Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 723-725
Received: 09-09-2017
Accepted: 10-10-2017

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Evaluation of antidiarrhoeal activity of polyherbal preparation

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Abstract
Antidiarrhoeal activity of polyherbal preparation containing hydro-ethanolic extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* was evaluated in mice using castor oil induced diarrhea. Acute toxicity study revealed no visible signs of toxicity at 500, 1000 and 2000 mg/kg body weight in mice within and after 24 hrs. The results showed that the polyherbal preparation (400 mg/kg and 800 mg/kg) produced significant ($p<0.01$) reduction in frequency of defecation up to 79.56% and 81.14%, respectively as compared to control group mice in castor oil induced diarrhoea. The antidiarrhoeal action exhibited by polyherbal preparation might be due to active phytoconstituents such as alkaloids, flavonoids, tannins, sterols and saponins present in the polyherbal extracts as evident from phytochemical analysis (qualitative) carried out in the present investigation. The study concluded that polyherbal preparation containing hydro-ethanolic extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* possess potent antidiarrhoeal activity.

Keywords: Antidiarrhoeal, *Aegle marmelos*, *Dalbergia sissoo*, *Holarrhena antidysenterica*, Castor oil, Mice

Introduction
Diarrhoea has been recognised as one of the most important health problems and leading cause of mortality and morbidity in the developing countries. It is not the disease but can a symptom of several diseases. It is associated with viral, bacterial, fungi infection, food poisoning and other disease conditions. 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 [9].

In veterinary field diarrheal is one of the leading cause of mortality in domestic animals of various species. The various treatments are available for diarrhoea such as loperamide, bismuth subsalicylate, racecadotril etc. However, these synthetic drugs possess various side effects like abdominal discomfort, dry mouth, nausea, constipation. Therefore, the world health origination (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices [1]. Thus it is very important to identify and evaluate commonly available indigenous herbal drugs as an alternative to currently used antidiarrheal drugs.

In India several drugs are mentioned in traditional system of medicine and widely used by traditional healers, however the most of the drugs have not been evaluated on scientific basis. There are many herbal plants such as *Aegle marmelos, Dalbergia sissoo, Holarrhena antidysenterica, Helicteres isora* etc which possess antidiarrheal activity, which acts by reducing the gastrointestinal motility and gastric secretion and possess lesser side effects than the conventional drugs and thus are safer to use [9]. The main chemical constituents in plants found to be responsible for antidiarrhoeal activity are tannins and tannic acid, flavonoids, alkaloids, diterpenes, terpenes and terpenoids etc.

The present study was undertaken to evaluate the antidiarrhoeal activity of polyherbal preparation containing extracts of unripen fruit of *Aegle marmelos*, bark of *Dalbergia sissoo* and *seeds* of *Holarrhena antidysenterica* against diarrhoea.

Materials and Methods
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

(Maharashtra State).

1. Preparation of Extract

The unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* were collected in month of Jan- Feb from the Nagarjun Van-aushadhi, Udhyan Dr. Punjabrao Deshmukh Krushi Vidyapeeth, Akola and local market of Akola District and campus of PGIVAS, Akola. All the procured plant material were identified and authenticated from expert botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S). The collected material was cleaned, dried under shed and processed to get fine powder with the help of pulverizing machine. Freshly prepared powder of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and 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 was evaporated to get solid to semisolid mass of extract and per cent extractability was determined. The extracts were stored in airtight screw cap vials and kept in the desicator until further use in this study.

2. Phytochemical Screening

The freshly prepared extracts were subjected for preliminary phytochemical analysis (qualitative) for identification of different phytoconstituents in twelve different solvents viz. acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether, and xylene for the presence of the active phytochemical constituents as per various test such as Test for Alkaloids (Dragendroff's reagent, Mayer's reagent (Potassium mercuric iodide reagent) and Wagner's reagent), Test for reducing sugars (Fehling's solution test and Benedict's reagent), Test for glycosides (Benedict's reagent and Fehling's reagent), Test for Tannins (Ferric chloride test and Lead acetate test), Test for sterol (Salkowski's reaction), Test for Anthraquinones (Borntrager's test), Test for flavonoids, Test for proteins (Biuret test and Xanthoprotein test), Test for amino acids (Ninhydrine test), Test for Saponins (Foam test) and Test of Phenolics [8].

3. Acute Toxicity Study

Acute toxicity study was performed according to the OECD-423 guide lines. Swiss albino mice (20 – 25 g) of either sex were used. The mice were procured from the recognized CPCSEA authorized laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola and the experimental protocol was approved from (312/CPCSEA) IAEC of PGIVAS, Akola. The animals were administered with 500 mg, 1000 mg and 2000 mg/kg of each extract of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* and combination of three extracts orally (p.o). The animals were observed for 24 h, then for further 14 days for deaths and manifestation of general signs and symptoms of toxicity.

4. Antidiarrheal activity

4.1 Castor oil induced diarrhoea

Castor oil induced diarrhoea model was used [6]. Twenty four albino mice (20 – 25 g) of either sex were divided into 4 groups of six animals each. The animals were fasted for 18 hrs before conducting the study. The first group (positive control group) received vehicle (0.2 ml normal saline) orally. The second group (standard control group) received Loperamide hydrochloride (3 mg/kg) orally as a standard drug. The third and fourth group (test groups) received polyherbal hydroethanolic extracts containing unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* at 400 mg and 800 mg/kg body weight orally, respectively. One hour after treatment, each mice were received 1ml of castor oil orally. All the animals were observed for defecation for the next 4hr. The presences of characteristic wet and dry diarrhoeal droppings were noted down on non-wetting paper sheet of uniform weight for period of 4 hr (Table 1). The per cent inhibition of defecation was calculated as a function of the castor oil control as per the following formula [6].

% Inhibition = (control – test) x 100%/control.

5. Statistical analysis:

The data obtained was analysed statistically by student t-test for comparison between control group against each test group separately using Web Based Agricultural Statistics Software Package (WASP), ICAR Research Complex for Goa, Ela, Old Goa, Goa. 403 402. India.

Results and Discussions

In Phytochemical (qualitative) analysis of hydroethonolic extract of unripe fruit of *Aegle marmelos*, Bark of *Dalbergia sissoo*, Seeds of *Holarrhena antidysenterica* were evaluated in twelve different solvents extracts to estimate the various active phytoconstituents [8]. The phytochemical analysis of hydroetholonic extract of *Aegle marmelos* revealed presence of alkaloids, protein, tannins, sterols, phenolic compounds and saponins. In related study [3] phytochemical analysis of ethanolic leaf and fruit extract of *A. marmelos* reported several compounds of confirmed biological activity such as presence of tannins, flavonoids, saponins and alkaloids. The aqueous and methanolic extracts of seeds of *A. marmeloes* contain alkaloids, carbohydrates, proteins, glycosides and phenolics qualitatively [11]. In another study [7] anthraquinone, glycosides, catechins, fixed oils, saponins, alkaloids, flavonoids and proteins were found in aqueous extract of leaves of *Aegle marmelos*. In the present study, flavonoids, tannins, glycosides, anthroquinon, phenolic compounds and saponins were found in the twelve different solvent extracts of *D. sissoo*. The presence of carbohydrate, tannins and saponins in aqueous extracts, whereas, flavonoids tannins, terpenoids and alkaloids in ethanol extract were reported in bark of *D. sisso* [6]. In simillar study [5] flavones, isoflavones, quinines and coumarins were reported in *D. sissoo* ethanolic leaves extract. The phytochemical analysis of hydroethonolic extract of *H. antidsenterica* seeds revealed presence of anthroquinone, glycosides, alkaloids, flavonoids, tannins, sterols, amino acids and phenolic compounds. In similar study, the presence of alkaloids, carbohydrate, flavonoids and phenolic compound in ethanolic extract of seeds of *H. antidysenterica* was demonstrated [10].

In acute oral toxicity studies, extracts of unripe fruit of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* and combination of these extracts were administered at 500,1000 and 2000 mg/kg body weight to mice. Acute toxicity study revealed no visible signs of toxicity in mice within and after 24 hrs at any of the doses administered. There were no lethality or mortality observed even at the highest dose of 2000 mg/kg body weight. The absence of toxicity symptoms suggest that hydroethanolic

extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo*, seeds of *Holarrhena antidysenterica* and combination of these 3 extracts (polyherbal extract) were non toxic and well tolerated at the doses employed in this study.

In present study, two doses of polyherbal antidiarrhoeal preparation containing *Aegle marmelos*, *Dalbergia sissoo* and *Holarrhena antidysenterica* viz 400 mg/Kg and 800 mg/Kg body weight orally were used. Loperamide at dose rate of 3 mg/kg was used as reference standard drug. Control group mice were treated with normal saline.

Table 1: Effect of polyherbal antidiarrhoeal preparation on Castor oil induced diarrhea in mice

Groups	Treatment	Dose	Total no. of feces in 4 hrs	% inhibition
I	Control	N.S	8.22 ± 0.065	-
II	Loperamide	3 mg/kg	1.15 ±0.022**	86.0
III	Polyherbal preparation *(A. marmeloes D. sissoo* and *H. antidysenterica)*	400 mg/kg	1.68 ±0.031**	79.56
IV		800 mg/kg	1.55 ±0.043 **	81.14

Values are expressed in mean ± S.E. (n=6), Student *'t'*-test, **P<0.01, when compared to control

In castor oil induced diarrhoea, the mice of vehicle control group showed diarrhoea after 30 min and continued up to observation period of 4 hours with signs of watery and frequent defecation. The mice treated with polyherbal extract (400 mg/kg b.w. and 800 mg/kg b.w.) and loperamides (3mg/kg b.w.) produced significant ($p<0.01$) reduction in the frequency of defecation in castor oil induced diarrhoea. Both the doses of polyherbal extracts significantly decreased ($p<0.01$) the total number of wet faeces produced by administration of castor oil. The mean per cent inhibition of frequency of defecation by polyherbal extract at 400 mg/kg and 800 mg/kg was found to be 79.56% and 81.14%, respectively. While per cent inhibition of frequency of defecation by loperamide at 3 mg/kg was 86.00% (Table 1). Thus, polyherbal extract showed potent antidiarrhoeal activity in castor oil induced diarrhoea in mice. Both the doses of extract (400mg/kg and 800mg/kg) did not differ significantly with each other.

Castor oil induced diarrhoea due to its active ingredient ricinoleic acid, which is liberated as a result of action of lipases on castor oil in the duodenum. The ricinoleic acid is poorly absorbed in small intestine and results in irritation and inflammation of the intestinal mucosa which leads to release of autocoids and prostaglandins which stimulates motility and alters electrolyte permeability of the intestinal mucosa leading to hypersecretions and diarrhoea [7].

In the present study polyherbal extracts significantly inhibited castor oil induced diarrhoea. It was evident by reduction in total number of wet faeces produced by polyherbal extracts treated animals. The antidiarrhoeal action exhibited by polyherbal preparation might be due to active phytoconstituents such as alkaloids, flavonoids, tannins, sterols and saponins present in the polyherbal extracts which might have inhibited the prostaglandin synthesis [2]. Earlier studies showed that antidysenteric and antidiarrhoeal properties of medicinal plants were due to alkaloids, flavonoids, tannins, sterol, saponins, titerpenes and reducing sugars [4, 6].

The study concluded that polyherbal preparation containing hydro-ethanolic extracts of unripe fruits of *Aegle marmelos*, bark of *Dalbergia sissoo* and seeds of *Holarrhena antidysenterica* possesses potent antidiarrhoeal activity and may be evaluated in clinical cases of diarrhea in livestock.

Conflict of Interest: All authors declare no conflict of interest.

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