi Sq.	FCC 0.000	FCC 88.450
d.f.	5	5
Sig.	1.000	0.000**

Table 2: The mean haemoglobin (g/dl), PCV (%), TLC ($\times 10^6$ /cu.mm), TEC ($\times 10^6$ /cu mm), neutrophils (%), lymphocytes(%), eosinophils (%) monocytes(%) and basophils (%) in group T_1 and T_2 before treatment and 5thday after treatment.

S. No	Groups	Intervals		Pooled mean	Paired 'T' Test		
		Before treatment	After treatment		T statistic	d.f	P value
		'0' Day	5 th Day				
Haemoglobin (g/dl)							
1.	Group (T_1)	11.40 \pm 0.15	11.32 \pm 0.17	11.3 \pm 0.11	0.488	9	0.318 NS
2.	Group (T_2)	8.64 \pm 0.28	8.70 \pm 0.28	8.67 \pm 0.20	0.447	24	0.329 NS
PCV (%)							
1.	Group (T_1)	32.30 \pm 0.61	33.40 \pm 0.61	32.85 \pm 0.44	1.673	9	0.064 NS
2.	Group (T_2)	25.84 \pm 0.99	28.80 \pm 0.93	27.32 \pm 0.70	3.414	24	0.001**
TLC ($\times 10^6$/cu.mm)							
1.	Group (T_1)	10.97 \pm 0.43	11.3 \pm 0.21	11.13 \pm 0.23	0.734	9	0.241 NS
2.	Group (T_2)	12.96 \pm 0.50	11.89 \pm 0.42	12.43 \pm 0.33	6.521	24	0.000**
TEC ($\times 10^6$/cu mm)							
1.	Group (T_1)	8.70 \pm 0.30	10.25 \pm 0.30	9.47 \pm 0.25	3.464	9	0.0035**
2.	Group (T_2)	7.83 \pm 0.24	8.23 \pm 0.25	8.03 \pm 0.17	2.740	24	0.0057**
Neutrophils (%)							
1.	Group (T_1)	44.60 \pm 3.10	36.60 \pm 1.30	40.60 \pm 1.87	2.579	9	0.0149*
2.	Group (T_2)	49.92 \pm 2.17	46.04 \pm 1.39	47.98 \pm 1.30	3.247	24	0.0017**
Lymphocytes (%)							
1.	Group (T_1)	49.10 \pm 3.54	53.60 \pm 2.03	51.35 \pm 2.05	1.359	9	0.104 NS
2.	Group (T_2)	44.00 \pm 2.00	46.76 \pm 1.57	45.38 \pm 1.27	3.123	24	0.002**
Eosinophils (%)							
1.	Group (T_1)	2.90 \pm 0.57	4.70 \pm 0.49	3.80 \pm 0.42	2.290	9	0.0239*
2.	Group (T_2)	4.96 \pm 0.26	3.60 \pm 0.20	4.28 \pm 0.18	7.141	24	0.000**
Monocytes (%)							
1.	Group (T_1)	3.00 \pm 0.29	3.40 \pm 0.26	3.20 \pm 0.20	1.078	9	0.155 NS

2.	Group (T ₂)	3.28 ± 0.25	3.20 ± 0.20	3.24 ± 0.16	0.569	24	0.287 NS
Basophils (%)							
1.	Group (T ₁)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	9	0.5 NS
2.	Group (T ₂)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	24	0.5 NS

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