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EVALUATION OF IN-VIVO LAXATIVE EFFECT OF LACTUCA SATIVA LINN. ON EXPERMENTALLY INDUCED CONSTIPATION IN MICE

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Keywords: *Lettuce, Lactuca sativa*

Abstract

Objective: Lettuce (*Lactuca sativa Linn*) is an important herb in Ayurved, reported to have a wide range of medicinal properties. In clinical practice it is usually prescribed in its various dosage forms. It is taken internally in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, rheumatic pain etc. Use of lettuce for various disorders is well documented in scientific literature however; no data is available on the effect of constipation. Hence, the study was carried out to evaluate the effects of ***Lactuca sativa*** extract on experimentally induced laxative activity by using in-vitro method.

Method: The laxative activity of *lactuca sativa* was studied against constipation induced by Loperamide in mice and Bisacodyl (20 mg/kg p.o) was used as standard. Faecal parameters measurement and small intestinal transit were recorded as index of constipation.

Result: The administration of LSEE to the constipated mice was effective in increasing the fecal number, fecal weight and fecal water content in loperamide induced constipation mice. And the SIT time was shortened by the treatment with LSEE in this constipation models.

Conclusion: The results indicated *Lactuca sativa Linn.* leaf extract to be effective in treatment of constipation thus suggesting a further scope of evaluation of these formulations as an adjuvant treatment for constipation. **Objective:** Lettuce (*Lactuca sativa Linn*) is an important herb in Ayurved, reported to have a wide range of medicinal properties. In clinical practice it is usually prescribed in its various dosage forms. It is taken internally in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, rheumatic pain etc. Use of lettuce for various disorders is well documented in scientific literature however; no data is available on the effect of constipation. Hence, the study was carried out to evaluate the effects of ***Lactuca sativa*** extract on experimentally induced laxative activity by using in-vitro method.

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Introduction

Constipation is a disorder in the gastrointestinal tract, which can result in the infrequent stools, difficult stool passage with pain and stiffness. Acute constipation may cause closure of the intestine, which may even require surgery.[1] Chronic constipation is a complicated condition among older individuals, which is characterized by difficult stool passage.[2] In this regard, this condition *has a close relationship with* the *patients' quality of life,*[3] and consuming health resources.[4,5] The causes are of two types: obstructed defecation and colonic slow transit (or hypomobility). About 50% of the people evaluated for constipation at tertiary referral hospitals have obstructed defecation. Causes of colonic slow transit constipation include diet, hormonal disorders such as hypothyroidism, side effects of medications, and rarely heavy metal toxicity behavioral, biological, and pharmaceutical factors.[6] Symptoms of constipation include feeling bloated, uncomfortable and sluggish.[7] Constipation causes many expenses for the community with an estimated prevalence of 1% to 80%, worldwide,[8] In elderly people living in care homes the rate of constipation is 50-75 percent.[13,14] About 29–50% patients referred to tertiary referral centres in the West may have functional outlet obstruction such as anismus or puborectal dyssynergia.[9-12] Because constipation is a symptom, not a disease, so effective treatment of constipation may require first determining the cause. Treatments include changes in dietary habits, laxatives, enemas, biofeedback, and in particular situations surgery may be required.[15-21]

Lettuce (*Lactuca sativa*) is an annual plant of the daisy family, Asteraceae.[22] It is most often grown as a leaf vegetable, but sometimes for its stem and seeds. Lettuce is used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps; it can also be grilled. Lettuce is a rich source of vitamin K and vitamin A and a moderate source of folate and iron. It consists of Glycosides, steroids, phenolics, tannins, resin, alkaloid, organic acid (oxalic acid, malic acid, citric acid). Lactuca sativa contain antioxidants flavonol, quercetin, and caffeic acid, ascorbic acid. Its having anti-inflammatory property,[23,24,25] protects neuronal cells, lowers cholesterol levels, induces sleep, antioxidant properties, antimicrobial properties and controls anxiety.[26,27]

Since the constipation was a huge impact on our lives; it is worth to look after the alternative forms of medicines which can be used for its treatment. Perusal of the literature survey indicates that there are no literatures or studies were done on the constipation activity of leaf extract of *Lactuca sativa (L).* So in this study, an effort was made to investigate the laxative effects of ethanolic extracts of leaves and compared with the control and standard drug-bisacodyl.

Materials and methods

2.1. Collection of plant materials:

Lactuca sativa (lettuce) (2kg) were collected in the month of December, 2017 procured locally from the vendors in Vijayawada. The plant was Authenticated by V.S.Raju kakatiya University and a voucher specimen herbarium with number KUW 4038was deposited at the faculty of Pharmacy, Kakatiya University, Warangal.

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Leaves of L.sativa


Dried material of L.sativa


Powdered material of L. sativa

2.2. Preparation of extract:
Aerial parts of *lactuca sativa* were dried under shade, powdered with a mechanical grinder, and passed through sieve no. 40. The sieved powder was stored in airtight container and kept in room temperature. Dried plant material (500 gm) was extracted with 1500ml of ethanol using a Soxhlet extractor (hot continuous percolation) for 72 hrs. The extract was filtered through Whatmann filter paper (no.1) and then concentrated in vacuum at 40c using a rotary evaporator. The extract was kept in the dark at 40^{o}c.

2.3. Drugs and Chemicals:
Lopiramide was procured from unit of I.G. pharma Ltd. Haridwar and Bisacodyl from Kemwell biopharma pvt.Ltd. used as positive control and standard respectively. All the other chemicals used for activity are phenyl red from Kemphasol, Mumbai, 0.9% Normal saline from Claris otsuka Ltd. Methyl cellulose and1%v/v Tween 80 obtained from Loba,Mumbai.

2.4. Experimental animals:
Swiss albino mice (weighing 20-25g) of either sex were used in this study. They were procured from Mahaveer Enterprises, Hyderabad. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained constant temperature at 27°C ± 2°C under 12 hrs dark / light cycle. They were fed with standard rat pellet feed and water *ad libitum.* The husk in the cages was renewed every day to ensure hygeinity and maximum comfort for animals. Experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No.: 1629/PO/a/12/CPCSEA and approval no 004/IAEC/NCPA) for the care and use of animals and were strictly followed throughout the study.

2.5. Experimental designs:
Animals were divided into five groups (n= 6); Group I served as control group and received only 0.9% Normal saline (10ml/kg i.p.) and feed ad libitum, Group II received Loperamide -5mg/kg p.o. treated as positive control, Group III received standard drug Bisacodyl (20 mg/kg p.o), Group IV and IV received *Lactuca sativa* ethanolic extract 100mg/kg and 200mg/kg. The dose was decided according to the acute toxicity studies done on the animals.

2.6. Test procedure
2.6.1. Faecal parameters measurements in normal mice
Mice, given food and water *ad lib*, were randomly divided into five groups (n=6 in each group): control and LSEE (two groups of different dosage). After either LSEE (100 and 200mg/kg body weight or saline was administered, the animals were immediately placed in clean transparent cages individually and allowed access to their standard lab chow and top water *ad libitium.* Then, faeces for each mouse were collected, counted and weighed at 0-8 h period. The number and weight of faeces were expressed in terms of the total number and wet weight per mouse.

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Faecal parameters measurement:
The number and weight of feces for each mouse in loperamide-induced constipation model mice were measured as the method of Lakino et al. The mice given food and water *ad lib*, were administered (p.o.) saline, standard drug or LSEE at 100 and 200mg/kg body weight, and then administered loperamide (5 mg/kg bw, dissolved in 1% v/v Tween80, p.o.) at 1h after LSEE treatment. Then, after no. of feces and faecal content was weighted, the wet feces for mouse were dried at 105^0C for 48 h. The water content of feces was calculated as: faecal **water content (%) = (feces weight before dried – feces weight after dried)/ feces weight before dried × 100.**

2.6.2. Determination of small intestinal transit:
Small intestinal transit (SIT) was determined using a phenol red marker meal (0.5 % phenol red indicator in 1.5 % methyl cellulose; 10 ml/kg body weight, i.g.). Administering of two doses of LSEE and std. After fasted for 14 h with water *ad libitium*, all mice were administered by p.o. with either LSEE or saline at 1 h before loperamide administration. After 30 min, a phenol red marker meal was administered to experimental mice. After 20 min, the mice were sacrificed by cervical dislocation under anesthesia with diethyl ether. The small intestine from the pylorus to the blind intestine was carefully removed.

The SIT (%) for each animal was calculated as: **distance traveled by phenol red marker meal/total length of the small intestine × 100.**

2.7. Statistical analysis:
All the data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data was assessed by one-way ANOVA followed by Tukey's model test using Graph Pad instant software. A probability level of less than 1% ($p < 0.01$) was considered as significant. Treated groups were compared with the normal and positive control groups.

Results

3.1. Results of preliminary phytochemical studies:
The preliminary phytochemical analysis of ethanolic extract of leaves of *Lactuca sativa* revealed the presence of flavanoids, terpenoids, carbohydrates, saponins, oils, tannins and phenolic compounds.

Table no: 3.2. Effect of LSEE on lopiramide induced constipation using mice (Avg no. of feces, Total weight of feces and % of water content)

GROUPS	TREATMENT	AVERAGE NO.OF FECES	TOTAL WEIGHT OF FECES(gm)	% OF WATER CONTENT
Group-l	Control	18.9	0.40	64.6%
Group-ll	loperamide	2.5	0.02	37.5%
Group-lll	Std (bisacodyl)	14.6	0.26	59.6%

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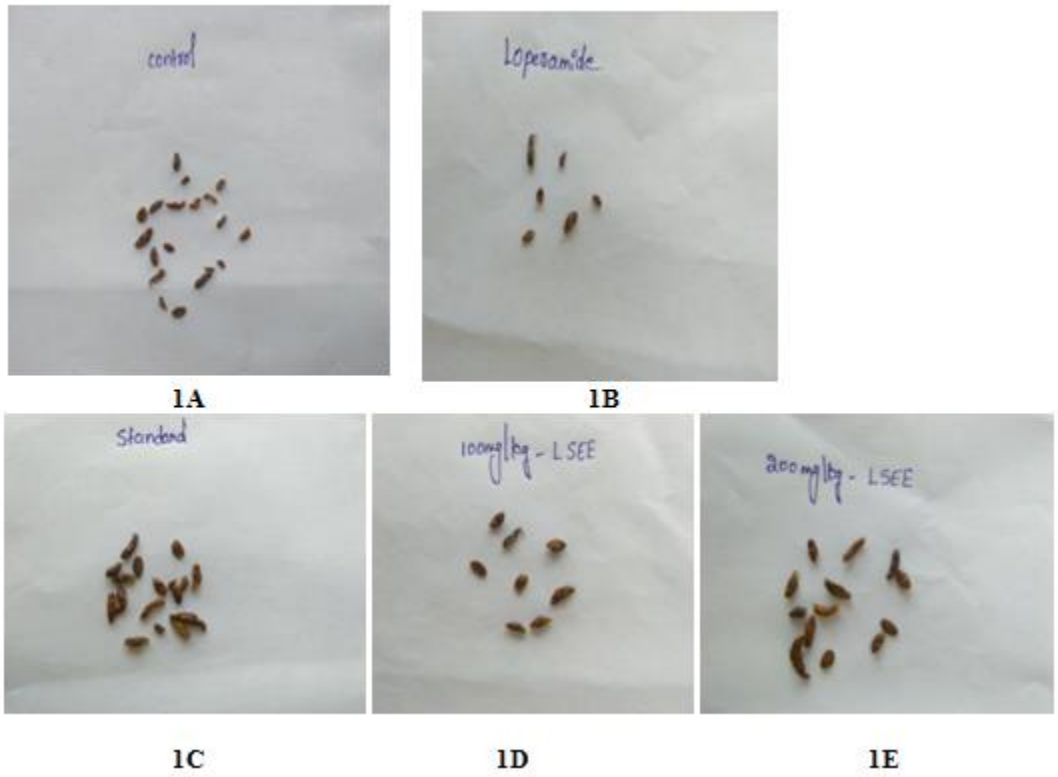
Group-lv	LSEE 100mg/kg	2.4	0.06	45.5%
Group-v	LSEE 200mg/kg	6.3	0.18	54.7%



1A 1B 1C 1D 1E

Fig no: 3.2.1– Faecal content data

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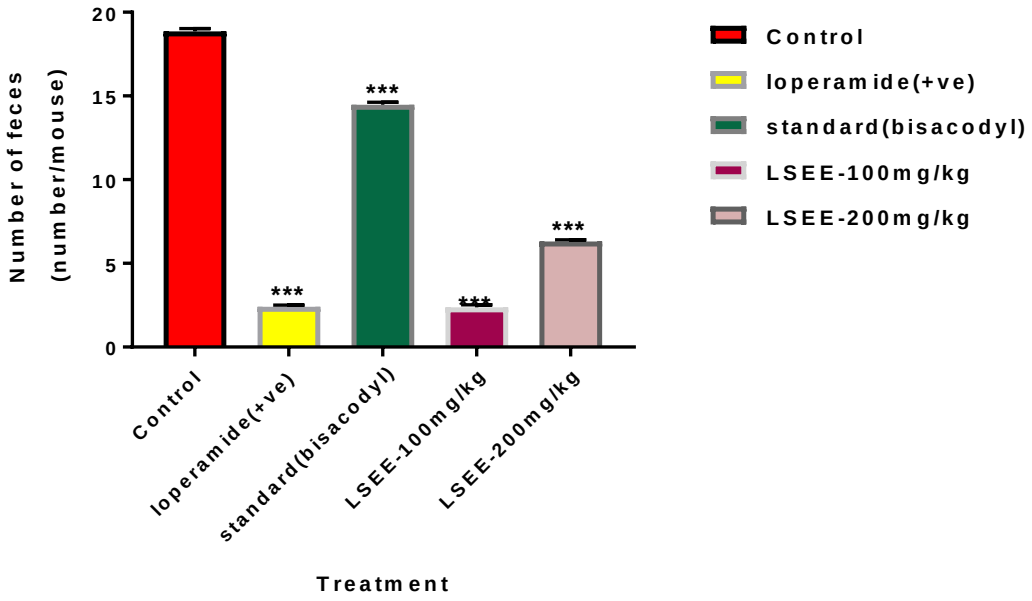
Graph no: 3.2.1



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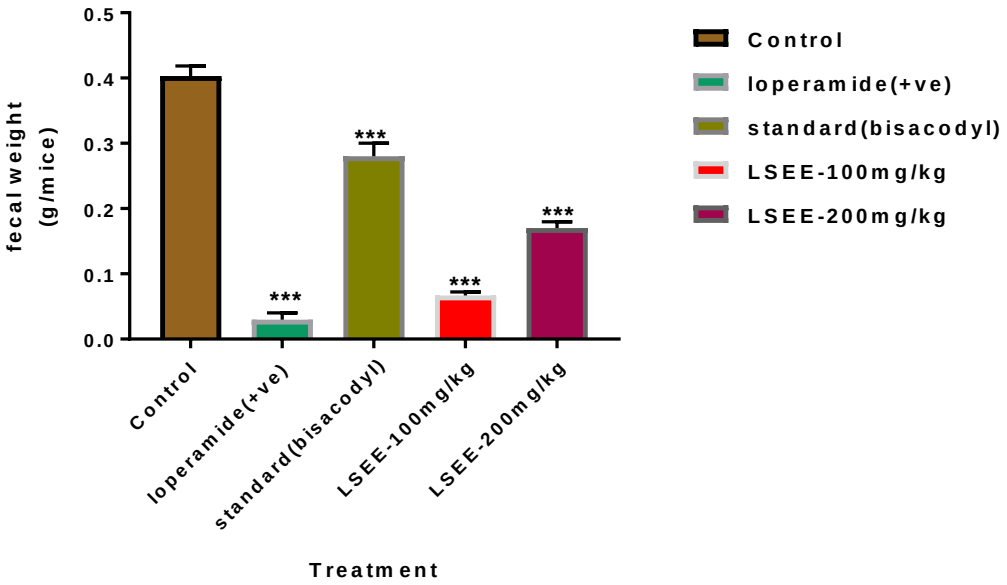

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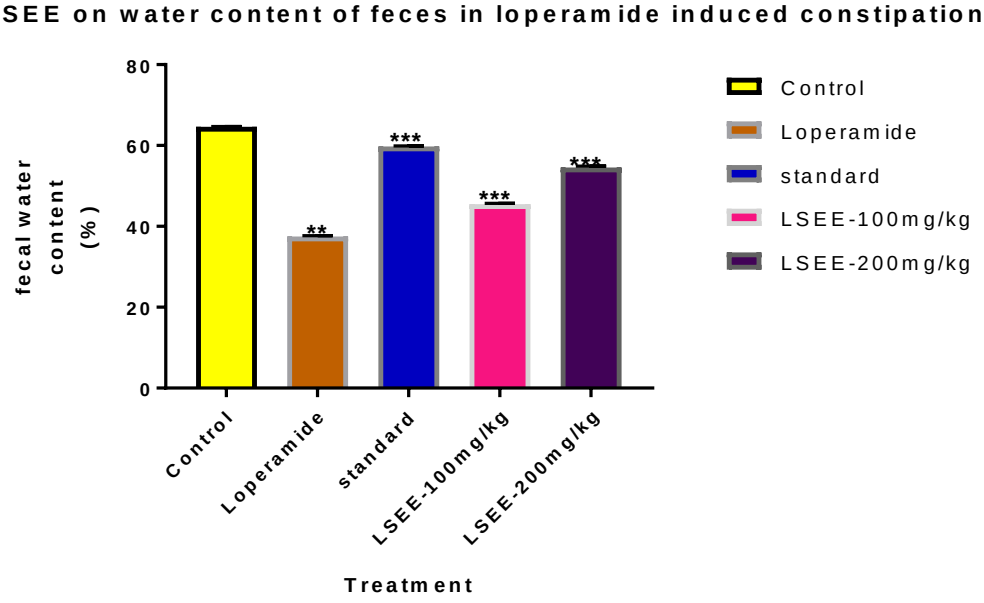
Graph no: 3.2.3



Increased fecal output in normal mice treated with LSEE. The effect of LSEE on number and weight of feces in normal mice were shown in figure 1A. Compered with the control treatment with LSEE (200mg/kg BW) produced a significant increase in no. of feces (figure 1E) and fecal weight.

LSEE improved fecal output character in constipated mice:
A model of spastic constipation was induced by lopermide. Loperamide obviously reduced number, weight and water content of feces (figuer 1B). LSEE counteracted the decrease in number and weight of feces induced by loperamide. The effect was significant starting from the 100mg/kg body weight oral dose. There was a significant restoration of fecal water content in LSEE group at 200mg/kg (54.7% of control) compared with the LP group (37.5% of control) (figure 1A).

Table no: 3.3 - Effect of LSEE on lopiramide induced constipation using mice in small intestinal transit time:

GROUPS	TREATMENT	% (SMALL INTESTINE TRANSIT TIME)
GROUP I	Control	84.32
GROUP II	Loperamide	29.79

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GROUP III	Standard (bisacodyl)	78.14
GROUP IV	LSEE (100mg/kg)	59.26
GROUP V	LSEE (200mg/kg)	71.25

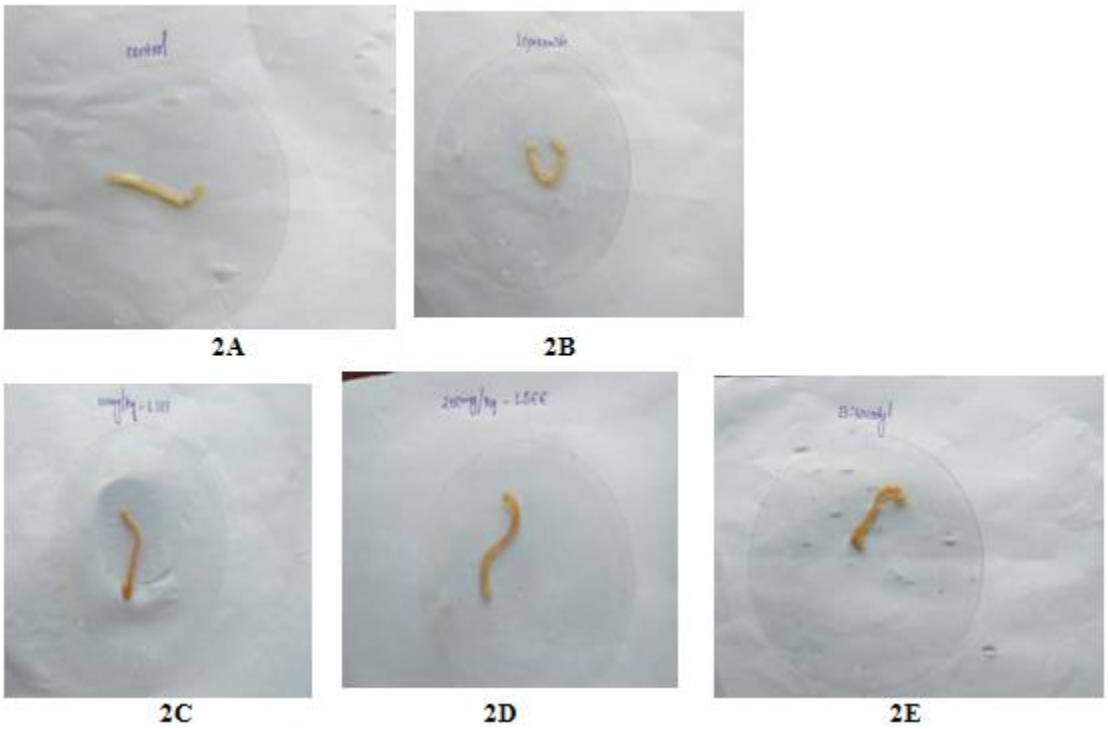

Fig.no: 3.3.1 – small intestine transit time

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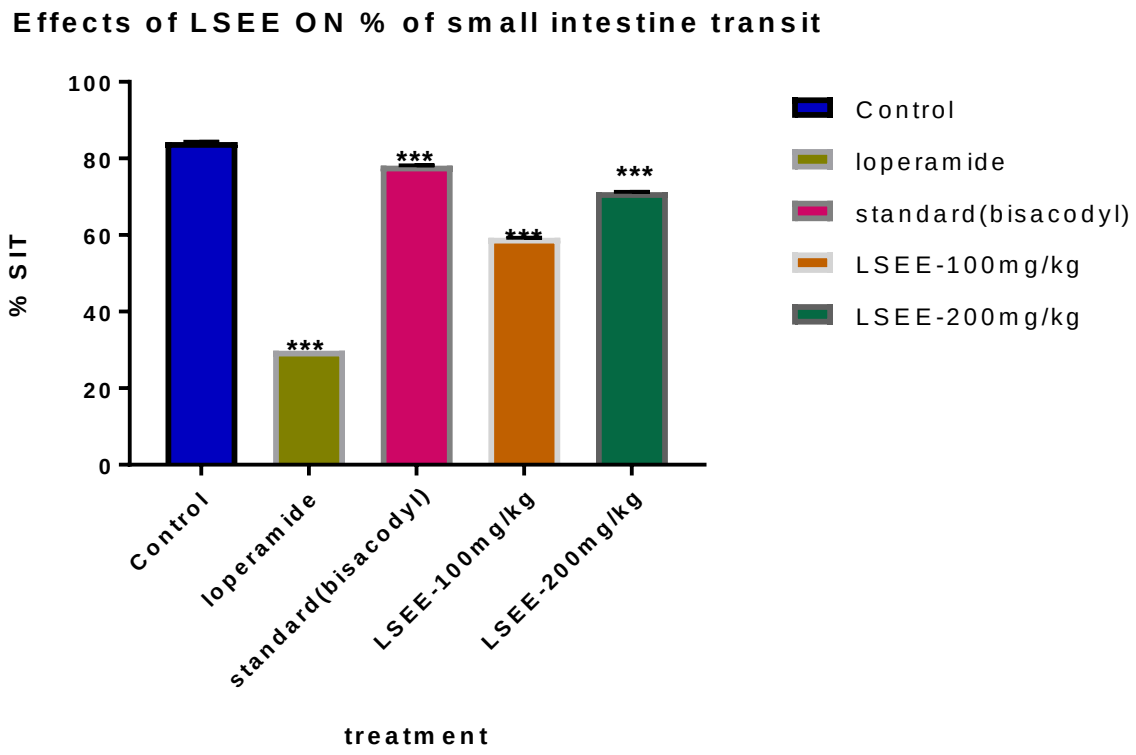
Graph no: 3.3.1



Effects of LSEE on the SLT:
After loperamide administration, the SIT was significantly inhibited (figure-2B). The treatment with LSEE increased the SIT in a dose dependent manner in loperamide induced constipation model mice.

Values are expressed as mean ±S.E.M. for n= 6 mice in each group. Data analysis was performed using One way ANOVA followed by Tukey's Multiple Comparison Test. A significant ($^{***}P < 0.0001$ vs. control) decrease in the duration of immobility was seen with the standard drug Lopiramide and LSEE in all the tested doses as compared to the control (group 1).

Discussion

Constipation is a highly prevalent functional gastro-intestinal disorder, affecting the quality of life in constipated persons, and the use of dietary fiber in the prevention and treatment of constipation is a common practice in many countries in the world. The present study has evaluated laxative effects of ***lactuca sativa (l.)*** **ethanolic leaf extract** on normal as well as on **loperamide** induced constipation mice.

As an agent for functional bowel disorders like diarrhea, loperamide used as constipation inducer is well documented. The drug inhibits intestinal fluid secretion and intestinal motility, leading to delay fecal evacuation time and intestinal luminal transit, and is used to induce a model of spastic constipation. The observed disease in the number and weight of feces by the treatment with this drug indication induction of constipation in mice. Similar observation was reported by Kakino et al.

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Lettuce is a rich source of Glycosides, steroids, phenolics, polysaccharides, tannins, resin, alkaloid, flavonol, quercetin, and ascorbic acid, vitamin K and vitamin A, and a moderate source of folate and iron. Contaminated lettuce is often a source of bacterial, viral and parasitic outbreaks in humans, its having anti-inflammatory, antioxidant, hypoglycemic activities etc. Furthermore, LSEE increased fecal weight and fecal number in normal mice in present study. Similar observation was reported by Nakamura et at.[28] where brewer's yeast cellwall, which was composed mainly of polysaccharides, significantly increased fecal frequency and weight in normal rats. Due to these properties, it is presumed that LSEE has favorable effects on prevention of constipation.

The effects of LSEE on constipation in this study were tested in loperamide induced constipation mice. The administration of LSEE to the constipated mice was effective in increasing the fecal number and fecal weight, which were indications of the laxative character of *Lactuca sativa Linn.* Treatment with LSEE significantly raised the fecal water content in loperamide induced constipation mice. And the SIT time was shortened by the treatment with LSEE in this constipation models. The laxative effectiveness of dietary fibers as bulk agents depends on their WHC and swelling force.

LSEE was proved to have a high WHC. It may thus be concluded that the fecal output character was affected by the WHC and swelling force of lectuca in small intestine. This swelling force serves as a stimulus of defecation. Xu et al.[29,30] reported that supplementation of the dite with partially fiber meal markedly decreased gastrointestinal transit time as well as increases fecal frequency and weight in constipated mice, which was dependent on luminal bulk. In addition, given the fact that soluble fibers may delay nutrients digestion and absorption by absorbing large quantities of water and forming gels in the gastrointestinal tract, LSEE might be possible to prevent the absorption of loperamide in these results.

Conclusion

Through our study it was found that results of *Lactuca sativa Linn.* leaf extract possesses significant laxative effect due to the presence of chemical constituents like carbohydrates, glycosides, flavanoids, terpenoids, phenols, tannins and alkaloids, which alleviates the symptoms of loperamide induced constipation. The LSEE extract can be regarded as a safe, economic, natural source for the discovery of new laxative activity. And because of its easy availability, lectuca could be recommended as a cost-effective alternative for constipation. Further studies on identification, isolation, purification of active principles of plants responsible for these therapeutic properties may lead to the new drug development.

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