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# Assessment of *in-vivo* anti-diabetic and anti-diarrheal effects of *Flemingia stricta* Roxb. leaf

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**ABSTRACT:** The objective of this study was to evaluate the anti-diabetic and anti-diarrheal activity of methanol extract of *Flemingia stricta* Roxb. (Fabaceae) leaf. In anti-diabetic study, the extract was administered to alloxan-induced diabetic mice at two concentrations (200 mg/kg and 400 mg/kg body weight) for acute (12 hours) and prolonged treatments (15 days) and blood glucose levels of diabetic mice were monitored at intervals of hours and days throughout the duration of treatment. Antidiarrheal test was conducted by castor oil induced diarrhea and enteropooling as well as intestinal motility in mice at three different concentrations (100 mg/kg, 200 mg/kg and 400 mg/kg body weight). Treatment of alloxan-induced diabetic mice with the extract caused a significant reduction in fasting blood glucose level of the diabetic mice both in acute (12 hours) and prolonged treatment (15 days) and it was determined that the *F. stricta* methanol extract at both concentrations (200 mg/kg and 400 mg/kg) showed the significant ( $P < 0.05$ ) hypoglycemic effect in comparison to the standard drug metformin. In the case of castor oil induced diarrhea test, enteropooling test and gastrointestinal motility test, the extract of *F. stricta* at 100 mg/kg, 200 mg/kg and 400 mg/kg has given significant effect ( $P < 0.05$ ) compared to the standard drug loperamide. But 400 mg/kg demonstrated the highest activity amongst the three doses. These results suggested that the methanol extract of *F. stricta* Roxb. possess promising anti-diabetic effect on alloxan-induced mice and significant antidiarrheal effect on castor oil induced diarrhea mice.

**Keywords:** *Flemingia stricta*; Anti-diabetic; Anti-diarrheal; Alloxan; Metformin; Loperamide; Castor oil.

## 1. INTRODUCTION

Plants are the great source of medicine. Plant derived medicine has tremendous efficacy as well as safe and cost effective. Therefore, traditional use of phytomedicine has been continuing for centuries around the world [1]. Traditional medicine has always played a pivotal role in the health care systems all over the world.

In developing countries, 80% people are still depending on traditional medicine [2]. Moreover, over 25% compounds of commonly used medicines are extracted from medicinal plants [3]. From this perspective, importance of medicinal plants is inevitable. *Flemingia stricta* Roxb. is a subshrub of Fabaceae family which

is widely distributed in southeast Asian country such as Bangladesh, Bhutan, China, India, Indonesia, Laos, Philippines, Thailand, Vietnam [4, 5]. It is generally found in Chittagong, Chittagong Hill Tracts and Sylhet area of Bangladesh. Moreover, it is traditionally known as Charchara (in Bangla) as well as called as various traditional names in local tribes of Chittagong, Bangladesh [6]. This plant species has various medicinal properties.

Hence, it is traditionally used for the treatment of various diseases such as bone fracture, cough, goiter and polio, asthma, and polio [7-9]. Diabetes is characterized by the destruction of beta cell caused by the high blood glucose level which in turn decreases or ceases the production of insulin in the human body. The number of people suffering from diabetes is increasing day by day.

As reported by International Diabetes Federation (IDF), approximately 425 million people are affected by diabetes in 2017 and will reach about 642 million in 2040 [10]. According to the study of researchers, diabetic patients will be increased in near future at an alarming rate in United States, China and India [11]. In United States, more than 30 million adults and children have diabetes. Besides, another individual is diagnosed with diabetes in every 21 seconds [12]. Due to the food habit and life style, it is also a major threat to a developing country like Bangladesh. By 2030, Bangladesh will have the 7th highest number of diabetic patients [13, 14].

Diarrhea is defined as abnormally loose or watery stools due to virus, bacteria or parasitic organisms. Despite of availability of several anti-diarrheal agents as well as great development in the pharmaceutical field, diarrheal diseases are still one of the leading causes of mortality and morbidity around the world which are correlated with approximately 1.3 million deaths annually [15, 16]. As reported by UNICEF, diarrheal diseases are responsible for about 8 percent of all deaths among children under age 5 in 2016 which means 1300 young children are dying every day or approximately 480,000 children in a year. Due to lack of education and hygiene awareness, developing countries in Africa and Asia are more vulnerable to diarrheal diseases [17, 18].

Therefore, this experiment was designed to evaluate the in vivo anti-diabetic activity and anti-diarrheal activity of *F. stricta* Roxb leaf extract against alloxan induced diabetic mice and castor oil induced diarrhea in mice respectively.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

*F. stricta* Roxb. leaves were collected from a local area (Bhatiary) of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Bangladesh.

### 2.2. Preparation of extract

The leaves were air dried under the shade and grounded. The ground (500 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use. Plant extract was diluted by using dimethyl sulfoxide (DMSO) to make stock solution.

### 2.3. Animals

In the present study, Swiss albino mice (male), which weighed between 35-45 g were used. The animals were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRDB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of  $25 \pm 2^\circ\text{C}$ . Animals were allowed free access to drinking water and pellet diet, collected from ICDDRDB Dhaka. Mice were acclimatized for 10 days in the laboratory environment prior to the study.

### 2.4. Materials and chemicals

Castor oil (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), Metformin (Square Pharmaceuticals Ltd., Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Castor oil (WELL's Health Care, Spain), were procured and used in the experiment. For the induction of diabetes, Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and used in the experiment. Glucometer kit was purchased from Accu-Check active, Roche Diagnostic GmbH, Mannheim, Germany. All chemicals used in this investigation were of analytical reagent grade.

### 2.5. Determination of median lethal dose (LD<sub>50</sub>)

The LD<sub>50</sub> of the extract was determined using Swiss albino mice. The extract was administered intraperitoneally (i.p.) and the method of Miller and Tainter [19] was adopted. This involved the administration of different doses of the extract (100-1000 mg/kg) to groups of five mice each. The animals were observed for physical manifestation of signs of toxicity.

### 2.6. Anti-diabetic activity

#### 2.6.1. Induction of diabetes

The animals (male mice) were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (130 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 mL of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. Seventy two hours later, mice with blood glucose levels above 26 mmol/dL were considered diabetic and selected for the experiment.

#### 2.6.2. Evaluation of anti-diabetic activity

The animals were randomly divided into four groups with five mice in each group and treated as follows:

Group I: Diabetic mice were administered with normal saline (10 ml/kg) p.o.

Group II: Diabetic mice were given metformin (10 mg/kg b.w.) p.o.

Group III: Diabetic mice were administered orally with *F. stricta* extract (200 mg/kg b.w.).

Group IV: Diabetic mice were administered orally with *F. stricta* extract (400 mg/kg b.w.).

The change in body weight and fasting blood glucose level of all the rats were recorded at regular intervals during the experimental period. For acute study, the blood glucose level were monitored after 1, 3, 5, 7, 10 and 12 h of administration of a single dose of the extract and at the end of 1, 3, 5, 7, 10, 15 days for prolonged treatments. The blood glucose levels were monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded [20].

## 2.7. Anti-diarrheal activity

Anti-diarrheal activity was performed by three tests which are castor oil-induced diarrheal test, castor oil-induced enteropooling test and lastly gastrointestinal motility test.

### 2.7.1. Castor oil-induced diarrhea in mice

Castor oil-induced diarrhea was done according to the method of Soba et al. [21] and Uddin et al. [22]. Mice were divided into five groups of five mice each. The animals were fasted for 18 h prior to the test. Group I was treated with normal saline (2 mL/kg), which served as control; while Group II received loperamide (5 mg/kg). Both groups III, IV and V received methanol extract at 100 mg/kg, 200 mg/kg and 400 mg/kg. All doses were administered orally. After 1 h, all groups received 1 mL of castor oil orally. Then animals were placed in cages lined with adsorbent papers and observed for 4 h for the presence of diarrhea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated as follows:

$$\% \text{ Inhibition of defecation} = [(A-B)/A] \times 100$$

where A indicated mean number of defecation caused by castor oil; B indicated mean number of defecation caused by drug or extract.

### 2.7.2. Castor oil-induced enteropooling

The castor oil-induced enteropooling was carried out according to the method of Robert et al. [23]. Mice were divided into five groups and fasted for 18 h prior to the test. Saline water was given to Group I as control (saline 2 mL/kg body weight, orally); Group II received standard drug (loperamide 5 mg/kg body weight, orally), and the rest groups (Groups III, IV and V) were given *F. stricta* methanol extract (at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight, orally). One hour later, all the mice were challenged with 1 mL of castor oil orally. After 1 h of castor oil received, the mice were sacrificed and the small intestine from the pylorus to the caecum was isolated. Then the intestinal contents were weighed and volume measured by graduated tube [23, 24].

## 2.8. Gastrointestinal motility test

This test was performed according to the method previously described using charcoal as a diet marker [25]. Animals were divided into five groups of five mice in each and fasted for 18 h before test. All groups received castor oil to produce diarrhea. One hour later, group I treated as control (saline 2 mL/kg body weight, orally); Group II received standard drug (loperamide 5 mg/kg body weight, orally) and the rest three groups received three different doses (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, orally) of *F. stricta* methanol extract. After 1 h of drug administration, all animals were received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, all animals were sacrificed, and the distance covered by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as percentage of distance moved [26].

## 2.9. Statistical analysis

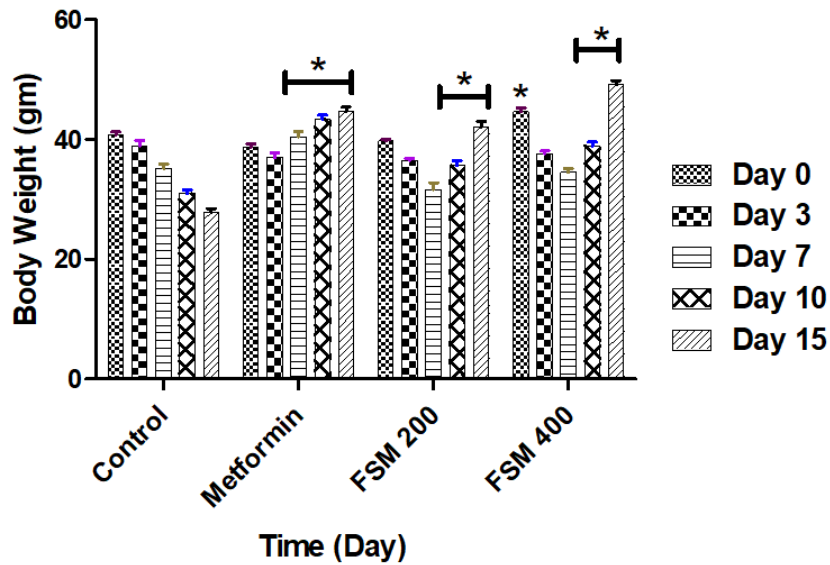
The data was expressed as mean  $\pm$  standard error of mean (S.E.M.). Statistical comparisons were performed using one-way ANOVA followed by post-hoc Dunnett's test with the SPSS program (SPSS 20.0,

USA). The values obtained were compared with the vehicle control group and were considered statistically significant when  $*P < 0.05$ . Graphpad Prism was used for the graphical representation of data.

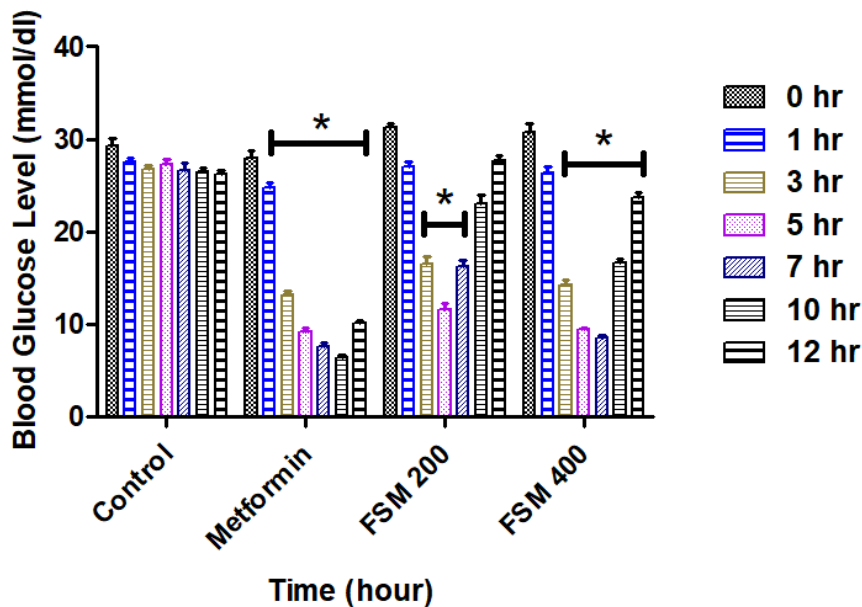
### 3. RESULTS

#### 3.1. Anti-diabetic activity

There were observable changes in the body weight of treated and untreated (control) diabetic mice. Treatment of diabetic mice with the leaf extract of *F. stricta* and metformin improved the weight gain compared to untreated diabetic mice whereas weight of control group was decreased (Fig. 1).



**Figure 1.** Effect of methanol leaf extract of *F. stricta* on body weights of alloxan-induced diabetic rats. Values are expressed as mean  $\pm$  SEM (n=5).  $*P < 0.05$  when compared with control group. FSM: *F. stricta* methanol extract.

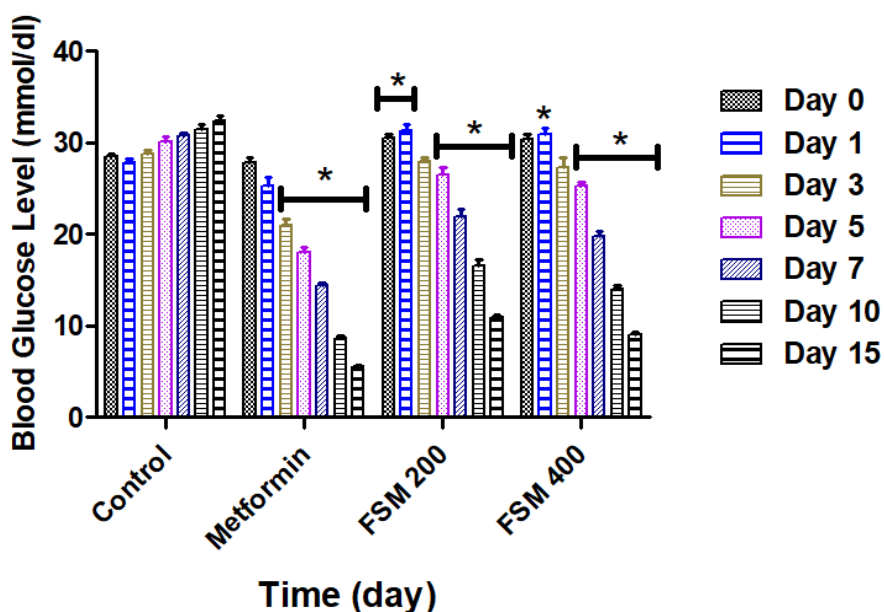


**Figure 2.** Anti-diabetic effect of methanol leaf extract of *F. stricta* on blood glucose level of alloxan-induced diabetic study (acute treatment). Values are expressed as mean  $\pm$  SEM (n=5).  $*P < 0.05$  when compared with control group.

FSM: *F. stricta* methanol extract.

In the case of acute treatment, a dose-dependent reduction in blood glucose level was observed in alloxan-induced diabetic mice treated with methanol leaf extract of *F. stricta*. After a single dose of the extract given to the alloxan-induced diabetic mice, there was a significant ( $P < 0.05$ ) reduction in blood glucose level of the diabetic mice within the period of acute study compared to control. The maximum effect was observed at 5 h for 200 mg/kg dose and 7 h for 400 mg/kg dose. However, the effect of extract was found significant compared to the control group (Fig. 2).

During prolonged treatment (15 days), the *F. stricta* extract produced a sustained significant ( $P < 0.05$ ) reduction in blood glucose level of the diabetic mice compared to control treated by saline (Fig. 3). The effects of the highest dose of the extract were near about to the standard drug, metformin, 10 mg/kg, on day 15.



**Figure 3.** Effect of methanol leaf extract of *F. stricta* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment. Values are expressed as mean  $\pm$  SEM ( $n=5$ ). \* $P < 0.05$  when compared with control group.

FSM: *F. stricta* methanol extract.

### 3.2. Castor oil induced diarrhea

In castor oil induced diarrhea test, *F. stricta* methanol extract showed considerable antidiarrheal effect in mice at three doses (100 mg/kg, 200 mg/kg and 400 mg/kg) whereas 400 mg/kg showed the highest activity. Methanol extract significantly inhibited the frequency of defecation when compared with placebo treated control mice ( $P < 0.05$ ). Three doses of the extract decreased the total number of wet feces produced upon administration of castor oil when compared to the placebo control (saline) rats. The results are shown in Table 1.

### 3.3. Castor oil induced enteropooling

The *F. stricta* plant extract at three different doses showed noticeable effect in castor oil induced enteropooling test in the mice (Table 2). The intestinal volume was decreased by 17.69%, 20.99% and 38.44% for three different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of methanol leaf extract. But FSM 400 mg/kg showed highest activity among three doses. The values were statistically significant compared to

control ( $P < 0.05$ ). The standard drug, loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (49.29%).

**Table 1.** Effect of methanol leaf extracts of *F. stricta* in castor oil induced diarrhea on mice.

Groups	Treatment (p.o)	Total number of feces	% inhibition of defecation	Total number of diarrheal feces	% inhibition of diarrhea
I	Saline (2 ml/kg)	16.2±0.95	--	14.8±1.36	--
II	Loperamide (5 mg/kg)	4.6±0.66	71.61*	3.2±0.59	78.38*
III	FSM (100 mg/kg)	8.4±0.65	23.46*	8.4±1.07	43.24*
IV	FSM (200 mg/kg)	5.2±0.86	67.91*	7.4±1.07	50.00*
V	FSM (400 mg/kg)	5±0.58	69.14*	5.2±1.16	64.86*

Values are expressed as mean±SEM (n=5). \* $P < 0.05$  when compared with control group. FSM: *F. stricta* methanol extract.

**Table 2.** Effect of methanol leaf extracts of *F. stricta* on castor oil induced enteropooling in mice.

Groups	Treatment (p.o)	Weight of intestinal content (g)	Volume of intestinal content (ml)	% of inhibition
I	Saline (2 ml/kg)	1.63±0.12	0.84±0.04	--
II	Loperamide (5 mg/kg)	0.61±0.07	0.43±0.03	49.29*
III	FSM (100 mg/kg)	1.03±0.02	0.69±0.03	17.69*
IV	FSM (200 mg/kg)	0.84±0.03	0.67±0.02	20.99*
V	FSM (400 mg/kg)	0.72±0.02	0.52±0.02	38.44*

Values are expressed as mean±SEM (n=5). \* $P < 0.05$  when compared with control group. FSM: *F. stricta* methanol extract.

### 3.4. Gastrointestinal motility test

The gastrointestinal distance traveled by the charcoal meal in the mice significantly ( $P < 0.05$ ) lessened by two doses (200 mg/kg and 400 mg/kg) of *F. stricta* methanol leaf extract compared with the placebo control group. Loperamide (5 mg/kg) produced a marked decrease (45.91%) in the propulsion of charcoal meal through gastrointestinal tract.

**Table 3.** Effect of methanol leaf extracts of *F. stricta* on small intestinal transit in mice.

Groups	Treatment (p.o)	Total length of Intestine (cm)	Distance travel by marker (cm)	% of Inhibition
I	Saline (2 ml/kg)	57.13±0.50	50.78±1.03	--
II	Loperamide (5 mg/kg)	57.12±0.58	27.47±0.70	45.91*
III	FSM (100 mg/kg)	53.98±0.61	46.20±1.28	9.03
IV	FSM (200 mg/kg)	53.02±0.42	39.20±0.86	22.81*
V	FSM (400 mg/kg)	55.26±0.32	32.40±0.92	36.20*

Values are expressed as mean±SEM (n=5). \* $P < 0.05$  when compared with control group. FSM: *F. stricta* methanol extract.

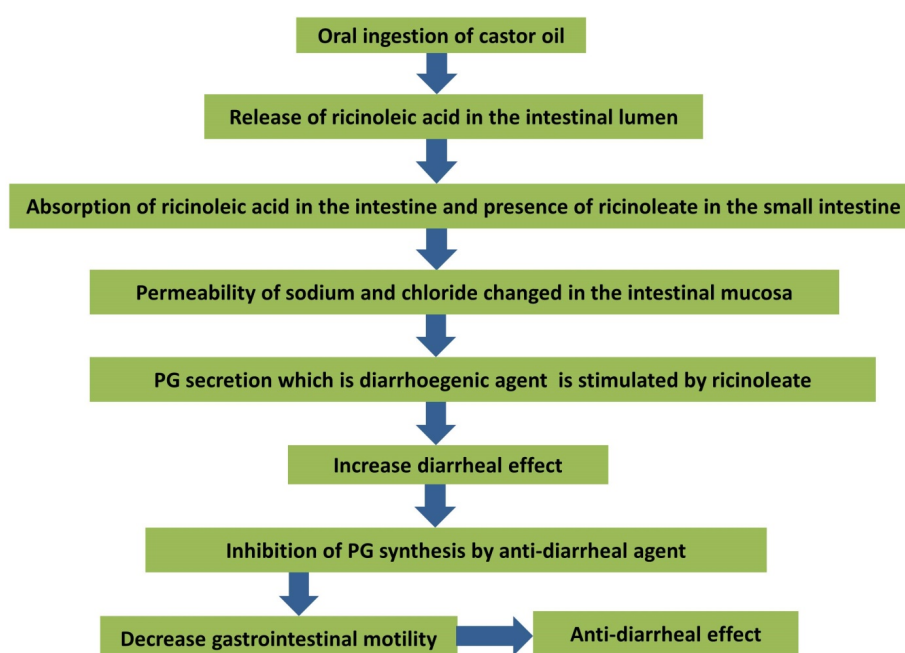
## 4. DISCUSSION

Alloxan induced diabetic test on mice was performed to assess the anti-diabetic activity of methanol extract of *F. stricta*. The methanol leaf extract of *F. stricta* was exhibited significant anti-diabetic activity in alloxan induced diabetic mice. There are a lot of reports implicating phytochemical compounds in plants as being responsible for their anti-diabetic activities [27-29].

Perhaps, presences of some phytochemical constituents are responsible for the observed significant activity of this plant extract either singly or in synergy, like standard drug metformin or other anti-diabetic agents are effective in diabetic state and ineffective in severe diabetic state where pancreatic cells are completely destroyed [30].

The observed reduction in blood glucose levels of the diabetic mice by metformin in this study displayed significant anti-diabetic activity as a standard drug. In addition, acute and prolong treatment with the methanol leaf extract of *F. stricta* for a period of 12 hours and 15 days respectively caused significant decrease in blood glucose levels of treated mice compared to untreated (control) diabetic mice.

The objective of anti-diarrheal test was to determine the effect of methanol extract of *F. stricta* on castor oil induced diarrhea. Castor oil is a triglyceride which is the combination of ricinoleic acid and hydroxylated unsaturated fatty acid. The responsible compound for the production of diarrhea is ricinoleate [31, 32].



**Figure 4.** Mechanism action of anti-diarrheal activity [33-38].

Many anti-diarrheal agents act by reducing the gastrointestinal motility and/or the secretions. In this experiment, the methanol extract of *F. stricta* exhibits significant ( $P < 0.05$ ) anti-diarrheal activity. Many reports are available on anti-diarrheal activity of different plant extract using this dose level [39]. Plant extract significantly reduced intestinal transit by decreasing the distance traveled by charcoal meal. According to this experiment, we found the plant extract suppressed the propulsion of charcoal meal. This suppression was possible due to the increased absorption of water as well as electrolysis.

From this point of view we can suggest that, *F. stricta* displays promising anti-diabetic and anti-diarrheal effects which can be a potential source of biologically important drug candidates.

## 5. CONCLUSION

In conclusion, the results of this experiment exhibited that methanol leaf extract of *F. stricta* possess significant anti-diabetic and anti-diarrheal properties. Henceforth, further analyses are required in order to decode the mechanism of this methanol leaf extract.



**Authors' Contributions:** MSB, MMR, MNA and MRI designed the whole study. MSB, MNA, AIC and MF collected the plant and arranged all the materials for laboratory experiments. MSB, MNA, MJA, AIC, MF, MIS and MMR performed all the laboratory experiments. MSB, MNA and MMR wrote the whole manuscript. All authors read and approved the final manuscript.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Ethical Approval:** The set of rules followed for animal experiment were approved by the institutional animal ethics committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh according to governmental guidelines.

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