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Investigation and Validation of Selected Herbal Formulations for Claimed Antidiarrheal Activity

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Abstract
Sharbat Habb-ul-Aas and Rooh-e-Kafoor syrups are the two branded herbal formulations marketed widely in Pakistan for their antidiarrheal properties. This study is focused to validate the antidiarrheal potential of both products and to provide experimental evidence for their use. Selected products were investigated against different types of diarrhea induced by castor oil (1 ml/kg), magnesium sulphate (2 mg/kg) and clarithromycin (100 mg/kg) using pigeon model. Change in GI transit time was also determined using charcoal activity. Both products demonstrated significant reduction in average weight of stool and reduction in intestinal motility. Excellent anti-diarrheal activity demonstrated by the products may be due to different crude drugs included in the formulations. These findings revealed that use of Sharbat Habb-ul-Aas and Rooh-e-Kafoor syrups for the control of diarrhea is justified and may be effective as alternative medicine for the control of diarrhea.

Key words: Sharbat Habb-ul-Aas, Rooh-e-Kafoor, Loperamide, Anti-diarrheal, Charcoal

Introduction

Diarrhea is a condition of having at least three times loose or liquid bowel movement per day. It usually ends after a few days and results in dehydration (Mahor, 2013). Diarrhea is the most common sign of gastroenteritis. It enhances gastrointestinal motility and secretion, and reduces fluid absorption, leading to loss of electrolytes. Loss of sodium and water is largely associated with high fatality among children (Chisti et al., 2011).

A number of drugs and medicines are available for the control of diarrhea both in allopathic and homeopathic systems of treatment. Loperamide is used as standard drug in allopathic system, however opiates like effect associated with the drug may cause severe depression of respiration especially in higher dose (Mukarram et al., 2016). Moreover, use of loperamide is discouraged in children by WHO and American Academy of Pediatrics due to safety concerns (Faure, 2013), where diarrhea is the second leading cause of death among children under 5 years old (Lanata et al., 2013). Similarly, difenoxin and atropine combination is also used in allopathic system for the treatment of loose stools. However, this combination has been reported to produce toxic

megacolon, CNS effects, dry mouth, urine retention and constipation (Scarlett, 2004). Moreover, this combination is limited for use in adult diarrhea only. The associated side effects and limitations with currently available remedies have created an opportunity for manufacturers to market herbal formulations for the control of diarrhea especially in developing countries which are largely dependent upon the traditional way of treatment (Singh, Verma, Singh, 2012). In many parts of the world including Pakistan, plants based traditional remedies are used without scientific validation. We have selected two different herbal products, Sharbat Habb-ul-Aas (SHA) by Ashraf Dawakhana and Rooh-e-Kafoor syrup (RKS) by Mumtaz Dawakhana available in the local market for investigation on scientific basis and to validate their claimed anti-diarrheal activity.

Materials and Methods

Chemicals and drugs

All chemicals and drugs were arranged from local market. Loperamide HCl Sigma-Aldrich, Castor oil Sigma-Aldrich, Clarithromycin BDH chemicals, Metronidazole donated by Stanley Pharmaceuticals.

Formulation SHA (Sharbat Habb-ul-Aas by Ashraf Dawakhana)

Common name	Botanical Name	Part used
Acacia	Acacia arabica	Peel
Pomegranate	Punica granatum	Flower
Myrtle	Myrtus communis	Seeds
Yellow Guava	Psidium guajava	Extract
Quince	Cydonia oblonga	Extract
Crab Apple	Pyrus malus	Extract
Pomegranate	Punica granatum	Extract

Formulation RKS (Rooh-e-Kafoor Syrup by Mumtaz Dawakhana)

Common name	Botanical Name	Part used
Kafur Syyal	Cinnamon camphora	Fruit
Damascena	Rosa damascena	Flower
Wild Mint	Mentha avernsis	Leaves
Rose red	Rosa santana	Flower
Fennel	Foeniculum vulgare	Seeds
Sharbat Ajowan	Trachyspermum ammi	Seeds

Experimental Animals

Healthy pigeons of either sex were purchased from local market and shifted to the animal house of Pharmacy Department, Abdul Wali Khan University, Mardan. Animals were kept in control environment and observed for one week. Only healthy pigeons with normal movement, drinking and feeding habits were included in the study. Free access to food and water was provided under standard laboratory condition of temperature (25 ± 2°C) humidity (55-65%) and 12-hour light/dark cycle. Ethical committee of the university approved the study protocols. Pigeons with abnormal movement or no drinking or feeding abnormality were excluded from the study.

Grouping and Dosing

Selected pigeons were divided randomly into six groups, each having eight pigeons. Negative control group was given distilled water while positive control group received standard drug loperamide. Rest of the groups received test samples in given doses.

Acute toxicity test

Acute toxicity test was performed for both herbal formulations in single as well as multiple doses in short period of time (Turtle et al., 1963). Pigeons were administered orally with test samples in doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg of body weight. Any possible change in their movement and feeding habit was observed for 4 h, where possible lethality determination was made for 24 h.

Induction of Diarrhea

Diarrhea was induced by magnesium sulphate (2gm/kg), castor oil (1 ml/kg) and clarithromycin (50 mg/ml) in pigeons already administered with distilled water (10 ml/kg), loperamide (1 ml/kg) and both products at 0.5 ml/kg, 1 ml/kg and 1.5 ml/kg doses. Birds were kept separately in cages having pre-weighed white paper at the base. Number of defecation, time for first drop, second drop and weight of stool was determined as per standard protocols (Shoba & Thomas, 2001). Percent inhibitory effect on diarrhea of each sample was calculated using the following relationship.

$$\%\ inibition = \frac{stool\ weight\ of\ control - stool\ weight\ of\ test\ group}{stool\ weight\ of\ control} \times 100$$

Gastrointestinal motility test

Five groups of pigeons were kept on fasting for 24 h with free access to water. Group 1 served as negative control and was administered with distilled water and castor oil where Group 2 served as positive control, received 1 ml metronidazole. Group 3, 4 and 5 served as test groups and were administered with test samples at different doses. After 5 minutes of sample administration all groups were provided with charcoal meal and allowed to drink water for about 30 minutes. Pigeons were sacrificed and intestine was removed for the determination of percent motility using reported method (Atta & Mouneir, 2005).

$$\%\ \text{Motality} = \frac{Distance\ travelled\ by\ Charcoal}{Total\ length\ of\ small\ intestine} \times 100$$

Statistical analysis

All experiments were carried out using 8 animals for each dose. Statistical analysis was finalized by one-way analysis of variation (ANOVA) followed by Dunnett's tests, p values <0.05 were considered statistically significant.

Results and Discussion

Acute Toxicity Test

Observations of acute toxicity test are given in Table 1. Both formulations showed nausea at lowest dose used. Sever vomiting was observed for both formulations at higher dose i.e. 10mg/kg. Half of the test animals died with higher dose of SHA, where some deaths were also recorded with the higher dose used for RKS during 24 hr observation.

Table 1: Acute toxicity of selected formulations

Gp	Dose (ml/kg)	Jerk	Vomiting	Deaths
DW	10	--	--	--
SHA	2.5	Yes	Yes	--
	5	Yes	Yes	--
	10	--	Severe	4
RKS	2.5	Yes	--	--
	5	--	Yes	--
	10	--	Severe	2

DW (Distilled Water), SHA (Sharbat Habb-ul-Aas), RKS (Rooh-e-Kafoor Syrup), n = 8

Effect of selected herbal formulation on Magnesium sulphate induced diarrhea

SHA and RKS displayed non-significant anti-diarrheal activity against magnesium sulphate induced diarrhea. The effect of all three doses used for both formulations on number of defecations remained insignificant. SHA significantly ($p < 0.05$) reduced diarrheal feces at 0.5 ml/kg while all other doses of both tested products significantly ($p < 0.01$) decreased diarrheal feces. Both brands significantly ($p < 0.001$) reduced average weight of stool at all doses used. SHA inhibited 05.52%, 12.87% and 19.82 % where RKS has 02.20%, 09.75%, and 13.99 % inhibition on average weight of feces at dose of 0.5 ml/kg, 1 ml/kg, and 1.5 ml/kg respectively.

Magnesium sulphate produces diarrhea by osmotic properties, preventing reabsorption of water ions, leading to increase in the volume of the intestinal content. It promotes the liberation of cholecytokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents absorption of sodium chloride and water (Afroz et al., 2006). In this study both the selected products showed non-significant activity against magnesium sulphate induced diarrhea, therefore, it may be assumed that osmotic properties of the GIT may not be involved in our study.

Table 2: Effect of selected formulations on magnesium sulphate induced diarrheal model

Gp	Dose (ml/kg)	No. of Defecation	Diarrheal Feces	Feces Ave. Wt (mg)	% Inhibition
DW	10	16.35±1.55	12.45±1.78	3563.75±2.5	-
SHA	0.5	11.53±1.76	3.75±1.65*	3366.80±4.69***	05.52
	1.0	10.03±2.30	2.75±4.21**	3105.0±4.63***	12.87
	1.5	09.14±1.73	1.34±0.71**	2857.25±5.02***	19.82
RKS	0.5	12.20±1.23	3.12±3.15**	3485.25±5.11***	02.20
	1.0	11.79±2.88	4.12±1.03**	3216.14±3.27***	09.75
	1.5	09.96±2.02	2.65±4.03**	3064.85±1.70***	13.99
Lop	1	6.33±1.44*	1.12±2.14**	1903.21±3.69***	46.59

Data are presented as mean ± standard error of the mean for groups (n = 8), one-way ANOVA followed by Dunett's test was applied for data analysis* ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) versus negative group

Effect of selected herbal formulation on Castor oil induced diarrhea

Both branded herbal formulations showed insignificant anti-diarrheal effect against castor oil induced diarrhea. Total no. of defecations and diarrheal feces were insignificantly reduced by both tested products at all doses. Average weight was significantly ($p < 0.001$) decreased by both brands at all doses used. SHA has 08.94%, 15.82%, and 19.19 % inhibition at 0.5 ml/kg, 1 ml/kg, and 1.5 ml/kg doses respectively, whereas RKS has 06.83%, 14.38% and 18.80 % inhibition on average weight using the same doses.

Castor oil-induced diarrhea model is considered as an appropriate method of the complex hyper-secretion process. Ricinoleic acid formed by hydrolysis in the upper small intestine, causes irritation and inflammation of the intestinal mucosa leading to prostaglandin release that changes water secretion and electrolyte transport, leading to a hyper-secretory response in the small intestine (Okere et al., 2015, Rao, Prakash, Kumar, 2006). The observed non-significant anti-diarrheal effect demonstrated by both selected formulations on castor oil induced diarrhea excludes the involvement of anti-secretory mechanism.

Table 3: Effect of selected formulations on castor oil induced diarrheal Model

Gp	Dose (ml/kg)	No. of Defecation	Diarrheal Feces	Average weight (mg)	%Inhibition
DW	10	16.35±1.55	12.45±1.78	3563.75±2.5	-
SHA	0.5	12.35±1.03	4.51±2.13	3245.12±2.88***	08.94
	1.0	11.34±1.15	3.65±2.40	2999.81±3.33***	15.82
	1.5	10.07±1.01	5.63±3.82	2879.54±3.31***	19.19
RKS	0.5	13.15±0.86	5.33±3.20	3320.34±3.21***	06.83
	1.0	11.91±1.21	4.71±3.03	3051.04±3.21***	14.38
	1.5	10.93±0.95	3.52±3.05	2893.41±2.36***	18.80
Lop	1	6.33±1.44*	1.12±2.14**	1903.21±3.69***	46.59

Data are presented as mean ± standard error of the mean for groups (n = 8), one-way ANOVA followed by Dunett's test was applied for data analysis* ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) versus negative group

Effect of selected herbal formulation on Clarithromycin induced diarrhea

SHA and RKS displayed insignificant anti-diarrheal effect against clarithromycin induced diarrhea. Both tested products do not significantly decreased total no. of defecations at all doses. SHA significantly ($p < 0.05$) reduced diarrheal feces at 0.5 ml/kg and 1.5 ml/kg while insignificant decrease was observed in diarrheal feces at 1 ml/kg dose. Average weight was significantly ($p < 0.01$) reduced by both herbal formulations at 0.5 ml/kg and 1 ml/kg doses while SHA significantly ($p < 0.01$) decreased average weight at 1.5 ml/kg dose. SHA inhibited 10.56%, 13.03% and 20.76 % at 0.5 ml/kg, 1 ml/kg, and 1.5 ml/kg doses respectively, where RKS induced 08.98%, 15.16% and 18.73% inhibition with the same doses.

Administration of clarithromycin causes alteration of normal colonic micro-flora resulting into diarrhea (Rafii, Sutherland, Cerniglia, 2008). The observed anti-diarrheal effect of the selected products may be attributed to their composition. These brands contain herbs with anti-diarrheal and antibacterial effect. Both formulations have interestingly attenuated the loose motion induced by various chemicals having different mechanism of action. Antibiotics induced diarrhea that may lead to non-compliance and disturbing effect on the duration and outcome of the therapy may be effectively controlled with such anti-motility formulations (George, Sutter, Finegold, 1977).

Table 4: Effect of selected formulations on clarithromycin induced diarrheal model

Gp	Dose (ml/kg)	No. of Defecation	Diarrheal Feces	Average Weight (mg)	% Inhibition
DW	10	16.35±1.55	12.45±1.78	3563.75±2.5	-
SHA	0.5	13.20±0.86	3.10±3.10*	3187.24±3.24***	10.56
	1.0	10.89±1.01	3.09±3.26	3099.04±3.11***	13.03
	1.5	08.62±1.12	3.40±3.10*	2823.62±3.40**	20.76
	0.5	13.85±0.75	3.70±3.63*	3243.40±4.01***	08.98

RKS	1.0	11.05±3.09	3.35±5.03	3023.26±4.13***	15.16
	1.5	09.01±1.21	3.15±3.63*	2896.21±3.12	18.73
Lop	1	6.33±1.44*	1.12±2.14**	1903.21±3.69***	46.59

Data are presented as mean ± standard error of the mean for groups (n = 8), one-way ANOVA followed by Dunett's test was applied for data analysis* ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) versus negative group

Gastrointestinal Motility Test

Both products decreased the normal intestinal movement of charcoal meal in a dose dependent way. SHA has 46.91%, 42.62% and 38.84% motility compare to control while RKS has 47.44%, 43.25%, and 39.58% motility at 0.5, 1, and 1.5 ml/kg dose respectively.

Table 5: Effect of selected formulations on GIT motility in pigeon model

Gp	Dose (ml/kg)	Distance travelled	Total distance	% motility
DW	10	26.08±3.09	43.13±4.23	60.44±3.09
Castor oil	01	22.69±3.42**	39.63±2.09	57.25±2.98
Metronidazole	01	8.52±4.38*	41.53±2.90	19.86±1.75
SHA	0.5	17.86±2.91**	38.07±1.53	46.91±4.01
	1	18.19±4.03**	42.67±2.08	42.62±0.80
	1.5	16.52±2.19	42.53±3.16	38.84±1.01
RKS	0.5	19.46±3.48*	41.02±2.71	47.44±2.06
	1	17.96±1.93**	41.52±3.07	43.25±0.86
	1.5	16.63±3.21**	42.01±5.01	39.58 ±1.06

Data are presented as mean ± standard error of the mean for groups, * ($p < 0.05$), ** ($p < 0.01$) (n = 8)

Conclusion

Findings of the study revealed that both herbal products have anti-diarrheal activity which may be associated with combined effect of the sub-crude drugs present in these formulations. These results also justified the claim of traditional drugs manufacturers and local medical practitioners about the efficacy of both formulations in diarrhea.

On the basis of these findings, exploration of natural herbal medicines is strongly recommended in order to provide scientific reasons for use of plant based local and traditional remedies used by poor communities due to wide availability of such herbs on affordable price. The study also recommends further investigation on tissues to evaluate the effect of such formulations on vital organs such as kidneys and liver.

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