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Investigation of Phytochemical Profile and Combine Synergistic Anti-Diarrheal Potential of *Peperomia Pellucida* Aerial Part and *Cyprus Esculentus Root Extracts*

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Research Article

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Abstract

Peperomia pellucida and *Cyperus esculentus*locally found plant used for anti-diarrhoeal activity using standard animal model. The aerial parts of *Peperomia pellucida*and *Cyperus esculentus*were collected from Trikaripur forest area, kasaragod district of Kerala, India, dried and extracted with ethanol (70%). Wistar albino rats were used for studies. Castor oil was used to induce diarrhoea and 200, 400 mg/kg of ethanol extract of *Peperomia pellucida* and *Cyperus esculentus* dual combination was used to test antidiarrhoeal activity. Results were calculated by student "t" test, to assess statistical significance.The extract showed dose dependant inhibition of frequency of defecation as well as reduction in number of wet feces. The percentage inhibitions of faecal with 200 and 400 mg/kg doses of ethanol extract were 69.15 and 76.21 respectively. The plant extracts contains tannins and flavonoids, which could have contribution to the anti-diarrhoeal activity. *Peperomia pellucida* and *Cyperus esculentus*combine hydro-ethanolic extractpossesses significant antidiarrhoeal activity compared with loperamide.

Keywords: *Peperomia pellucida*; *Cyperus esculentus*; Tannins; Castor oil induced diarrhoea; Antidiarrhoeal

Introduction

Diarrhoea is the passage of stools more than three times in an hour period [1]. It occurs due to an imbalance in the absorption and secretory mechanisms in the intestinal mucosa [2], which results in an increase in fluid and electrolyte loss into the gut lumen, leading to the production of unformed, liquid faeces [3]. Diarrhoea is one of the leading causes of mortality and morbidity in developing countries especially in children under five years. It is most commonly caused by gastrointestinal

infections, which kill around 1.8 million people globally each year [4].

Plant medicines are consisting of a multiplicity of chemical components that act synergistically to make active constituents bio-available and preventing adverse effects. It has thus been established that combination drug therapy can deliver greater therapeutic effect than a single conventional medicine. Numerous studies have validated the traditional use of anti-diarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion [5]. All these properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids[6]. The anti-diarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions [7]. Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion [6]. All these evidences explain the importance of plants in diarrhoeal management in modern day healthcare.

Cyperus esculentus(L)(Figure 1) belongs to the Family of Cyperaceae, commonly known as yellow nut sedge, watergrass, tiger nut, nutgrass etc. Usually it is a tough, single-stemmed, erect, perennial graminoid; up to 3 ft. (9 dm) tall. Underground, along with fibrous roots, are many slender rhizomes which form a tuber at each end. Rhizomes are slender, scaly; long rhizomes terminating with one tuber can occur up to 14 in. (36 cm) below the soil surface. Tubers are unevenly globose, 0.1-0.8 in. (0.3-1.9 cm) in diameter, black to brown, hard, smooth (scales shed with maturity), with buds at the apex only. Tubers taste mildly almond-like.

Peperomia pellucida (L) HBK (Figure 2) belongs to the Family of Piperaceae commonly known as Shiny Bush, Slate pencil plant, pepper elder, rat's ear, shiny bush, silver bush etc. Itis a common fleshy tropical annual, shallow-rooted herb, usually growing to a height of about 15 to 45 cm. It is characterized by fibrous roots, succulent stems, shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. It has a mustard-like odor when crushed.

Figure1: *Cyperus esculentus* L (Photo Credit: Clint Shock, Malheur Agricultural Experiment Station)

Figure 2: *Peperomia pellucida* (Photo Credit: SurajitKoley@hooghly)

Materials and Methods

Collection of plant materials Peperomia pellucida and Cyprus escalates

Peperomia pellucida whole plant and *Cyprusescalates*roots were collected from the road sides of Trikaripurkerala, India, in the month of August 2012 in a quantity sufficient for all the experiments in a single batch.Both the plants were washed under running tap water, cut into small pieces of 2-3cm and shade dried (30^{o}C, 50 ± 5% relative humidity) for 15days. The shade dried plant material was powdered using a dry grinder to get the coarse powder (sieve no. 10/44). The powder was stored in air tight container for further use.

Preparation of extracts

The shade dried plant powder of *Peperomia pellucida* and *Cyprus esculentus* was subjected to solvent extraction individually. The powder material was macerated with ethanol (90%) and distilled water, for 72 hrs in batches of 100g each. Both the extracts obtained by the above techniques were concentrated in vacuum under reduced pressure using a rotary flash evaporator.

Preparation of polyherbal formulation

Both the concentrated extracts were mixed together in similar ratio (1:1).

Preliminary phyto-chemical screening of dual herbal formulation

The preliminary phytochemical screening was carried out according to the recommended standard procedures [8-10] as follows:

Test for Carbohydrates

Molisch's test: to theextractadded few drops of Molisch's reagent and 1-2 ml Conc. sulphuric acid slowly through the sides of the test tube. Development of a violet ring at the junction, indicate the presence of carbohydrates.

Fehling's test: Extract on boiling with equal proportions of Fehling's A and Fehling's B solution gives yellow to brick red coloured precipitate.

Benedict's test: Extract on boiling with Benedict's reagent gives green, yellow or red colour.

Barfoed's test: Extract when boiled with few ml of Barfoed's reagent shows brick red precipitate

Test for Proteins

Biuret test: Extract on treatment with 4% Sodium hydroxide and 1% Copper sulphate solution gives violet or pink colour.

Ninhydrin test: Appearance of blue colour when the extract was treated with Ninhydrin reagent indicates the presence of proteins.

Millon's test: Extract on heating with Millon's reagent on a water bath gives white/yellow precipitate.

Xanthoproteic test: Extract treated with concentrated sulphuric acid gives white precipitate. After boiling, precipitate shows yellow and turns orange when Ammonium hydroxide was added.

Test for Alkaloids

Mayer's test: Extract treated with Meyer's reagent gives cream coloured precipitate.

Dragendorff's test: Addition of Dragendorff's reagent to the extract gives reddish brown coloured precipitate.

Hager's test: Extract treated with Hager's reagent gives yellow precipitate.

Wagner's test: Extract with Wagner's reagents gives reddish/brown coloured precipitate.

Test for Saponins

Foam test: Specified quantity of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. formation of 1cm foamy layer indicate the presence of saponins.

LibermannBuchard Test: Addition of Acetic anhydride and concentrated sulphuric acid (19:1) to the extract gives violet purple colour.

Test for Tannins

Ferric chloride test: Extract on treatment with Ferric chloride solution give bluish colour.

Lead acetatetest: Extract on treatment with Lead acetate solution gives white precipitate.

Gelatin test: Addition of gelatin solution to the extract gives white precipitate

Test for Falvonoids

Shinoda test: Extract on boiling with few pieces of Magnesium ribbon and few drops of concentrated hydrochloric acid give pink colour.

Ferric chloride test: Extract treated with Ferric chloride solution gives green to black colour.

Mineral Acid test: Extract on treatment with Sulphuric acid gives yellow orange colour.

Lead acetate test: Extract treated with Lead acetate solution gives yellow precipitate.

Test for Steroids

Libermann-Burchard Sterol Reaction test: Addition of few drops of acetic anhydride and concentrated sulphuric acid through the sides of test tube shows formation of a brown ring at the junction of two liquids.

Salkowski Reaction: Extract when treated with concentrated sulphuric acid gives red colour.

Test for Triterpenoids

Salkowaski test: extract was shaken with a few drops of concentrated sulphuric acid and allowed to stand for a few minutes. Development of yellow colour in the lower layer, indicate the presence of triterpenoids.

Liebermann-Burchard test: Extract was treated with a few drops of acetic anhydride. Addition of concentrated sulphuric through the sides of the test tube shows the formation of deep red colour.

Acute toxicity studies

Fasted overnight rats were randomly divided into six animals into four groups. Different groups of Rats were administered with increasing doses (250, 500, 1000, 2000 and 2000 mg/kg b.w.) of the Poly Herbal drug. The animals were observed individually for first one hour for any gross behavioural changes like drowsiness, restlessness, writhing, convulsions and symptoms of toxicity and mortality if any, and then periodically for the next 24 h, and then at every 24 h for any signs of acute toxicity over a period of 14 days. The acute toxicity study was done as per OECD guideline-425.

Anti -Diarrheal activity[11]

Animals

Wistar albino rats weighing between 150-200g were maintained under standard laboratory conditions on 12-day/night cycle with free access to food and water being *adlibitum*. The animals were acclimatized to laboratory conditions prior to experimentation. The activity was carried out in between 09.00 hr to 17.00 hr at ambient temperature. The animals were drawn at random for the study. All the experiments were performed according to current guidelines for the care of the laboratory animals and the ethical guidelines.

Castor oil induced diarrhoea

Before the experimental study, the animals were fasted overnight with free access to water. The experimental animals were grouped into four, each group containing six Wistar albino rats.

Group I received vehicle (1%CMC), orally and served as control.

Group II received hydro-ethanol poly-herbal extract (200 mg/kg).

Group III received hydro-ethanol poly-herbal extract (400 mg/kg).

Group IV received Loperamide (2 mg/kg) and served as Standard.

All test preparations and standard drug were administered 1hr prior to Castor oil (10 ml/Kg). Each rat was then housed separately in the cages and observed for diarrhoeal episode, for a period of 4hr. During that period, number and weight of diarrhoeal feaces were taken after every half an hour. Using mean diarrhoeal episodes, percentage diarrhoea and percentage protections were calculated.

Mean no. of defecation caused by castor oil–Mean no. of defecation caused by poly-herbal formulation

% Protection =Mean no. of defecation caused by castor oil x 100/1

Castor oil induced enteropooling

Rats were fasted 24h prior to the experiment. Then the drugs were administered accordingly as per the groupings. After 1h 2ml/rat castor oil was given orally to all the groups. 2h later the rats were sacrificed. Small intestine from pyrolus to caecum was isolated. Their intestinal contents were collected by milking into graduated tube. Volume was measured in ml.

Statistical analysis

Results were calculated by student “t” test, to assess statistical significance and data summarized as mean ±SEM

Result and Discussion

In the present study of poly herbal formulation were subjected to preliminary phyto-chemical, and pharmacological investigation. Preliminary phyto-chemical screening which is performed to establish a chemical profile of a crude drug is a part of chemical evaluation. Herbal combination was taken for phyto-chemical investigation by different phyto-chemical tests to check the presence or absence of a group of phyto-chemical constituents. These phyto-chemical tests showed the presence of carbohydrates, alkaloids, tannins,

steroids, triterpenoids, flavonoids etc (Table 1). In acute toxicity study, hydro alcoholic combined extract did not show any mortality up to the dose of 2000 mg/kg in rats, also there was no change in general behaviour and morphological profile.

Sl no.	Chemical Constituents	Hydro ethanol extract
1	Carbohydrates	+++
2	Proteins	-
3	Alkaloids	++
4	Saponins	+
5	Tannins	++
6	Flavonoids	+++
7	Steroids	+++
8	Triterpenoids	+++
9	Lipids	+++
10	Glycosides	_
11	Volatile oil	-

+ = Present, - = Absent

Table1: Preliminary phytochemical analysis of polyherbal formulation.

The polyherbal formulation showed dose dependant inhibition of frequency of defecation as well as reduction in number of wet faces (Table 2). However, this value was more significant at 400 mg/kg dose. The Loperamide has shown significant reduction in frequency of defecation and wet faeces. The percentage inhibitions of faecal with 200 and 400 mg/kg doses of dual herbal combination were 69.15 and 76.21 respectively (Figure 3). Castor oil induced enteropooling inhibition also found to be significant (Table 3)in the dose of 400mg/kg compared with control. The percentage inhibition of standard and tests were 81%, 51.02% and 77.06 respectively (Figure4).

Group no	Group	Watery Diahhroea no.	Protection (%)	Mean weight of stools
I	Vehicle (1%CMC)	5.11±0.33	0	5.66±0.32
II	Polyherbal extract (200 mg/kg)	2.6±0.13	69.15	2.63±0.51
III	Polyherbal extract (400 mg/kg)	1.2±0.23	76.21	1.44±0.11*
IV	Loperamide(2 mg/kg)	1.3±0.41	77.99	1.11±0.09*

Values are expressed as mean ± SEM (N= 6 animals in each group), the sign (*) indicated values significantly different from the diabetic control group at $p < 0.05$.

Table 2: Castor Oil Induced Diarrhoea.

Group no	Group	Volume (ml)	% inhibition
1	Normal saline	7±0.11	-
2	Loperamide (5mg/kg po)	1.8±0.56	81±0.21*
3	Herbal formulation 200mg	4.1±0.37	51.02±0.15
4	Herbal formulation 400mg	2.1±0.52	77.06±0.01*

Values are expressed as mean ± SEM (N= 6 animals in each group) the sign (*) indicated values significantly different from the diabetic control group at $p < 0.05$.

Table 3: Castor oil induced enteropooling.

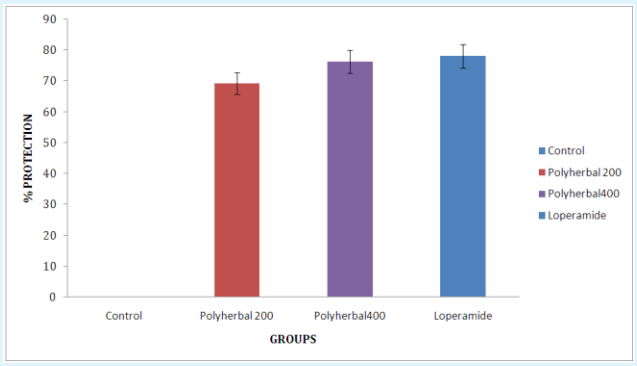

Figure 3: Percentage Protection of various polyherbal formulations and Standard drug.

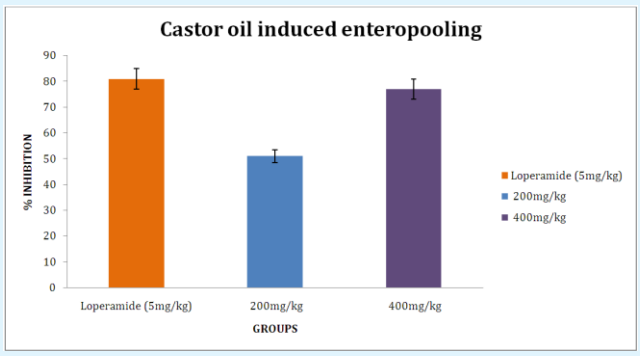

Figure 4: Castor oil induced enteropooling.

In the present study anti-diarrhoeal activity of dual herbal combination was evaluated in terms of percent protection using model of castor oil induced diarrhoea in rats. It is well known that ricinoleic acid, an active component of castor oil, induces changes in mucosal permeability, electrolyte transport and intestinal peristalsis, leading to hypersecretory of the intestinal mucosa, leading to prostaglandin's release, which causes an increase in net secretion of water and electrolytes into the small intestine [12]. Ricinoleic acid causes irritation and inflammation biosynthesis delay castor oil induced diarrhoea. It has been shown that E type of prostaglandins causes diarrhoea in experimental animals as well as in human beings. The mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [13]. PGE2 also inhibits the absorption of glucose, a major stimulus to the intestinal absorption of water and electrolytes [14].

Phytochemical screening on the plant extract revealed the presence of flavonoids and tannins (Table 1). These compounds are reported for their antidiarrhoeal activity [15,16]. Tannins can evoke an anti-diarrhoeal effect and these substances may precipitate proteins of the electrolytes, reduce peristaltic movement and intestinal secretion [16].

The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion [17], which is known to be altered in intestinal condition. *In vitro*and *in vivo*experiments have shown that flavonoids are able to inhibit the intestinal secretary response induced by prostaglandin E2 [18]. In addition, flavonoids possess antioxidant properties, which are presumed to be responsible for the inhibitory effects exerted upon several enzymes, including those involved in the arachidonic acid metabolism.As an alternative approach to the costly and lengthy development of a new chemical entity, here this investigation is the possibility of effective, natural-product anti-secretory therapeutics which may already be available, but unappreciated.The poly-herbal may act on diarrheal as described in the schematic diagram (Figure5).

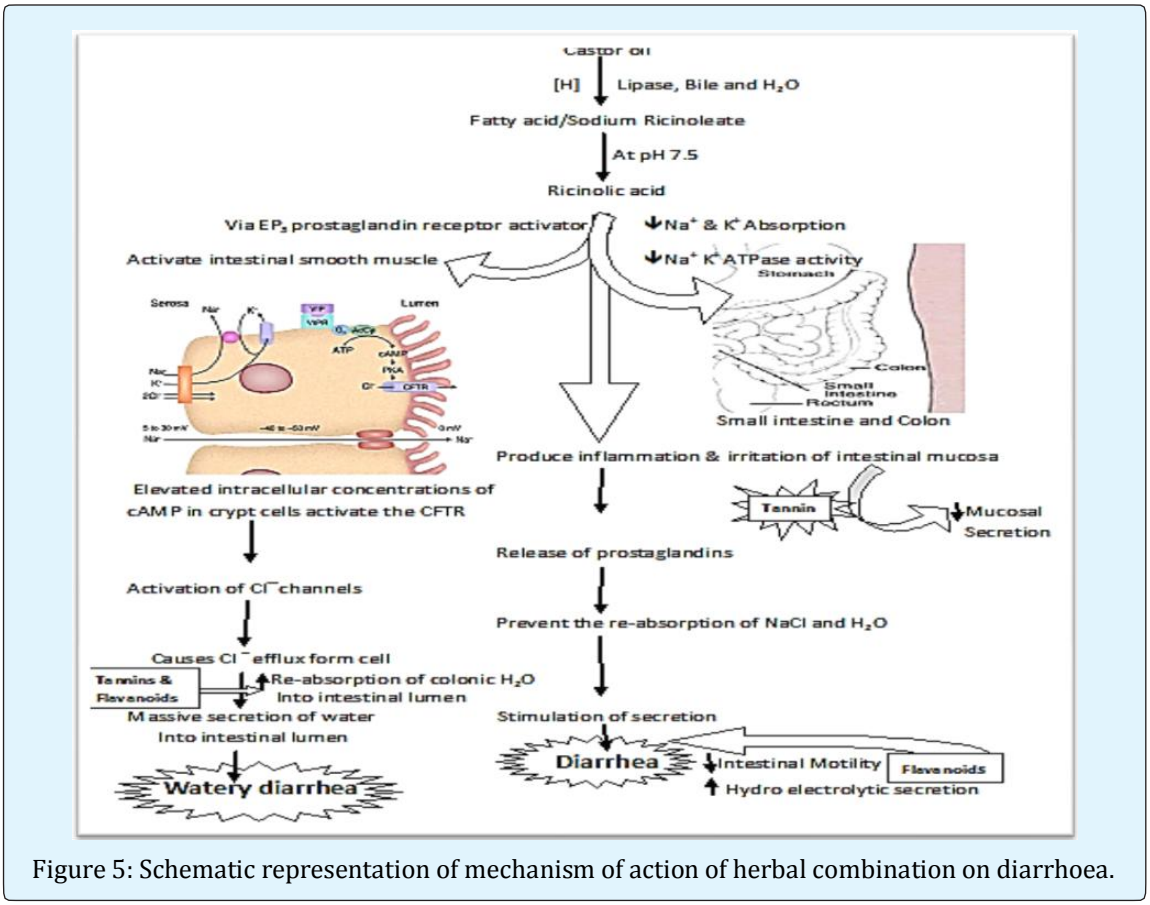

Figure 5: Schematic representation of mechanism of action of herbal combination on diarrhoea.

Conclusion

The dual herbal combination contains a good quantity of tannins and flavonoids, which could have contribution to the anti-diarrhoeal activity in experimental rats or anti-diarrhoeal activity in rats, may be the result of herbal drug synergism effect of several phyto-constituents.

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