Evaluation of Anti Diarrheal Activity of Methanolic Rhizome Extract of *Drynaria quercifolia* (Linn.) J. Smith in Albino Rats

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ABSTRACT

Exploration on therapeutic screening of Pteridophytes is comparatively scanty. Drynaria quercifolia (Linn.) J. Smith (family: Polypodiaceae), a newly reported pteridophyte from South Odisha, is an important herbal of antidiarrheal potential used extensively as a folk remedy in Ganjam- Gajapati districts of South Odisha (India). The study was aimed to scientifically validate the folklore therapeutic claim of antidiarrheal potential of its methanolic rhizome extract. The antidiarrheal activity of methanolic extract of the rhizome was evaluated in live system of castor oil induced diarrhea, along with gastrointestinal motility, enteropooling in albino rats. The methanolic rhizome extract (MEDQ) was given to the animals orally at the doses of 100 mg/kg and 200 mg/kg body weight. Loperamide was used as standard antidiarrheal agent. Oral administration of MEDQ in 100 and 200 mg/kg body weight (BW) showed a significant dose dependent inhibitory activity ($p<0.05$) against the castor oil induced diarrhea, gastrointestinal motility in charcoal meal test and enteropooling in albino rats.The severity of diarrhea was reduced to 54.21% and 74.94% at the dose level of 100 and 200 mg/kg body weight respectively; whereas, 75.99% inhibition was found for standard drug Loperamide (5 mg/kg). Castor oil-induced GIT motility was significantly ($p<0.05$) reduced to 42.79% and 65.05%; whereas castor oil-induced enteropooling was found to be reduced to 55.79% and 67.39% at 100 and 200 mg/kg BW dose of MEDQ, respectively. The folklore claim of the plant as an antidiarrheal agent was scientifically validated. The tested extract with their various Phyto-constituents render antidiarrheal property through hindering and /or controlling with varied causes of diarrhea and this fact is in conformity with the traditional claim was established.

Keywords: *Drynaria quercifolia* (Linn.) J. Smith, Antidiarrheal, Loperamide, Castor oil, Intestinal motility, Enteropooling.

INTRODUCTION

Diarrhea is a gastrointestinal disorder in which frequent discharge of liquid faeces occurs from the bowels. The disorder causes increase in the volume, frequency and liquidity of the stool (Guerrant *et al*. 2001) accompanied with spasmodic stomach cramps, abdominal pain, gas and depletion of electrolytes leading to dehydration. Sometimes, it becomes life threatening particularly in children, elderly and immune suppressed people if not treated early. According to the report of 'Rehydration Project of 19th March 2004' diarrhea kills approximately 2.2 million children every year which shows that children are more susceptible to this disease and accounted as the second leading cause of death in children of less than five years age group (Saralaya *et al*., 2010). It emerged as one of the leading health problems in developing countries, causing for death of millions of people each year (Carlos *et al*., 1990). Many factors, such as infection by bacteria, protozoa and virus, food poisoning, excessive intake of fatty foods and laxatives, malnutrition in children, deficiency of niacin and folic acid, adverse effect of some synthetic drugs *etc* construed as are the causes of diarrhea. In addition to this, diarrhea also found as symptom with patients suffering from colon cancer, AIDS, irritable bowel syndrome, Crohn's disease and ulcerative colitis, diabetes mellitus *etc* (Benson *et al*., 2004; Farthing, 2000; Feldman and Schiller, 1983).

Among the different causes of diarrhea, infection by microbial agent is the most prevalent one. It becomes challenging due to emergence of multidrug resistant strains of Enterobacteriaceae, because of their high mutation frequency and horizontal gene transfer (Le *et al*., 1996). Depending on the mechanism and microbial agent involved diarrhea may be of three types. Type-I: toxigenic diarrhea caused by the action of microbial toxins produced by *ETEC, Vibrio cholera etc*; Type-II: the invasive diarrhea caused by microbes *Salmonella, Shigella, Yersinia* and *Campylobacter etc* that invade the gut epithelium and Type-III: the epithelial diarrhea, where causative agents constitute rota virus, parvo virus and *Giardia lambia*, which targets the enterocytes of epithelial villi severely affecting the intestine.

To counter and provide symptomatic relief to diarrhea, medicinal plants have been screened and these are found to be promising source of antidiarrheal drugs (Maikere-Faniyo *et al*., 1989). A study pertains to phytotherapy for diarrheal diseases in Ganjam and Phulbani districts of south Odisha, India shows that roots of the herbals are extensively used by diverse folklore and aboriginals cure diarrhea and dysentery (Girach, 1992; Mohanty *et al*., 1996). Herbals seem to be significant alternative because of their rich extractable potent bioactive compounds that inhibit microbial growth and complementing body and organ systems in detoxification and strengthening immune function. In addition to this, limited availability, high cost and increasing failure of synthetic drugs towards multidrug resistant microbial strains, urge to search for plants with antidiarrheal activity. Several studies have been evaluated the effectiveness of herbals in treatment of diarrhea in different continents (Mukherjee *et al*., 1998, Rani *et al*., 1999, Zavata *et al*., 1998). Increased use of herbals has tempted the WHO to encourage studies pertain to treatment and prevention of diarrheal diseases and to expedite this; it has also launched a Diarrhea Disease Control (DDC) programme worldwide using traditional medical practices (Syder *et al*., 1982; Park, 2000).

Drynaria quercifolia (Linn.) J.Smith, locally called 'Goruda' (in Odia) belongs to a Cryptogrammic plant group 'Pteridophyte'. It is inhabited infrequently in hill tops of Kerandimal Mountains, especially in the Taptapani-Chandragiri areas of undivided Ganjam district. Study of biological activities and chemistry of the rhizome extract was reported earlier as: anti-microbial (Kandhasamy *et al*., 2008); acute toxicity study (Khan *et al*., 2007); anti-inflammatory (Anuja *et al.,* 2010); neuro-pharmacological (Khan *et al*., 2009); anti ulcer (Soni *et al*., 2012) and profiles of the isolated compounds (Ramesh *et al*., 2001). Since, a survey of literature revealed that folklore use of this lower plant for diarrheal diseases are evident and no scientific approach has been made to study the biological activity of this plant on GI-tract disorder, the present study was undertaken to find out the *in vivo* and *in vitro* antidiarrheal activity of the methanolic rhizome extract of the herbal species.

Therapeutic drugs, available in the market to treat diarrhea, are with various side effects. Therefore alternate source is gaining potential day by days and uses of plant products are being preferred due to several advantages which include lower toxicity compared to synthetic compounds. Natural lead compounds in the recent years have been found to be significant for treatment of diarrhea. Research on natural products can contribute to the discovery of new active compounds with novel structures which may serve as leads prescriptions to development of new antidiarrheal drugs. Phytochemical screening of plant extracts (through organic solvents or water) may reveal the presence of numerous lead active compounds.

There are several plants known for their antidiarrheal activity, with different mode of action and phyto-constituents. The need for newer, more

effective, and most importantly, cheaper antidiarrheal drugs has become a paramount issue to tackle this present situation (Alam *et al*., 2008).The natural drugs are used as antidiarrheal drugs, which may not always be free from adverse effects (Syder *et al*., 1982). Therefore, the search for safe and more effective agents are being continued to be an important area of active research (Maiti *et al*., 2007). This is an effort to streamline the phyto-constituents of specific family with specific mode of action to diarrhea.

MATERIALS AND METHODS

Plant materials

The rhizomes of the petriphytic cum epiphytic plant *Drynaria quercifolia* (here in after referred as DQ) were collected from the hill tops of Mahendragiri hills spread through Kerandimals of undivided Ganjam district at Taptapani - Chandragiri areas of Odisha during the month of August and December 2010-14. The herbal after collection from its venue was identified by Dr S.K. Dash, Professor and Head, PG Department of Biosciences, CPS and there after authenticated and conformed by Botanical Survey of India (BSI), Howrah *vide* no. CNH/TECH/2014/187. Voucher herbarium specimens vide no. Ranjan / 08 /2008 and live specimens for undertaking *ex situ* conservation and *in vitro* studies of the specimen were planted at the suitable nearby Mohuda hills and deposited in the Museum of College of Pharmaceutical Sciences, Berhampur of Ganjam district, Odisha, respectively for future study and reference as well.

Preparation of the plant extract

The collected rhizomes were shade dried for six weeks and powdered mechanically for size reduction and then was subjected to successive extraction with solvents like n-Hexane, petroleum ether, chloroform and methanol, and finally with distilled water in the increasing order of polarity using Soxlet extractor. The extracts were filtered and the filtrates were dried using rotary evaporator to get dried crude fractional extracts. The yields of the concentrated crude extracts were estimated. There after crude extracts were subjected to preliminary Phytochemical screening (Kokate, 1999).

Qualitative preliminary phytochemical studies

The dried extracts of the rhizome were subjected to qualitative tests in order to identify the nature of Phytochemical present. Phytochemical screening of the extracts was carried out prescribed treatises (Harborne, 1998)

Animal system used:

Albino rats (*Rattus norvegicus*) of Wister strain weighing 160–200gm of either sex were used for the study. Animals were procured and maintained in animal house of College of Pharmaceutical Sciences (at.Mohuda) Berhampur, at least of 2 weeks prior to the study. For acclimatization the animal house was well maintained under standard conditions, at a temperature (22 ± 20C), room humidity (60 ± 10%) with 12 hours day and night cycle. They were all provided with commercial food pellets and tap water *ad libitum*. They were fasted for at least 18 hours prior to the experiments; but, allowed free access to drinking water. Cleaning and sanitation was done daily, giving due importance. Paddy husk was provided as bedding material and was changed every day. All animal experiments were carried out as per CPSCEA committee of CPS, Mohuda: Regd. No. 1170/AC/08/CPCSEA and 1479/GO/c/11/ CPCSEA.

Drugs and Chemicals

All the chemicals and solvents used in this study were of analytical grade. The marketed drug *viz*; Loperamide (Torrent Laboratory, Ahmadabad) and Castor oil (Dabur Pharma, India) were used in the study.

Acute toxicity study (LD_{50} / LC_{50})

The acute toxicity was performed in albino mice, maintained under standard conditions. Overnight fasted animals were used as per the protocol. Fixed dose (OECD Guideline no. 423, Annexure 2d) method of CPCSEA was adopted for toxicity studies (Veerarghavan, 2001). The tested extract was administered orally. The sign of mortality was observed at 2000 mg/kg in all the cases (OECD, 1997). Common side effects such as loss of weight and depression of treated groups of animals were recorded within the 7 days observation period.

Study of anti-diarrheal activity

Castor oil induced diarrhoea:

The standard method of Shoba *et al*., (2001); Uddin *et al*., (2005) and Nwafor and Bassey (2007) was followed to induce diarrhoea in albino rats. Animals were fasted for 24h before commencement of experiment but allowed free access to water. They were randomized into four groups of six rats each. Group 1 (control) received normal saline (2 ml/kg) by p.o, groups 2 (standard) treated with 5 mg/kg of loperamide (p.o), group 3 and 4 were administered with 200 mg/kg and 400 mg/kg of methanolic extracts of rhizome of DQ (here after referred as MEDQ) orally. After 1 hour of drug pre-treatment, each animal received 2 ml castor oil (p.o). The animals were kept in separate cages with a plain sheet of butter paper placed on floor to collect their droppings and observed for the presence of characteristic diarrhoea dropping and dry stools. The consistency of faecal matter and the frequency of defecations counted for 4 hours. The total diarrheal faeces for the control group were considered to be 100%. The absence of stool was considered as protection from diarrhoea and the % protection was calculated as follows.

$$\% \text{ of diarrheal inhibition} = (T_0 - T_1/T_0) * 100$$

Where, T_0 = number of wet faeces in Control group.T_1 = number of wet faeces in Test group.

The ricinoleic acid formed by hydrolysis of the castor oil induces diarrhea which changes the transport of water and electrolytes resulting in a more intensified secretion and also sensitizes the intramural neurons of the gut.

GIT motility test:

This test was performed according to the method previously described using charcoal as a diet marker (Meite *et al*., 2009). Animals of either sex were fasted for 18h being allowed free access to water. They were randomized into four groups of six rats each. All groups received 2 ml of castor oil (p.o) to induce diarrhea. One hour later, Group 1 (control) received normal saline (2 ml/kg) by p.o; Groups 2 (standard) treated with 5 mg/kg of loperamide (p.o); Group 3 and 4 were administered with 100 mg/kg and 200 mg/kg of MEDQ orally. After one hour of drug administration, all animals received one ml of marker (10% charcoal suspension in 5% gum acacia) orally. One hour later, all the animals were sacrificed and the distance travelled by charcoal meal in intestine from the pylorus to caecum was measured and expressed as % of distance moved (Morona *et al*, 2004).

Castor oil induced enteropooling:

The castor oil induced enter pooling was carried out using standard method (Robert *et al,* 1976 and Qnais *et al*, 2007). Rats of either sex were divided into four groups and fasted for 18 hours prior to the experiment. Group 1 (control) received normal saline (2 ml/kg) p.o; Group 2 (standard) treated with 5 mg/kg of Loperamide (p.o); Group 3 and 4 were administered with 100 mg/kg and 200 mg/kg of MEDQ orally. After 1 hour of respective above drug treatment, all groups received 2 ml castor oil (p.o) to induce diarrhoea. After 1 hour of castor oil induction, the animals were sacrificed and small intestine from the pylorus to the caecum was isolated. There after the intestinal contents were

weighed and volume measured through graduated tubes.

Statistical analysis

The experimental data were expressed as mean ± SEM of six animals. The significance of difference between the control and treated groups was determined by using one-way analysis of variance (ANOVA) followed by Dunnet's t-test. *P* value < 0.05 level were considered as significant. The statistical analysis was carried out using Graph Pad Prism 6 software.

RESULTS

Castor oil induced diarrhoea

Castor oil induced diarrhoea lasted up to 24 hours in the vehicle treated control group. The MEDQ exhibited pronounced anti diarrheal effect in a dose depended manner following oral pre-treatment on castor oil induced diarrhoea as compared with control. The fractioned extract prolonged the onset time of diarrhoea, 75.55 min 135.45 min, at the dose of 100 and 200 mg/kg, respectively. The effect is significant and more comparatively similar with the loperamide 5mg/kg (145min). MEDQ significantly (p< 0.05) inhibited both the frequency of defecation as well as the wetness of stools of animal. Treatment with MEDQ in the dose of 100 and 200 mg/kg reduced the weight as well as the frequency of defecation compared with the control group. The inhibition was 61.89 and 66.25%, with the dose of 100 and 200 mg/kg body weight respectively as that of standard drug Loperamide (5 mg/kg) produced an inhibition of 83.1%. The results are shown in Table-1.

Gastrointestinal motility test

The gastrointestinal distance travelled by the charcoal meal in the experimental rats significantly (P<0.05) lessened by both the test groups as compared with control group. Distance travelled by the charcoal meal was reduced to 36.08% and 54.88% w/w in treated groups. Loperamide (5 mg/kg) on the other hand, produced a marked decrease (72.74%) in the propulsion of charcoal meal through gastrointestinal tract. The results are shown in Table-2.

Table 1: Effect of MEDQ on castor oil induced diarrhoea in albino rats.

Groups	Treatment	Total no. of faeces before diarrhoea	Total no. of diarrheal faeces	% inhibition of diarrheal faeces	Total weight of stools (in gm)	% inhibition of total weight of faeces
Control	Normal saline (2ml/kg) + Castor oil (2ml p.o)	7.66± 0.95	26.67± 0.98	--	9.46± 0.29	--
Standard	Loperamide (5mg/kg) + Castor oil (2ml p.o)	6.50± 0.67	7.00± 0.63****	83.10	2.48± 0.13****	69.97
Test-1	MEDQ (100mg/kg) + Castor oil (2ml p.o)	8.5± 0.56	10.17± .65****	61.89	5.75± 0.21***	39.21
Test-2	MEDQ (200mg/kg) + Castor oil (2ml p.o)	8.83±0.47	9±0.44****	66.25	3.91±0.32***	58.66

*Values are expressed as mean ± SEM (n=6). *P < 0.05, **P < 0.01 and ***P < 0.001when compared to control group.*

Castor oil induced enteropooling test

Castor oil induced a significant increase in the fluid volume of rat intestine in control group (Table-1). MEDQ inhibited castor oil induced enteropooling significantly (*P*<0.05) in rats by both the doses. The percentage of reduction of enteropooling was 57.69% and 66.92% w/w with the dose of 100 and 200 mg/kg of MEDQ respectively in comparison to control (Table-3). The standard drug, Loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (74.61%) (*P*<0.05) and the fraction was almost potent in comparison to the standard drug.

Table 2: Effect of MEDQ on charcoal induced GIT motility on albino rats.

Groups	Treatment	Total length of intestine (cm)	Distance traveled by charcoal meal (cm)	% inhibition
Control	Normal saline (2ml/kg) + Castor oil (2ml p.o)	97.67±2.86	88.67±3.21	-----
Standard	Loperamide (5mg/kg) + Castor oil (2ml p.o)	95.33±0.95	24.17±1.53****	(72.74%)
Test-1	MEDQ (100mg/kg) +Castor oil (2ml p.o)	96.17±1.90	56.67±1.66***	(36.08%)
Test-2	MEDQ (200mg/kg) + Castor oil (2ml p.o)	97.67±0.91	40±2.23****	(54.88%)

*Values are expressed as mean ± SEM (n=6). *P < 0.05, **P < 0.01 and***P < 0.001and ****P < 0.001, when compared to control group.*

DISCUSSION

The result of acute oral toxicological study confirms the MEDQ is non toxic at the dose of 2000 mg/kg. Hence, in this study, LD_{50} is greater than 2000 mg/kg. Since, published report suggested that ≥200 mg/kg body weight of extracts for *in-vivo* studies and ≥ 200µg/ml of extract concentrations in in-vitro are likely to be artefact despite of yielding reproducible effects. Even worse, such high concentrations may trigger non-physiological effects resulting in artefacts (Jurg, 2009). So, dose of 100 and 200 mg/kg were chosen for initiated activity.

The results of present study show that the MEDQ produced statistically significant (P<0.05) reduction in severity and frequency of diarrhoea produced by castor oil. It is also noted that MEDQ significantly (P<0.05) inhibited intestinal fluid accumulation and the volume of intestinal content in dose dependent manner as compared to standard drug loperamide (Figure-1).

Plants synthesize specific Phytochemical which may be harmful or beneficial to human beings. These can have complementary and/or overlapping actions including antioxidants, modulation of detoxification enzymes, stimulation of immune system, reduction of inflammation, modulation of steroid metabolism, antibacterial, anthelmintic and antiviral effects (Johana, 2003). The antidiarrheal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydro electrolytic conditions (Venkatesan *et al.*, 2005).

Table 3: Effect of MEDQ on castor oil induced enteropooling in albino rats.

Groups	Treatment	Mean weight of intestine before milking (gm)	Mean weight of intestine after milking (gm)	Volume of intestinal content (gm)	% inhibition
Control	Normal saline (2ml/kg) + Castor oil (2ml p.o)	5.483±0.21	4.1±0.22	1.3 ± 0.06	-----
Standard	Loperamide (5mg/kg) + Castor oil (2ml p.o)	5.517±0.18	5.28±0.277	0.33 ± 0.042***	(74.61%)
Test-1	MEDQ (100mg/kg) + Castor oil (2ml p.o)	5.817±0.12	6.45±0.32*	0.55 ± 0.042**	(57.69%)
Test-2	MEDQ (200mg/kg) + Castor oil (2ml p.o)	5.950±0.13	5.85±0.37*	0.43 ± 0.06***	(66.92%)

*Values are expressed as mean ± SEM (n=6). *P < 0.05, **P < 0.01 and***P < 0.001and ****P < 0.001, when compared to control group.*

Flavonoids and reducing sugars obtained from selected traditional medicinal plants in Bangladesh and some parts of the world exhibit antidiarrheal properties (Rahaman *et al* 1991, Palombo, 2006). Flavonoids present in the plant extracts are reported to inhibit release of autacoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil (Veiga *et al*, 2000).

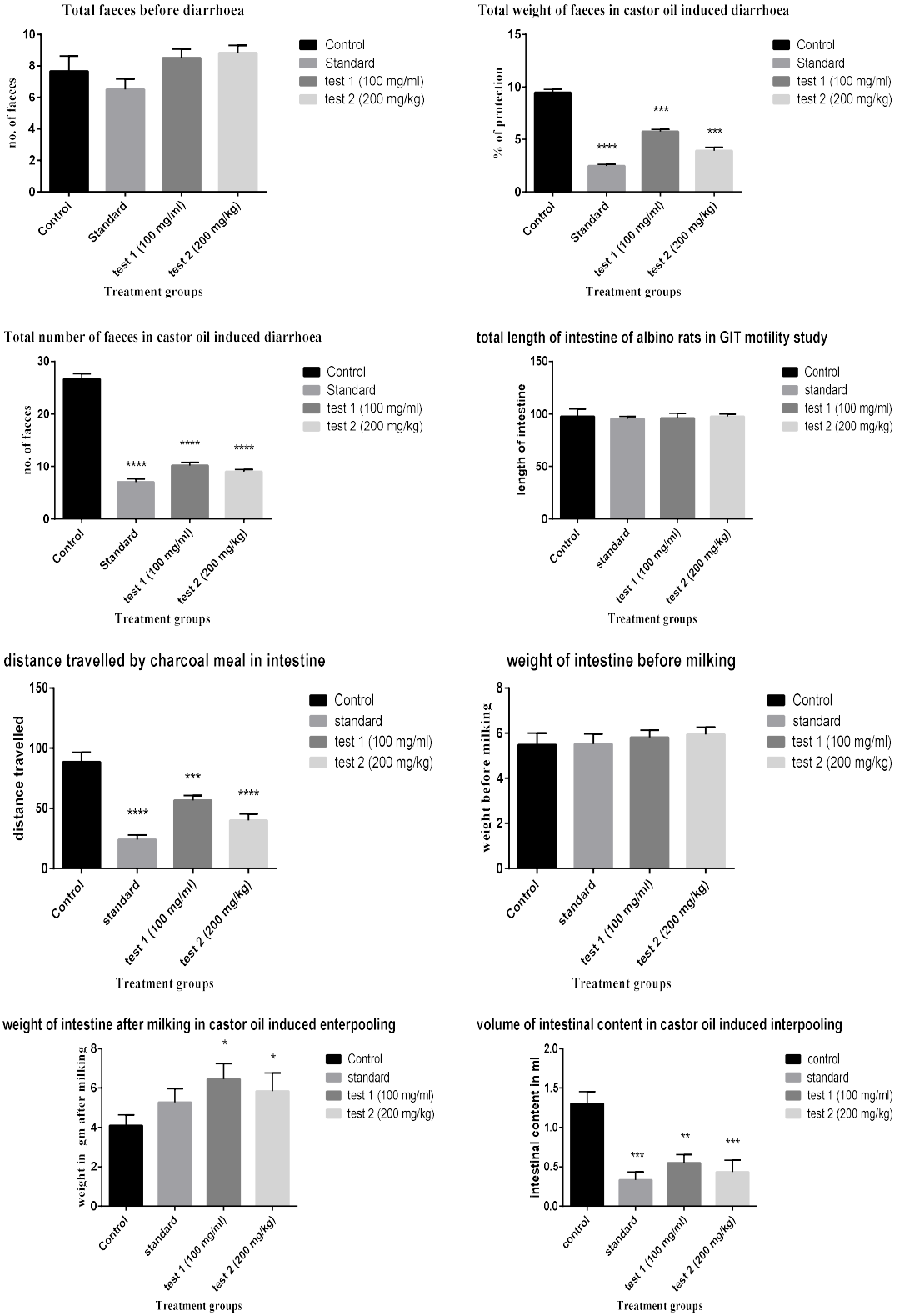

Figure 1: Graphic representation of antidiarrheal activity of MEDQ in Albino rats.

Tannins and tannic acid present in antidiarrheal plants denature proteins in the intestinal mucosa by forming protein tenants which make the intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray *et al*, 2004). Longanga *et al.,* 2000) screened a number of medicinal plants and showed that antidiarrheal activities of these plants were due to tannins, alkaloids, saponins, flavonoids, terpens and glycosides contained in them. Therefore, the presence of these phytoconstituents in MEDQ may be the reason for its antidiarrheal effect.

Diarrhoea results from altered motility and fluid accumulation within the intestinal tract, causing an excess loss of fluid in faeces. In some cases the secretary components predominates, while some are characterized by hyper motility. The objective of diarrhoeal test is to determine the effect of MEDQ on castor oil induced diarrhoea. Castor oil is a triglyceride characterized by a high content of the hydroxylated unsaturated fatty acid *i.e*. ricinoleic acid. About 90% of ricinoleate present in castor oil is mainly responsible for diarrhoea production (McKeon *et al*, 1999). After oral administration of castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic are absorbed in the intestine (Watson *et al.*, 1962). Presence of ricinoleate in small intestine causes increase in the peristaltic movement, as a result of which there is permeability of Na^+ and Cl^- changed in the intestinal mucosa (Palombo, 2006). Secretion of endogenous prostaglandin is also stimulated by ricinoleate (Yoshio *et al.*, 1999). Some Prostaglandins are considered to be good diarrheal agents in experimental animals and in human beings, as well. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil induced diarrhoea (Sorin *et al.*, 2012). Prostaglandins are associated with changes in the bowel that stimulate diarrhoea. Recent study shows that the laxative effect of ricinoleic acid present in castor oil is due to the induction of contraction of intestinal smooth muscle which is mediated by the activation of specific receptors on intestinal smooth muscle (Brijesh *et al.,* 2009). Many antidiarrheal agents act by reducing the gastrointestinal motility and/or the secretions.

The MEDQ exhibited antidiarrheal effect at both the experimental doses taken. The given effects were noticeable considering with standard drug, Loperamide at 5 mg/kg body weight. In our study, we have shown the experimental data for two doses *i.e.* 100 and 200 mg/kg of fractionated extract. There are many reports on antidiarrheal activity of plant extracts using these dose levels (Singh *et al*., 2013). The standard drug Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine; reduce the colon flow rate and consequently have any effect on colonic motility (Camilleri, 2004 and Brown, 1996). Both the doses moderately reduced intestinal transit by the decrease in the distance travelled by charcoal meal. Particularly anti muscarinic drug and atropine decreased the propulsive movement in the charcoal meal study due to its anti cholinergic effect (Brown, 1996). From the result, we found that MEDQ suppressed the propulsion of charcoal meal by increasing the absorption of water and electrolytes.

CONCLUSION

From the present study it is concluded that methanolic extracts of rhizome of *Drynaria quercifolia* (MEDQ) possess potent antidiarrheal activity mainly due to the presence of major amounts of flavonoids, glycosides, saponins, reducing sugars, tannins and tannic acids. This study also scientifically justifies; rather, validates the traditional claim of usefulness of this plant against diarrhoea. Further research is needed to fully investigate the mechanisms involved. The isolated compounds may serve as useful prototypes of antidiarrheal drugs of natural origin.

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