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# Alteration In Gut Serotonin Level Induced by Ethanolic Ripen Fruit Extract of Ficus Carica and Its Mechanism Against Constipation in Animal Models

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**Abstract**

Constipation is a common, long-term gastrointestinal motility problem. Because of their long-term side effects, the drugs available to treat constipation are restricted. *Ficus carica* belongs to the mulberry family Moraceae, commonly known as fig. It is traditionally used as an emollient, laxative, aphrodisiac, cough suppressant, hemorrhoids suppressants, antiulcer, and hypercholesterolemia. Several animal and human studies have reported the anti-constipation activity and the mechanism of ethanolic fruit extract of *Ficus carica* [EFF]. But, serotonin's role behind gut motility is not yet reported. Therefore the current research was conducted to evaluate the role of EFF in gut serotonin and its mechanism against constipation in animal models. The extract's total phenolic and flavonoid content was determined using a UV-visible spectrophotometer. The charcoal meal test and the loperamide-induced constipation model were used to assess EFF's anti-constipation effectiveness. The gut serotonin was measured by spectrofluorimeter and gut serotonin transporter (SERT) by ELISA. The total phenolic and flavonoid content was found as 326.9 ± 9.2 mg of GAE/g and 22.94 ± 0.4 mg of RT/g respectively. When compared to control animals, the results from these models demonstrate a significant dose-dependent increase in peristalsis index and moisture content. The EFF-treated animals showed reduced gut serotonin and SERT in the sigmoidal colon. The gut serotonin binds on the 5-$HT_4$ receptor in colon smooth muscle and accelerates colon motility. The EFF decrease the reuptake of serotonin in enterocyte by reducing SERT. The tryptophan in EFF increases the synthesis of serotonin. More research, such as serotonin content in feces and tryptophan hydroxylase-1 in the gut, is needed to confirm the mechanism behind serotonin release. The oral administration of EFF was proven to be useful in the treatment of constipation in this trial.

**Keywords:** *Ficus carica;* serotonin; SERT; flavonoid; constipation; castor oil; loperamide.

## 1. INTRODUCTION

Constipation is an acute or chronic gastrointestinal motility disorder that affects 20% of the world population [1, 2]. Constipation can be caused by many conditions including a lack of fiber in the diet, a change in eating habits, and lifestyle, ignoring the desire to pass stools, not drinking sufficient fluids, and side effects of some drugs [3, 4]. It is characterized by infrequent, incomplete bowel evacuation, hard pellet-like dry feces, and difficulty during defecation [5, 6]. It leads to abdominal pain, distension, restlessness, gut obstruction, perforation, discomfort, and affects the quality of life [7]. Long-term constipation leads to hemorrhoids, fecal impaction, anal fissure, and rectal prolapse [8]. Currently, a variety of treatments are available including drugs, a more fiber diet, and exercise, etc [9]. However,

few drugs are more potent and efficacious such as plecanatide and prucalopride act as stool softener that alters colonic motility by stimulation of 5-$HT_4$ receptor [10]. The senna act as a stimulant laxative, polyethylene glycol, and magnesium salts act as an osmotic laxative. But its usage is limited because of its cost and undesirable side effects, severe dehydration induced by plecanatide [11], abdominal pain by prucalopride [12] development of melanosis coli and colorectal cancer by senna [13], bloating, diarrhea, cramping induced by osmotic laxatives [14]. Hence, the new potent therapeutic drug with fewer side effects is needed to treat constipation.

5 hydroxytryptamine (5 HT), sometimes known as serotonin, is a neurotransmitter, majorly (95%) produced from mucosa and muscle layer of the gut. It is stored and released from enterochromaffin cells and regulates gastrointestinal function [15]. Gut serotonin plays a crucial role in GI motility; its levels are higher in diarrhea, irritable bowel disease, and celiac disease, and lower in constipation [16]. Recently, herbal drugs and medicinal foods have special attention and act as novel therapeutic drugs to treat constipation.

*Ficus carica* (FC) is also known as fig, is a small deciduous tree that belongs to the mulberry family Moraceae [17]. FC is a temperate species, widely cultivated for its fruit, originating from southwest Asia and the Mediterranean region in Europe [18]. It is an edible fruit eaten as fresh, dried, or jam. Its fruits are traditionally used as an emollient, laxative, aphrodisiac, antipyretic, cough suppressant, emmenagogue, hemorrhoid suppressant, antiulcer, and hypercholesterolemia [19]. It contains a high amount of soluble dietary fiber, vitamins (thiamin (B1), riboflavin (B2), vitamin C, and vitamin A) [20], minerals (iron, calcium, potassium, copper, and magnesium) [21], polyphenols (gallic acid, catechin, epigallocatechin, procyanidin, caffein, vanillin, quercetin, epicatechin, and kaempferol) [22], anthocyanins [23], alkaloids, saponins, and coumarins [24]. Several animal studies [25, 26] and human trials [27, 28] reported fruit paste of FC used to treat effectively against constipation. The different mechanisms of fig including high cellulose increased colon mucin, and acetylcholinesterase inhibitor activity is responsible for action against constipation. There are no studies that reported the fig-induced gut serotonin alteration and its mechanism. Hence, the research was conducted to study the role of ethanolic fruit extract of *Ficus carica* (EFF) on gut serotonin level and its mechanism against constipation.

## 2. MATERIALS AND METHODS

### 2.1. Drugs and Chemicals

Castor oil, Loperamide, Folin–Ciocalteu reagent, Diethyl ether, and Reddot biotech kit were obtained from Sigma Aldrich Chemical Pvt Ltd. The other medicines and chemicals were purchased commercially as analytical quality.

### 2.2. Animals and Housing

Wistar rats (weighing 200–250 g) were housed in sterile polypropylene cages with rice husk as bedding. The animals were kept at a temperature of 25°C and a humidity of 30–60%. The light and dark cycle was set to 12:12 h. Food and water was freely available to the animals. Following the standards of the IAEC, the Institutional animal ethics committee (688/PO/Re/S/02/CPCSEA. Proposal No: NCP/IAEC/2019-20/022) reviewed all of the experimental techniques and protocols utilized in this work.

Animal care was provided per the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were cremated when the experiment was completed, according to IAEC guidelines.

**2.3. Plant material**

The ripen fruits of *Ficus carica* were collected from erode and authenticated by the botanist, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. For future reference, voucher specimen no. 289 was deposited in the herbarium. For extraction, the fruits were dried in the shade and ground into powder.

**2.4. Method of Extraction**

The fruit powder was macerated with 70% aqueous ethanol for 24 hours. The filtrates were then evaporated in a rotary evaporator. Phytochemical analyses and animal investigations were carried out using the dried extract.

**2.5. Screening of phytochemicals in Extract**

Standard assays were used to identify numerous phytochemical components such as phenolic compounds, saponins, carbohydrates, glycosides, alkaloids, tannins, terpenoids, steroids, proteins, gums, and mucilage in the crude ethanolic fruit extract of *Ficus carica* (EFF)[29].

**2.6. Total phenolic content**

The total phenolic content of extracts was measured by Folin–Ciocalteu colorimetric method as described by Singleton *et al.* [30]. 1ml of extract (varying concentrations) mixed with 5 ml of 10% Folin–Ciocalteu reagent (FCR), and 4 ml of 7% $Na_2CO_3$. The mixture was incubated for 30 min at 40°C to oxidize phenols in extracts and produce a dark blue color. Then, the absorbance was measured by a UV-visible spectrophotometer at 760 nm against the blank. The total phenolic content of the sample was measured using a gallic acid standard curve and the results were represented as mg of gallic acid equivalents per gram (GAE/g) of the sample.

**2.7. Total flavonoid content**

The total flavonoid content was determined by the aluminum chloride colorimetric method by Jing *et al*. [31**].** 1 ml of varying concentration of the extract was mixed with 4 ml of distilled water, 0.3 ml of 5% $NaNO_2$, and 10% of 0.3 ml of $AlCl_3$. Then, 5 min later, 2 ml of 1 M NaOH was added, followed by 10 ml of distilled water. The spectrophotometer was used to measure the absorbance at 510 nm. The total flavonoid content was represented as rutin equivalents using the linear equation (mg of RT/g) derived from the calibration curve.

**2.8. Charcoal meal test**

A castor oil-induced hyperperistalsis test was used to assess the laxative effect. The animals were accessed free of food and water. The animals were kept into five groups, each with six animals. Group I was given normal saline orally as a control, Group II was given castor oil 1ml/kg as a positive control, and Groups III, IV, and V were given the test medication (EFF) orally at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. The extract suspension was produced with 0.5 percent

carboxymethylcellulose (w/v). All rats were given a dose of 1ml/kg of 5 percent charcoal suspension orally one hour following the treatment. The rats were euthanized after 30 minutes by cervical dislocation, and the entire length of the intestine (from the pylorus to the caecum) was taken and laid on white paper. The entire length of the intestine and the distance traveled by the charcoal meal were also measured. Each group's outcome was given as a percentage of GIT inhibition (percent) [32]. Gastrointestinal propulsion (percent) = (D/L) 100 was used to calculate small intestinal transit. Where D (cm) is the distance traveled by charcoal and L (cm) is the length of the small intestine.

**2.9. Loperamide induced constipation model**

The loperamide hydrochloride at a dose of 5 mg/kg/day in 0.9% (w/v) sodium chloride was administered orally for 7 days for induction of constipation. Bodyweight and fecal characteristics, such as moisture content and stiffness, were evaluated during the induction phase to ensure constipation. The rats in group I was given saline solution (10 ml/kg, p.o.) as a control. Each of the six constipated rats was separated into groups II, III, IV, V, and VI. Group II acted as a loperamide-treated negative control group (Lop). The group III animals were given the standard medicine, sodium picosulphate orally at a dose of 5 mg/kg and loperamide. The test medication EFF was given orally to the constipated rats in groups IV, V, and VI at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg (weighed extract was produced suspension with 0.5 percent w/v carboxymethylcellulose), respectively, for 7 days along with loperamide. During the trial phase, food intake was monitored three times a week [33]. On the seventh and fourteenth days, the expelled feces of each rat were collected. Fecal samples were weighed, and water content was evaluated by drying fecal pellets in an oven at 70°C for 24 hours, and the weight difference between before and after drying was calculated [1]. The water content of fecal matter was determined by using the formula: Fecal water content (%) = [(fecal wet weight − fecal dry weight)/fecal wet weight] × 100. The spectrofluorimetric procedure was used to evaluate serotonin levels in the proximal section of the small intestine and the sigmoidal colon.

**2.10. Estimation of Serotonin**

The Wistar rats were divided into 2 sets of 4 groups each consisting of 6 animals. Food pellets were provided two times per day at 6 pm and 9 am. The excess food pellets were removed at 5 am and food was denied for 4 hours. Group I- animals treated with normal saline solution at a dose of 10 ml/kg, p.o., and served as the control. Group II, III, and IV rats were treated orally with EFF at a dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively for 7 days after one hour of food supplementation (10 am). The first set of rats was sacrificed at 4 pm for serotonin estimation. The second set of animals was sacrificed on the $8^{th}$ day for serotonin measurement and follows the same procedure. The proximal part of the small intestine (duodenum and jejunum) and sigmoidal colon were separated for serotonin estimation. Serotonin was determined by the method of Schlumpf *et al.* [34]. Intestinal tissues were mixed with 5 ml HCl–butanol (0.85:1000 v/v) and homogenized for 1 min. The homogenized tissue was then centrifuged (10 min, 3000 rpm, 4 C) and collected the supernatant solution. The 2.5 ml heptane and 0.31 ml of 0.1 M HCl were added to 1ml of supernatant and centrifuged (10 min, 3000 rpm, 4 C) to separate the phases1.25 ml of a 20 mg percent o-phthalaldehyde solution was combined with 1 ml of the aqueous phase. In a water bath, the mixture was then heated to 100 C for 10 minutes. The fluorescence of each sample was measured using a spectrofluorometer at 360 nm excitation and 470

nm emission after the samples were cooled to room temperature. Serotonin was made serial dilutions and used for the standard calibration curve. The serotonin level was calculated by the extrapolation method.

**2.11. Estimation of SERT**

The proximal part of the small intestine and sigmoidal colon was removed, flushed with phosphate buffer solution (PBS 0.01 mol/l pH 7.2), and washed again. The mucosa of the intestine was scraped by using a glass slide. The mucosa (10% W/V) was mixed with PBS and homogenized with Teflon pestle. The homogenate was centrifuged for 5 min at 5000 x g and the supernatant liquid was used for estimation of the serotonin transporter. The SERT was measured by using a sandwich enzyme immunoassay. The microtiter plates were pre-coated with a SERT antibody. The test samples (100 µl) were added to microtitre plates with a biotin-conjugated polyclonal antibody specific for SERT. Avidin conjugated to horseradish peroxidase (HRP) was added to each microtitre plate and incubated. After addition of TMB substrate causes color change due to the presence of SERT. The enzyme-substrate reaction was stopped by sulphuric acid solution addition. A spectrophotometer was used to measure the color change at a wavelength of 450 nm. The SERT level in the sample was estimated by comparing the O.D. of the samples to the standard.

**2.12. Statistical analysis**

The mean and standard deviation are calculated for each group of six animals (SD). A one-way analysis of variance (ANOVA) was used in the statistical analysis, followed by Dunnett's test. The graphs were created using GraphPad Prism software. Statistical significance was considered as a P value of less than 0.05.

**3. RESULTS**

**3.1. Preliminary phytochemical screening**

The phytochemical analysis of crude ethanolic extract showed the presence of phenolic compounds, saponins, carbohydrates, glycosides, alkaloids, tannins, terpenoids, steroids, and proteins.

**3.2. Estimation of Total phenolic and flavonoid content**

The phenolic content of EFF was 326.9 ± 9.2 mg of GAE/g. The flavonoid concentration of EFF was found to be 22.94 ± 0.4 mg of RT/g.

**3.3. Charcoal meal test**

**3.3.1. Effect of the EFF on gastrointestinal motility**

The results of the charcoal meal gastrointestinal motility test are represented in figure 1. The EFF at a dose of 100 and 200 mg/kg showed a mild increase in gastric propulsion of the charcoal meal through the gastrointestinal tract. The EFF at high dose 400 mg/kg showed a significant ($P < 0.05$) elevation (69.92%) in the movement of the charcoal meal than the control group (56.24%). Castor oil-treated groups showed a significant ($P < 0.01$) elevation (91.18%) in gastrointestinal motility than control. But castor oil-treated rats showed the highest motility than a high dose of EFF treated animals.

### 3.4. Loperamide-induced constipation model

#### 3.4.1. Bodyweight changes

The loperamide-treated animals showed a significant ($P < 0.05$) reduction in body weight (table 1) changes on the day of induction of constipation and 14$^{th}$ day when compared to control animals. The control animals showed 10.8 g and the constipated animals showed 9.2 g bodyweight changes on the 14$^{th}$ day. The EFF 100, 200 mg/kg treated rats showed a mild increase in body weight changes compared to constipated rats. The EFF high dose (400 mg/kg) treated rats attenuated the loperamide-induced body weight changes. The sodium picosulphate ingested rats showed 10.3 g bodyweight changes which were higher than EFF treatment.

#### 3.4.2. Feed intake changes

Table 2 shows the feed intake of all experimental animals on the first day of treatment, day 7, day 14, and changes in feed intake. The constipated animals (2.4 g) showed a significant ($P < 0.05$) reduction in feed intake than normal (3.2 g) animals. The EFF 100 and 200 mg/kg treated groups showed a reduction in food intake than loperamide treated rats. The EFF at a dose of 400 mg/kg treated rats showed significant elevation in feed intake than constipated rats. The SPS treated rats attenuated the loperamide-induced alteration in feed intake.

#### 3.4.3. Fecal parameters

The loperamide-induced constipation was evidenced by fecal morphology. The constipated rats showed a small-sized, pellet-like hard fecal matter with decreased moisture content. The normal rats showed spindle-shaped and soft stools. The SPS-treated animals exhibited smooth and viscous stools. The EFF-treated animals showed a smooth and more viscous mass of stools. The percentage moisture content in the fecal matter was shown in figure 2. The constipated rats (30.35%) showed a significant reduction in moisture content ($P < 0.01$) than normal rats. The EFF 100, 200, and 400 mg/kg treated rats showed a significant increase ($P < 0.01$) in stool weight and moisture content than loperamide treated rats. The SPS administered rats showed 69.17% of moisture content, which was lesser than EFF treated rats.

### 3.5. Gut serotonin level

The serotonin level in the loperamide-induced constipation model was shown in figure 3. The animals treated with loperamide, standard drug SPS, and EFF were not shown significant alteration in serotonin levels in the small intestine. The EFF at a dose of 100, 200, and 400 mg/kg treated animals showed a significant ($P < 0.01$) reduction (1.4 ±0.5, 1.26±0.3, and 1.17±0.1) in serotonin in the colon. We found wide individual variations in serotonin levels. So the experiment was conducted under controlled food timings and serotonin was measured on 1$^{st}$ and 8$^{th}$-day exposure of EFF (figure 4). The animals showed a mild reduction in serotonin level in the small intestine on 1$^{st}$ exposure to EFF in a dose-dependent manner. But on the 8$^{th}$ day, EFF treated animals did not show significant alterations in serotonin levels. The EFF-treated animals showed a significant ($P < 0.01$) reduction in colon serotonin levels on the 1$^{st}$ and on the 8$^{th}$ day.

### 3.6. Gut SERT level

The small intestinal SERT level in loperamide, SPS, and EFF treated rats were not shown a significant ($P > 0.05$) difference compared to control rats (figure 5). The EFF-treated rats did not show alteration in SERT on the 1st and 8th day in the small intestine. The EFF at a dose of 100, 200, and 400 mg/kg treated rats showed significant ($P < 0.01$) reduction in SERT level by 0.8, 0.76, and 0.71 ng/g of tissue simultaneously in loperamide induced constipation model. The EFF treated rats showed dose dependant reduction in colon SERT level on the 1st and 8th day when compared to control.

**4. DISCUSSION**

In the present study, we have explored the role of fruit extract of FC in gut serotonin level and its mechanism on rat constipation models. Our preliminary phytochemical studies reported the presence of phenolic compounds, saponins, carbohydrates, glycosides, alkaloids, tannins, terpenoids, steroids, proteins, gums, mucilage, and quantified the total phenolic acids and flavanoids in fig extract coexist with several earlier investigations [24,25,35,36]. The present study indicated that the castor oil-treated rats showed increased gastric motility of charcoal meal. The castor oil and its active metabolite ricinoleic acid initiate hyperperistalsis by irritating the gut and increasing prostaglandin biosynthesis [37]. The EFF extract 100, 200 and 400 mg/kg treated rats showed a mild elevation in gastric motility in dose dependant manner, but lesser than castor oil. The insoluble cellulose in fig is responsible for propulsive activity in the small intestine by increased water content, viscosity, the bulkiness of intestinal content [38, 39].

The current results indicated that the animals treated with loperamide demonstrated a reduction in body weight and feed intake than control animals. Loperamide induced constipation by acting on mu receptor and decreased bowel motility, increased the time for reabsorption of water and electrolyte. The constipation was evidenced by the fecal morphology [40]. The SPS treatment showed a mild elevation in body weight and feed intake than loperamide treatment. The SPS has converted into 4, 4'-dihydroxy diphenyl-(2-pyridyl) methane as active metabolites formed by colon bacteria and enhance colon motility [25]. The EFF-treated animals attenuated the body weight and feed intake changes induced by loperamide. The moisture content of fecal matter was high in EFF treated animals than loperamide, but lesser than SPS treated animals.

Several animal and human studies reported that fig extract has a high efficacious drug for the treatment of constipation [26, 41]. The several mechanisms including soluble cellulose in fig increase the fermentation and acid production by coliform bacilli. The high acidity in the colon accelerates colon motility [20]. The phenolic compounds in EFF have potential health benefits via modulation of the gut microbiota and increasing probiotics including *Prevotella, Bacteroides, Butyricicoccus, and Coprococcus*, and increased fermentation process in the colon [35]. The fiber in the FC undergoes fermentation and increased the production of short-chain fatty acids in the colon (SCFAs). The SCFAs increased the fecal weight by enhancing water holding capacity [42]. **B**utyrate is an SCFA formed from fig fermentation in the colon; it strengthens the gut barrier, suppresses inflammation, and releases neurotransmitter serotonin from the intestinal enterochromaffin cell [43]. The current data also evidenced the release of serotonin in the gut by fig. Loperamide treatment reduced colonic mucus secretion by decreasing mucosal layer thickness. Fig fruit has insoluble fiber that increases colonic mucus by decreasing the mucinase activity in the distal colon and decreasing mucus degradation [44]. The intracellular calcium is

responsible for colonic smooth muscle contraction. The fig extract contains calcium it either increases intracellular calcium or promotes the release of intracellular calcium for muscle contraction [45]. The fig also reported anticholinesterase activity and promote cholinergic mediated colonic motility [46].

The gut is the human body's largest immunological organ, forming the biological barrier. The microbiota (trillions of microorganisms that live on and within the human body) has been found to play a function in human health and disease. The important amino acid tryptophan can be metabolized by gut bacteria as a precursor for the manufacture of indole, serotonin, and melatonin, limiting tryptophan availability for the host [47]. Serotonin or 5 hydroxytryptamine (5 HT) is a neurotransmitter, majorly synthesized, stored, and released from enterochromaffin cells of the gut, and regulates gastrointestinal function. Gut serotonin plays a crucial role in GI motility, its level increased in diarrhea, and celiac disease, irritable bowel disease, decreased in constipation. The gut and neuronal 5 HT are identical, but the synthesis is regulated by enzymes such as tryptophan hydroxylase-1, (TpH-1) expression in gut and tryptophan hydroxylase-2, (TpH-2) in nerves [48]. The major difference between TPH1 and TPH2 is site and play different roles in the gut and neurons [49]. 5-HT is reuptake intracellularly by the serotonin transporter (SERT) present in neurons and intestinal mucosa [50]. 5-HT reuptake requires active co-transport with $Na^+$ ($Na^+$ $K^+$ATPase in basolateral) by the SERT [51]. The SERT mRNA expression is higher in small intestine than colon (ileum >> duodenum >> jejunum) [52]. The SERT in human enterocytes is responsible for removing gut serotonin. SERT expression is high in the order to circulatory platelets and enterocytes taken high concentration of 5 HT from the gut. 5-HT is metabolized to 5-hydroxy indole acetic acid (5-HIAA) in platelets by monoamine oxidase (MAO) and excreted through urine. The platelets took up 5HT for either distribution to other sites or metabolized [53]. Selective serotonin reuptake inhibitors (SSRIs) decrease the activity of SERT, thus rising the availability of 5-HT. The 5 $HT_4$ receptors are distributed in the whole gut but have a high concentration in the proximal and distal colon [54]. The circular muscle of the distal colon has more 5 $HT_4$ receptors than in the proximal colon. The distal colon is responsible for the propulsion of contents and the proximal colon for the storage of contents. The $5HT_4$ agonists such as cisapride and metoclopramide are stimulated primarily upper GI motor activity, and act as prokinetic effects in the colon [55]. Two new novel enterokinetic compounds are tegaserod and prucalopride used to treat constipation effectively, but tegaserod has withdrawn because of the increased risk of heart attack and stroke [56]. Prucalopride stimulates the colon more selectively without affecting small bowel function, but it causes abdominal pain. The study focuses on the role of EFF on gut serotonin levels and their functions.

The present study measured the gut serotonin level in the proximal part of the small intestine and sigmoidal colon because 5 $HT_4$ receptors are high concentration in this region. In the loperamide-induced constipation model, we identified more individual variations in serotonin levels in drug, extract-treated animals, and also in control group animals. EFF treatment showed dose dependant reduction in serotonin and SERT levels in the sigmoidal colon. But no alteration in serotonin and SERT in the small intestine. The results indicate that the serotonin release is increased in the colon after EFF treatment and it is mixed with fecal matter. We measured serotonin in the gut that includes stored serotonin, but not measured serotonin binds on the $5HT_4$ receptor and mixed with stools. The low SERT level in the colon also supports the increased serotonin level in the colon by preventing the reabsorption of serotonin in the enterocyte. Our pilot studies and loperamide-induced constipation model showed that food consumption and its timing alter the gut serotonin level. So the rats were denied food for 4 hours

to maintain the uniform timing for food consumption to minimize the individual variations. The intestine and colon were cultivated 6 hours after EFF administration because extract have taken 6 hours to reach the colon. The serotonin was measured on 1st day and 8th-day treatment with EFF. The mild reduction in serotonin level and no alteration in SERT indicate that a small amount of serotonin is released in the small intestine on the first-day exposure of EFF. But on the 8th day of EFF treatment showed no alteration in serotonin level due to EFF providing tryptophan for the synthesis of serotonin, so the loss of serotonin is compensated with synthesis or upregulation of tryptophan hydroxylase-1. The SERT level was decreased on the 8th day of EFF treatment in the colon which indicates a high release of serotonin in the colon and accelerates colon motility. Several studies also reported that fig fruit extract has antidepressant activity by altering 5HT, noradrenaline in the brain due to its phenolic compounds [57, 58]. The antidepressant activity may be due to tryptophan in fig or upregulation of TpH-2 or downregulation of SERT. The flavonoids and their metabolite in fig extract can shape gut microbiota by an increased beneficial pathogen of *Bacteroidetes* and *Actinobacteria* and improve gut health by a reduction in endotoxin production. It decreased harmful pathogen *proteobacteria* [59]. Gut bacteria influenced serotonin levels. Pseudomonas uses tryptophan to generate serotonin, which it uses for virulence and intercellular signaling. The reduced circulatory tryptophan by gut microbiota alters serotonergic neurotransmission in the central and enteric systems [60]. Several factors are responsible for the alteration of gut serotonin levels including food, gut microbiota, phenolic compounds, flavonoids, amino acid tryptophan, etc. The EFF at a high dose of 400 mg/kg showed a mild increase in peristaltic activity in the small intestine and significantly accelerated colon motility. But a low dose of EFF only accelerates colon motility and is especially used to treat diet-induced constipation. The EFF is used as an efficacious drug to treat constipation by altering serotonin levels in the gut.

## 5. CONCLUSION

The present study concludes that EFF showed significant anti-constipation activity by using different models. We found individual variation in gut serotonin level, even though; EFF significantly increased peristaltic activity in the small intestine and also accelerated colon motility by altering gut serotonin level and SERT. EFF is an economic, nutrient, safe and efficacious drug to treat constipation. Further studies to be recommended by using more animals in each group to minimize variations and measure serotonin in fecal matter, gut TpH-1 level, and TpH-1 knockout animals to confirm the release of gut serotonin.

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**Conflict of interest:** Nil

**Animal Ethics committee approval:** The Nandha College of Pharmacy's Institutional Animal Ethics Committee (688/PO/Re/S/02/CPCSEA) accepted all experimental protocols. Proposal No. NCP/IAEC/2019-20/022, prepared under IAEC guidelines.

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**Table 1**. Bodyweight changes in loperamide-induced constipated rats during the experimental period.

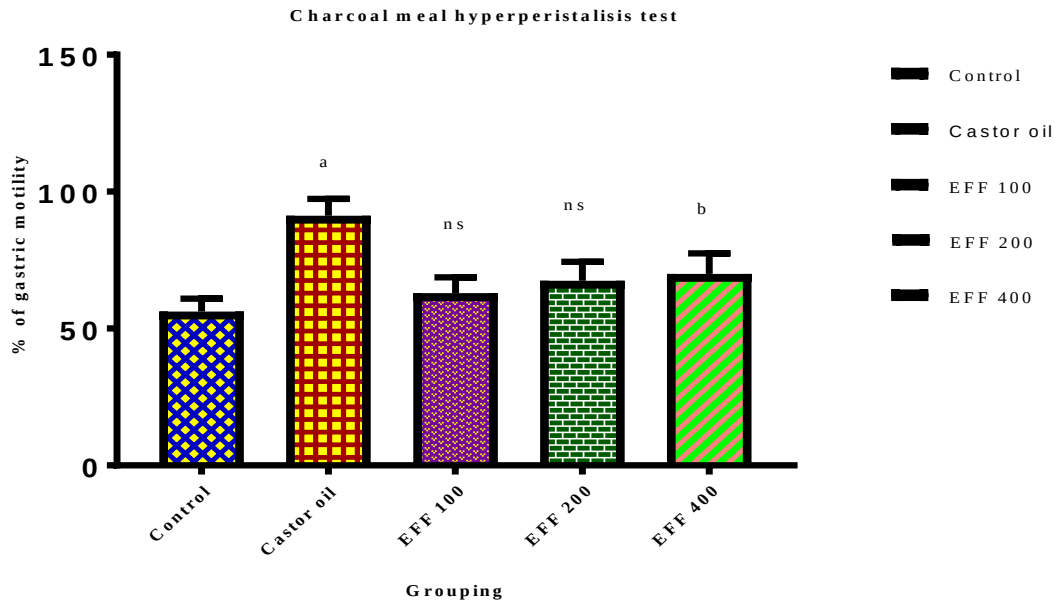
| Group | Bodyweight in g | | | Bodyweight changes on the 14$^{th}$ day |
|---|---|---|---|---|
| | 0 day | 7$^{th}$ day | 14$^{th}$ day | |
| Control | 210.8±5.6 | 216.1±3.4 | 221.6±4.5 | 10.8±0.4 |
| Lop | 218.2±4.7 | 222.9±4.1 | 227.4±3.8 | 9.2±0.6$^{a}$ |
| Lop+SPS | 220.5±3.4 | 220.5±2.8 | 230.8±2.4 | 10.3±0.5$^{b}$ |
| Lop+EFF-100 | 214.1±5.8 | 219.2±5.2 | 223.5±4.9 | 9.4±0.6 |
| Lop+EFF-200 | 224.3±5.2 | 229.5±4.6 | 233.9±3.4 | 9.6±0.5 |
| Lop+EFF-400 | 227.6±4.5 | 233.4±3.2 | 237.5±2.8 | 9.9±0.3$^{b}$ |

Values are expressed as mean ±S.D; n=6. $^{a}P < 0.05$ when compared to control. $^{b}P < 0.05$, when compared to loperamide, treated animals. One-way ANOVA was used to evaluate the data, followed by Dunnett's test.

**Table 2**. Feed intake changes in loperamide-induced constipated rats in the experimental period.

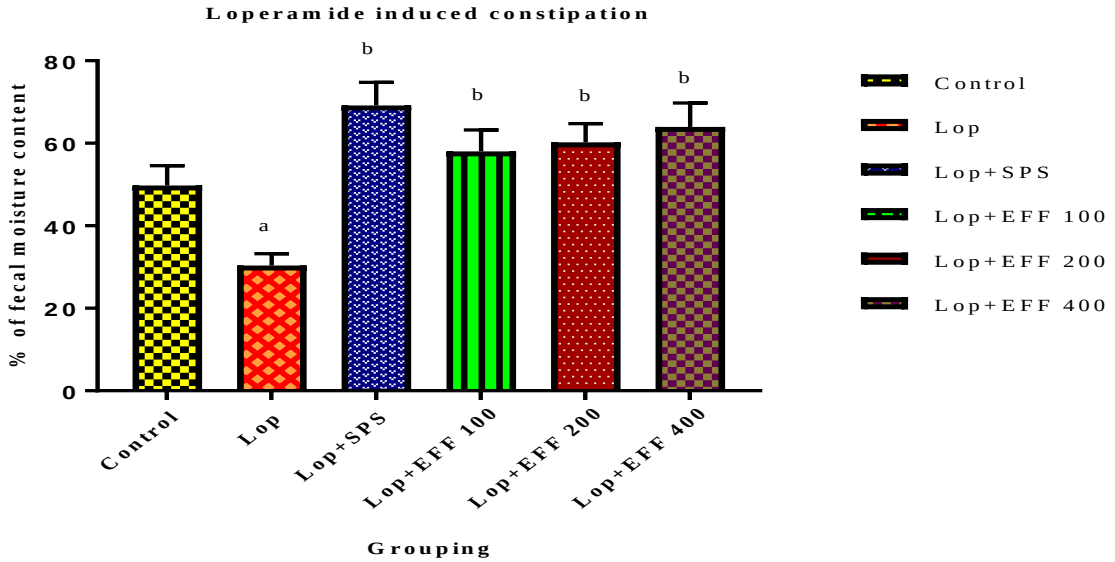
| Group | Feed intake in g | | | Changes in feed intake on 14th day |
|---|---|---|---|---|
| | 0 day | 7th day | 14th day | |
| Control | 19.4±0.8 | 20.9±0.9 | 22.6±0.7 | 3.2±0.2 |
| Lop | 20.5±1.2 | 21.8±1.0 | 22.9±0.9 | 2.4±0.1[a] |
| Lop+SPS | 21.3±0.9 | 22.5±0.7 | 24.3±0.5 | 3.0±0.3[b] |
| Lop+EFF-100 | 19.9±0.6 | 21.3±0.4 | 22.4±0.6 | 2.5±0.2 |
| Lop+EFF-200 | 21.5±1.0 | 23.1±1.2 | 24.2±0.8 | 2.7±0.3 |
| Lop+EFF-400 | 21.8±0.9 | 23.5±0.6 | 24.7±0.7 | 2.9±0.2[b] |

Values are expressed as mean ±S.D; n=6. [a]P < 0.05 when compared to control. [b]P < 0.05, when compared to loperamide treated animals. One-way ANOVA was used to evaluate the data, followed by Dunnett's test.



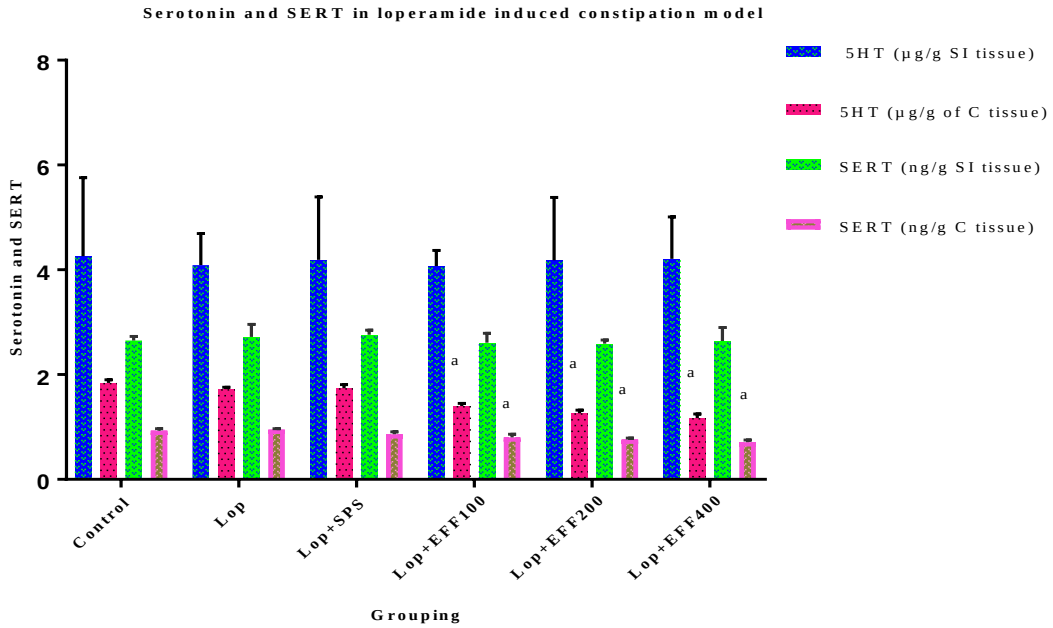

Values are expressed as mean ±S.D; n=6. [ns]P > 0.05, [a]P < 0.01, [b]P < 0.05 when compared to control. One-way ANOVA was used to evaluate the data, followed by Dunnett's test.

**Figure 1**. Effect of the EFF on gastrointestinal motility.



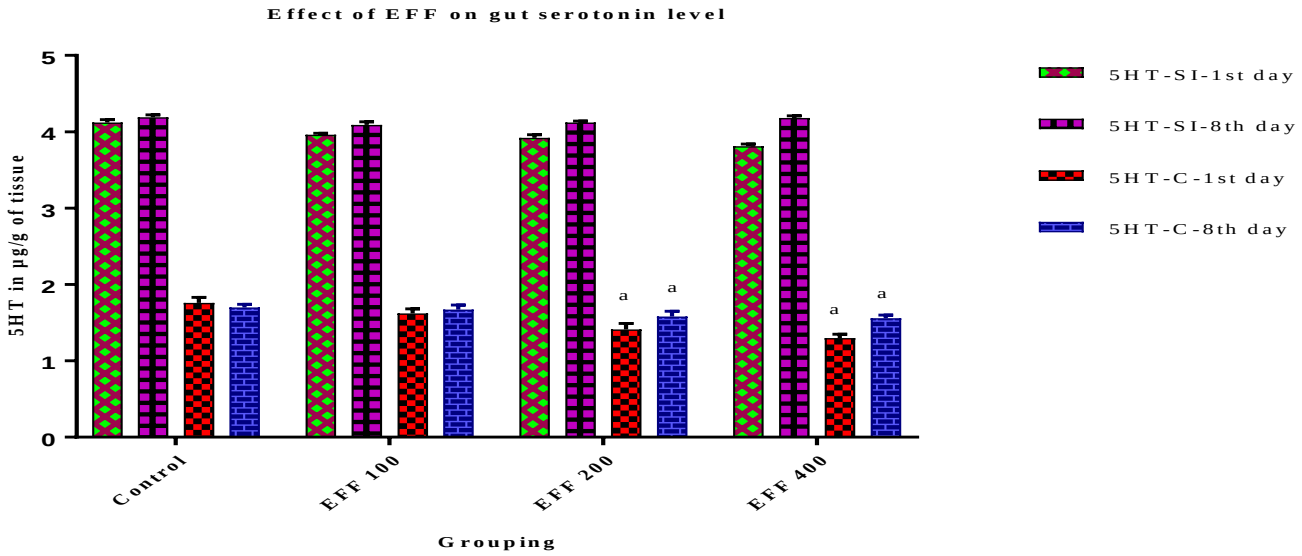

Values are expressed as mean ±S.D; n=6. [a]P < 0.01 when compared to control. [b]P < 0.01 compared to loperamide treated rats when compared to control. One-way ANOVA was used to evaluate the data, followed by Dunnett's test.

**Figure 2**. Effect of the EFF on fecal moisture content.



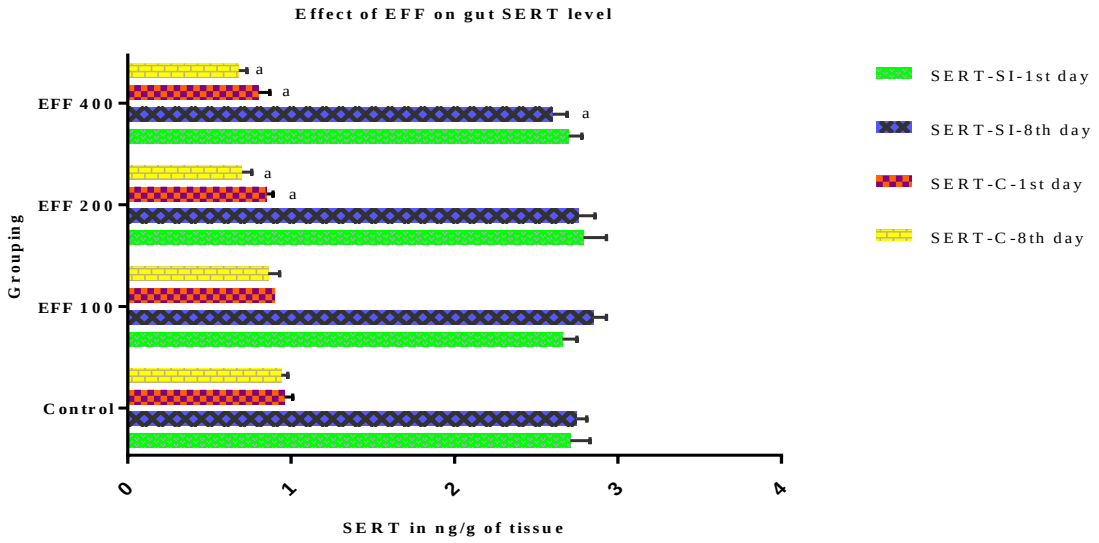

Values are expressed as mean ±S.D; n=6. [a]P < 0.01 when compared to control. One-way ANOVA was used to evaluate the data, followed by Dunnett's test. SI-small intestine, and C-colon.

**Figure 3.** Effect of the EFF on serotonin and SERT in loperamide-induced constipation model.




Values are expressed as mean ±S.D; n=6. $^{a}P < 0.01$ when compared to control. One-way ANOVA was used to evaluate the data, followed by Dunnett's test. SI-small intestine, and C-colon.

**Figure 4.** Effect of the EFF on gut serotonin level.




Values are expressed as mean ±S.D; n=6. $^{a}P < 0.01$ when compared to control. One-way ANOVA was used to evaluate the data, followed by Dunnett's test. SI-small intestine and C-colon.