

# Evaluation of Potentiality of *Salvia hispanica* in the Management of Irritable Bowel Syndrome in Wistar Albino Rats

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## ABSTRACT

**Introduction:** Globally, approximately 11% of the population has been affected by the irritable bowel syndrome. Recent advancement in medical area have made it easily possible to find out the proper pathophysiology behind the generation of symptoms of irritable bowel syndrome followed by its treatment. **Objective:** The aim of this research is to check the effect of mucilage of *Salvia hispanica* on gastrointestinal tract symptoms (IBS) as a result of excessive fibers availability in the mucilage. **Materials and Methods:** The anti-IBS activity was seen in rats by using the various animal experimental models. The anti-IBS activity of *Salvia hispanica* mucilage (100 mg/kg, p.o.) in rats was checked by using the Water Avoidance Stress Model (WAS), Food Related model of IBS and Croton oil Induced Hemorrhoids. For the estimation of effect of monoamine on GIT fluorimeter was used to check the level of serotonin and iliac motility of rats were also checked. **Results:** It was found out that *Salvia hispanica* mucilage had contractile action on iliac motility that leads to the shortening the evacuation time of bowel and relief in constipation. From the data collected by using above methods. **Conclusion:** It was concluded that the *Salvia hispanica* mucilage revealed to possess activity against IBS significantly ( $****p < 0.0001$ ) at a dose of 100 mg/kg. Activity against IBS was supported by enhanced serotonin level in the gastrointestinal tract.

**Keywords:** IBS, *Salvia hispanica* mucilage, Water Avoidance Stress Model, Serotonin, Food Related Stress Model of IBS.

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## INTRODUCTION

It is a GI tract disruption that involves stomach discomfort, pain, change in bowel movement and abdominal cramps.<sup>1</sup> Patient can suffer either with constipation, diarrhea or alternative constipation, diarrhea and often present with bloating and incomplete defecation.<sup>2,3</sup> Globally, approximately 11% of the population has been affected by the irritable bowel syndrome.<sup>4,5</sup> Specific pathophysiology of IBS is not known but there are asserted important factors which defines the cause of disease. There is no specific test for the confirmation, diagnosis is symptomatic termed Rome criteria.<sup>6</sup>

Significantly a wide percentage of IBS patients experiences with other diseases or syndrome like dyspepsia, angina, headaches, and fibromyalgia.<sup>7</sup> Recent advancement in medical area have made it easily possible to find out the proper pathophysiology

behind the generation of symptoms of IBS like; previous Gastrointestinal Infection (GI), visceral intolerance, regulating of gut-brain interaction, dysbiosis, alteration in the percentage of GI hormones, abnormality in the growth of gut microbiota in gastro-intestine tract and autonomic nervous system.<sup>8,9</sup>

*Salvia hispanica* is a biennially grown plant, family (Labiatae), superfamily Spermatophyta and plant order Plantae. Prominently, *Salvia hispanica* cultivated for its seeds, but it also makes white or violet blooms. The seed comprises 24-40% of oil with 60% of it being Omega-3 and 20% omega-6. Dietary fiber mainly soluble fiber is also present in huge amount approx. 25-35% in Chia. For the good health and nutritional power both the ingredients are necessary, and these are not synthesized artificially.

The mechanism of action of fiber in gastrointestinal disorders is that insoluble fiber increases faecal bulk and quickens intestinal transit by mechanically stimulating/irritating the colonic mucosa and causing an increase in secretion and peristalsis.<sup>10</sup> The large intestine's bacteria digest soluble food fiber, increasing the biomass and producing small-chain fatty acids and gas that add weight to the faeces.<sup>11</sup> Various therapeutic uses of chia seeds in the



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treatment of Parkinsonisms and chia seeds oil as neuroprotective had been reported by researchers too.<sup>12</sup>

## MATERIALS AND METHODS

### Materials used

Bisacodyl (Elder Pharmaceutical Pvt. Ltd.) Hydrocortisone (AGIO Pharmaceutical Ltd., Pune, India and Marketed by Ipca Laboratories Ltd., Mumbai, India), Croton oil (Devinez, Pacific computech Pvt. Ltd., C-Block, Sushant Lok-1, Gurugram, India).

### Preparation of Chia Mucilage

100 g of entire seed sample were introduced at a proportion of 1:40 to containers with distilled water.<sup>13</sup> The temperature controller was used for maintaining the temperature at  $80 \pm 1.5^\circ\text{C}$  and pH was adjusted and kept at 8. The combination was hydrated for 2 hr while being magnetically agitated. This suspension was then poured out onto a drying plate and left in the heat for 48 hrs at  $50^\circ\text{C}$ . To separate the seed mucilage, it was sieved using a 40-mesh screen, and the pulp's weight was then determined.<sup>14</sup>

### Animals used

Wistar albino rats of either sex weighing 200-250 gm were selected for the induction of IBS. Animals were selected from Central Animal House of NIET Pharmacy Institute, Greater Noida. The Institutional Animal Ethics Committee (CPCSEA Reg No: 1845/Re/S/16/CPCSEA) approved the experimental animals (Protocol no: IAEC/NIET/2022/01/08). The animals were procured in polypropylene cages under standard conditions for 90 days in animal house. The temperature of the animal house were maintained at  $25 \pm 20^\circ\text{C}$ , 12:12 hr light: dark cycle and 40-70% relative humidity.

### Water Avoidance Stress Model

A functional gastrointestinal disorder's symptoms might develop and worsen due to constant chronic stress. The alterations in intestinal mobility and faecal count were observed in rats subjected to chronic psychological stress for 10 days in order to better comprehend the processes underpinning this association. For 10 days straight, male wistar rats underwent daily 1-hr WA stress tests. Fecal pellet was counted as a marker after placing the animal to stress condition and found that the pellet count was decreasing continuously till the last day of the experiment. On the 10 days, no fecal pellet excreted by the animal which implies that animal is suffering from the constipation condition which is due to the disturbance in the psychological behavior.

The animals was separated into three groups comprised of six animals each. All the animals were put into the tank having platform for 1 hr each day. Water avoidance stress model include a plexiglas tank ( $45 \times 25 \times 25$ ) CM along with a section ( $10 \times 8 \times 8$ )

**Recto anal coefficient = Weight of recto anal tissue (mg) / Body weight (gm)**

cm fixed into the middle of the tank. Water was poured into the tank until platform should be 1 cm above to the surface of the water. According to the chronic stress protocol, the animals were kept on the platform for 1 hr of 10 days.<sup>15</sup>

Eighteen wistar albino rats were selected and divided into three groups and 6 animals in each group. Group 1 selected as control group was given saline solution (0.9%) orally. Group 2 which is mentioned as standard was given with bisacodyl (20 mg/kg) orally. Group 3 selected as test group was administered with *Salvia hispanica* mucilage (100 mg/kg) by oral route. The animals were examined for 10hr to check the effect of the drug. The fecal material was counted every two-hour interval and found that the counting had increased, and animals become normalized. The frequency of fecal output of test group was lower than the standard group.<sup>16</sup>

### Croton oil induced hemorrhoids

Croton oil (20 mg/kg) were administered to the animals by anal route for the induction of hemorrhoids. Hydrocortisone was selected as a standard drug (20 mg/kg) for the treatment of hemorrhoids.

Applying a croton oil mixture (diethyl ether, pyridine, deionized water and six percent croton oil in diethyl ether in proportion of 5: 4: 1: 10) caused haemorrhoids in all groups. All animals were fasted for the previous night, sterile cotton swabs (4 mm in diameter) were placed into their anus (the part of the anus that is located 20 mm from the anal entrance) and held there for 10 sec. Up to 7-8 hr following the administration of croton oil, a linear progression of inflammation was seen. Evans blue dye (30 mg/kg) was administered by the tail vein to the animals 30 min before the croton oil application to cause the haemorrhoids. Group 1 is mentioned as control, was given 0.9% saline solution orally. Group 2 which is mentioned as standard group was administered with hydrocortisone (20 mg/kg) and group 3 is mentioned as test was administered with *Salvia hispanica* mucilage (100 mg/kg) by oral route. Animals from the various groups received 5 days of treatment starting 24 hr after the induction. Under severe isoflurane anesthesia, all animals were exsanguinated to cause their death. Then isolate the tissue of recto-anal region, weighed for histology.<sup>17-19</sup> For histological examination, the tissue was placed into 10% unbiased formalin solution for settled, weighed and severity score. The histological examination was followed by staining the tissue with hematoxylin and eosin. The Recto-anal Coefficient (RAC) was calculated by utilizing the following formula.

### Food related stress model for IBS

Discomfort of abdomen is preferably related with the alteration in digestion of food, allergic reaction to some food items and certain food intolerance. Genetic predisposition, stress and illness may link with the oral tolerance, that can play a vital part

in reducing some immunological reaction towards food antigens. All of this can result in IBS. Certain elimination diet strategies like FODMAP or diet free from gluten and some food molecules resulting in IBS, are beneficial in some patients.<sup>20,21</sup>

Food is a significant factor in animal digestive problems. The gluten in the diet was intolerable to rats. Food containing gluten makes rats constipated. Grain varieties like wheat, rye, spelt, and barley all contain the protein known as gluten. The grain that contains the greatest gluten is by far wheat. The two main proteins in gluten are glutenin and gliadin. The majority of gluten's harmful health effects are caused by gliadin.

Eighteen healthy rats were used for this food-related model of IBS, which were grouped into 3 groups with 6 animals each. Grouping all animals into 4 categories. Normal saline was used as the control group in Group I, 20 mg/kg bisacodyl was used as the standard in Group II, 100 mg/kg of *Salvia hispanica* mucilage were used in Group III. Each rat is placed in its own cage (one rat in each cage). Following that, animals were fed gluten-containing food (bread) in place of their regular diet. This process continues for 14 days, and each time we count the rat faeces. Food containing gluten, makes rats constipated. Standard and test medications were administered at the end of 14 days and monitored the faecal count for next 24 hr. A brain-gut interaction is involved in IBS and we, observed the level of serotonin in the rat's brain.<sup>22</sup>

### Serotonin estimation

In the gut, serotonin is a key signaling chemical that targets enterocytes, smooth muscle, and enteric neurons. Enterochromaffin cells house the maximum level of serotonin of all body. Serotonin activates both the afferent neurons (extrinsic and intrinsic) to begin peristaltic and secretory actions, respectively and to initiate and transfer the message to the CNS.

All animals of Water Avoidance Stress Model and food related model of IBS were randomly assigned to three groups for individual model. All the animals in these models were found to have the severe constipation with IBS. The drug was administered to the groups like:

For Water Avoidance Stress Model we used a control group (0.9% Saline solution), standard group (20 mg/kg bisacodyl) and test group (100 mg/kg *Salvia hispanica* mucilage).

For Food Related Stress Model of IBS: Control group (0.9% saline solution), Standard group (20 mg/kg bisacodyl) and test group (100 mg/kg *Salvia hispanica* mucilage). For checking the effect of serotonin in constipation with IBS, all animals were executed with under deep isoflurane and brain was dissected. In a Teflon tissue homogenizer set at 0°C, brain was homogenized with 0.1 N HCl. After being placed on ice, the tissue homogenates were centrifuged for 15 min at 14000 rpm at 0°C. Add 10% ZnSO<sub>4</sub> (1mL) and 1N NaOH solution (0.5 mL) to the clear supernatant

solutions. The tubes were shaken for five minutes before being centrifuged at 2500 rpm for 20 min. A quartz cuvette containing 0.3 mL (2N) of HCl, and 1 mL of the clear supernatant solution were combined. Utilizing a fluorimeter (SYSTONIC fluorimeter S-9150) at the wavelength [excitation (nm)/ fluorescence (nm)] 290/550, the fluorescence intensity in the resulting solution was measured.<sup>23</sup>

## RESULTS

### Phytochemical investigation

The seeds of *Salvia hispanica* have the mucilage and percentage yield of the mucilage was found to be 33%. On phytochemical investigation of mucilage showed the presence of proteins, alkaloids, tannins, phenolic compounds, steroids and flavonoids.

### Water Avoidance Stress Model

The saline treated animals (control group) were estimated for 10 hr and found that very less increment in the fecal count. The fecal count was increased exponentially in the standard drug (bisacodyl) treated group due to increase the level of serotonin. Oral administration of *Salvia hispanica* mucilage (100 mg/kg) exhibit significant ( $****p < 0.0001$ ) rise in the frequency of fecal material and animal become normalized after 10 hr as evident from Table 1. These results proved that the mucilage of *Salvia hispanica* possess potentiality to manage constipation which is major reason of IBS.

### Croton Oil Induced Haemorrhoids

Upon application of croton oil in anal region of animals produce variation in RAC and severity score. The test group treated with 100 mg/kg chia mucilage that results approx. 60% of lamina propria was seen normal and other part were destroy. A remarkable variation was seen in RAC and severity score in all groups. The RAC value for standard group was found to be (0.97 ± 0.009). The RAC value for control treated and test group treated at dose (100 mg/kg) was found to be 1.45 ± 0.012 and 1.13 ± 0.011 ( $****p < 0.0001$ ) respectively.

Upon histological investigation the severity score of all the groups were compared in Table 2 and it was found that control group have maximum degradation (2.83 ± 0.30) of tissue while hydrocortisone treated group have minimum severity of the tissue (1.00 ± 0.25), but test group have moderate tissue degradation (2.00 ± 0.25) ( $**p < 0.001$ ) that confirms the significant activity of *Salvia hispanica* in management of haemorrhoids. The disease hemorrhoids have been cured in the standard and test groups, but the extent of treatment was different in both the groups.

### Food related stress Model of IBS

After examining all the groups for 10 hr at every 2 hr interval, the faecal material was counted, noted, and compared in Table 3. It was found that the test group have increased in faecal material in

**Table 1: Faecal count for Water Avoidance Stress model.**

Sl. No.	Group	Drug with dose	Cumulative faecal counts at different time interval				
			2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
1.	Control	Saline solution (0.9% w/v)	0.333 ± 0.211	0.833 ± 0.167	1.167 ± 0.167	2.000 ± 0.000	2.333 ± 0.211
2.	Standard	Bisacodyl (20 mg/kg)	8.000 ± 0.365****	14.500 ± 0.428****	22.500 ± 0.719****	29.833 ± 0.307****	34.833 ± 0.601****
3.	Test	<i>Salvia hispanica</i> mucilage (100 mg/kg)	4.000 ± 0.365***	9.833 ± 0.477****	16.500 ± 0.428****	24.667 ± 0.333****	30.000 ± 0.365****

The data were mentioned as mean ± SEM, with (\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ ) and each group containing six animals. The statistical calculation was accomplished by using two-way ANOVA. The faecal count in control and test group animals were increased as (2.333 ± 0.211) and (30.000 ± 0.365) (\*\*\*\* $p < 0.0001$ ) respectively and the *Salvia hispanica* was significantly effective at dose of 100 mg/kg.

**Table 2: RAC and severity score in Croton oil induced haemorrhoids.**

Sl. No	Groups	Recto-anal coefficient	Severity score <sup>#</sup>
1.	Control (saline 0.9% w/v)	1.45 ± 0.012	2.83 ± 0.30
2.	Standard (hydrocortisone)	0.97 ± 0.009****	1.00 ± 0.25***
3.	Test ( <i>Salvia hispanica</i> mucilage 100 mg/kg)	1.13 ± 0.011****	2.00 ± 0.25**

The facts were mentioned as mean ± SEM, with (\*\* $p < 0.001$ , \*\*\* $p < 0.0001$  and \*\*\*\* $p < 0.0001$ ) each group containing six animals. The statistical calculation was accomplished by using two-way ANOVA.

**Table 3: Faecal count for food related stress model of IBS.**

Sl. No	Groups	Cumulative faecal counts at different time interval				
		2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
1.	Control Normal saline (0.9% w/v)	0.167 ± 0.167	1.000 ± 0.000	1.167 ± 0.167	1.833 ± 0.167	2.333 ± 0.211
2.	Standard Bisacodyl (20 mg/kg)	8.000 ± 0.365****	15.000 ± 0.447****	25.300 ± 0.803****	32.000 ± 0.516****	37.000 ± 0.365****
3.	Test <i>Salvia hispanica</i> mucilage (100mg/kg)	4.333 ± 0.333****	9.333 ± 0.494****	15.333 ± 0.422****	23.333 ± 0.667****	29.167 ± 0.601****

comparison with normal group which explains the effectiveness of the drug.

The facts were mentioned as mean ± SEM, with each group containing six animals. The statistical calculation was accomplished by using two-way ANOVA. In comparison with the control group the test drug was found effective (\*\*\*\* $p < 0.0001$ ) after every 2 hr difference.

### Effect of *Salvia hispanica* mucilage on brain serotonin

In the present estimation, the control group had decreased the serotonin content for water avoidance stress model and food related stress model of IBS (67.16 ± 1.302 and 66.00 ±

1.633 ng/mL of brain tissue respectively) which had resulted to the development of constipation in control group (Table 4). The standard groups were treated with the standard drug (hydrocortisone and Bisacodyl) for both the models respectively and found that serotonin level has increased (111.33 ± 1.563 and 112.80 ± 1.579 ng/mL respectively), and animals become normalized. The test group was treated with *Salvia hispanica* mucilage 100 mg/kg (99 ± 1.065 and 97 ± 1.438 respectively) for both the models exhibited enhancement in the concentration of serotonin significantly (\*\*\*\* $p < 0.0001$ ) in comparison with control group.

From the results, it was seen that the serotonin level in control group for water avoidance stress model was found to be

**Table 4: Serotonin level in IBS models.**

Sl. No.	Groups	Serotonin Levels (ng/mL)	
		WAS Model	Food Related Model
1.	Control	67.17 ± 1.302	66.00 ± 1.633
2.	Standard	111.33 ± 1.563****	112.80 ± 1.579****
3.	Test	99.00 ± 1.065****	97.00 ± 1.438****

The facts were mentioned as mean ± S.E.M, with each group containing six animals. The statistical calculation was accomplished by using two-way ANOVA. The serotonin level found in both the models was significant (\*\*\*\* $p < 0.0001$ ) for the test group as compared to the control group.

(67.16 ± 1.302 ng/mL). But in case of standard and test group, administrated with the standard and test dose, the serotonin level was found to be (111.3 ± 1.563 ng/mL) and (99.00 ± 1.065 ng/mL) comparatively higher than the Control group. Thus, this observation shows that test formulation was significantly (\*\*\*\* $p < 0.0001$ ) effective at a dose of 100 mg/kg of *Salvia hispanica* mucilage.

The serotonin level in control group for food related stress model was found to be (66.00 ± 1.633 ng/mL). but in case of standard and test group, administered with standard and test dose, the serotonin level was found to be (112.8 ± 1.579 ng/mL) and (97.00 ± 1.438 ng/mL) comparatively higher than the control group. Thus, this observation shows that test formulation was significantly (\*\*\*\* $p < 0.0001$ ) effective at a dose of 100 mg/kg of *Salvia hispanica* mucilage.

### Effect of *Salvia hispanica* mucilage on iliac motility

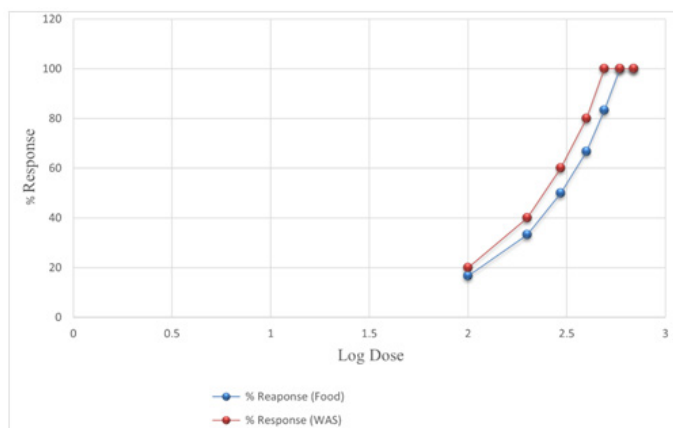
The mucilage of *Salvia hispanica* exhibited contraction on iliac motility in WAS model that result enhancement of iliac motility and the percentage response was found 100% at dose of 500 µg/mL.

The mucilage of *Salvia hispanica* exhibited contraction in iliac motility in Food related stress model of IBS that result enhancement of iliac motility and the percentage response was found 100% at dose of 600 µg/mL (Figure 1).

In the above two animal models i.e., WAS model as well as Food related stress model of IBS, there is the presence of IBS symptoms (constipation) the iliac motility of the animal had changed and evaluate this change in motility the ileum of the animal was isolated and was experimented on organ bath and found out that mucilage of *Salvia hispanica* showed contractive effect on rat's ileum.

## DISCUSSION

IBS stands out as the most prevalent issue that the general population has experienced and is also a reason to see a gastroenterologist. Since it may be brought on by a variety of factors, like food or stress which eventually ties it to the brain, its etiology is still unknown. Therefore, we might conclude that IBS may have physical or psychological causes. Basically, it consists

**Figure 1:** Effect of *Salvia hispanica* mucilage on iliac motility.

of a number of issues, including hemorrhoids, constipation, diarrhea, and stomach discomfort. Because of this, there is no effective treatment for any of these issues; instead, each one must be handled separately. In light of this, the current study outlines the effects of chia seed mucilage on IBS symptoms and how to treat them. Three models were run for this study project—Croton oil induced hemorrhoids, Water avoidance stress model and Food related model of IBS. In the first model, the WAS model, it was observed that faecal count significantly decreased in the control group, but that it returned to normal in the standard and test groups after 10 hr. using bisacodyl as the standard and chia seed mucilage 100 mg/kg as the test drug. The importance of chia seed mucilage in the fight against hemorrhoids was further demonstrated by the second model—croton oil-induced hemorrhoids. Hydrocortisone was the conventional medication, while chia seed mucilage was tested at a dosage of 100 mg/kg. Recto-anal coefficient shown to be significant of chia seed mucilage. The rat brain serotonin levels were also calculated, and it was shown that they were lower in constipation related conditions. Consequently, when test medications at 100 mg/kg were compared to controls in both models found significant. All three models included estimations of the animals' iliac motility which revealed contraction on the isolated ileum. It was determined from the foregoing discussion that the chia seed demonstrated its efficacy against IBS indication in every model and that it produces favorable results when compared to present medications.

## CONCLUSION

Afunctional bowel illness called irritable bowel syndrome is characterized by bloating, irritation, irregular stools and pain in the abdomen. IBS frequently co-exists with other somatic comorbidities (such pain, syndrome, overactive bladder, and severe headache), mental disorders (depression and anxiety) and visceral sensitivity.

This study presents that mucilage was prepared from chia seeds, which was confirmed by chemical testing. An experimental study was designed for the evaluation of the potentiality of mucilage against the irritable bowel syndrome. Some experimental models were followed like Water Avoidance Stress Model, Food related Stress model of IBS and croton oil induced haemorrhoids. On performing these models, animals were found with the IBS symptom as constipation. But mucilage has the fiber content which helps for relief in the constipation by decreasing the defecation time.

Water Avoidance Stress Model is a procedure which disturbs the gut motility. The drug showed its highest significance after 8 hr ( $****p < 0.0001$ ) of test drug treatment at dose 100 mg/kg. The gastrointestinal tract is directly connected with CNS. The gut motility is regulated by the serotonin neurotransmitter.

Food Related Stress Model of IBS is a testing model for the evaluation of anti-constipation activity of *Salvia hispanica* mucilage. As animals are not able to digest the gluten protein that prolongs the defecation time that leads to the development of constipation condition. Present study revealed that *Salvia hispanica* mucilage (100 mg/kg) possess the positive activity by enhancing the frequency of faecal matter. The significance ( $****p < 0.0001$ ) of test drug was compared with that of control group after 8 hr of treatment.

Croton oil is a phlogistic agent which causes the irritation and inflammation in the recto-anal route. The lining of anal region becomes swells, and it is a condition of haemorrhoids. In this study animals were treated with their dose and found that mucilage of *Salvia hispanica* (100 mg/kg) were significantly effective for recto-anal coefficient and severity score of recto-anal tissue ( $****p < 0.0001$  and  $**p < 0.001$ ) in comparison to control group.

In the estimation of serotonin level in *Salvia hispanica* mucilage treated animals showed an increment in the serotonin in GIT in comparison to control group significantly but also found that the level was slightly low in comparison to standard group.

Iliac motility was decreased in the Water avoidance model due to stress. The *Salvia hispanica* mucilage showed the contractile action on isolated iliac and the maximum response (100%) was observed at log dose 2.69.

For Food related stress model, the iliac motility was checked on isolated ileum of the rat by using different concentration of *Salvia*

*hispanica* mucilage that showed the 100% response at log dose 2.77 which was contractile action on motility.

So, it is concluded that this study on chia mucilage, extracted from the seed certainly suggested to possess Anti-IBS activity. The chia seeds can be explored in future for various other therapeutic uses of chia seeds too. One such promising activity which can be explored in future includes its use in the control of gastric problems.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**IBS:** Irritable bowel syndrome; **WAS:** Water Avoidance Stress Model; **GIT:** Gastro intestinal tract; **WAS:** Water avoidance stress; **RAC:** Recto-anal coefficient; **SEM:** Standard error of Mean; **ANOVA:** Analysis of Variance.

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