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Research Article

LAXATIVE ACTIVITY OF *RAPHANUS SATIVUS L.* LEAF

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ABSTRACT

Methods: Aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus L.* were prepared and evaluated by preliminary phytochemical test. Laxative action of the extract at a dose of 250, 500 and 750 mg/kg (p.o) was evaluated using wistar albino rats, weighing 200-300 g, in different experimental model such as loperamide induced constipation, laxative activity test, gastrointestinal motility test, water and electrolyte secretion test.

Results: Preliminary phytochemical studies carried for AqRS extract and FreRS juice of *R.sativus* leaves revealed the presence of carbohydrates, tannins, phytosterols, flavonoids and saponins. The FreRS juice & AqRS extract at 750mg/kg showed significant increased in fecal output in loperamide induced constipation ($p<0.05$) and laxative activity test ($p<0.01$). Both extract and the juice increased the distance covered in charcoal meal test ($p<0.001$) and increased the water-ion secretion in electrolyte secretion test ($p<0.001$) indicating laxative activity of *Raphanus sativus* leaf.

Conclusion: The present study revealed that the aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus L.* at a higher dose of 750 mg/kg exhibit significant laxative activity.

Keywords: Laxative, *Raphanus sativus*, Loperamide, Constipation

INTRODUCTION

Constipation is one of the most frequent gastrointestinal disorders in the recent years. It is found to be more prevalent in women and adults of age (65 & above) than in children [1]. In India, about 53% population is suffering from constipation [2]. The major factors responsible for causing constipation are lack of fibre in diet, lack of physical activity, insufficient amount of liquid present in the diet, consumption of certain medicines, and changes in lifestyle or routine etc [1].

Lack of fibre is one of the major causes of constipation. Diet rich in fibres and fluids can act as good natural bulking agents and help to prevent constipation. *Raphanus sativus*, commonly known as radish is known as a plant containing fibre, roughage along with plenty of water.

Raphanus sativus Linn. belonging to the family Brassicaceae (Cruciferae) is an annually cultivated herb found at an altitude of 3000-4000 m. Generally found in hilly areas of Himalayas region [3]. It is also commonly grown in India and is used as a functional food. It is long known for its gastronomic and therapeutic purposes. Since 10th century, *R. sativus* have been documented in India for its use in diseases like scurvy, gonorrhoea, urinary infections, piles, gastrodynia, and other gastric infections [4].

Various pharmacological studies have been carried out on *Raphanus sativus* which suggest its use as an antiseptic, antibacterial [5] antifungal [6], antioxidant [7], antirheumatic [8], appetite stimulant, diuretic, diaphoretic, rubefacient, liver protective [9], and also effective in asthma and other chest problems [10]. The aqueous leaf extract was found effective in controlling blood glucose level [11]. The aqueous extract of the plant has shown antiurolithiatic activity [12]. They are used as an alternative for the treatment of various disorders like whooping cough, cancer, liver problems, constipation, gastric discomfort, kidney stones and many more GIT disorders [13].

The roots are used a tonic to stimulate the appetite and digestion. The laxative effect of roots was found to be on intestines by indirectly stimulating the production and flow of bile thereby aiding digestion [14]. The roots are antiscorbutic, antispasmodic, and astringent. When crushed can be used as a poultice for burns, bruises and smelly feet [10]. *Raphanus sativus* root and seeds have been used for the alleviation of symptoms and treatment of diseases related to liver & Gall bladder [15]. The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints. The seeds are carminative, diuretic, expectorant, laxative and stomachic [16]. Radishes are also an excellent food remedy for stone, gravel and scorbutic conditions [17]. Glucosinolate is an important chemical constituent generally found in roots, leaves and seeds [18]. Apart from this the leaves of *Raphanus sativus* is found to be a good source of protein, having biological value of 76.6 and digestibility co-efficient as 73.5%. It shows presence of some biochemical substances like methins, sapogenins, levon; enzymes such as phosphatase, catalase; histaminergic component and a weak spasmolytic factor; amino acids such as lysine, methionin etc.; polyphenolics such as protocatechuic acid, vanillic acid; and antibacterial substances such as sulphoraphene and raphanin; are also present [13]. Phytochemical screening of plant has shown the presence of triterpenes, alkaloids, saponins and coumarins [19].

Ethnomedicinal claim strongly suggests the use of *Raphanus sativus L*, in constipation. Traditionally it is suggested to take fresh juice of leaves internally in the treatment of indigestion, wind, acid regurgitation, diarrhoea. The juice of the fresh leaves is claimed to be an excellent diuretic and laxative [20].

According to the homeopathic treatment, *Raphanus* is considered to be one of the best and natural remedy for constipation especially in case of blotting of abdomen, absence of gases and prompt satiety which may be caused either due to overeating or because of sydentry lifestyle [21]. Gilani et al has reported gut stimulatory activity and has correlated it with the presence of, biochemical substance, histaminergic components and a weak spasmolytic factor [22].

However there is no scientific evidence or pharmacological study carried out to evaluate the laxative activity of the same. The present research was therefore undertaken to study the laxative activity of the of *R.sativus* leaf extract.

MATERIAL AND METHODS

Drugs and Chemicals

Loperamide HCl tablet (Ridol) & Sodium Picosulphate tablet (Cremalax) was procured from chemist shop. Charcoal, Sodium chloride, Potassium chloride, Calcium chloride was procured from Rankem analytical laboratory.

Plant extraction

Aqueous extract

Fresh leaves of the plant *Raphanus sativus L*. was bought from the local Shirpur market, Maharashtra and authenticated from Agharkar Research Institute, Pune. The fresh leaves were cleaned with water, weighed, and 500g of the leaves was air-dried and powdered. The course powder was subjected to extraction process with distilled water in soxhlet apparatus at 60- 80°C for 2-3 days. The extract was concentrated in a rotary vacuum evaporator at 40 °C to yield a greenish brown color, extract with the percent yield of 2.036%

Fresh juice

500g of fresh leaves were cleaned, washed with water and grinded in grinder with addition of 50 ml distilled water. The thick, green colored paste was passed through muslin cloth to obtained green colored fresh juice of *Raphanus sativus* weighing 78ml (41.38 gm). The percent yield of fresh juice was found to be 8.276%.

Phytochemical Investigation

The aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus L.* were subjected to preliminary, qualitative phytochemical investigation using standard method of analysis [23].

Experimental animals

Male wistar albino rats, weighing 200-300 g, were used and housed in standard environmental conditions i.e. room temperature 22°C ± 3°C, with relative humidity 50-60%. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/OCT-11/03/011) of NMIMS, SPTM, Dhule, Maharashtra and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity studies:

The acute toxicity of both AqRS and FreRS was determined in male wistar albino rats, (200-300 g) as per OECD guideline 423. Both AqRS extract and FreRS juice were given up to the maximum dose of 5000mg/kg. Immediately after dosing, the animals were observed continuously for the first 24 hours for any behavioral changes and mortality. Thereafter, they were kept under observation up to 14 days after drug administration to find out the mortality if any.

Laxative activity test

Laxative activity was carried as per the method described by Capasso *et al* [24]. Animals were placed individually in cages lined with clean filter paper. Rats were divided in eight groups with six animals in each group. All the animals were kept on fasting for 12 hr. The first group acted as the normal control and received saline (5 mL/kg, p. o.) The second group received sodium picosulfate (5 mg/kg, p.o), and served as standard. The third, fourth and fifth groups received 250, 500 and 750 mg/kg (p.o) of the aqueous extract (AqRS) and sixth, seventh & eight group received 250, 500 and 750 mg/kg (p.o) of fresh juice (FreRS) of the *Raphanus sativus* leaves. The faeces production in all eight groups was monitored for 16 hrs.

Laxative activity in loperamide induced constipation:

This study was carried out, as described by Takahura *et al* [25]. Male wistar rats were placed individually in cages lined with clean filter paper, and fasted for 18 hours and divided into eight groups of six animals each. The first group received normal saline (5 mL/kg, p.o) act as control. The second group received the standard drug sodium picosulfate (5 mg/kg). The third, fourth and fifth groups received 250, 500 and 750 mg/kg of the aqueous extract (AqRS) of *Raphanus sativus L.* and sixth, seventh & eight received fresh juice (FreRS) of *Raphanus sativus L* at a dose of 250, 500 and 750 mg/kg respectively. After 1 hour, all the animals received loperamide constipation inducing agent (5 mg/kg, p.o.) by gavage. The faeces production in all eight groups was monitored for 8 hrs.

Gastrointestinal motility tests in rats

Mascolo *et al* developed a method for carrying out gastrointestinal motility tests in rats [26]. According to this the male wistar rats were divided into eight groups and fasted for 18 hours before the experiment. First group served as control treated with normal saline (5 ml/kg, p.o.) and second one was administered castor oil (2ml/rat, p.o.), a laxative agent, as the positive control or standard. Groups four, five & six were treated orally with (250, 500 and 750 mg/kg) of the *Raphanus sativus L.* aqueous extract (AqRS) and the rest three (6, 7, & 8) were given (250, 500 and 750 mg/kg) of fresh juice (FreRS) serving as the test groups. After 30 minutes, the animals were given 1 ml of freshly prepared charcoal meal *p.o.* (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal). Following 30 minutes of charcoal administration, the rats were sacrificed by cervical dislocation and the abdomen immediately cut open, to excise the whole small intestine from pylorus region to ceacum region.

Evaluation was done by measuring the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal for obtaining the charcoal transport ratio or percentage.

Water and electrolyte secretion

The method of Robert *et al.* was used. Male wistar rats were used for study. The first group received normal saline solution (5 ml/kg, p.o). The second group received castor oil (2 ml/rat, p.o), this served as the positive control or standard. The third, fourth and fifth groups received 250, 500 and 750 mg/kg of the *Raphanus sativus L*. groups received aqueous extract (AqRS) and sixth, seventh, & eighth group received 250, 500 and 750 mg/kg of the *Raphanus sativus L*. fresh juice (FreRS). After 2 hours, the rats were sacrificed and the small intestine from the pylorus to caecum was cut open and extracted. The intestinal contents were collected by milking into a graduated tube. Volume of intestinal contents was measured. The fluid samples were analyzed for Na+, K+, Cl^-, Ca2+ concentrations [27].

Statistical evaluation

Data obtained are presented as means ± standard error of mean (S.E.M.) for the number of animals in each group (n = 6). The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P<0.05$ was considered as significant. For Ion secretion model, groups were compared using two-way analysis of variance (ANOVA) followed by Bonferroni test.

RESULTS

From the various phytochemical studies carried out it was found that aqueous extract and fresh juice contains alkaloids, flavonoids, tannins, triterpenoids, proteins, amino acids, vitamin C, calcium, magnesium, carbohydrate and saponins.

In acute toxicity studies, no sign of behavioural changes and mortality was recorded both in aqueous extract (AqRS) and fresh juice (FreRS) up to the maximum dose of 5000 mg/kg. Hence $1/10^{th}$ of the maximum dose tested was selected for the laxative activity.

In the study of laxative activity, the aqueous extract (AqRS) and fresh juice (FreRS) showed dose dependant increase in fecal output of rats when compared to the control group. The effects of *Raphanus sativus L* at doses of 500 and 750 mg/kg (p.o.) increased the fecal output of rats significantly compared to control group ($p < 0.01$-0.001) (Table 1).

In the loperamide-induced constipation test, the aqueous extract & fresh juice of *Raphanus sativus* at the doses of 500 and 750 mg/kg (p.o.), increased the total number of faeces in a dose dependent manner, and the results were statistically significant ($p < 0.05$-0.001) (Table 2). There was less effect seen with the dose of 250 mg/kg (p.o.) of the fresh juice as compared with control.

Table 1: Effect of fresh juice (FreRS) and aqueous extract (AqRS) of *Raphanus sativus L.* on faeces output

S.No.	Groups	Dose mg/kg	Faeces output (g)	
			0-8 hrs	8-16 hrs
1	Control	5 mg/kg	0.745±0.091	0.745±0.091
2	Sod. Picosulphate	5 mg/kg	4.475±0.174**	4.475±0.174**
3	FreRS	250 mg/kg	1.582±0.078**	1.565±0.093**
4	FreRS	500 mg/kg	2.927±0.104**	2.75±0.100**
5	FreRS	750 mg/kg	3.902±0.081**	3.857±0.096**
6	AqRS	250 mg/kg	1.530±0.1202*	1.560±0.097**
7	AqRS	500 mg/kg	2.88±0.107**	2.812±0.069**
8	AqRS	750 mg/kg	3.868±0.089**	3.858±0.065**

Values are given as (mean ± SEM), n = 6 (no. of animals). Values are statistically significant at *P<0.01, one-way analysis of variance (ANOVA) followed by Dunnett's. Values are statistically significant at **P<0.001 compared with control group.

Table 2: Effect of aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* on loperamide induced laxative activity

S.No.	Groups	Dose mg/kg	Faeces output (g)	
			Aqueous Extract	Fresh juice
1	Control	5 mg/kg	0.956±0.051	0.956±0.051
2	Sod. Picosulphate	5 mg/kg	4.048±0.2128***	4.048±0.2128***
3	*R. Sativus*	250 mg/kg	1.450±0.0577*	1.398±0.1144
4	*R. Sativus*	500 mg/kg	2.780±0.0789***	2.835±0.0760***
5	*R. Sativus*	750 mg/kg	3.418±0.1689***	3.473±0.1476***

Values are given as (mean ± SEM), n = 6 (no. of animals). Values are analyzed using, one-way analysis of variance (ANOVA) followed by Dunnett's test. Values are statistically significant at *P<0.05, **P<0.01 & ***P<0.001 compared with control group.

The results of gastrointestinal motility test are reported in (Table 3). The aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* increased momentum of the charcoal meal through the gastrointestinal tract in a concentration dependant manner (Figure 1.). Little effect was observed at the dose of 250mg/kg of the aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus.*

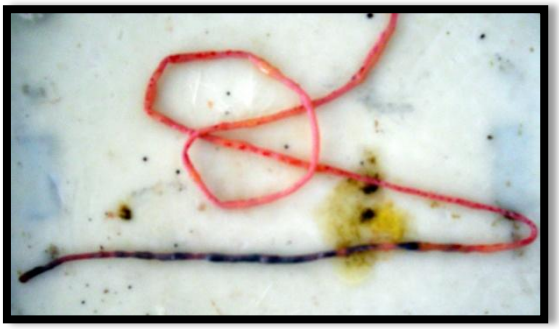

Fig 1: Small intestine showing charcoal transport region

Table 3: Effect of aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* on GI motility test

S. No.	Groups	Dose mg/kg	Percentage of distance (%)	
			Aqueous Extract	Fresh juice
1	Control	5	50.77±1.926	50.77±1.926
2	Castor oil	2 ml	87.59±2.013***	87.59±2.013***
3	*R. Sativus*	250	54.13±1.131	54.16±1.568
4	*R. Sativus*	500	67.68±2.92***	69.60±0.759***
5	*R. Sativus*	750	81.29±1.09***	82.99±1.091***

Values are given as (mean ± SEM), n = 6 (no. of animals). Values are analyzed using, one-way analysis of variance followed by Dunnett's test. Values are statistically significant at *P<0.05, **P<0.01 & ***P<0.001 compared with control group.

The results of the volume of intestinal fluid analysis and ion secretion by aqueous extract (AqRS) and fresh juice (FreRS) are shown in (Table 4, 5 & 6). Both the extract and fresh juice at dose (250 mg/kg) produced negligible effects on intestinal water secretion. However, the fluid volume of the rat intestine was significantly increased by the extract at the dose 750 mg/kg (p.o.) ($p < 0.001$) There was little effect seen with the dose of 500 mg/kg (p.o.) of the extract as well as juice on intestinal Ca2+,K+ secretion compared to control group. At the dose of 750 mg/kg, the aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* increased significantly Na+, K+, Cl- and Ca2+ ($p < 0.05$-0.001) secretions through the gastrointestinal tract compared with control group.

Table 4: Effect of aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* on water secretion in rats

S. No.	Groups	Dose mg/kg	Volume of intestinal fluid (ml)	
			Aqueous extract	**Fresh juice**
1	Control	5	0.675±0.091	0.675±0.091
2	Castor oil	2 ml	2.750±0.160***	2.750±0.160***
3	*R. Sativus*	250	0.900±0.157	0.917±0.117
4	*R. Sativus*	500	1.383±0.1447**	1.483±0.0872**
5	*R. Sativus*	750	1.983±0.083***	2.050±0.187***

Values are given as (mean ± SEM), n = 6 (no. of animals). Values are analyzed using one-way analysis of variance (ANOVA) followed by Dunnett,s test. Values are statistically significant at *P<0.05, **P<0.01 & ***P<0.001 compared with control group.

Table 5: Effect of aqueous extract (AqRS) of *Raphanus sativus* for ion secretion in rats

S.No.	Groups	Dose (mg/kg)	Na^+ (mg/L)	Cl^- (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)
1	Control	5	1.948±0.068	10.878±0.251	0.158±0.009	0.470±0.062
2	Sod. Picosulphate	5	3.695±0.039***	25.073±0.062***	0.360±0.004	0.754±0.003*
3	AqRS	250	2.115±0.021	11.030±0.075	0.188±0.008	0.569±0.016
4	AqRS	500	2.490±0.097***	16.558±0.095***	0.236±0.004	0.593±0.011

5	AqRS	750	3.048±0.034***	22.623±0.123***	0.304±0.003	0.703±0.005

Values are given as (mean ± SEM), (n= 6 (no. of animals). Data were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni test. Values are statistically significant at *P<0.05, **P<0.01 & ***P<0.001 compared with control group.

Table 6: Effect of fresh juice (FreRS) of *Raphanus sativus* for ion secretion in rats

S. No.	Groups	Dose mg/kg	Na^+ (mg/L)	Cl^- (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)
1	Control	5	1.913±0.053	10.702±0.130	0.155±0.010	0.435±0.035
2	Castor oil	2ml	3.645±0.155***	25.203±0.150***	0.342±0.017	0.761±0.013*
3	FreRS	250	2.060±0.040	11.042±0.053*	0.189±0.005	0.567±0.010
4	FreRS	500	2.477±0.144***	16.588±0.111***	0.266±0.007	0.593±0.011
5	FreRS	750	3.055±0.076***	22.632±0.125***	0.312±0.004	0.769±0.016*

Values are given as (mean ± SEM), n = 6 (no. of animals). Data were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni test. Values are statistically significant at *P<0.05, ***P<0.001 compared with control group.

DISCUSSION

The laxative activity of *Raphanus sativus* was studied in rats. The results showed that an oral administration of the aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* produced prominent and dose dependant increase in the faeces output of rats. Both the extract & fresh juice increased the stimulation of gastrointestinal motility and increased the water-ion secretion in electrolyte secretion test. The effects of aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus* at high dose of 750 mg/ kg were comparable to that produced by castor oil & sodium picosulphate (standard drug).

Sodium Picosulphate is a member of the polyphenolic group of stimulant laxatives. It converts itself into an active form through the action of bacterial enzymes [28]. This results in stimulation of peristaltic movement and reduces water reabsorption leading to softening of stools. These aforesaid results suggest that the laxative activity of aqueous extract & fresh juice of *Raphanus sativus* may be because of presence of polyphenols as phytoconstituents which may possibly work in this way. *Raphanus sativus* leaves and roots are found to be rich in dietary fibre contents, which facilitate digestion, decreases reabsorption of water and help to relieve constipation [20]. The laxative effects of dietary fibre have been recognized since Hippocrates in 430 BC. It has been reported that about 9.6 gm of fibre per 100 gm were present in leafy part [29]. Thus we can say that the laxative effect of *Raphanus sativus* extract as well as fresh juice is also because of high content of dietary fibres. Thus they can act as good natural bulk forming laxative and also as an osmotic laxative, as they can pass through the small intestine and form much of the bulk in the large intestine by holding water effectively, resulting in a softer, larger stool. Increase in bulk of stool not only helps in reducing transit time in colon and preventing the constipation but also helps in preventing from the detrimental effect of microflora causing toxicity [30]. It also helps to speed up transit time through the colon [31] and thus helps to prevent constipation. It stimulates the production of bile thereby aids in digestion. All these effect together becomes effective in the treatment of constipation and piles.

There is enough evidence for histamine being an important cellular messenger of the gastrointestinal tract [32] and stimulates various smooth muscles including the gut tissues through activation of H1 receptors which increase gastrointestinal motility [33]. It is evident that the presence of the histaminergic components along with weak spasmolytic factors provides a resonatic mechanistic source in curing constipation and is used in the traditional system of medicine [22]. It has been observed that both aqueous extract and fresh juice shows stimulant effect on intestinal ion content. The gastrointestinal motility effect may be because of existence of histamine-like spasmogenic component in the plant which shows its conventional use in constipation [22].

This study has also shows that *Raphanus sativus* had stimulated Na^+, K^+ and Cl^- ion secretion. Most of the natural laxatives exert their effects on the colonic epithelium by stimulating Cl^- secretion and/or inhibiting Na^+ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility [34]. Apart from these Raphanus contains various nutrients like magnesium, calcium and vitamin C. Magnesium is found to be pharmacologically very active mineral to cure the symptoms of constipation, it works by increasing water in intestines thus leading to an increased amount of water in the colon helps in softening of the stool, and making them to pass easily and helps in initiating peristalsis. It even relaxes the muscles in the intestines which help in establishing a smoother rhythm that eliminates constipation. However, magnesium is present in a very small amount, but it provides a synergistic effect with calcium as different surveys recommended that calcium should be taken with magnesium. These nutrients when present in colon, combines with excess bile and help in decaying fat to form a harmless insoluble soap, which is excreted with the stool, thus helps in cleaning the colon. *R.sativus* is found to be a good source of vitamin C, which interacts with a fibre called chitosan, thus helping it in binding with food digested in the intestinal tract, which makes the stool smoother and easier to eliminate. Beside this the extract and fresh juice of *Raphanus sativus* shows presence of alkaloids, tannins, flavonoids, polyphenols, sterols and carbohydrates. Hence, it could be concluded that these constituents may be responsible for the laxative activity of *Raphanus sativus.*

CONCLUSION

The present study revealed that the aqueous extract (AqRS) and fresh juice (FreRS) of *Raphanus sativus L.* has a significant laxative activity and supports its traditional use in herbal medicine.

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CONFLICT OF INTEREST STATEMENT

Authors don't have any conflict of interest.

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