

In vivo acute toxicity, laxative and antiulcer effect of the extract of *Dryopteris Ramose*

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Abstract: *Dryopteris ramosa* (*D. ramosa*) is one of the most traded medicinally important plants of Himalayan region. Apart from other uses, *D. ramosa* is traditionally also used to treat gastric ulcers and as a laxative. The present study was designed to investigate the role of methanolic crude extract of *Dryopteris Ramosa* (MEDR) in acute toxicity, against loperamide induced constipated mice model, antiulcer effect of methanolic extract of *D. Ramosa* and cholinomimetic like effect of methanolic extract of *D. Ramosa*. The crude extract was investigated for the presence of active compounds (secondary metabolites) such as alkaloids, flavonoids, carbohydrates, glycosides, terpenoids, phenolic compounds, saponins, and tannins following the standard methods. The antiulcer effect was investigated in mice using the ethanol induced ulcer model at various doses i.e. 50 mg/kg, 100 mg/kg and 200 mg/kg doses. Constipation was induced in the mice via loperamide (3mg/kg body weight). The control group received normal saline. Different doses of plant extracts (50, 100, 150 and 200 mg/kg body weight/day) were administered for 7 days. Various parameters like feeding characteristics, gastrointestinal transit ratio, body weight, fecal properties and the possible mechanism of action of *D. Ramosa* on intestinal motility were monitored. Various Phytochemicals like saponins, glycosides, flavonoids, tannins, phenols, carbohydrate, alkaloids and triterpenes were found in *D. Ramosa*. The acute toxicity study showed that MEDR was associated with no mortality except mild and moderate sedation at the highest tested doses (1500 and 2000 mg/kg). MEDR also showed significant antiulcer activity against ethanol-induced ulcerogenesis. The extract enhanced the intestinal motility, normalized the body weight of constipated mice and increased the fecal volume which are indications of laxative property of the herb. The 200 mg/kg body weight dose of the extract was found effective. The presence of various Phytochemicals such as flavonoids, glycosides and tannins might be responsible for the antiulcer activity of *D. Ramosa*. This study provides the scientific background for the folkloric use of *D. Ramosa* as antiulcer agent. The laxative action of the extract compares positively with Duphalac, (standard laxative drug). These findings have therefore evidence scientific background to the folkloric use of the herb as a laxative agent.

Key words: *Dryopteris ramosa*; extract; Phytochemical analysis; Acute toxicity; Anti-ulcer activity; Constipation; Laxative effect.

Introduction

In the recent years, constipation becomes one of the most widespread gastrointestinal disorders. About 1/5th of the general population of world suffers from this disorder at some stage in their life (1). Constipation is three or few bowel movements per week, accompanied with symptoms like hard stool, infrequent defecation, feeling of incomplete evacuation, pain and straining (2, 3). Constipation can cause discomfort and affects the individual's quality of life. Constipation causes discomfort as well as cause restlessness, gut obstruction, vomiting, abdominal distension, perforation and fatal pulmonary embolism (4). Factors causing constipation are generally chronic illness, physical inactivity, metabolic problems, side effects of drugs and low intake of dietary fibers and fluids (1, 3, 5). Laxatives are agents which increase the frequency and ease of defecation by retaining water in the bowel lumen and add bulk to intestinal content due to their osmotic effects, increasing intestinal secretions or stimulating intestinal motility

(6). Synthetic laxatives can cause various side effects such as hyperphosphatemia, hypercalcemia, hypokalemia, bloating, cramping and diarrhea (7).

In chronic cases they can further causes harm rather than healing or treatment due to their various side effects such as saline purgatives can cause dehydration, fever, tachycardia and loss of colon motility (8). Majority of the patients are not satisfied from these drugs due to their high costs and undesirable effects such as abdominal pain, stomach problems and slow relieving action (9-11). The use of plants as a source of medicine has been practiced for centuries (12). The attention towards the use of medicinal plants is increasing in people due to their belief that plant extracts are fast acting, readily available, cheap and have fewer side effects as compared to other choices (11, 13, 14). Apart from these qualities the use of medicinal plants as treatment for constipation also have the advantage of some control in the medication choice (15).

Medicinal Plants are traditionally used since ancient times against various diseases including constipation

(16-18). Ferns are used since ancient times for the welfare of human beings as treatments for various ailments like burns, trauma, bleeding, cold, diarrhea, constipation, gastric ulcer and many more (19). The medicinal values of ferns have been reported as early as 300 B.C. by a Greek philosopher Theophrastus (20). The pteridopytes which consists of ferns and fern allies have been known to human for their medicinal purposes for more than 2000 years. Pteridopytes comprises of 12000 species in the world flora. In different systems of medicines such as homeopathic, ayurvedic, unani and other medicine systems, pteridopytes have been used to treat different ailments.

Dryopteris ramosa (*D. ramosa*) is one of the most traded medicinally important plant of Himalayan region (21). It is a perennial herb (22). It grows in moist shady places (23). *D. ramosa* is also known as *Nephrodium ramosum* (24). Local names in different areas of Pakistan are Longer, pakha, ateer etc (25). It belongs to dryopteradaceae family (21, 26) which is the largest family of ferns and *Dryopteris* (third largest genus) in the family. *Dryopteris* species are found worldwide primarily in Eastern Asia and secondarily in North America, South Africa and eastern Europe (27). *D. ramosa* is a terrestrial fern which grows on the damp and shady floors of elevated forests. It is found in Afghanistan, Tibet, Nepal, India and the northern areas of Pakistan like Galiyat, Swat, Neelum valley and Kashmir. Its flowering period is from December- march (28). Ferns contain various useful Phytochemicals such as flavonoids, alkaloids, steroids, phenols, tri terpenoids, fatty acids and various amino acids (29). *D. ramosa* is traditionally used as an astringent, antibacterial, febrifuge (23, 24, 30). It is also used to treat gastric ulcers and as a laxative (22, 31, 32). It is also used as diuretic, as stomachic *Dryopteris cochleata* of the same family, is also used for ulcers and wounds (33, 34). The phytochemical analysis of *D. ramosa* shows the presence of flavonoids, glycosides, volatile oils, proteins and free amino acids (35, 36).

This study was aimed to detect and provide scientific surroundings for the laxative potential of *D. ramosa*, antiulcer and cholinomimetic like effect of *D. ramosa*. In this study the methanolic extract of *D. ramosa* was used in loperamide induced constipated experimental animals to evaluate the laxative activity and the laxative potential was compared with Duphalac, a standard drug for constipation. This study also provides the minimum required dosage of methanolic extract of *D. ramosa* that will provide the laxative effect.

Materials and Methods

Chemicals used

Loperamide was a product of Johnson & Johnson (Pvt) Ltd (Pakistan), Duphalac® from Abbott Laboratories Pakistan, Atropine sulphate (Atropine®) from Siza International (Pvt) Ltd. Femme® was a product of Batala Pharmaceutical Industries Ltd (Pakistan), Normal saline was procured from Shazeb Pharmaceutical Industries Ltd (Pakistan). Analytical grade chemicals were used in this study.

Plant material

Methanolic extract of *D. ramosa* (7gm) was gifted by Syed Hurmat Ali Khan, M.Phil Scholar at pharmacy department of COMSATS Institute of Information Technology, Abbottabad Campus. The plant was collected by Syed Hurmat Ali Khan from Galiyat in the month of September 2017. The aerial parts, roots, rhizome plant was identified by a botanist, Dr. Manzoor Hussain and was placed (BDHU-D.r-088/17) in the herbarium at the botany department of Hazara University Mansehra for ready reference.

Animals used

For the laxative activity, healthy Swiss albino mice ranging in weight from 25-30 gram of either sex were purchased from the Veterinary Research Institute (VRI) Peshawar, Pakistan. The experimental mice were housed in cages in properly ventilated animal house of the department of pharmacy, Garden campus Abdul Wali Khan University, Mardan at a temperature of (22±1 °C). Before the experiment the test animals were acclimatized for one week with light and dark cycles of 12 hours. Standard rodent pellet diet and fresh water were provided to animals. All the experimental measures and studies were carried out according to the guidelines of the care and use of laboratory animals, as adopted and promulgated by the ethics committee, department of pharmacy, Garden campus Abdul Wali Khan University Mardan.

Phytochemical tests

Different preliminary phytochemical tests are performed for the detection of phytochemical groups in plant extract (37, 38).

Acute toxicity test

Before performing the experiment, acute toxicity of *D. ramosa* extract was determined in test mice. Swiss albino mice were grouped into 4 groups of which each group consist of 6 animals. *D. ramosa* extract was administered orally in different doses (gradually increasing) of 500, 1000, 1500 and 2000 mg/kg. After extract administration mice were examined within 24 hours to check the symptoms of toxicity, changes in behavior and mortality. Also they were observed up to 14 days after dosing to note the mortality, if present (39).

Antiulcer activity

Five groups having 8 mice per group were used. Animals were fasted 24 hours before the experiment and allowed access to water. Animals of group 1, 2 and 3 were administered with MEDR as 50, 100 and 200mg/kg body weight, 4th group received 20 mg/kg body weight famotidine (standard drug) and 5th group received normal saline. The treatment was given intraperitoneally in 0.5 ml vehicle. After 30 minutes, all the groups were administered with 1 ml of 70% ethanol. After one hour of ethanol administration, animals were sacrificed and stomach was separated, opened alongside the larger curvature and washed with normal saline. Freshly excised stomachs were laid flat and mucosal lesions were measured by tracing on plain acetate paper. Coarse mucosal lesions or injury were recognized as linear breaks or hemorrhage with damage to the mucosa. The total area

of stomach and coarse lesions were calculated by planimetry with the help of simple magnifier. The result was expressed as the term (40).

$$\text{Ulcer index (\%)} = \frac{\text{total ulcer area}}{\text{total gastric area}}$$

Induction of constipation

Constipation was induced by one ml of loperamide administration to all the groups' animals (3mg/kg body weight in normal saline) for three consecutive days except control group. The 6th group (normal control) received only regular normal saline. Hard, dry and reduced fecal pellets are passed which shows that constipation is induced in mice. The animals in Group one were selected as negative control (normal saline) and Group 2nd as positive control (constipated). These groups received only distilled water. Animals in 3rd, 4th and 5th group (constipated mice) received the methanolic extract of *D. ramosa* as 50 mg, 100mg and 200 mg/kg body weight /day. Group 6 consisting of constipated mice, were administered with Duphalac (standard drug). The feed intake, water intake and changes in body weight (loss and gain) of all the animals were observed and recorded throughout the whole experimental period. The extract treatment continued for seven days (11).

The faeces (pellets) excreted by each of the animals were daily collected on appropriate time (after 24 hours) throughout the experimental duration. The dry weight, overall number and water content of pellets were examined. Water content was calculated from the difference of dry and wet weights of the pellets (41).

Gastrointestinal transit ratio

On the Seventh day of extract dosing, one ml of carmine (3gram of carmine suspended in fifty milli liter of 0.5 percent carboxymethylcellulose) was administered to the test animals (all 6 groups). After one hour of marker administration, test mice were dissected and their small intestine was detached. The total travelled distance by marker (carmine) and the total intestinal length was measured. The gastrointestinal transit ratio

was showed as the percent of distance travelled by the marker as compared to the overall length of small intestine (42).

Cholinergic receptor agonist

The cholinomimetic effect of *D. ramosa* extract was investigated in test animals. The extract's effect was antagonized by an anticholinergic agent (atropine) to confirm the cholinergic receptor agonist mechanism of action of the extract. Four groups of mice (overnight fasted) having 6 mice per group were used. Animals of group 1 received DW as (10 ml/kg), group 2 received atropine as (5 mg/kg), and group 3 and 4 received DW and atropine respectively in the same dose as group 1st and 2nd, ninety minutes before the sacrifice. After 30 minutes, animals of group 1 and 2 received DW while group 3 and 4 received *D. ramosa* extract as 50mg/kg body weight. Then after 30 min of extract and DW administration, charcoal (0.2 milli liter, 3% in DW) was administered to the animals of all 4 groups. Animals were sacrificed after 30 min of treatment. The experimental duration was 90 minutes. Small intestine was removed carefully from pyloric end through the ileocaecal junction. The travelled distance by the charcoal with reference to the overall intestinal length was calculated and expressed as the percentage of distance travelled (43).

Statistical analysis

Data are expressed as mean \pm SEM (n=6). One-way analysis of variance (ANOVA) followed by the Duncan multiple range tests were performed to determine significant differences in all the parameters. Values were considered statistically significant at $P < 0.05$.

Results

Phytochemical tests

The phytochemical analysis for MEDR was done and the result is illustrated in table 1.

Acute toxicity test

Table 1. Phytochemical Analysis of MEDR

S. No	Chemical Compound	Reagent used	Precipitate	Result
1	Flavonoids	Shinoda test	Pink or red color	+
		Alkaline reagent test	Yellow color	+
		Wagner Reagent	Reddish brown ppt's	+
2	Alkaloids	Dragendroff reagent	Reddish brown ppt's	+
		Mayer reagent	Creamy precipitate	+
		Froth test	Frothing test	++
3	Saponins	Salkowski test	Reddish brown color	++
4	Triterpenes	Fehling's test	Brick red ppt's	+
5	Tannins	FeCl ₃ test	Black color	+
		Baljet test	Yellowish orange color	+
6	Glycosides	Keller-Killiani test	Brown ring at interface	+
		Legal's test	Pinkish red color	+
7	Phenols	FeCl ₃ test	Color change	++

Key; Absent (-), Present (+), Ppt (precipitate).

Table 2. Toxicity Profile and Percentage Mortality of MEDR in Animal Model.

Dose (extract)	Animals	Animals survived	Mortality
500 mg/kg	6	All	0
1000 mg/kg	6	All	0
1500 mg/kg	6	All	0
2000 mg/kg	6	All	0

Table 3. Physical Behavior of Test Animals.

Dose	Sedation	Immobility	Convulsion	Photo sensitivity	Writhing
500mg/kg	–	–	–	–	–
1000mg/kg	–	–	–	–	–
1500mg/kg	+	–	–	–	–
2000mg/kg	++	–	–	–	–

There were no signs of behavioral changes except mild sedation at 1500mg/kg and 2000mg/kg body weight and no case of mortality was observed after the administration of MEDR at 2000 mg/kg body weight in mice (table 2).

Animals were examined for various physical behaviors. No convulsions, immobility, writhing and photosensitivity were seen during 24 hours (table 3). Mild sedation was observed at 1500 and 2000mg/kg but animals were awakened by tapping the cages.

Antiulcer activity

The administration of 70% ethanol causes the occurrence of several mucosal lesions in the mice stomach (table 4). Pretreatment with MEDR and famotidine inhi-

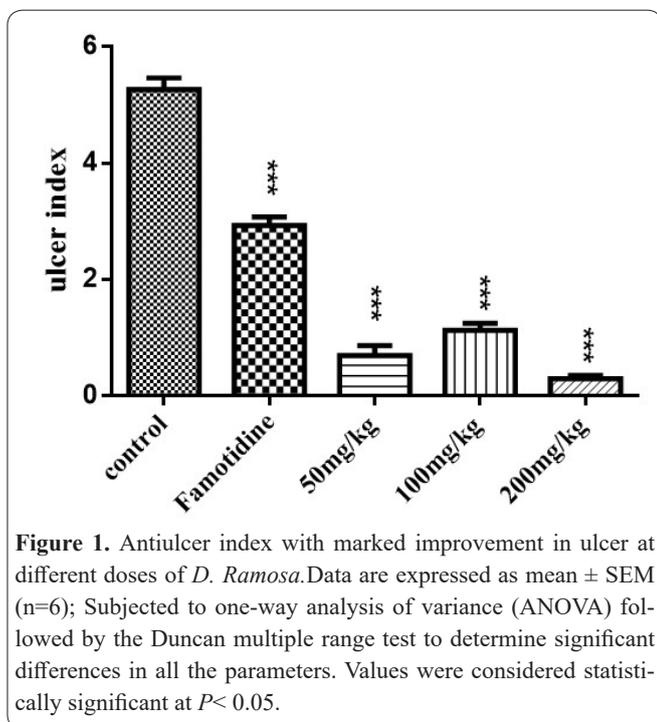


Figure 1. Antiulcer index with marked improvement in ulcer at different doses of *D. Ramosa*. Data are expressed as mean ± SEM (n=6); Subjected to one-way analysis of variance (ANOVA) followed by the Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $P < 0.05$.

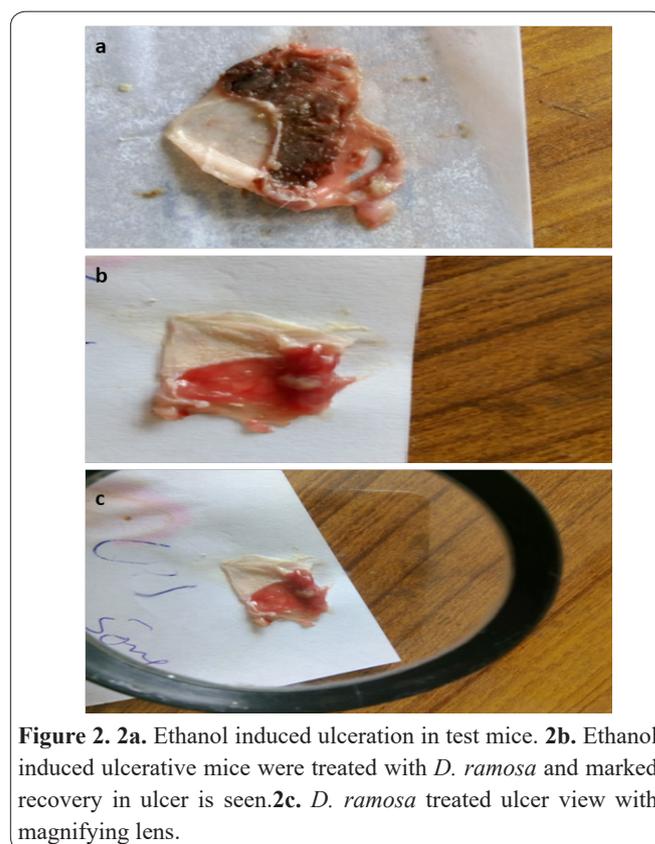


Figure 2. 2a. Ethanol induced ulceration in test mice. 2b. Ethanol induced ulcerative mice were treated with *D. ramosa* and marked recovery in ulcer is seen. 2c. *D. ramosa* treated ulcer view with magnifying lens.

bited the gastric mucosal lesions induced by the ethanol treatment (figure 1). Gastroprotective role of 50 mg/kg, 100 mg/kg and 200 mg/kg MEDR doses was observed in a dose-dependent manner. Famotidine was also found to significantly inhibit the gastric lesions (figure 2). Ulcer indices are mentioned in Table.4.

Laxative activity

Administration of loperamide decreased water intake, amount of water, total count (number) and the overall weight of the faeces (pellets). This shows the induction

Table 4. The action of Famotidine and Various Doses of MEDR on the Ethanol Induced Ulcerogenesis/ Gastric Mucosal Injury.

Groups	Ulcer index	% decrease of injury
Control	5.27±0.20	0
Famotidine	2.93±0.15***	32
50mg	0.70±0.17***	66
100mg	1.13±0.12***	62
200mg	0.09±0.01***	73

of constipation in the mice. There was no considerable variation in the food (feed) intake of constipated and the control animal groups. Water intake was reduced in the constipated control group while the administration of the MEDR increased the water intake in constipated mice. There was no considerable difference between the feed intakes of all the animals. The administration of MEDR considerably enhanced the amount of water (water content), the number and the weight of faeces of constipated mice in a dose dependent mode. The weight of constipated animals became normal after the admi-

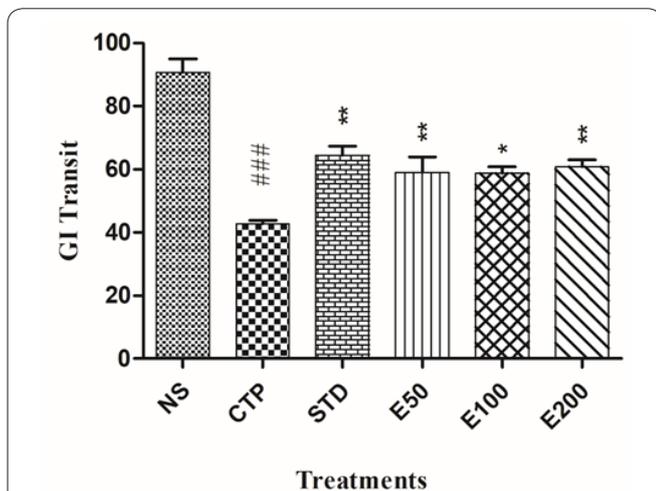


Figure 3. Effect of MEDR on GIT Motility. Data are expressed as mean \pm SEM, ### P <0.001= highly significant when compared against normal saline (control), * P <0.05= significant, ** P <0.01= highly significant when compared against constipated group. Normal saline (negative control), Loperamide (positive control) and extract was administered in different doses. The animals were sacrificed and intestinal motility was determined by GIT transit ratio. NS= normal saline, CTP= constipated, STD=standard (duphalac), E50= 50 mg dose of MEDR, E100=100mg dose of MEDR, E200= 200 mg dose of MEDR.

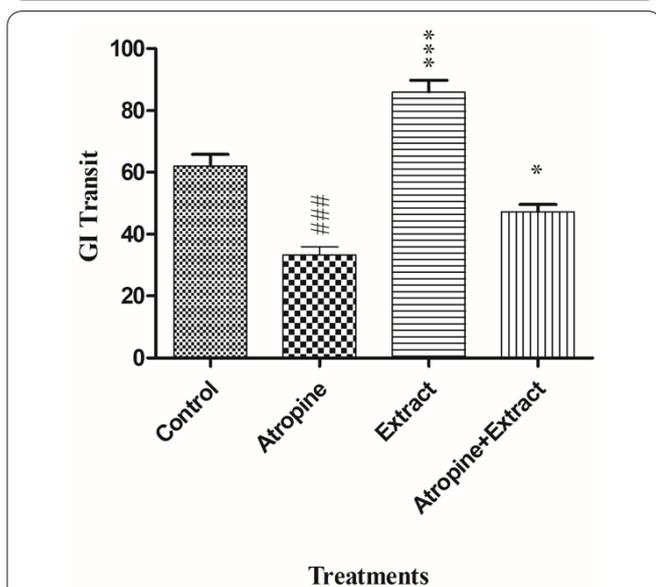


Figure 4. Cholinomimetic Effect of MEDR. Data are expressed as mean \pm SEM, ### P <0.001= highly significant when compared against normal saline (control), * P <0.05= significant, *** P <0.001= highly significant when compared against atropine+ D/water group. Distilled water was administered as negative control, atropine was given as anticholinergic agent, extract and combination of extract and atropine was given to check the cholinomimetic activity (antagonistic activity).



Figure 5. Photograph of intestinal dissection for gastric transit ratio.

nistration of MEDR.

GIT transit ratio

Administration of loperamide significantly reduced the GIT motility in the constipated mice (untreated) while the extract treatment enhanced the GIT motility in a dose dependent mode which was well compared with Duphalac, a standard laxative drug (figure 3).

Cholinergic receptor agonist

There was significant decrease in intestinal motility by atropine in untreated mice while *D. ramosa* increased the GIT motility rate. The GIT motility enhancing action of *D. ramosa* was significantly inhibited by atropine (figure 4). The distance travelled by activated charcoal was shown in figure 5.

Discussion

Ferns have been used for a very long time for the wellbeing of human in the form of treatments for various ailments like burns, trauma, bleeding, cold, diarrhea, constipation and many more (44). The phytochemical analysis of crude extract is important as it can help pharmaceutical research in identifying the novel agents for potential managements of various diseases. The phytochemical study of MEDR proved the existence of various medicinally active constituents such as alkaloids, tannins, flavonoids, triterpenes, glycosides, saponin, carbohydrate, phenols (table 1). These compounds may be responsible for the medicinal outcome in the traditional uses of this plant. As terpenoids and tannins are used as an antibacterial agent, flavonoids as antioxidant and antiulcer (30).

Acute toxicity test is a preliminary screening test for the detection of toxic manifestation and the safety profile of a compound or crude extract. Acute toxicity studies are necessary for any drug proposed for human use. Acute toxicity is the only type of study where mortality is considered as an endpoint. It is used to determine the therapeutic index of therapeutic agent as greater the index safer the therapeutic agent. Acute toxicity study may be used as an aid for dose selection but in many cases it is no longer used for selection of dose (45). The acute toxicity test was done to investigate the safety and toxic effects of MEDR. Different doses of 500, 1000, 1500 and 2000 mg/kg body weight were checked in experimental animals but there were no signs of toxicity and mortality or morbidity up to the highest dose of 2000 mg/kg body weight (table 2). The phytochemical analysis of *D. ramosa* shows the presence of carbohydrates, tannins, flavonoids, glycosides, triterpenes,

saponins, phenols. Baloch and his coworkers reported for the first time the antioxidant effect of *D. ramosa*. They found that methanolic extract of *D. ramosa* has significant DPPH scavenging activity (46).

Our study has also proved the antiulcer effect of *D. ramosa* (figure 1). The ulcer induced was significantly treated by extract of *D. ramosa*. The GIT protective role of tannins is experimentally proved. Tannins cause protein precipitation and vasoconstrictions, flavonoids stimulate the prostaglandin, mucus and bicarbonate secretion. Glycosides and triterpenoids also has antiulcer effect (47). Phenolic compounds are the renowned antioxidants and acting as antiulcer agents (48). Saponins also has pronounced antiulcer activity (49). Farrag and his coworkers found that the phenolic compounds have pronounced antiulcerogenic effect. They established that the phenolic compounds have significantly reduced the ulcer indices, gastric volumes, severity and number of lesions and total acidity. They also reported that phenolic compounds possess antioxidant activities as these compounds have elevated the reduced glutathione levels significantly. Thus the antiulcerogenic and antioxidant activity of phenol compounds has been proved scientifically (50). Herbal remedies are preferred in such cases, when there is a need of chronic or long term therapy. Herbal drugs reduces the offensive factors and are cheap, safe, globally competitive and have good tolerance (47).

The present study also demonstrated that MEDR has laxative or anti-constipation potential, which is favorably equivalent to duphalac (lactulose), a standard laxative drug. The decline in the number of faeces, water content and weight of the faeces after the loperamide treatment indicated the constipation induction in the mice. The decreased water intake of mice (constipated) might be due to the result of loperamide, resulting in decreased water content of faeces (51). Loperamide is well documented for the induction of constipation by delaying the intestinal transit via blockage of smooth intestinal movement and secretions of water. In various studies, administration of loperamide causes reduction in the water consumption and feed intake, while in some cases contrary results have been reported (52). In our study, feed intake was not affected while water consumption was affected by loperamide treatment. Same observation was established by Wintola *et al.*, 2010. MEDR effectively improved the defecation rate, colon motility and fecal volume in the constipated mice. These are the signs of laxative activity of the plant extract.

Phytochemical analysis of the plant extract shows the presence of tannins, alkaloids, flavonoids, glycosides and carbohydrates. The laxative effect of the MEDR may be due to the existence of these compounds. Thaina *et al.*, reported the laxative effect of glycosides in *Senna Alata*, they also compared the laxative potential of aglycone (400 mg/kg) and glycoside (800 mg/kg). They proved that the laxative potential of both aglycone and glycosides was same but slower onset of action in aglycone treated mice (53).

Although the ingestion or the intake of feed did not vary between all groups but increase in the weight of their bodies were high in the control (constipated) group in comparison to the groups which were treated with the extract. This might be owing to the accumula-

tion of faeces in the animal bodies causing raise in the weight. Similar investigations were found by Ashafa *et al.*, 2011. Hwang and his coworkers found that natural products containing tannins have potent laxative effect. They found that *Mareya micrantha* (rich source of tannins) increased the intestinal water secretions, stool output, intestinal ion secretions and gastrointestinal motility. The laxative effect was found same as that of sodium picosulfate. Thus Hwang proved the laxative effect of tannins (54). This shows that the plant extract enhances the gastric propulsive motility in the constipated mice. In this study, the laxative action of *D. ramosa* was dose dependent. The activity of MEDR at maximum dose coincides with duphalac. GIT transit process reflects the overall GIT motor activity. So the rise in gastrointestinal transit ratio by MEDR reflects the improved or increase in colonic peristalsis and intestinal motility in the animals (42). Measuring the GIT transit time is helpful in irritable bowel syndrome constipation and abdominal bloating. This study also gives information about detection and characterization of abnormalities in colon transit, assessment of the disease severity and the reaction to the treatment (51). Measurement of the GIT transit ratio helps in the diagnosis of constipation as decline in GIT transit ratio shows constipation. In this study, elevation in the GIT transit ratio is seen in the extract treated animals thus evidenced that MEDR possess good laxative potential.

In this study, the extract increased the intestinal motility hence enhancing the colonic peristalsis in test animals. The increase in the movement of marker is most likely due to the increase in peristalsis in test animals GIT tract due to the activation of cholinergic receptors by extract (55). On the basis of observation that atropine pretreatment blocked the effect of *D. ramosa* extract, it can be speculated that the stimulatory effect of *D. ramosa* was mediated through the interaction with post synaptic cholinergic (muscarinic M3) receptors, the major muscarinic receptors subtype mediating the contractions in gut muscles (smooth muscles). The two other receptors are also found as M1 and M2 which modulates the acetylcholine release from the nerves of cholinergic origin through negative feedback. When these receptors are stimulated, they block the acetylcholine release from cholinergic nerves and when these receptors are blocked, they facilitate the acetylcholine release (56). This reveals that *D. ramosa* may have 2 type of effects as direct synergistic (cholinomimetic) effect on the M3 and blocking effect on M1 and M2 (muscarinic auto receptors).

Our research reveals that the phytochemical investigation of the methanolic extract of *D. ramosa* confirmed the existence of various chemical groups like flavonoids, glycosides, tannins, saponins, alkaloids, phenolic compounds, carbohydrates and triterpenes. The acute toxicity test was done to detect the safety of the extract and toxic effects. The crude extract was found to be safe up to 2000mg/kg as no toxicity signs and mortality were seen for 24 hours. The crude extract of *D. ramosa* can be considered safe as it did not cause any mortality or adverse changes in behavior of test animals in acute toxicity study up to the dose of 2000mg/kg body weight. Thus MEDR was found safe up to the highest dose of 2000mg/kg. MEDR justifies its role as antiulcer agent

by significantly inhibiting the ulcerogenesis induced by ethanol treatment. The result of our study shows that the MEDR have antiulcer potential.

The present study also revealed that the methanolic extract of *D. ramosa* exhibit the laxative potential in constipated mice induced by loperamide in a dose dependent approach. It has pronounced GIT motility enhancing potential. It exhibited the better laxative activity at 100 and 200mg/kg as compared to 50mg/kg which is comparable to duphalac, a standard laxative drug. Hence our study supports the scientific basis for the use of *D. ramosa* as a laxative agent. The GIT motility promoting effect of MEDR was significantly inhibited by atropine. The inhibition of the laxative effect of MEDR by atropine suggests that the plant extract may contain certain compounds that have the same effects as acetylcholine (cholinomimetic properties) but further scientific investigation is needed for the finding of those compounds.

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Interest conflict

The authors of this article have no conflict of interest.

Authors Contribution

Sana Nazir, Waqas Alam, and Rukhsana Ghaffar carried out the experimental work and draft the initial MS. Sajjad Ali Khan help in the finalizing the MS draft while the overall project was designed and supervised by Haroon Khan.

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