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Original Research Article

AN INVITRO STUDY ON *EUGENIA JAMBOLANA*PLANT EXTRACTIN ISOLATED RABBIT ILIUM SHOWING SPASMOLYTIC EFFECTS

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ABSTRACT

Gastrointestinal smooth muscle spasm is the major cause of symptoms of different gastrointestinal disorders. This study was undertaken to examine the effects of Jamun fruit (***Eugenia jambolana)*** extract. The experiment was performed on forty rabbits; properly dissected. Study was performed on Power Lab System. Acetylcholine, histamine, serotonin, and calcium were used in increasing doses to obtain significant contractions in the ilium until the ceiling effect was observed. Thereafter, these drugs were used in the presence of different doses of Jamun extract. Shift of different concentration-response curves in each group was obtained. Percentage inhibition was determined in each group.

Statistical analysis showed that in the presence of antagonist dose response curve in acetylcholine and calcium group were more significant than serotonin and histamine, while low doses of serotonin and histamine showed initially spasmogenic effects. Muscle contraction was measured using isometric transducer. Difference in proportions was examined by using ANOVA. Before and after using the drugs, the changes were compared and significance of the study was judged.

It can be concluded from current results that Jamun can provide useful spasmolytic effect in different intestinal disorders.

***Keywords*:** Jamun fruit, *Eugenia jambolana*, acetylcholine, histamine, serotonin, calcium induced contraction, isolated rabbit ileum

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INTRODUCTION

The history of herbal medicine practice is as old as the civilization itself; nearly two hundred years ago the Pharmacopoeias of the world were dominated by herbal medicines[1]. It is

estimated by the world health organization that a huge population in the developing countries depend on plant based medicines for their primary health care. [2]. The World health organization encourages the exploration and evaluation of medicinal plants[1].

Gastrointestinal motility disorders have diverse symptoms and signs that affect the entire gut.[1]Much research has been carried out in elucidating the underlying mechanism causing motility disorders of the gut [3]As a result of these developments, the quantity of research literature on these plants has been growing steadily worldwide.[4]. The plants and plant products are frequently used as drugs, especially in developing countries, which provide a basis for studies on drugs obtained from plant sources.[5].

It is reported that fruits of *Eugenia jambolana* contain raffinose, fructose, glucose, mallic acid, citric acid and gallic acid. [4]In Pakistan, it is used as the main constituent in many traditional medicines for the treatment of diabetes mellitus. The fruit extract have been proved to be antihyperglycemic in experimental diabetes mellitus[6]. Its flowers contain oleanolic, quercetin, and kampferol. Myrcetin is also present in small amounts. Myrcetin in small amount have been reported to be the major glycoside extracted[4]. The fruit is stated to be astringent, carminative, diuretic, and antioxidant[7], the juice of ripe fruit may be administered in case of enlargement of spleen and urine retention.[8] Its bark is used for sore throat, bronchitis, asthma, and dysentery.[4]Seeds are used for treatment of diabetes mellitus[9] and to reduce cholesterol level in blood[10].Its fresh juice given with milk is used for treating diarrhea in children. The extract of jamun seeds lowers blood pressure significantly [11].

It has been found that its bark extract reduces diarrhea by inhibiting gut motility.[12]. Furthermore, it is also suggested that tannins extracted from its bark have protective effect on gut and promote healing of gastric ulcers.[13]. Flavonoids existing in the extracts have spasmolytic and antidiarrheal activities[14-16].

It has also been reported that Quercetin and Myrcetin are flavonoids present in jamun extract that inhibit the activation of necrosis factor (NF)and cyclooxygenase II (COX-II) enzyme that forms the basis of its gastro protective property.[17].

Much of the research carried out on this plant with respect to its antidiabetic effect in Pakistan. The aim of the present study is to unravel its potential spasmolytic effects.

METHODOLOGY

It was a comparative interventional experimental study carried out in the Department of Pharmacology and Therapeutics, University of Health Sciences, Lahore. Data were recorded using Force Transducer attached with Power lab. The measurements were made at range of 20 millivolts; low pass 5 Hz × 10 gain using input 1 and the rate was 40/seconds. Materials used were Bridge Amp 118ML, Isotonic force calibrated transducer (F- 60), sensitivity 30mV/V having Single Tissue bath. Other chemicals used were Carbogen gas and Tyrode's solution. Ethanol extract of *Eugenia jambolana* fruit pulp prepared from PCSIR**,** Acetylcholine chloride, Histamine dihydrochloride, EDTA and Serotonin creatinine sulphate, were obtained from Sigma Chemicals, Germany. $CaCl_2$and KCL was obtained from Merck Chemical.

I. Preparation of Extracts of the *Eugenia Jambolana* fruit:

Two kilograms fresh fruit of *Eugenia jambolana* (Jamun) was procured in the months from June to August. The extract of the fruit of the *Eugenia Jambolana* was prepared by sequential extraction with water and ethanol. The extract was then stored in laboratory at -20°C until performing experiment and the standardization of extract was carried out in PCSIR (Photochemistry & Analytical labs).

Note: For preparation of extract of fruit pulp of *Eugenia jambolana* 300 ml of 95% alcohol was used.

II. Preparation of different Doses of Jamun Extract

Stock solution: 1g/ml of extract = 100mg/ml

Further dilutions are prepared from stock solution.

III. Dose calculation of Acetylcholine

0.1816g in 10ml = $1x10^{-1}$ M

Further dilutions are prepared from stock solution by dilution i.e,1ml of $1x10^{-1}$ M of ACh dissolved in 9ml D.W = $1x10^{-2}$ M. Likewise other concentrations of ACh were prepared.[18, 19]

IV. Dose Calculation of Histamine

18.407mg of Histamine dihydrochloride dissolved in 10ml D.W = 0.01 M= $1x10^{-2}$ M

1ml of $1x10^{-2}$ M of Histamine dihydrochloride dissolved in 9ml D.W = $1x10^{-3}$ M

Different concentrations of Histamine dihydrochloride were prepared likewise. [18, 19]

V. Dose Calculation of Serotonin

0.045mg of Serotonin creatinine sulphate dissolved in 10ml D.W = 0.01M= $1x10^{-2}$M

1ml of $1x10^{-2}$ M of serotonin creatinine dissolved in 9ml D.W = $1x10^{-3}$M

Further dilutions of serotonin creatinine were prepared likewise [18, 19]

VI. Dose Calculation of Calcium

1.10g of $CaCl_2$ dissolved in 1L = 0.001M= $1x10^{-2}$ M

1ml of $1x10^{-2}$ M of $CaCl_2$ dissolved in 9ml D.W = $1x10^{-3}$ M

Further dilutions of were prepared likewise.[18, 19]

Formula for Dose Calculation

Dilution Factor = Stock concentration / Final bath concentration

Volume to be injected = Final bath volume / Dilution factor

Pharmacological Activity on Power Lab/Kymograph System

Group A: Effect of Acetylcholine

1. Tissue was stabilized using sub maximal dose of ACh (0.3 μmol). The tissue was considered stabilized when 2-3 doses gave equal response. Tissue was washed after each response.

2. Starting with lowest concentration of Ach. Following cycle was repeated for each dose till ceiling effect was obtained.

Step 1: 0 min	Start normal record
Step II: 30 sec	Add Ach and record stimulatory effect
Step III: 30 sec	washed three times and wait
Step IV: 30 sec	Normal record
Step V: 30 sec	Add jamun extract and record
Step VI; 30 sec	Add Ach and observe effect [19, 20]

(Step V timing could be changed for maximum time (15-20 min) to observe maximum inhibition).

3. Following cycle was repeated in presence of 1, 2, 3 and 5mg/ml of jamun extract.
4. Effect was measured in height of contraction (Mean ± SEM) and expressed as % age of maximum response with Ach (n = 5)
5. Graded dose response curves were plotted on semi log scale.
6. Inhibition of response with each dose of extract was expressed as percentage and significant was calculated using excel and Graph pad Prism.

Group B: Effect of Histamine

1. Tissue was stabilized using sub maximal dose of histamine (0.3 μmol).[19] The tissue was considered stabilized when 2-3 doses gave equal response. Tissue was washed after each response.
2. Experiment was performed in each step as by standard procedure with Ach.

Group C: Effect of Serotonin

1. Tissue was stabilized using sub maximal dose of serotonin (0.3 μmol).[19] The tissue was considered stabilized when 2-3 doses gave equal response. Tissue was washed after each response.[20]

2. Experiment was performed in each step as by standard procedure with Ach.

Group D: Effect of Calcium

1. Effect of extract was seen on Ca^{++} induced contractions in this group.

2. Tissue was pre-treated with K^{+} (50mM) in order to depolarize the tissue, and lower dose (1mg/ml) of plant extract was added in presence of different concentration of K^{+} solution and changes were noted. Dose of extract of Jamun was increased successively obtain maximum inhibitory responses.

3. To make it calcium free the Tyrode's solution was replaced with calcium free Tyrode's Solution.

4. The tissue was pre-treated with different doses of plant extract and waited for 20-30 minutes.

The dose response curve of Ca^{++} was constructed with different doses of extract.

5. Experiment was performed further as by standard procedure with Ach.

RESULTS

When Contraction in rabbit ilium was induced by Acetylcholine:

Results in table 1 shows that contractile response of acetylcholine in absence of jamun extract is 81.248 ± 4.598 while in the presence of Jamun 1mg/ml, contractile response of acetylcholine was decreased; i.e. S.E.M of acetylcholine is 65.250 ± 6.423. On successively increasing the concentration of Jamun extract the response decreased significantly as is shown by the values in table 1.

Table 1: **Effect of Jamun fruit extract on acetylcholine induced contractions**

Parameter	**A** Ach alone(mm) (n = 5)	**B** ACh+ Jamun1mg/ml(mm)	**C** ACh+ Jamun 2mg/ml(mm)	**D** ACh + Jamun 3mg/ml(mm)	**E** ACh + Jamun 5mg/ml(mm)
Contractions Mean ± SEM	81.248 ± 4.598	65.250 ± 6.423	55.916 ± 7.029	47.416 ± 7.437	42.707 ± 6.498

Table 1: Contractile response of acetylcholine alone and with extracts of *Eugenia jambolana* extract

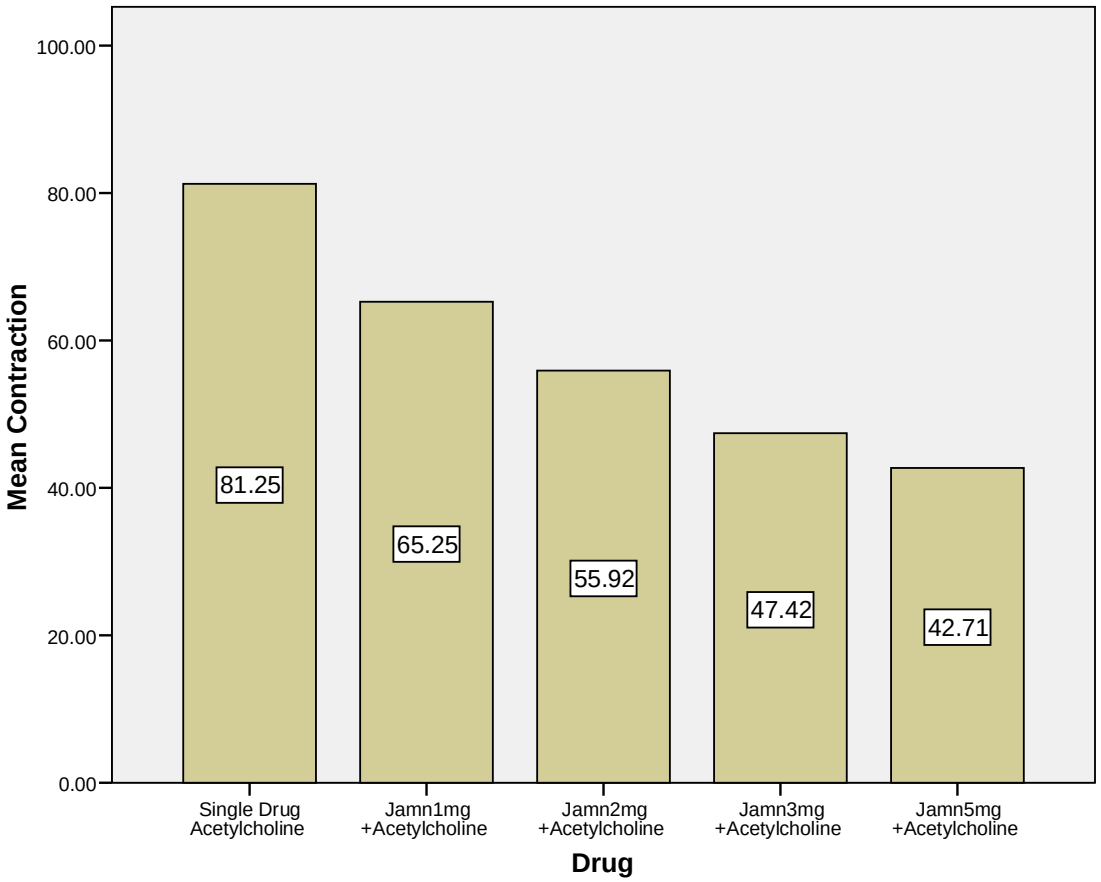
Graph1 shows the inhibitory response of different doses of Jamun fruit extract on acetylcholine induced contractions. Increasing doses of jamun extract reduced the effect of acetylcholine induced contractions. The maximum response of the agonist was regarded as 100% and the subsequent response in the presence of jamun extract was calculated as a % of the control response. It is evident from the table that at high doses, jamun extract completely reversed the effect of ACh.

Table 2 shows the multiple comparisons of the inhibitory effect of Ach and different doses of Jamun extract. Higher doses of jamun extract clearly showed inhibitory effect on the Ach mediated contractions with a $P < 0.05$.

When Contraction in rabbit ilium was induced by Histamine:

Data in the table 3 show the contractile response with histamine in absence of jamun extract, which is 64.501 ±7.053. However, in the presence of increasing concentrations of Jamun, contractile response with histamine is not decreased significantly. Some of the relaxant effect was only observed when excessive dose of jamun was used. When jamun extract dose was increased, some effect was observed. i.e.; P value is 0.166 which is not significant as shown in

Table 3



Graph 1: The inhibitory response of different doses of Jamun fruit extract on acetylcholine induced contractions.

Figure 1shows the effect of increasing concentrations of *Eugenia Jambolana* extracts on the graded dose response sigmoid curves of acetylcholine in rabbit ileum.

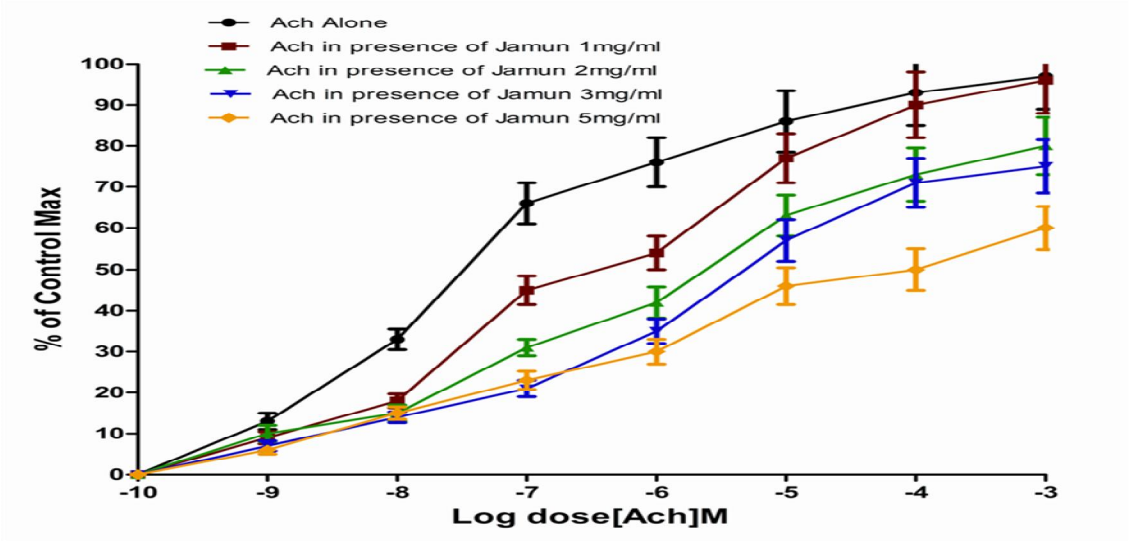


Fig. 1: Response observed on increasing concentrations of the extracts.

Table 2: Multiple comparisons of the inhibitory effect of Ach and different doses of Jamun extract

Multiple Comparisons

Dependent Variable: Contraction
Tukey HSD

(I) Drug	(J) Drug	Mean Difference (I-J)	Std. Error	Sig.
Jamn1mg +Acetylcholine	Jamn2mg +Acetylcholine	9.33375	9.15138	.844
	Jamn3mg +Acetylcholine	17.83375	9.15138	.312
	Jamn5mg +Acetylcholine	22.54250	9.15138	.123
	Single Drug Acetylcholine	-15.99875	9.15138	.419
Jamn2mg +Acetylcholine	Jamn1mg +Acetylcholine	-9.33375	9.15138	.844
	Jamn3mg +Acetylcholine	8.50000	9.15138	.884
	Jamn5mg +Acetylcholine	13.20875	9.15138	.605
	Single Drug Acetylcholine	-25.33250	9.15138	.064
Jamn3mg +Acetylcholine	Jamn1mg +Acetylcholine	-17.83375	9.15138	.312
	Jamn2mg +Acetylcholine	-8.50000	9.15138	.884
	Jamn5mg +Acetylcholine	4.70875	9.15138	.985
	Single Drug Acetylcholine	-33.83250*	9.15138	.006
Jamn5mg +Acetylcholine	Jamn1mg +Acetylcholine	-22.54250	9.15138	.123
	Jamn2mg +Acetylcholine	-13.20875	9.15138	.605
	Jamn3mg +Acetylcholine	-4.70875	9.15138	.985
	Single Drug Acetylcholine	-38.54125*	9.15138	.001
Single Drug Acetylcholine	Jamn1mg +Acetylcholine	15.99875	9.15138	.419
	Jamn2mg +Acetylcholine	25.33250	9.15138	.064
	Jamn3mg +Acetylcholine	33.83250*	9.15138	.006
	Jamn5mg +Acetylcholine	38.54125*	9.15138	.001

. **Table 3: Effect of Jamun fruit extract on histamine induced contractions**.

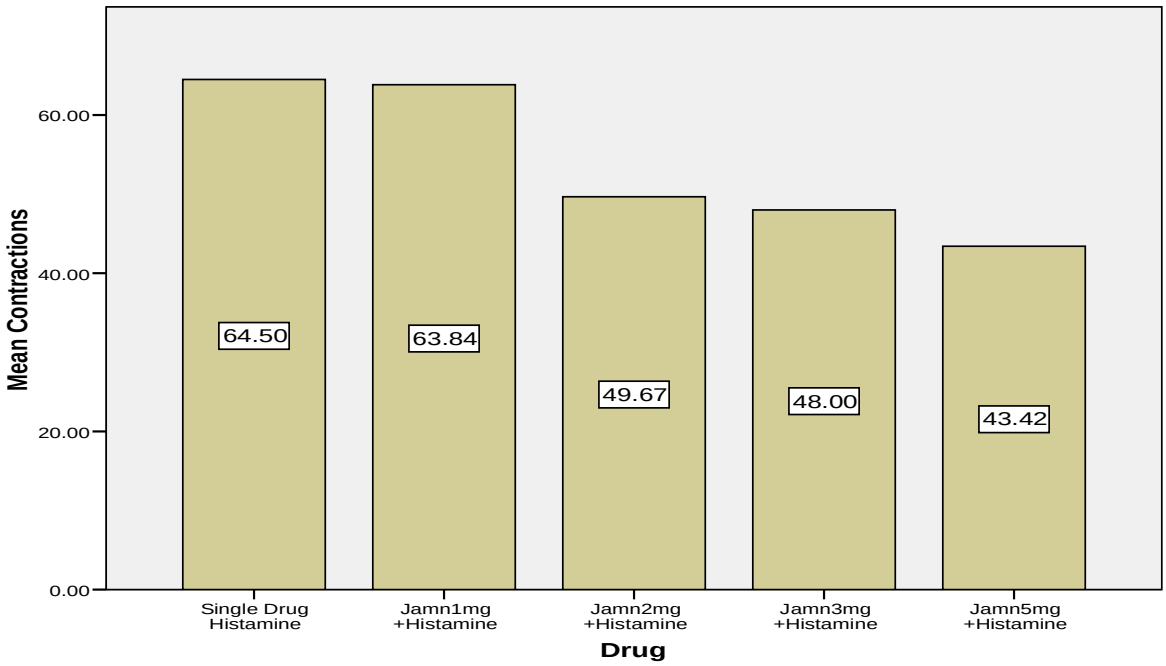
Parameter	**A** (Mm)	**B** (mg/ml)	**C** (2mg/ml)	**D** (3mg/ml)	**E** (5mg/ml)
	Histamine Alone(n=5)	Histamine +Jamun	Histamine +Jamun	Histamine +Jamun	Histamine +Jamun
Contractions Mean ± EM	64.501 ± 7.053	63.835 ± 6.149	49.667±9.432	48.00±7.279	43.416±6.40

Graph 2 shows the inhibitory response of different doses of Jamun fruit extracton histamine induced contractions. P value is greater than 0.05, which represents and insignificant effect but a trend of inhibition is present with larger doses.

The response observed with different concentrations of the extracts on the cumulative dose response sigmoid curves of histamine in rabbit ileum is expressed in figure 2. In the presence of histamine, the inhibitory effect of jamun is significant only at very higher doses of extract. Jamun 3mg/ml & 5mg/ml showed some relaxation of the ileum; spontaneous contractions with the respective value of EC_{50} of 1.9mg/ml (1mg/ml-5mg/ml) with the %age inhibition of 28 ± 3%.

Table 4 shows the multiple comparison of the inhibitory effect of different doses of Jamun fruit

extract. It is evident from the table that only higher doses of jamun extract showed some inhibitory effect, on contractile response induced by histamine.



Graph 2: The inhibitory response of different doses of Jamun fruit extract on histamine induced contractions

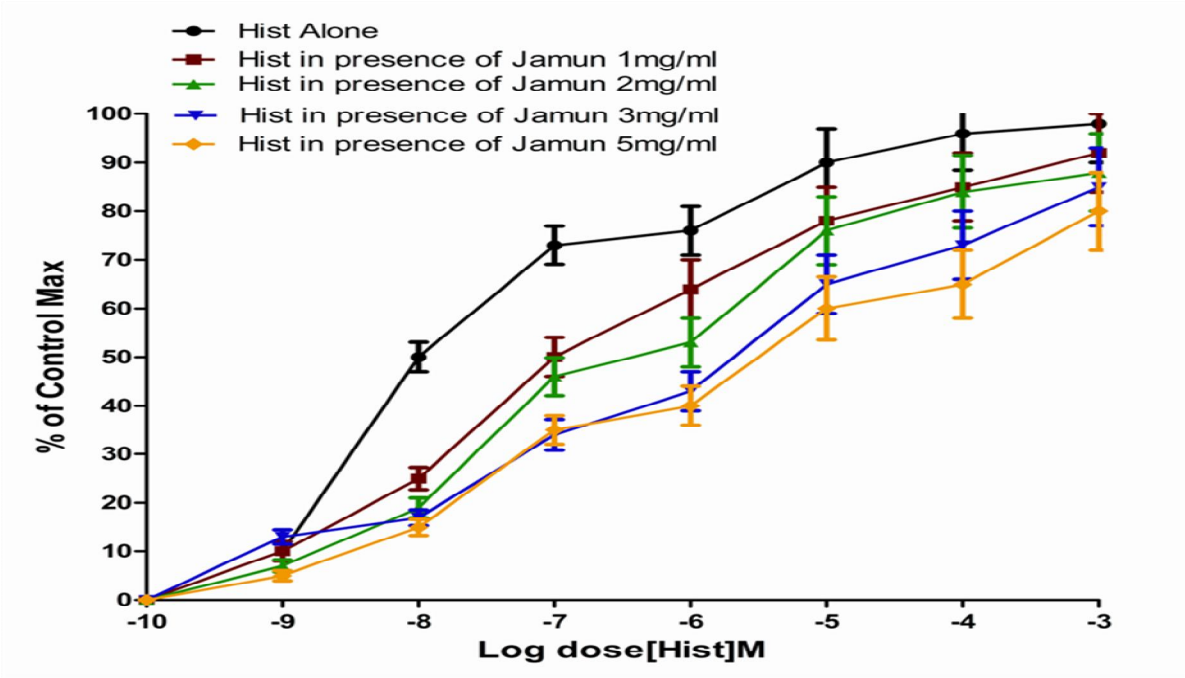


Fig 2: Effect of increasing concentrations of ethanolic extract of *Eugenia Jambolana.*

Multiple Comparisons

Dependent Variable: Contractions
Tukey HSD

(I) Drug	(J) Drug	Mean Difference (I-J)	Std. Error	Sig.
Jamn1mg +Histamine	Jamn2mg +Histamine	14.16750	10.40161	.655
	Jamn3mg +Histamine	15.83500	10.40161	.555
	Jamn5mg +Histamine	20.41875	10.40161	.305
	Single Drug Histamine	-.66625	10.40161	1.000
Jamn2mg +Histamine	Jamn1mg +Histamine	-14.16750	10.40161	.655
	Jamn3mg +Histamine	1.66750	10.40161	1.000
	Jamn5mg +Histamine	6.25125	10.40161	.974
	Single Drug Histamine	-14.83375	10.40161	.615
Jamn3mg +Histamine	Jamn1mg +Histamine	-15.83500	10.40161	.555
	Jamn2mg +Histamine	-1.66750	10.40161	1.000
	Jamn5mg +Histamine	4.58375	10.40161	.992
	Single Drug Histamine	-16.50125	10.40161	.516
Jamn5mg +Histamine	Jamn1mg +Histamine	-20.41875	10.40161	.305
	Jamn2mg +Histamine	-6.25125	10.40161	.974
	Jamn3mg +Histamine	-4.58375	10.40161	.992
	Single Drug Histamine	-21.08500	10.40161	.275
Single Drug Histamine	Jamn1mg +Histamine	.66625	10.40161	1.000
	Jamn2mg +Histamine	14.83375	10.40161	.615
	Jamn3mg +Histamine	16.50125	10.40161	.516
	Jamn5mg +Histamine	21.08500	10.40161	.275

Table 4: Multiple comparison of the inhibitory effect of different doses of Jamun fruit extract

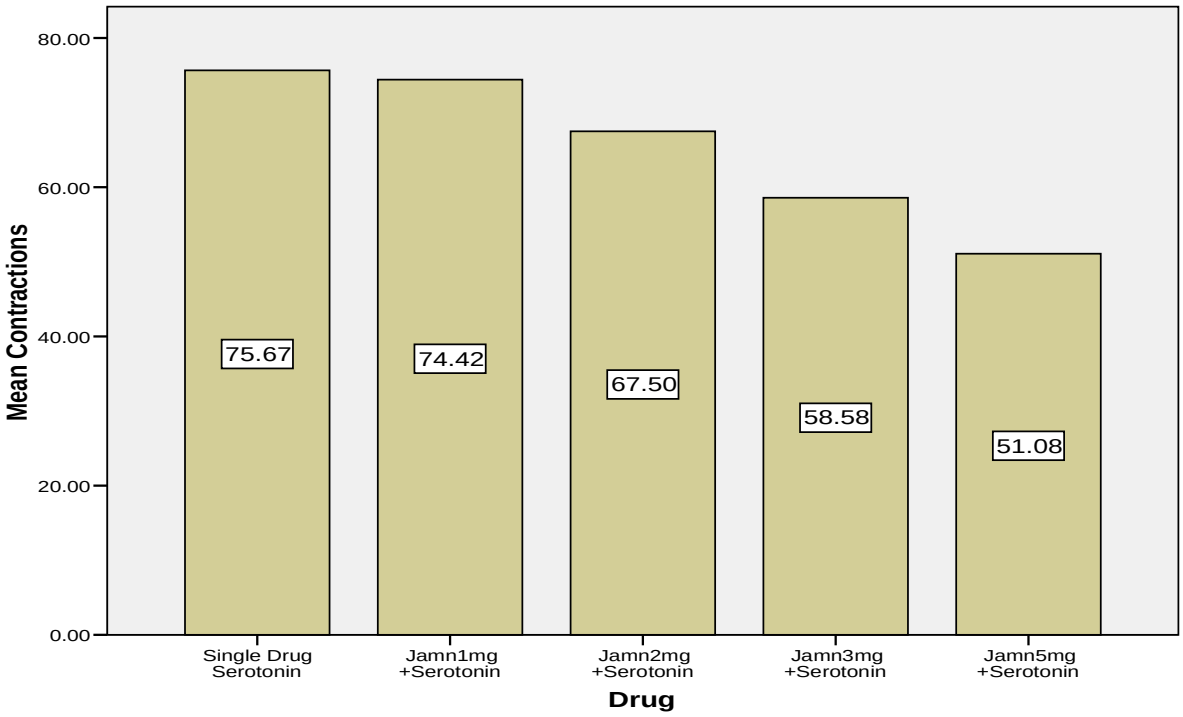
When Contraction in rabbit ilium was induced by serotonin:

Table 5 shows the contractile response with serotonin alone and in the presence of jamun extracts. Some of the relaxant effect was observed only when excessive dose of jamun was used.

Table 5: **Effect of Jamun fruit extract on serotonin induced contractions**

Parameter	**A(mm)**	**B1mg/ml**	**C 2mg/ml**	**D 3mg/ml**	**E 5mg/ml**
	Serotonin Alone	Serotonin + Jamun	Serotonin + Jamun	Serotonin + Jamun	Serotonin + Jamun
Contractions Mean± SEM	75.666 ± 5.736	74.416 ± 5.254	67.496 ± 6.448	58.583 ± 7.151	51.083 ± 8.075

Graph 3 shows the inhibitory response of different doses of Jamun fruit extract on serotonin induced contractions.P values greater than 0.05 represents insignificant effect but a trend of inhibition is present with larger doses statistically different from control.



Graph 3: The inhibitory response of different doses of Jamun fruit extract on serotonin induced contractions.

The response observed with different concentrations of the extracts on the cumulative dose response sigmoid curves of serotonin in rabbit ileum is expressed in figure 3. In the presence of serotonin the inhibitory effect of jamun is not significant but showing inhibition at high doses of extract. Jamun 3mg/ml & 5mg/ml have shown some relaxation of the ileum. Table 6 shows the multiple comparison of the inhibitory effect of different doses of Jamun fruit extract; only higher doses of jamun extract have shown some inhibitory effect, on contractile response induced by serotonin.

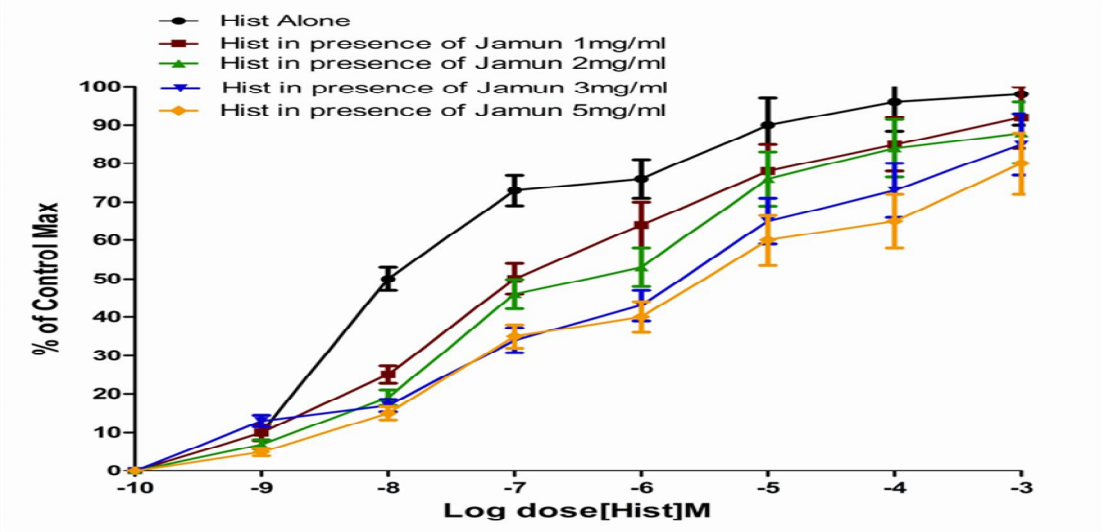


Fig 3: Effect of increasing concentrations of the extracts

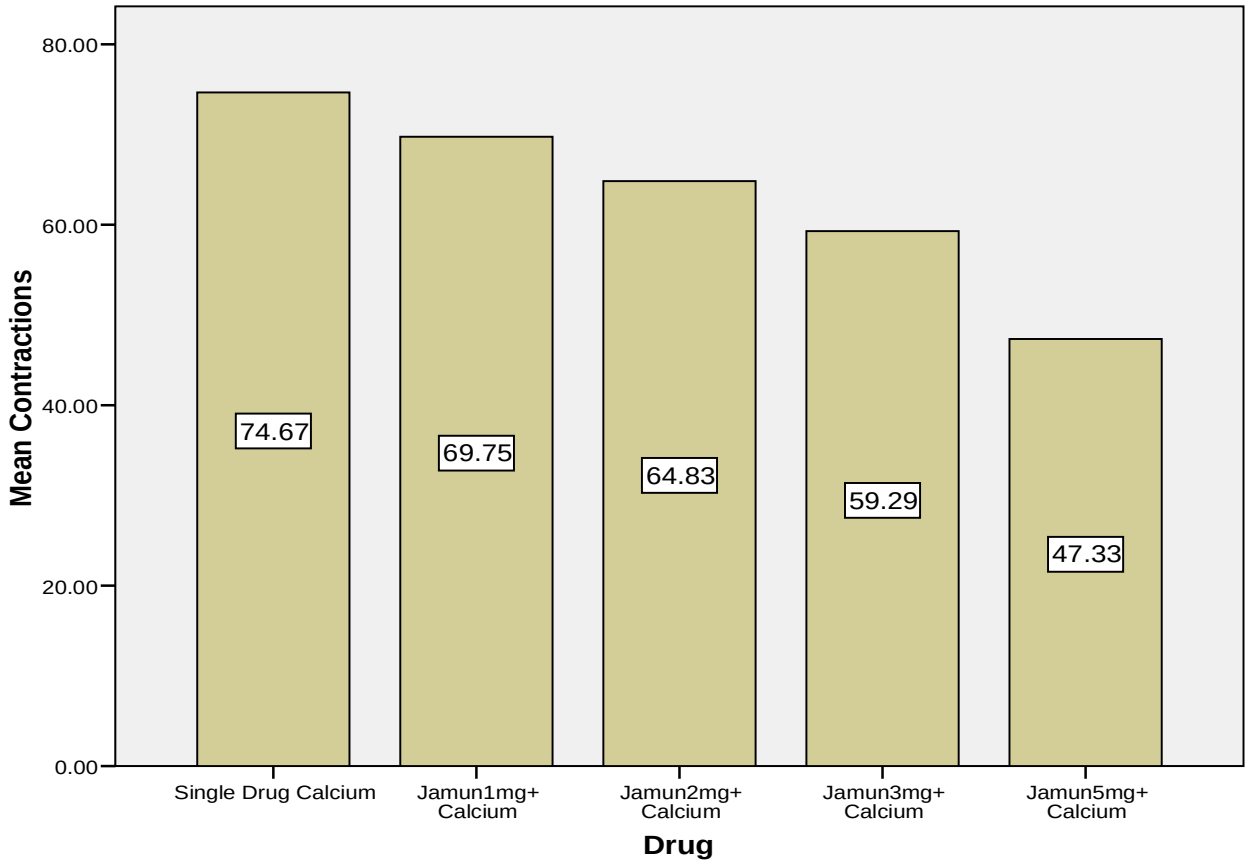
When Contraction in rabbit ilium was induced by Calcium:

The contractile response of calcium with different concentrations of Jamun extract is outlined in table 6. More relaxant effect was observed when excessive dose of jamun was used. The inhibitory response of jamun 3mg/ml & 5mg/ml was more significant i.e. P value is 0.007.

Table 6: **Effect of Jamun fruit extract on calcium induced contractions.**

Parameter	**A (mm)**	**B 1mg/ml**	**C 2mg/ml**	**D 3mg/ml**	**E 5mg/ml**
	calcium alone	calcium+ Jamun	calcium+ Jamun	calcium+ Jamun	Calcium+ Jamun
Contractions Mean± SEM	74.666 ± 6.336	69.748 ± 5.595	64.832 ± 3.797	59.290 ± 3.770	47.332 ± 5.557

Graph 4 shows the inhibitory response of different doses of Jamun fruit extract on calcium induced contractions.



Graph 4: The inhibitory response of different doses of Jamun fruit extract on calcium induced contractions

Figure4 shows that the high dose of jamun extract completely reversed the effect of calcium on rabbit ilium. Table 6 shows the multiple comparisons of different concentrations of jamun extract on calcium induced contractions.

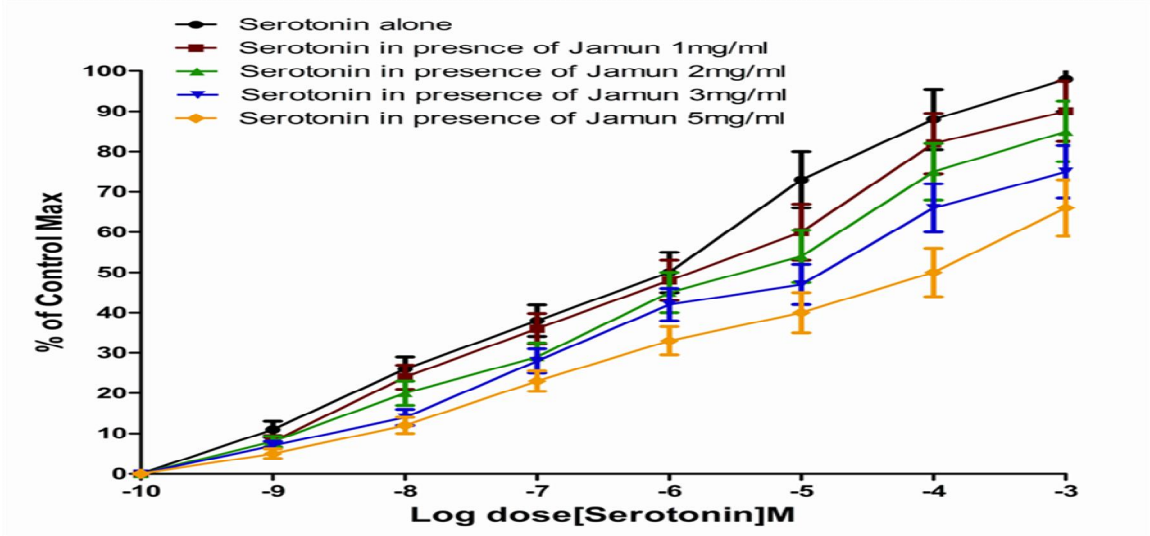
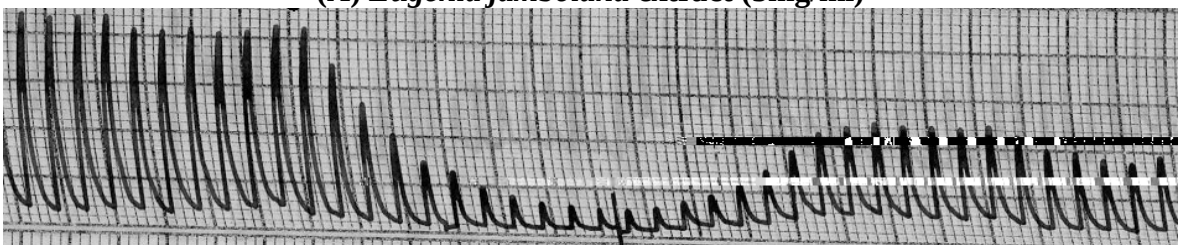


Fig 4: Effect of increasing concentrations of ethanolic extract of *Eugenia Jambolana*

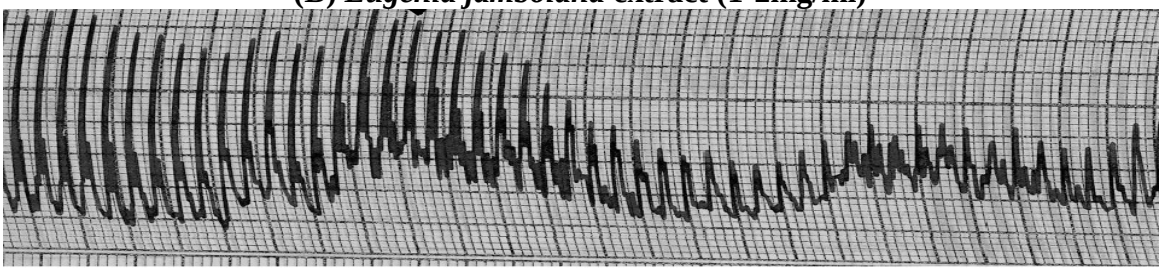
Effect of jamun extract on contractions in ilium induces by Acetylcholine and Calcium:

(A) *Eugenia jambolana* extract (5mg/ml)



Calcium (1x10-5M) (Jamun extract 3mg/ml to 5mg/ml)

(B) *Eugenia jambolana* extract (1-2mg/ml)



Ach (1x10-5M) (Jamun extract 1mg/ml to 5mg/ml)

Fig. 5 A, Dose dependent effect of *Eugenia jambolana* (5mg/ml) on calcium induced contractions showing more inhibition of contractions. B, with low dose i.e., 1-5 mg/ml of extract, and lower effect on acetylcholine induced contractions.

The figure 5shows the analysis of the dose-response curve for contractions and it also shows a significant quantitative difference in the action of the *Eugenia jambolana* on intestinal contractions. Concentrations used were (1mg/ml to 5mg/ml).

Figure 6A shows the graded dose response of different concentrations (1mg/ml-5mg/ml) of ethanolic extract of *Eugenia jambolana* on Ach induced contractions. High dose of extract clearly have shown more inhibition induced by Ach. While figure 6 **B** shows the graded dose response of different concentrations (1mg/ml-5mg/ml) of the extracts on Calcium induced contractions. High dose of extract clearly have shown more inhibition induced by calcium.

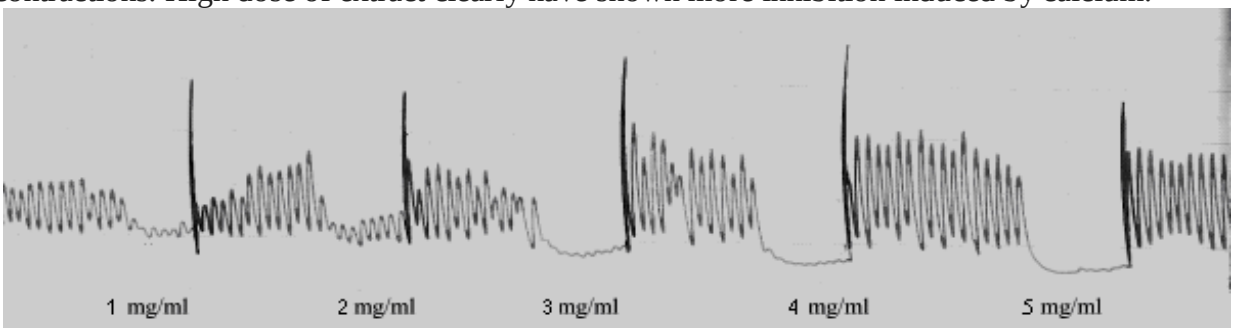
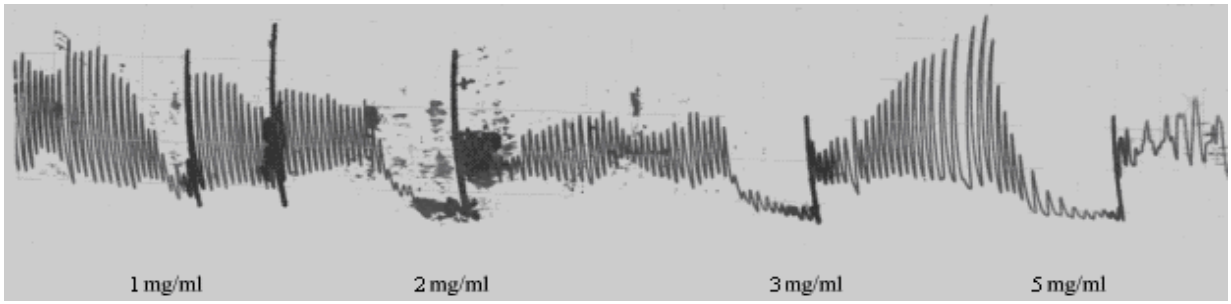


Figure 6A) Graded dose response of different concentrations (1mg/ml-5mg/ml) of *Eugenia jambolana* extract on Ach induced contractions.



B. Graded dose response of different concentrations (1mg/ml-5mg/ml) of *Eugenia jambolana* extract on Calcium induced contractions.

DISCUSSION

Gastrointestinal spasm is the primary marker of various gastrointestinal disorders. Various plant species show the antispasmodic activity by different mechanisms. *Eugenia jambolana* is a locally occurring species of family *Myrtaciae*. It is found commonly in our local flora. This plant is known for its antidiabetic, antidiarrheal, diuretic, anti-inflammatory and cholesterol lowering properties.[4] Phytochemical investigation on gastrointestinal effects has been done on this plant. Keeping these aspects in mind the comparative study of the antispasmodic activity of jamun has been investigated.

Eugenia jambolana fruit extract significantly reduced the GIT spasm and produced a relaxant effect on isolated intestinal tissues of experimental animals. This investigation was done on easily available intestinal segments of locally bread rabbits in the laboratory. In this study we found that *Eugenia jambolana* fruit extract significantly reduced the acetylcholine, histamine,

serotonin & calcium induced contractions on rabbit ileum.

Eugenia jambolana fruit pulp extract was investigated for its possible actions on gut against acetylcholine induced contractions. The fruit extract significantly reversed these contractions, which suggests that the inhibitory action of the extract is due to its interaction with muscarinic receptors. The spasmolytic activity was further confirmed by plotting the acetylcholine response curve with different concentrations. In ileum, jamun fruit extract shifted the dose response curve of acetylcholine. The displacement of Ach dose response curve to the rightward showed a relaxant effect with even a lower dose (1mg/ml) of jamun fruit extract, while more inhibitory response was observed when the dose of extract was increased. In Fig 5 B, with low dose i.e, 1-5 mg/ml of extract, a lower effect on acetylcholine induced contractions was observed. The analysis of the dose-response curve for contractions showed significant quantitative differences in the action of the *Eugenia jambolana* on intestinal contractions.

Eugenia jambolana fruit extract has also shown some inhibitory response against histamine induced contractions. This effect was not significant, when dose response concentration of histamine was constructed against low dose of jamun extract. Some effect was seen when excess dose of jamun was used. EC_{50} was noted as 1.9 mg/ml, showing inhibitory response with higher dose of fruit extract. These finding with jamun extract was justified by Muruganandan, 2001[21]. It is also noted that *Eugenia jambolana* shows inhibitory actions in inflammatory response to histamine, serotonin & prostaglandin 2.

In further investigations, jamun fruit extract was also applied to find out some inhibitory effect on serotonin induced contractions. For this purpose the calculated dose of extract was applied to rabbit ilium but it showed no effect on the tissue, which suggests that the extract doesn't interact with the serotonergic receptors of the ileum.

In further investigations the extract was tested for high K^+ induced accompanied by calcium induced contractions. *Eugenia jambolana* fruit extract reversed both of these contractions. From this observation, it can be inferred that the inhibitory action of the extract is because of its interaction with calcium. The presence of inhibitory activity against calcium was confirmed through constructing the calcium response curve with its different concentrations. The fruit extract shifted the calcium curves to the right ward. The observed response was similar to that observed by Nefidipine, a standard Ca^{++}channel blocker[22]. The fruit extract in the concentration range of l-5mg/ml caused an inhibition of contractions of ileum induced by different concentrations of calcium. Figure 5 A shows a dose dependent effect of *Eugenia jambolana* (5mg/ml) on calcium induced contractions, showing more inhibition of contractions. Similarly, Verapamil also showed a similar inhibitory response, which justifies findings of jamun fruit extract.[23, 24] In order to test the mechanism of spasmolytic effect, a high dose of K^+ that is 50 mM was used. Addition of jamun extract caused a dose dependent inhibition of the pre-treated ileum. This observation was quite similar to that of the action of Verapamil[24].

In calcium group, displacement of calcium dose-response curve was shifted to the right but with greater dose, this inhibitory response was more significant, i.e., respective value of EC_{50} was 1.33mg/ml. In this case percentage inhibition was more i.e., 38% shown as compared to the Inhibition produced by ACh which was 34%. Inhibition in calcium group was more as compared to any other group, possibly giving us a proposed mechanism of jamun effect on isolated tissues as calcium antagonistic activity, which suggests that the inhibitory action of the extract is caused by both its interaction with muscarinic receptors and also by calcium. Both of effects indicate