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Ethanol extract of *Ficus Racemosa* l. Stem bark moderates diabetic and diarrhoeal activities in wistar rats

S. Mazumdar[1]*, R. Akter[1]

1. Pharmacology Research Division, BCSIR Laboratories, Chittagong, Chittagong-4220, Bangladesh.

ABSTRACT

The present study scrutinizes the antidiabetic and the antidiarrhoeal effects of ethanolic extracts of *Ficus racemosa* L. stem barks (EEFB) in Wistar rats. Oral glucose tolerance test (OGTT) model and alloxan induced diabetic model (AIDM) were performed to assess antidiabetic activity of EEFB at doses of 750mg/kg, 500 mg/kg and 250 mg/kg, and 2g/ kg respectively. For antidiarrhoeal effects of EEFB, castor oil-induced diarrhoeal (COID) model and gastrointestinal motility test with barium sulphate milk (BSM) model were also assessed at doses of 750 mg/kg and 500 mg/kg, and 250mg/kg respectively. Administration of EEFB resulted low blood glucose levels in OGTT model at doses 750 mg/kg and 500 mg/kg significantly ($P<0.01$). However, dose at 250 mg/kg also showed positive result ($P<0.05$) of the mentioned same antidiabetic model. In AIDM, Wistar rats showed worthy result at 2g/kg dose ($P<0.001$). After administration of EEFB at doses 750 mg/kg and 500 mg/kg inhibited significantly positive in COID model at $P<0.01$and $P<0.05$ respectively. However, at the dose of 250 mg/kg illustrated positive but not significant result in BSM model.

Keywords: *Ficus racemosa,* bark extract, antidiabetic, antidiarrhoea and Wistar rats

*Corresponding Author Email: mazumdarsantosh@gmail.com

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INTRODUCTION

Ficus racemosa L. (Family: Moraceae) is medium tall, growing 10-16 meters in height. This is native to Australia, South-East Asia and the Indian subcontinent. It is frequently found throughout Bangladesh near streams and canals and it also cultivated in different places of the country (Yusuf *et al*., 2009; Joseph and Raj, 2010)[1,2]. Various plant parts of this plant are popular in indigenous system of medicine like Ayurveda, Siddha, Unani and Homoeopathy (Paarakh, 2009)[3]. Especially, the bark of this plant is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, leprosy, dysentery, asthma and piles, antiseptic, antipyretic and vermicidal and the decoction of bark is used in the treatment of various skin diseases and ulcers (Paarakh, 2009; Ahmed *et al.*, 2010, Zulfiker *et al.* 2011; Mishra and Tiwari, 2013)[3,4,5,6].

Diabetes is a metabolic disorder characterized by fast elevation of blood sugar level. It is a major disease about 10% of the total population. According to World Health Organization (WHO) projections, the diabetic population is likely to increase to 300 million or more by the year 2025 (Joy and Kutton, 1999; Sy *et al.*, 2005)[7,8]. In addition, diarrhoeal disease is one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year (Carlos and Saniel, 1990)[10]. It is estimated that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. The WHO encouraged studies for the treatment and prevention of diabetes and diarrheal diseases depending on traditional medical practices (Marles and Farnsworth, 1995; Atta and Mouneir, 2004)[11,12].

In Bangladesh, tribes and traditional medicine practitioners use local plants for treatment of various diseases (Faruk and Uddin, 2011)[12]. A few researchers namely Hamid *et al.* (2011)[14], Hosain *et al.* (2011)[13] and Zulfiker *et al.* (2011)[5] worked on different pharmacological screenings on different parts except ethanolic extract of stem barks of *F. racemosa* in Bangladesh. The objects of the present study is to evaluate the antidiarrhoeal and antidiabetic effects of ethanol extracts of *F. racemosa* stem barks in Wistar rats.

MATERIALS AND METHOD

Plant materials: The stem barks of *F. racemosa* were collected from Kulgaon village, Panchlaish, Chittagong, Bangladesh. The plant was taxonomically identified by Professor Dr. Mostafa Kamal Pasha, Department of Botany, University of Chittagong and the voucher specimen (Pharma-0025/2012) was deposited at Pharmacology Research Division, BCSIR Laboratories, Chittagong, Bangladesh. The crude bark extraction was collected through some consequence steps namely

washing, chopping, drying, grinding powdered, extraction with 98% ethanol, and concentrated by using Rotary vacuum evaporator flasks were performed . The yield of the extract was 2.45% (w/w, in terms of dried starting material) which was kept in refrigerator at 4 °C.

Experimental animals: Healthy Male Wistar rats weighing between 180±10g were provided from animal house of BCSIR Lab. Chittagong to assess aforesaid pharmacological tests. All animals were kept and maintained under standard laboratory conditions. The animals were fed with standard diet and allowed to drink water, along with all aspects of animal care were complied with the ethical guidelines of Pharmacology research division, BCSIR Lab. Chittagong, Bangladesh.

Chemicals: Commercially available analytical grade chemicals and drugs like alloxan tetrahydrate (Merck, India), barium sulfate (Merck, India Ltd.), castor oil (Shengyang Kaiyingsheng Chemical Co. Ltd., China), diethyl ether (Sigma–Aldrich, India), glibenclamide [Marion Roussel Ltd., (Aventis, Bangladesh)], glucose powder (Dextrose monohydrase, GlaxoSmithKline, Chittagong, Bangladesh Ltd.), loperamide (Opsonin, Bangladesh) and Tween 80 [Polyoxyethylene . Loba Chemie Pvt. Ltd., India] were used for this experiment.

Experiments: Antidiabetic

A. *Oral glucose tolerance test* (OGTT) *model*

The oral glucose tolerance test was performed in overnight fasted normal Wistar rats as per reported method (Barik *et al.*, 2008)[15]. The rats were randomly divided into five groups marked as Group I to V. Each group contains five rats. Group I and Group II assigned as control and positive control group respectively. Groups III to V recognized as treated group for EEFB treatment. Blood was collected from the tip of tail and blood glucose level (BGL) was measured of all groups of rats with the help of a blood glucose meter (Accu-chek active, Roche Diagnostics, Germany). Afterwards only 2 ml/rat distilled water was supplied for control group and reference antidiabetic drug glibenclamide was given orally at a dose of 4.15 mg/kg body weight for positive control group. Groups III, IV and V of rats were treated orally with EEFB at doses of 750 mg/kg, 500 mg/kg and 250 mg/kg body weight respectively. After 30 min of water, drug and extract administration BGL was measured. Then 10 g/kg (body weight) glucose solution was provided orally for all these animals and BGL was observed of all rats after 30, 60, and 120 min of glucose administration.

B. *Alloxan induced diabetic model* (AIDM)

Due to induce diabetes, alloxan tetrahydrate (100 mg/kg i.p.) was provided in overnight fasted male Wistar rats with intraperitoneal injection. After 24 h BGL was measured

and separated them which were contained BGL more than 15 mmol/L and assigned as diabetic rats. These rats were used for the experiment. Diabetic induced rats were randomly divided into three experimental groups marked as Group II to IV, and each with five rats,. Group II indicated as diabetic control supplied only distilled water (2ml/rat). Reference antidiabetic drug glibenclamide (4.15 mg/kg) was provided for Group III marked as positive control. Group IV was treated with EEFB at the dose of 2g/kg. Previously selected Group I marked as control which had no diabetes. At first, BGL of all groups of rats was measured before administration of water, extract and drug which indicated the time 0 h, after then water, drug and extract were provided according to mentioned group and BGL were determined after 3, 6 and 9 h respectively by following above mentioned system. During blood collection from experimented rats, diethyl ether was used for anaesthesia. Blood was withdrawn from the tip of tail just prior to, and BGL was estimated by a blood glucose meter Accu-chek active, Roche Diagnostics, Germany.

Experiments: Antidiarrhoea

***A. Castor oil-induced diarrhoea* (COID model)**

Antidiarrhoeal activity of EEFB was estimated with COID model by following Awouters *et al.* (1978) with moderate modifications. Wistar rats were divided into four groups by random selection and each group contained five rats. Group I assigned as control. Group II marked as positive control and the rest of Groups III to IV recognized as treated groups. At first, extract and drugs were provided orally and after 1 h castor oil (2 ml/rat) was provided for inducing diarrhoea. Only distilled water (2 mL/rat) was rendered for the control group and standard drug loperamide (2 mg/kg) was provided for the positive control group. Treated Groups III

to IV recommended EEFB at doses of 750 mg/kg and 500 mg/kg body weight respectively. Separate cages were used for each rat and sheets of paper were placed below the cage for collection of fecal matters. The presence of stool with fluid material that stained the paper was placed beneath the cages indicated diarrhoea. At every hour the numbers of both hard and soft pellets were counted up to 6 h period for each rat and finally moisture content of all faeces of each group was measured.

B. Gastrointestinal Motility test with Barium Sulphate ($BaSo_4$) milk* (BSM) *model

BSM model was carried out by reported method, Solanki and Nagori, 2012[16]. Overnight fasted fifteen Wistar rats were randomly divided in to three equal groups (n=5). Control group received only distilled water 2 ml/rat orally. Positive control group received commercially available antidiarrhoeal drug loperamide (2 mg/kg) orally. Treated group received EEFB 250 mg/kg body weight orally. After that, thirty minutes later 2 ml `of 10% barium sulfate solution were

administered in all groups of rats. Lastly, after 30 min rats were sacrificed. Finally, the distance traveled by $BaSo_4$ milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction).

Statistical analysis

All the values of antidiabetic and antidiarrhoeal, tests were expressed as mean ± SEM (Standard error of the Mean). Statistical differences between the mean of the various groups were analyzed by using Students"t" test. Probability (p) value of 0.05, 0.01 or 0.001 was considered as significant. All the graphical presentation and statistical calculations were prepared using "Microsoft Excel-2007". Mean values were considered significantly different if P< 0.05, P<0.01and P< 0.001.

RESULTS AND DISCUSSION

Effect of EEFB of OGTT model

In the current study, the results of OGTT of positive control and different doses of ethanol extract of *F. racemosa* barks (EEFB) on Wistar rats are obtained in Table1. Mean and SEM for each of the doses of EEFB and positive control were determined. All the doses of EEFB and positive control were effective to the decreased blood glucose level (BGL) with the increasing exposure time. The BGL of the positive control were 4.28, 3.26, 10.08, 7.9 and 3.82 mmol/L at fasting where as the BGL of the three treated doses, 750mg/kg, 500mg/kg and 250mg/kg were 3.94, 4.88, 14.92, 10.08 and7.44; 3.86, 4.64, 18.04, 13.74 and8.44, and 4.08, 4.66, 19.66, 15.02 and 10.48 respectively at different times mentioned in Table 1. The results indicated a significant ($p < 0.05$) BGL reduction at 750mg/kg and 500mg/kg doses of EEFB.

Effect of EEFB on AIDM

The mean blood glucose concentration of control, diabetic control, positive control and plant extract for AIDM were estimated on rats at 0, 3, 6 and 9 hours respectively as shown in Table 2. After the administration of alloxan the blood glucose level (BGL) increased gradually in all observation periods. The mean of blood glucose level in alloxan induced diabetic rats were 19.46, 22.72, 24.9 and 29.03 mmol/L. Whilst, 21.44, 16.14, 9.3 and 5.36 mmol/L and 19.5, 15.28, 11.34 and 6.66 mmol/L were recorded in positive control and treated extract in aforesaid observation periods respectively. After 3 hours of the administration of positive control and plant extracts the BGL of treated rats gradually decreased. For instance EEFB and positive control were almost equally effective in reduction of blood glucose level in the alloxan induced diabetic rats (P<0.001 and P<0.01).

Effect of EEFB on COID model

The results of antidiarrhoeal effect of loperamide and EEFB in castor oil induced diarrhoea on Wistar Albino rats are illustrated in Table 3. Compared to the control group the results shown that EEFB at the dose of 750 mg/kg and 500 mg/kg were effective to inhibit the frequency of wet faeces ($P < 0.01$) and defecation as well as inhibit the moisture content of total faeces.

Effect of EEFB on BSM model

The results of BSM on Wistar rats have been illustrated in Table 4. At 30 min study, the highest reduction of gastrointestinal motility was for loperamide at dose of 2 mg/kg and inhibition of the distance travelled by BaSO4 milk was 38% compared with control. It was observed that the plant extracts decreased but not significant the distance of gastrointestinal motility of rats that was 9% at the dose of 250mg/kg extract compared with control.

DISCUSSION

Effect of *EEFB* on antidiabetic activities

The alcoholic extract of *F. racemosa* barks significantly reduces blood glucose in diabetic rats at the 6 h after ethanolic extract administration ($P < 0.001$) (Patil *et al*., 2010)[17.] In the present study, the antidiabetic activity was comparable to that of the effect produced by a standard antidiabetic agent, glibenclamide ($P<0.001$). The results of EEFB showed significant difference ($p<0.05$) which was observed between experimental and diabetic control Wistar rats in lowering fasting blood glucose level (Table1 and Table 2). At a dose of 100 mg/kg body weight, the extracts significantly lowered blood glucose level and showed maximum reduction of 33.85% on day 14. The extracts at 200 mg/kg body weight produced maximum reduction of 41.91% on day 14 whereas inhibition of 45.49% was found for metformin on day 14 as a peak on the effect of ethanolic fruit extracts of *F. racemosa* on blood glucose in experimental animals (Jahan *et al*., 2009)[18]. The present observations support with the almost similar observations reported earlier. According to Kar *et al.* (2003)[19] the ethanol extract (250mg/kg) of *F. racemosa* lowered blood glucose level within 2 weeks in the alloxan diabetic albino rats. Sophia and Manoharan (2007)[20] also mentioned that EEFB (100-500mg/kgbw) has potent antidiabetic and hypolipidemic effects in alloxan-induced diabetic rats and these effects were much comparable to that of the standard reference drug, glibenclamide which has been supported by the present study. The present findings also endure Ahmed and Urooj (2010)[21] as well as Shiksharthi and Mittal (2011)[22] in the regards of hypoglycemic potential of EEFB.

Table 1: Effect of EEFB on OGTM

Test samples groups		Dose	Mean blood glucose concentration (mmol/L)				
			Fasting BGL (Pretreatment)	BGL After 30 min extract administration	BGL after glucose administration 30 min	60 min	120 min
Control (Group I)		2ml/rat	4.14±0.22	4.66±0.31	19.7±0.44	17.24±1.1	14.02±0.94
Positive Control	(Group II)	4.15mg/kg	4.28±0.29	3.26±0.09	10.08±0.87***	7.9±1.0**	3.82±0.3***
EEFB treated	(Group III)	750mg/kg	3.94±0.39	4.88±0.22	14.92±0.52**	10.08±0.33**	7.44±0.38**
	(Group IV)	500mg/kg	3.86±0.18	4.64±0.16	18.04±1.7	13.74±1.95	8.44±0.6**
	(Group V)	250mg/kg	4.08±0.23	4.66±0.18	19.66±2.32	15.02±1.73	10.48±1.5*

Values are expressed mean ± SEM; *: P < 0.05; **: P < 0.01; ***: P < 0.001 mean significant difference compared positive control and extract with control.

Table 2:Effect of EEFB on AIDM

Groups	0 hrs	3 hrs	6 hrs	9 hrs
Group I (Control)	6.78±0.29	6.54±0.51	6.7±0.38	6.14±0.22
Group II (Diabetic control)	19.46±0.59###	22.72±0.99###	24.9±1.06###	29.03±1.81###
Group III (Positive control)	21.44±1.52	16.14±1.74	9.3±1.81**	5.36±0.59***
Group IIII (EEFB treated	19.5±2.00	15.28±1.68**	11.34±1.77***	6.66±1.28***

Values are expressed as mean± SEM; ###: *P*<0.001 means significant difference compared diabetic control with control. *: *P*<0.05, **: *P*<0.01 and ***: *P*<0.001 refer to positive control and extract with diabetic control.

Table 3:Effect of EEFB on COID model.

Group	Dose	Total feaces in 6 h	% inhibition of defecation	No. of wet feaces in 6 h	% inhibition of defecation	Water content of total feaces (g)	inhibition (%) of water content	Moisture content of total faeces (%)
Control (Water)	2mL	21.6±2.07		18.6±4.1		5.714		16.91
Positive Control (Loperamide)	2mg/kg	8.4±1.14***	61	4.2±0.84**	77	2.9	49.25	9.71
EEFB	750mg/kg	14.6±2.41**	32	10.2±1.3*	45	2.94	48.55	8.56
	500 mg/kg	14.8±3.96*	31	10.8±1.3**	41	4.812	15.79	12.78

Values are expressed mean ± SEM; *: P < 0.05; **: P < 0.01; ***: P < 0.001 mean significant difference compared positive control and extract with control.

Table 4:**Effect of EEFB on gastrointestinal motility with BSM model on Wister rat**

Group	Dose	Length of GIT (cm)	Distance passed by $BaSO_4$ cm	BaSO4 transverse (%)	Inhibition (%)
Control (Water)	2mL	110.8±3.6	70.4±5.14	64.2	
Positive Control (Loperamide)	2mg/kg	108±2.95	42.2±4.43	39.21	$38^{\downarrow}$
EEFB	250mg/kg	119.8±0.97	69.6±1.17	58.11	$9^{\downarrow}$

Values are expressed mean ± SEM; *: P < 0.05; **: P < 0.01; ***: P < 0.001 mean significant difference compared positive control and extract with control.

GIT: Gastro intestinal tract (from pylorus to the ileo-cecal junction).

Effect of EEFB Antidiarrhoeal activity

The ethanolic extract of *F. racemosa* stem barks significantly ($p<0.001$) inhibited the mean number of defecation when compared to control group and treated group of diarrhoea induced by castor oil was observed. The number of stools between 1st to 6th hours for ethanol extract treated groups 750mg/kg was significantly ($p<0.01$) decreased, and at $p<0.05$ for dose 500mg/kg as comparable to the effect of standard antidiarrhoeal drug loperamide (Table 3).

The EEFB has shown significant inhibitory activity against castrol oil induced diarrhea and Prostaglandin E2 induced enter prolong in rats and also showed a significant reduction in gastrointestinal mobility in charcoal real test in rats (Mukherjee *et al*., 1998; Paarakh, 2009)[23.]

These extracts also showed a significant reduction in gastrointestinal motility in charcoal meal tests in rats. The results obtained established its efficacy as anti-diarrhoeal agent (Mukherjee *et al*., 1998; Shiksharthi and Mittal, 2011).[22, 23]

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