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***In-vitro* Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn**

Gupta Daksha¹, Chandrashekar^{1*}, Richard Lobo¹, Yogendra² and Gupta Nilesh¹

¹Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India

²Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India

ABSTRACT

The objective of present work was to evaluate the antidiabetic activity of petroleum ether and aqueous extract of stem bark of *Bauhinia purpurea* L. The samples were studied for their effect on inhibition of glycosylation of haemoglobin, glucose transport across yeast cells and α -Amylase inhibition. Inhibition of glycosylation of haemoglobin and α -Amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. From the results of the study, it is inferred that, *B. purpurea* stem bark possesses antidiabetic activity. However, these effects need to be confirmed using in vivo models and clinical trials for its effective utilization as therapeutic agents.

Keywords: *Bauhinia purpurea*, antidiabetic, Acarbose, Metronidazole.

INTRODUCTION

The well-known and well established genus *Bauhinia* comprises of trees and shrubs that grow in warm climate. It is rare in southern most districts, 5-7m tall tree in deciduous forests which is often planted in gardens along roadside for its large purple beat flowers. The leaves are 10–20 cm long and broad, rounded, alternate and bilobed at the base and apex. The flowers are conspicuous, pink, and fragrant, with five petals. The fruit is a pod 30 cm long, containing 12 to 16 seeds and have long seeds as pea. Flowers and fruits appear in the month of December. Synonyms/Common names of plant *Bauhinia purpurea*-Purple Orchid tree, Mandaram, etc. [1, 2].

Geographical distribution- *B. purpurea* is native to South China (which includes Hong Kong) and South-eastern Asia and it is found throughout India, ascending to an altitude of 1300m in Himalaya [3].

The different species of *Bauhinia* viz., *B. reticulata*, *B. rufescens* and *B. variegata* have been traditionally used to treat roundworm infections, conjunctivitis, anthrax, ulcerations, dysentery, blood-poisoning, leprosy, lung and skin diseases in Africa; while in India, extracts of the bark of *B. variegata* is used for treatment of cancer.

Leaves are used as a plate for food and fodder during lean period [4], bark used as fibre, in dyeing and tannin extraction and its decoction is used in diarrhoea. The decoction of root is used for expelling gases, flatulence and gripping pain from the stomach and bowels. The decoction of flower works as a maturant for boils and abscesses^[5]. Root bark of *Bauhinia purpurea* L. contains flavones glycoside [5].

The present work was under taken to explore the *in-vitro* antidiabetic potential of the stem bark of *Bauhinia purpurea* (Fabaceae). It has been reported that the pharmacological significance was noted due to the presence of various bioactive compounds in the *Bauhinia* species such as flavonoids [6], sesquiterpenes [7], steroidal glycosides,

lactones, saponins, and tannins [8]. The aerial parts of the plant *Bauhinia purpurea* are reported to contain flavone glycosides, foliar flavonoids, 6-butyl-3-hydroxy flavonone, amino acid, phenyl fatty ester, lutine and β sitosterol [9, 10, 11, 12, 13, 14]. Leaves of *Bauhinia purpurea* showed the presence of flavonoids mainly dimeric flavonoids (biflavonoids) and reported to have the antioxidant property due to the presence of phenolic ring in the moiety.

MATERIALS AND METHODS

The stem bark of *Bauhinia purpurea* L. were collected from local area of Manipal, Karnataka, India during August 2011 and were authenticated by Dr.Chandrashekar KS, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal.

Preparation of extracts [15]

Petroleum ether extract- The stem bark of *Bauhinia purpurea* L. were dried in hot air oven at 50-60° C for 5-6 days and then grinded to a fine powder in a grinder. The powdered plant material (2.5 kg) was subjected to maceration using petroleum ether for 4 days, then filtered with muslin cloth and evaporated to dryness. Extract was kept in desiccator.

Aqueous extract- The stem bark of *Bauhinia purpurea* L. were dried in hot air oven at 50-60° C for 5-6 days and then grinded to a fine powder in a grinder. The powdered plant material (250g) was subjected to maceration using distilled water for 4 days, then filtered with muslin cloth and evaporated to dryness. Extract was kept in desiccator.

In-vitro Antidiabetic activity

1. Non-enzymatic glycosylation of haemoglobin method [16]

Antidiabetic activity of stem bark of *Bauhinia purpurea* L. were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. Petroleum ether extract of stem bark of *Bauhinia purpurea* L. was weighed and dissolved in DMSO to obtain stock solution and then 1-5 μ g/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Troxal) was used as a standard drug for assay. % inhibition was calculated as-

$$\% \text{ inhibition} = \frac{A_s - A_c}{A_s} * 100$$

A_c is Absorbance of Control

A_s is Absorbance of Sample

Statistical Analysis- All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at $p \leq 0.05$. Graphs were plotted using Origin 8.1 software.

2. Glucose uptake in Yeast cells [17]

Yeast cells were prepared according to the method of Yeast cells [18]. Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000 \times g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 μ l of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 \times g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula-

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} * 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Statistical Analysis- All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at $p \leq 0.05$. Graphs were plotted using Origin 8.1 software.

3. A- Amylase inhibition[19, 20]

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by Fuwa 1954[21] and later employed by others for determination of amylase activity in plant extracts[22] with some modifications. In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of drug solution (Acarbose std drug/ petroleum ether extract/ aqueous extract) of four different concentration such as 250, 500, 750 and 1000 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water).

The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy.

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} * 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Statistical Analysis- All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at $p \leq 0.05$. Graphs were plotted using Origin 8.1 software.

RESULTS AND DISCUSSION

In-vitro Non-enzymatic glycosylation of haemoglobin method

The stem bark of *Bauhinia purpurea* L. shows a good antidiabetic activity. The percentage inhibition of glycosylation is dose dependent, as dose increases, inhibition increases (figure 1). Because as the concentration of drug increases formation of glucose-haemoglobin complex decreases and free haemoglobin increases, which show the inhibition of glycosylated haemoglobin. The activity of petroleum ether and aqueous extracts of stem bark of *Bauhinia purpurea* L. was found to be better than standard drug acarbose (figure 1). This test is not important to detect diabetes. It is more important to judge the control of diabetes. The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose and form an abduct Alc. The greater the blood-glucose concentration, the greater is the amount of glucose-bound (called glycosylated) haemoglobin. Such glucose haemoglobin linkage is quite stable and lasts for 60 to 120 days (the life-span of red blood corpuscles). Thus the amount of glycosylated haemoglobin is a sure guide to the concentration of glucose in the blood (i.e., the degree of control over the disease achieved). Amount of Glycated haemoglobin should not be more than 12%.

Table 1: *In-vitro* Non-enzymatic glycosylation of haemoglobin method

S. No	Conc. ⁿ (µg/ml)	Blank	STD		PE		AE	
			Abs	% inh	Abs	% inh	Abs	% inh
1	0.00	Abs(0.057 ± 0.002)	0.089 ± 0.004*	35.9	0.207 ± 0.004*	74.8	0.175 ± 0.002*	70.2
2	250		0.093 ± 0.005*	44.0	0.353 ± 0.005*	85.2	0.225 ± 0.004*	76.8
3	500		0.103 ± 0.003*	49.5	0.483 ± 0.005*	89.2	0.265 ± 0.005*	80.3
4	750		0.131 ± 0.005*	60.0	0.634 ± 0.005*	91.7	0.392 ± 0.004*	86.7
5	1000							

STD- Standard, PE- Petroleum Ether extract, AE- Aqueous extract, Abs- Absorbance, % inh- % inhibition, Conc.ⁿ- Concentration.

* $P < 0.05$ when compared to control, Values are expressed as mean ± SEM.

In-vitro Glucose uptake in Yeast cells method

The rate of glucose transport across cell membrane in yeast cells system is presented in Fig. 2, 3 and 4. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the 3 glucose concentrations. The petroleum ether extract exhibited significantly higher activity than aqueous extract at all concentrations. However the highest uptake of glucose was seen in 10mM Glucose concentration.

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds/ medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is

by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose[18, 24, and 25].

Figure 1: Non-enzymatic glycosylation of haemoglobin

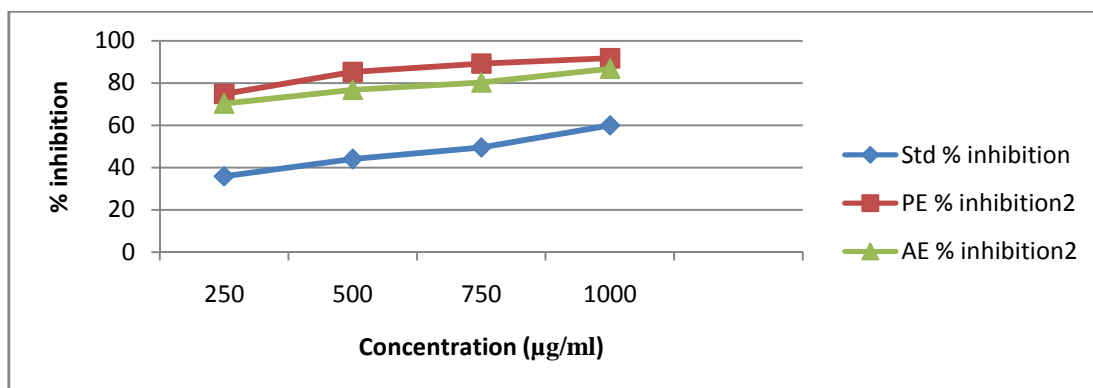


Table 2: % inhibition of Glucose uptake in 20mM glucose concentrations

Glucose 20mM							
Blank	Conc. ⁿ	STD		PE		AE	
	µg/ml	Abs	% inh	Abs	% inh	Abs	% inh
Abs(0.046± 0.008)	100	0.145± 0.007*	68	0.261± 0.005*	82	0.256± 0.003*	82
	200	0.182± 0.004*	74	0.520± 0.01*	91	0.334± 0.006*	86%
	300	0.212± 0.006*	78	1.135± 0.002*	95.9	0.394± 0.008*	88.3
	400	0.246± 0.005*	81	1.207± 0.005*	96.1	0.610± 0.005*	92.4
	500	0.354± 0.002*	87	1.240± 0.005*	96.3	0.608± 0.01*	92.4

*P<0.05 when compared to control, Values are expressed as mean ± SEM

Table 3: % inhibition of Glucose uptake in 10mM glucose concentrations

Glucose 10mM							
Blank	Conc. ⁿ	STD		PE		AE	
	µg/ml	Abs	% inh	Abs	% inh	Abs	% inh
Abs(0.066± 0.002)	100	0.208± 0.004*	68.2	0.432± 0.005*	84.7	0.489± 0.004*	86.5
	200	0.259± 0.01*	74.5	0.706± 0.002*	90.6	0.558± 0.004*	88.1
	300	0.288± 0.001*	77	1.476± 0.01*	95.5	0.612± 0.004*	89.2
	400	0.358± 0.004*	81.5	1.318± 0.004*	94.9	0.941± 0.005*	92.9
	500	0.417± 0.01*	84.1	1.619± 0.005*	96	0.987± 0.004*	93.3

STD- Standard, PE- Petroleum Ether extract, AE- Aqueous extract, Abs- Absorbance, % inh- % inhibition, Conc.ⁿ- Concentration.

*P<0.05 when compared to control, Values are expressed as mean ± SEM

Table 4: % inhibition of Glucose uptake in 5mM glucose concentrations

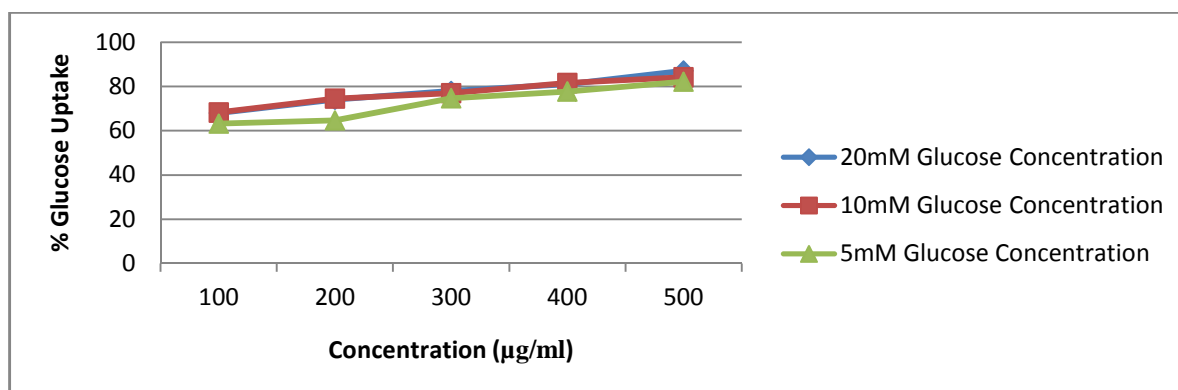
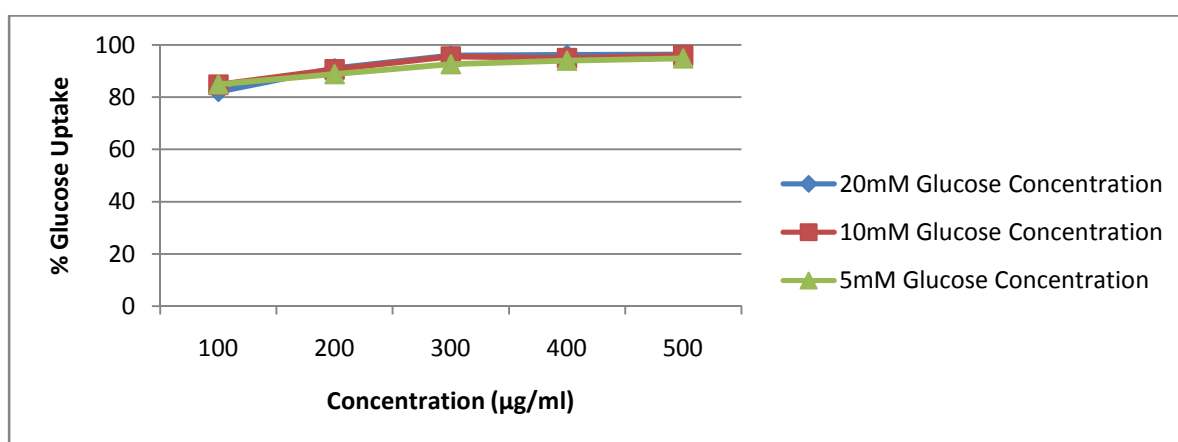
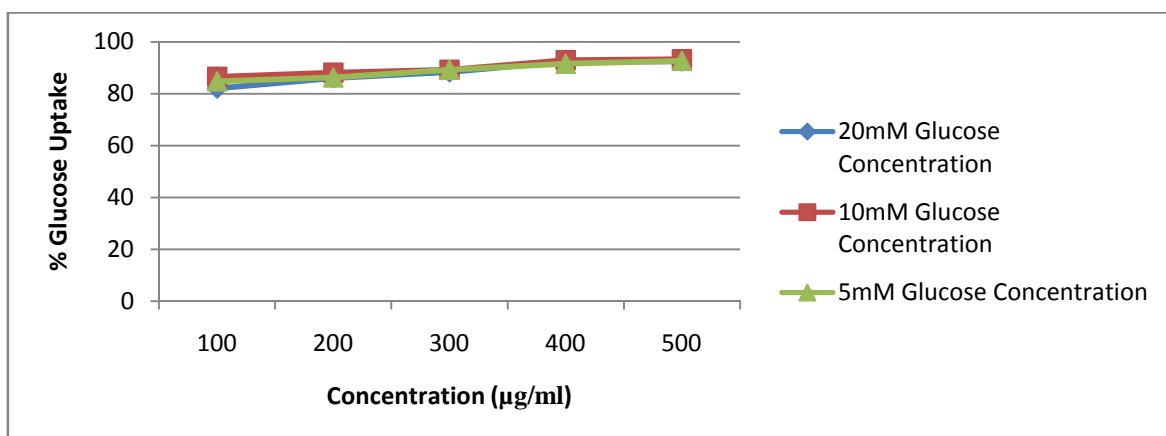
Glucose 5mM							
Blank	Conc. ⁿ	STD		PE		AE	
	µg/ml	Abs	% inh	Abs	% inh	Abs	% inh
Abs(0.085± 0.007)	100	0.231± 0.001*	63.2	0.564± 0.002*	84.9	0.561± 0.005*	84.8
	200	0.241± 0.004*	64.7	0.761± 0.004*	88.8	0.621± 0.005*	86.3
	300	0.260± 0.009*	74.6	1.519± 0.005*	92.6	0.789± 0.007*	89.2
	400	0.381± 0.015*	77.6	1.433± 0.005*	94	1.022± 0.003*	91.6
	500	0.480± 0.005*	82.2	1.641± 0.005*	94.8	1.168± 0.01*	92.7

STD- Standard, PE- Petroleum Ether extract, AE- Aqueous extract, Abs- Absorbance, % inh- % inhibition, Conc.ⁿ- Concentration.

*P<0.05 when compared to control, Values are expressed as mean ± SEM

Alpha- amylase inhibition method

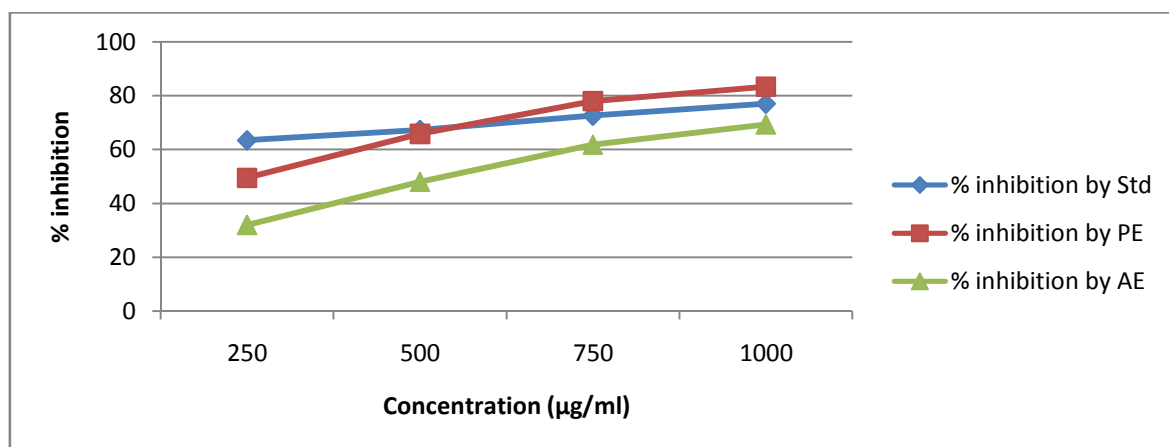
Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. As the result shows petroleum ether and aqueous extract of *B. purpurea* shows significant activity as compared to acarbose standard drug, and 500 and 1000 µg/ml concentration of petroleum ether extract shows greater activity than Acarbose.

Figure 2: Absorbance of Standard drug in different glucose concentration**Figure 3: Absorbance of Petroleum ether extract of *Bauhinia purpurea* in different glucose concentration****Figure 4: Absorbance of Aqueous extract of *Bauhinia purpurea* in different glucose concentration****Table 5: Alpha Amylase inhibition**

S. No	Conc. ⁿ (µg/ml)	Blank	STD		PE		AE	
			Abs	% inh	Abs	% inh	Abs	% inh
1	0.00	Abs(0.053 ± 0.002)						
2	250		0.145 ± 0.005*	63.4	0.105 ± 0.009*	49.5	0.078 ± 0.002*	32.0
3	500		0.162 ± 0.002*	67.2	0.155 ± 0.004*	65.8	0.102 ± 0.005*	48.0
4	750		0.194 ± 0.07*	72.6	0.242 ± 0.003*	78.0	0.139 ± 0.005*	61.8
5	1000		0.227 ± 0.004*	77.0	0.318 ± 0.005*	83.3	0.173 ± 0.003*	69.3

STD- Standard, PE- Petroleum Ether extract, AE- Aqueous extract, Abs- Absorbance, % inh- % inhibition, Conc.ⁿ- Concentration.
*P<0.05 when compared to control, Values are expressed as mean ± SEM

Figure 5: Alpha amylase inhibition method



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