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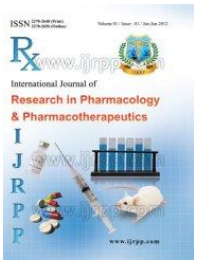

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Research article ***Open Access***

Formulation and evaluation of herbal tablets incorporated with *Strychnos potatorum and Punica granatum* extracts for antidiarrhoeal activity

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ABSTRACT

Plants are always an exemplary source of drug. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. Diarrhoea affects people of all ages, due to several causes-malnutrition, infection (bacterial, viral and parasitic organisms), zinc deficiency, etc. Severe diarrhoea becomes life-threatening, particularly in young children and people who are malnourished or have impaired immunity. The current study was aimed for the formulation and evaluation of herbal tablets incorporated with *Strychnos potatorum and Punica granatum extracts* for antidiarrhoeal activity. The tablets were prepared by wet granulation method using above plant extracts. The prepared tablets were subjected to physical evaluation like weight variation, hardness, thickness, friability and disintegration test. The accelerated stability study for the prepared formulation was carried out and reported. *Invivo* anti-diarrhoeal activity was evaluated by using castor oil induced diarrhoea in rats. The standard drug used for study was loperamide. Animals was divided in to five groups of 6 animals each and diarrhea was induced by administration of castor oil at a dose of 1ml/kg b.w and the tablet formulation was administered at 50 & 100mg/kg dose levels orally. The antidiarrhoeal activity was evaluated by estimating the total number of droppings, mean weight of droppings, number of wet feaces, onset time of diarrhoea. Results were analysed by oneway ANOVA followed by Dunnet's test. Studies revealed that prepared tablet formulation at doses 50 & 100 mg/kg b.w p.o showed significant results in a dose dependent manner comparable with that of standard Lopramide 5mg/kg b.w.

KEY WORDS: *Strychnos potatorum, Punica granatum,* Castor oil, wet granulation, stability studies, antidiarrhoeal activity.

INTRODUCTION

Plants are always an exemplary source of drug. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes(1). The World Health Organisation (WHO) statistics states that diarrhoea is the cause of 10% of all child deaths globally, under the age five(2). Diarrhoea affects people of all ages, due to several causes-malnutrition, infection (bacterial, viral and parasitic organisms), zinc deficiency, functional bowel disorders, food intolerances & sensitivities and reaction to medicines. Severe diarrhoea becomes life-threatening, particularly in young children and people who are malnourished or have impaired immunity. Other symptoms that accompany diarrhoea are cramping, abdominal pain, nausea

and loss of bowel control(3). Modern medication is still a challenge for a vast majority of the population in the third world countries. Traditional herbal remedies are an integral component of people's cultural beliefs and also represent a part of struggle of the people to meet essential drug needs(4). The dependence on plants as source of medicine is still relied upon in many parts of the world. The knowledge of traditional medicine and its practice has been passed down through generations. The antidiarrhoeal activity of medicinal plants has been attributed to presence of various constituents like alkaloids, flavonoids, saponins and tannins. *Strychnos potatorum* Linn belong to family Loganiaceae (Strychnaceae) and *Punica granatum* Linn belong to Punicaceae(5) are some plants used in folklore medicine to treat various gastrointestinal disorders like diarrhoea, abdominal pain, flatulence and as stomachics(6, 7). To date, the folklore claim of antidiarrhoeal activity of these plants has not been validated scientifically/pharmacologically. Therefore, the objective of this present study was formulation and evaluation of herbal tablets incorporated with *Strychnos potatorum and Punica granatum extracts* for antidiarrhoeal activity.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

The selected crude plant materials i.e *Strychnos potatorum* (seeds) *and Punica granatum* (rind) were collected from Erode and Madurai district of Tamilnadu. All the samples were identified and authenticated from Botanical Survey of India, Coimbatore. After authentication the materials were dried and coarsely powdered and used for further studies.

EXTRACTION

The collected plant materials were cleaned, shade dried and powdered by mechanical means. About 200 gm each standardized powder of *Strychnos potatorum* (seeds) *and Punica granatum* (rind) were subjected to extraction by Soxhlation with various solvents starting from nonpolar to polar. After the extraction, the extract was filtered and concentrated at room temperature by using Buchi rotary vacuum evaporator (8, 9). The biologically potent methanol extracts of both the plants was prepared for herbal tablet formulation(6, 7). The extracts was subjected to qualitative method of preliminary phytochemical analysis by adopting standard procedure(10).

FORMULATION OF HERBAL TABLETS

The tablets were prepared by using by non-aqueous wet granulation method containing drug i.e *Strychnos potatorum* seed extract 50 mg, *Punica granatum* rind extract 50 mg, spray dried lactose 137 mg, Starch 40 mg, Polyvinyl pyrrolidone 10 mg, Talc 10 mg, magnesium sterate 3 mg were triturated into fine powder. Then the granules were prepared by adding sufficient quantity of granulating liquid containing Polyvinyl pyrrolidone and Isopropyl alcohol to make a damp mass. This wet mass was passed through sieve no 16 and dried to get uniform granules. The prepared and evaluated granules were compressed into biconvex shaped tablets using a Rotary Tablet Compression machine (Cadmech) with average weight of 300 mg. The tablets were evaluated for the average weight, hardness, thickness, friability and disintegration test (11, 12).

POWDER CHARACTERISTICS

Herbal powders are of wide range with varied physical properties and micromeritic properties. Powdered solids are heterogenous because they are composed of individual particles of widely differing sizes and shapes randomly interspersed with air spaces. It is more complicated in case of herbal powders to convert into tablet.

ANGLE OF REPOSE

Flow properties of the physical mixtures of all the formulations were determined by calculating angle of repose by fixed height method. A funnel with 10 mm inner diameter of stem was fixed at a height of 2 cm. over the platform. About 10 gm of sample was slowly passed along the wall of the funnel till the tip of the pile formed and touches the steam of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured. Angle of repose was calculated from the average radius using the following formula.

$\theta = \tan^{-1} (h/r)$

Where,

θ = Angle of repose

h = Height of the pile

r = Average radius of the powder cone

BULK DENSITY

Bulk densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The volumes occupied by the sample were recorded.

TAPPED DENSITY

Tapped densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated.

COMPRESSIBILITY

It is also one of the simple methods to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr's compressibility.

HAUSNER'S RATIO

It provides an indication of the degree of densification which could result from vibration of the feed hopper.

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density}$$

Lower Hausner ratio Better flow ability
Higher Hausner ratio Poor flow ability

EVALUATION OF TABLETS

PHYSICAL EVALUATION TEST

The prepared tablets were subjected to physical evaluation like weight variation, hardness, thickness, friability and disintegration test as per I.P., 1996.

STABILITY TESTING OF PREPARED HERBAL TABLET FORMULATION

The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of 25^0C/60% RH, 30^0C/60% RH and 40^0C/75% RH for 6 months.

INVIVO ANTI DIARRHOEAL ACTIVITY

CASTOR OIL-INDUCED DIARRHOEA METHOD

The method described by Shobha and Thomas (2001) was followed with minor modification(13). The animals were divided into five groups of 6 each. Each animal was placed in an individual cage, the floor of which was lined with blotting paper(14). The floor lining was changed every hour. The control group received vehicle 1% Tween 80 in water at the dose of 10 ml/kg. Thirty minutes following the administration of loperamide and test extracts at doses 50 and 100 mg/ kg b.w p.o each animal was administered 0.3 ml castor oil orally. The parameters observed for a period of 4 hours were: onset time of diarrhoea, the total number of faeces as well as the number of diarrhoeic faeces excreted by the animals in 4 hours and the total weight of diarrhoeal stools in that period of time. A numerical score based on stool consistency were assigned as follows: normal stool=1, semi-solid stool=2 and watery stool=3. The onset was measured as the time interval in minutes between the administration of castor oil and the appearance of the first diarrhoeal stool (wet faeces that leave a halo on the filter paper). The percent protection against diarrhoea was calculated with respect to number of wet faeces.

STATISTICAL ANALYSIS

Data were analysed using Graph pad Prism Software version 6.0 (Graph Pad Software, La Jolla, USA). All the values were expressed as mean ± standard error of mean (SEM). The significance of difference between two groups for antidiarrhoeal activity was analysed using one-way analysis of variance (ANOVA) followed by post hoc Dunnet's tests. For statistical analysis, $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical investigations of all the plant extracts were carried out by standard protocols. Presence of alkaloids in *Punica granatum* is observed, both the extracts showed positive results for tannins & phenolic compounds. Presence of terpenoids was observed for *Punica granatum* and the results obtained was reported in Table1.

Table2: Results of Preliminary Phytochemical Investigations

S.No	Tests	*Strychnos potatorum*	*Punica granatum*
1	Alkaloids	-ve	+ve
2	Carbohydrates	+ve	+ve
3	Proteins	+ve	-ve
4	Amino acids	+ve	+ve
5	Glycosides	+ve	-ve
6	Steroids & Sterols	+ve	+ve
7	Flavonoids	+ve	+ve
8	Tannins& Phenolic compounds	+ve	+ve
9	Triterpenoids	-ve	+ve
10	Saponin	-ve	+ve
11	Fixed oil	-ve	-ve

+ve + Present, -ve = Absent

The micromeritics properties were determined for all the physical mixtures of *Punica granatum and Strychnos potatorum***.** The results of angle of repose, and Hausner ratio shown in table 2 indicated that the powder mixtures possess good flow properties and good packing ability. The physical properties of tablet were determined and the results of the uniformity of weight, hardness, and friability of the tablets are given in Tables 3.

All the samples of the test product complied with the official requirements of uniformity of weight. The drug content was found to be close to 100% in all formulations. The low friability indicates that the herbal tablets are compact and hard. The results are reproducible, even on tablets that had been stored at different storage conditions i.e 25 ºC , 30 ºC , 40 ºC for 3 months at 60% relative humidity and the results are shown in table 4

Table 2: Micromeritic parameters of physical mixture containing Plant extract

S.no	Property	Value
1	Angle of repose(^{0})	32.2
2	Bulk density(gm/ml)	0.46
3	Tapped density(gm/ml)	0.50
4	Compressibility%	23.52
5	Hausner's ratio	1.3

Table 3: Physical properties of compressed tablet

S.no	Property	Value
1	*Hardness* (kg/cm^2)	±5.5
2	*Friability%*	0.46
3	Weight Variation%	5.1±0.4
4	Disintegration test	9' 40"

Table 4: Stability data of the prepared formulation

Time	% Drug content at different storage conditions		
	25 ^{0}C/60% RH	30 ^{0}C/60% RH	40 ^{0}C/60% RH
1st Month	99.71	99.58	99.44
3rd Months	99.50	98.36	98.62
6th Months	99.37	98.23	97.39

EFFECT OF HERBAL TABLET ON CASTOR OIL INDUCED DIARRHEA

Castor oil treated animals showed significant increase in the total number of droppings, mean weight of droppings, number of wet feaces, onset time of diarrhoea when compared with that of vehicle control, pre-treatment with Lopramide 5mg/kg b.w and herbal tablet at doses 50 & 100 mg/kg b.w showed reduce in the total number of droppings, mean weight of droppings, number of wet feaces, onset time of diarrhoea significantly in a dose dependent manner when compare with negative control as shown in Table 5 and Fig 1, 2,3 & 4.

Table 5. Antidiarrhoeal activity of prepared tablets by castor oil induced diarrhea

GROUP	Onset time of diarrhoea (min)	Total No. of faeces	Number of wet faeces	Total weight of wet faeces (mg)
Vehicle control	48.4 ± 5.25	6.6 ± 0.40	2.2 ± 0.86	568.6 ± 16.28
Negative Control	65.4 ± 5.88	11.8 ± 0.3	4.8 ± 0.37	722.4 ± 14.79
Standard	220.8 ± 8.07***	1.6 ± 0.24***	2.6 ± 0.24*	526.6 ± 20.34**
Formulation (50mg/kg b.w.)	88 ± 10.56**	8.2 ±0.86***	1.4 ± 0.40**	438 ± 12.85***
Formulation (100mg/kg b.w.)	109.8 ± 4.08***	6.4 ± 0.24***	0.6 ± 0.60***	348.6 ± 10.23***

Values are mean ± SEM N=6; $P < 0.05$ as compared to vehicle control and positive control by one way ANNOVA followed by Dunnet's test

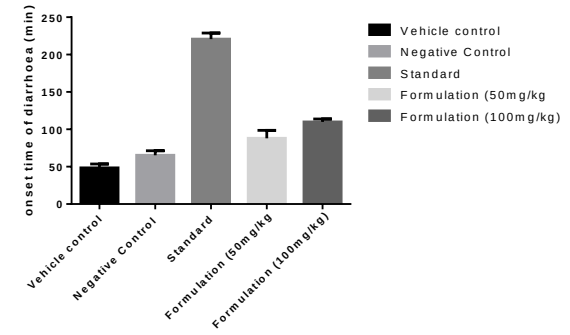

Fig:1 Effect of Formulated Tablet on onset of Diarrhoea

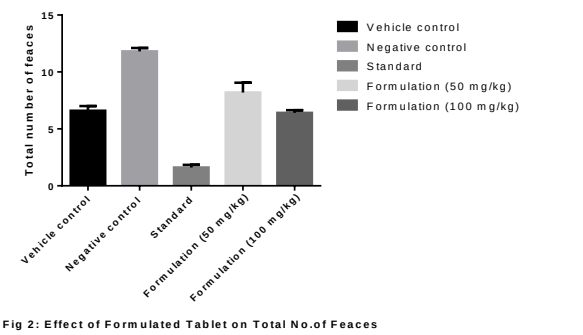

Fig 2: Effect of Formulated Tablet on Total No.of Feaces

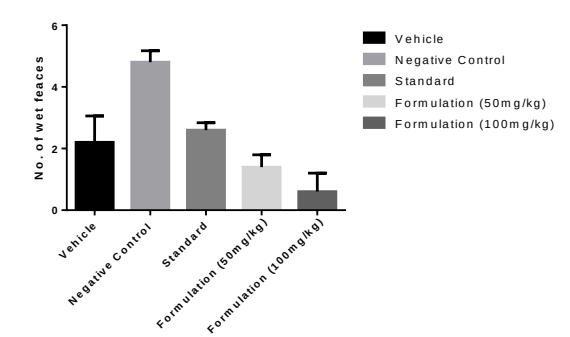

Fig3: Effect of Tablet formulation on No. of wet feaces

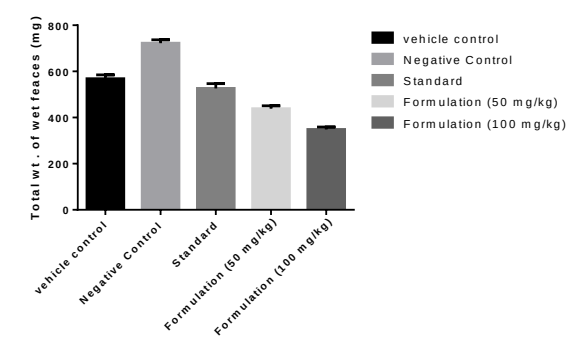

Fig4: Effect of Tablet formulation on Total Wt. of Wet feaces

Castor oil-induced diarrhoea method is considered as a suitable model of the complex hypersecretion process. Ricinoleic acid formed by hydrolysis in the upper small intestine, causes irritation and inflammation of the intestinal mucosa leading to prostaglandin release. This induces change in the net secretion of water and electrolyte transport, resulting in a hypersecretory response in the small intestine (15, 16). The total number of wet faeces

was significantly lower ($P<0.05$ and $P<0.01$) in the treatment groups as compared to the control.

CONCLUSION

Herbal products may contain a single herb or combinations of several different herbs believed to have complementary and/ or synergistic effects. Preliminary phytochemical studies of the plants used in the current study showed the presence of various phytoconstituents like alkaloids, carbohydrates, triterpenoids, flavonoids etc. The results of study demonstrate that tablet formulation of the extracts of *Punica granatum and Strychnos potatorum* showed significant increase in the total number of droppings, mean weight of droppings, number of wet feaces, onset time of diarrhoea when compared with that of vehicle control, pre-treatment with Lopramide 5mg/kg b.w and herbal tablet at doses 50 & 100 mg/kg b.w showed reduce in the total number of droppings, mean weight of droppings, number of wet feaces, onset time of diarrhoea significantly in a dose dependent manner when compare with negative control.

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