ANTIHYPERGLYCEMIC AND LIPID LOWERING ACTIVITIES OF ETHANOLIC EXTRACT OF *ERIOBOTRYA JAPONICA* SEEDS IN ALLOXAN INDUCED DIABETIC RATS

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Abstract:
Diabetes is a metabolic disorder characterized by hyperglycemia, hypertriglyceridemia and hypercholesterolemia, resulting from defects in insulin secretion or action or both. India is known as the diabetic capital of the world. The study of plants having antihyperglycemic and hypolipidemic activities gives a new approach in the treatment of diabetes mellitus. The study was carried out to evaluate the antidiabetic and hypolipidemic activity of ethanolic extract of seeds of *Eriobotrya japonica* (EBJS) in alloxan induced diabetic albino rats. Diabetes was induced in albino rats by administration of alloxan monohydrate (120 mg/kg) by intraperitoneal route. Rats were divided into 6 groups of 6 animals each. Group I served as non-diabetic control, Group II as diabetic control, Group III[rd] received antidiabetic standard drug (10 mg/kg of glibenclamide) Group IV and Group V received 100 and 200 mg/kg b.w of EBJS. Blood samples were analysed for blood glucose on day 1,4,7 and day 10 [th] and lipid profile was analysed on day 10. All the values are expressed as Mean±SEM. The results were subjected to statistical analysis using one-way ANOVA followed by students t test. $p<0.001$ was considered highly significant. The ethanolic extract of EBJS at the dose of 200 mg/kg body weight showed highly significant reduction in blood glucose and serum lipid profile levels in alloxan induced diabetic rats. It is concluded that ethanolic extract of EBJS is effective in controlling blood glucose levels and in improving lipid profile in diabetic rats.

Key Words: Diabetes mellitus, blood glucose level, Lipid Profile, *Eriobotrya japonica* seeds.

Introduction

Diabetes mellitus is a major degenerative disease in the world today affecting at least 15 million people. Diabetes mellitus is associated with long term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others. It is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells It is ranked third among the leading causes of death when its fatal complications are taken into account.Today in India alone there are more than 4.00 crore diabetics and the number is going to be around 9.00 crore by 2030. Over 7.20 lakh Indians die every year due to diabetes. People with diabetes are 2-4 times more likely to develop heart diseases [1]
Efforts are ongoing to understand and manage diabetes mellitus because the disease and disease related complications are increasing day by day. In spite of presence of large number of medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat and this disease. India has 45,000 plant species and several thousand have medicinal properties. More than 800 plant species have anti- diabetic activity. There has been great demand for plant products due to low cost, easy availability and lesser side effects. For this plant materials are continuously scrutinized and explored for their effect as antidiabetic agents.

One of the plants is ***Eriobotrya japonica*** locally known as loquat, has been used since olden times in the ethno medicine for treating diseases. The plant is reported to possess antioxidant[2,3,4]antiviral[5,6] cytotoxic[7] hepatoprotective[8] anti-inflammatory/antitussive activity [9,10,11.]
There is dearth of reports on the antidiabetic and hypolipidemic effects of the seeds of this plant.

The present study was aimed to investigate antidiabetic and hypolipidemic activity of ethanolic extract of *Eriobotrya japonica* seeds in alloxan induced diabetic rats.

Materials And Methods

Plant Material:

The fruits of *Eriobotrya japonica* (family Rosaceae) were collected from Shalimar area of the district, Srinagar, during the months of April to June and authenticated by a plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar. The identification was done on the basis of the characters described by Kirtikar and Basu, 1935. A sample of the plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under voucher specimen number 1012(KASH) dated 15-09-2008 for future reference. The seeds were separated from fruits, and dried in a well ventilated room with outside temperature ranging between 18 to 32^0 C .

Preparation of the extract:

The seeds were coarsely powdered and 500 gm of the material was allowed to macerate for 48 hrs with 50% ethanol, with occasional shaking. After 48 hrs, the ethanolic extract was filtered through Whatmans filter paper. The plant material was then macerated again with fresh 50% ethanol and the filtrate obtained from the first and the second maceration was then combined and the solvent was recovered . After the recovery of alcohol, the extract was then evaporated to dryness and the yield was noted. The procedure was repeated with 500 gm of the material again. The extract was refrigerated at 4^0 C for future use in experimental studies.

Phytochemical Screening:

The extract obtained was subjected to qualitative tests for identification of different constituents like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids , by using simple and standard qualitative methods described by Trease and Evans[12]

Pharmacological Study:

Animals

Healthy albino rats of either sex weighing about 180-210 g were used during the study. The animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu & were housed in clean polypropylene cages. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature ranging from 18 to 32^o C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Ashirwad Industries) and water *ad-libitum* under strict hygienic conditions. All procedures were performed in accordance to CPCSEA guidelines after approval from the Institutional Animal and Ethics Committee (IAEC) of the Department of Pharmaceutical Sciences, University of Kashmir[No. F-IAEC (Pharm.Sc) APPROVAL/2008/4 Dated Oct 23rd,2008]

Induction of diabetes:

Alloxan monohydrate was obtained from S.D Fine Chemical, Mumbai, India. All the other chemicals used were of analytical grade and were acquired from commercial sources. A single dose (120mg/kg, b.w, i.p) of alloxan monohydrate in sterile saline was used for the induction of diabetes in rats after overnight fasting. After one hour of alloxan administration, the animals were fed standard pellets and water *ad libitum*. After 5 days of alloxan administration, animals showing blood glucose levels above 250 mg/dl were selected for the study. Extract of EBJS was administered at two dose levels 100 and 200 mg/kg[13]

Experimental design:

Rats fasted overnight for 12 hrs were randomly divided into 6 groups of 6 rats per group.The various groups were:-

Group I- Served as normal control and received only 0.2 ml of 2% aqueous gum acacia

Group II- Served as diabetic control and received only alloxan monohydrate and 2% aqueous gum acacia.

Group III- Alloxan monohydrate + Glibenclamide (10 mg/kg, p.o) and served as Standard Antidiabetic drug.

Group IV- Alloxan monohydrate + 50% Ethanolic extract of EBJS (100 mg/kg, p.o)

Group V- Alloxan monohydrate +50% Ethanolic extract of EBJS (200 mg/kg, p.o)

The treatment (p.o) of the ethanolic extract was started the same day except normal control and diabetic control groups which received only 0.2 ml of 2% aqueous gum acacia for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated on 1st , 4th, 7th and 10th day of the treatment.

Sample Collection:

Blood samples were collected by pricking the tail from overnight fasted rats and blood glucose levels were estimated using One Touch Ultra glucose strips (Johnson & Johnson Ltd) on 1st, 4th, and 7th day.

Estimation of biochemical parameters:

On day 10th , blood was collected from overnight fasted rats under ether anesthesia by cardiac puncture and was kept aside for 30 min for clotting. By centrifuging the same sample at 6000 rpm for 20 min, the serum was separated and was analyzed for blood glucose[14], total cholesterol[15], triglycerides[16], HDL cholesterol[17] and LDL cholesterol[18]

Statistical analysis:

All the values are expressed as mean±SEM. The results were subjected to statistical analysis using one-way ANOVA followed by students t test.$p<0.001$ was considered highly significant.

Results

Phytochemical analysis:

Phytochemical analysis of the extract showed the presence of alkaloids, flavonoids and glycosides **(Table-1)**

Antihyperglycemic study:

Blood glucose levels showed a highly significant decrease in groups III,IV,V ($p<0.001$) as compared to group II (Diabetic control). A highly significant increase in blood glucose levels was seen in diabetic group as compared to normal control group I ($p< 0.001$). **(Table 2)**

Effect of 50% ethanolic extract of *Eriobotrya japonica* seeds on biochemical parameters in alloxan induced diabetic rats.

Serum total cholesterol levels showed a highly significant decrease in groups IV and V ($p<0.001$) as compared to diabetic control (Group II). Serum triglyceride levels showed a highly significant decrease in groups IV and V($p< 0.01$) . HDL levels showed a non significant increase in groups III,IV and V. LDL levels showed a significant decrease in groups IV and V groups**.(Table 3)**

Effect of 50% ethanolic extract of *Eriobotrya japonica* seeds on body weight in alloxan induced diabetic rats

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight after 10 days. ($p< 0.001$) Alloxan mediated body weight reduction was reversed by the ethanolic extract in dose dependant fashion 100 mg/kg and 200 mg/kg b.w showed a highly significant increase in body weight ($p <0.001$) . The effect of extract at 200 mg/kg on body weight of the animals was also found statistically significant. Results are shown(**Table 4)**

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. It has a destructive effect of the beta cells of the pancreas[19] Alloxan causes a massive reduction in insulin release by the destruction of beta-cells of the islets of Langerhans thereby inducing hyperglycemia[20]. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose and increased lipid profile.

The results of the present study found that 50% ethanolic extract of *Eriobotrya japonica* seeds reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present investigation 50% ethanolic extract of *Eriobotrya japonica* seeds demonstrated the significant antihyperglycemic activity. The results from the present study also indicate that ethanolic extract can reduce the levels of serum lipids. The antihyperglycemic effect of the ethanolic extract may be due to the enhanced secretion of insulin

from the beta cells of pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity.

Elevated plasma total cholesterol, triglycerides and LDL cholesterol are the major risk factors of cardiovascular diseases. Diabetic rats showed elevated plasma cholesterol, triglycerides and LDL cholesterol. Ethanolic extract in the dose of 200 mg/kg reduced the lipid profile along with the reduction in the blood glucose levels.

The literature reports reveal that flavonoids present in the plant extract known to possess antihyperglycemic and hypolipidemic activity. In the present investigation also the observed antihyperglycemic and hypolipidemic potential of test extract may be due to presence of similar phytoconstituents which was evident by preliminary phytochemical screening. Since many antihyperglycemic drugs do not correct dyslipidemia, the observed hypolipidemic effects of the plant extract in diabetic rats makes EBJS quite important in the management of diabetes. Since there is a strong well-established link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease, effect of the plant extract on weight loss/gain needs to be explored on scientific base.

Conclusion

From the study, it can be concluded that the 50% ethanolic extract of *Eriobotrya japonica* seeds has beneficial effects on blood glucose levels as well as improving hyperlipidemia and other metabolic aberrations. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as an therapeutic target in diabetics research.

Acknowledgement

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Table -1. Phytochemical Results of *Eriobotrya japonica* seeds
Table 1

S.No	Phytoconstituents	Results
1	Tannins	_
2	Alkaloids	+
3	Saponins	_
4	Glycosides	+
5	Terpenes	_
6	Phenolics	_
7	Flavonoids	+
8	Carbohydrates	_
9	Proteins	_
10	Steroids	_

Table-2 Effect of 50% ethanolic extract of *Eriobotrya japonica* (EBJS) seeds on fasting blood glucose level
(mg/dl) in alloxan induced diabetic rats

Table 2

Group	Treatment	1st day	4th day	7th day	10th day
I	Normal control 0.2 ml of 2% aqueous Gum acacia	85.07± 4.35	86.16±4.43	84.82±5.96	84.71±6.11
II	Diabetic control (Vehicle) 0.2 ml of 2% aqueous gum acacia	261.47±8.37	264.28±8.29	268.03±8.48	271.33±8.18***
III	Alloxan monohydrate + glibenclamide (10 mg/kg)	200.37±5.25	141.18±2.43	124.52±2.00**	114.84±3.21***
IV	Alloxan monohydrate+ 50% Ethanolic extract (EBJS,100 mg/kg)	204.27±4.25	156.99±2.48	156.21±2.85**	155.54±2.54***
V	Alloxan monohydrate+ 50% Ethanolic extract(EBJS,200 mg/kg)	202.95±4.95	144.67±1.56	128.22±5.55**	120.74±6.13***

Animal: Albino Rats Alloxan: 120 mg/kg.i.p
Extract: p.o.

Values are Mean ±S.E.M n=6; except in Group V where n=5
**p<0.01 significant
***P< 0.001 highly significant

Groups III,IV,V, vs Diabetic Control (Group II) and Group I vs Group II on 10th day

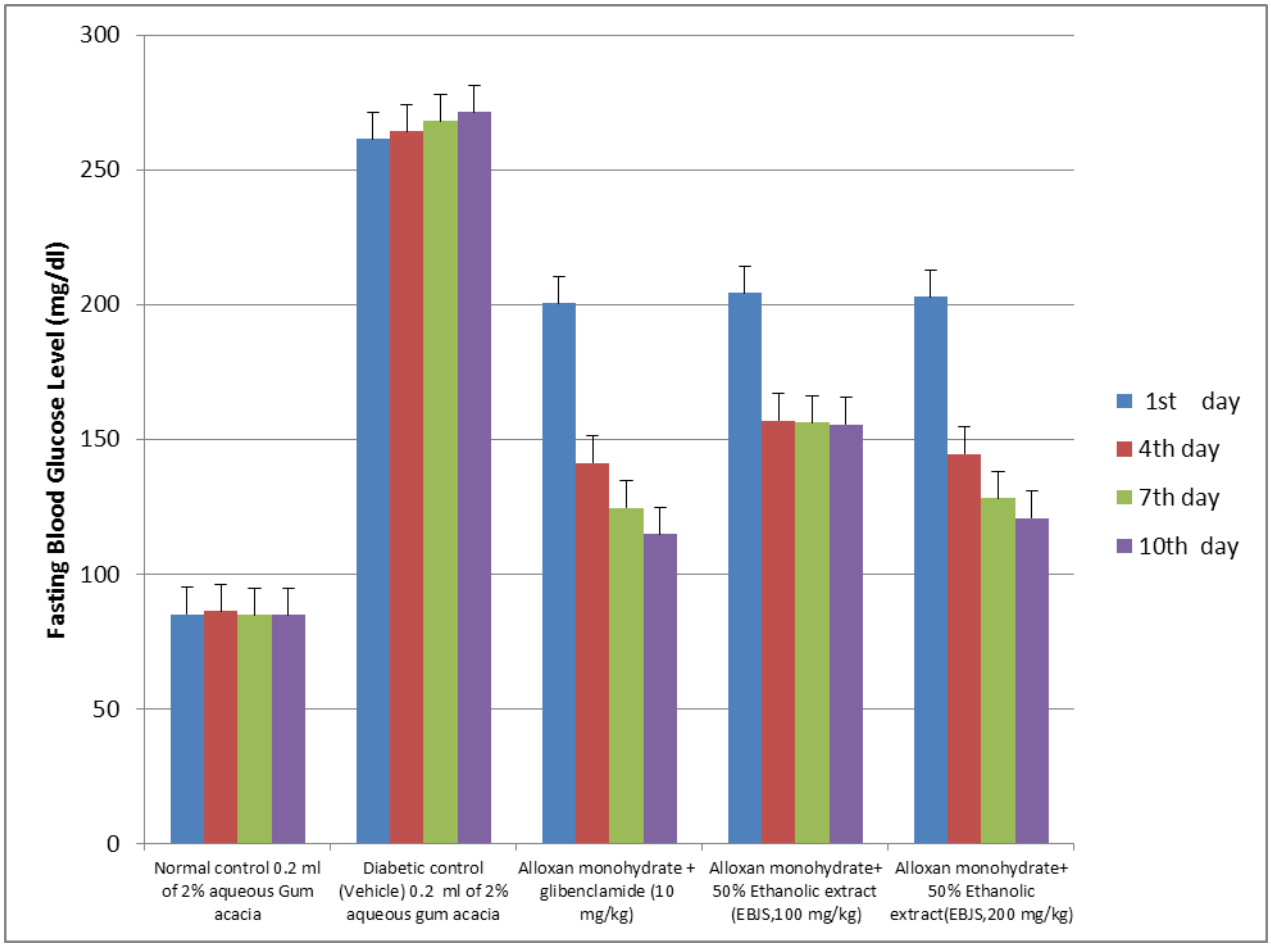

Fig 1: Effect of 50% ethanolic extract of *Eriobotrya japonica* (EBJS) seeds on fasting blood glucose level (mg/dl) in alloxan induced diabetic rats after 10 days of dosing.Each bar represents the mean ± SEM.

Table-3. Effect of 50% ethanolic extract of *Eriobotrya japonica* seeds on lipid profile in alloxan induced diabetic rats.

Table 3

Group	Treatment	Serumtotal Cholesterol mg/dl	Serumtriglyceride mg/dl	SerumHDL Cholesterol mg/dl	SerumLDL Cholesterol mg/dl
I	Normal control 0.2 ml of 2% aqueous gum acacia	85.25±8.51	80.71±9.38	24.54±6.49	52.03±3.21
II	Diabetic control 0.2 ml of 2% aqueous gum acacia	218.15±23.79***	194.56±14.99***	21.11±1.45	89.59±13.82
III	(Alloxan monohydrate +standard drug glibenclamide 10 mg/kg)	206.35±6.11*	184.30±9.68*	29.03±3.16*	85.39±7.24*
IV	Alloxan monohydrate + Ethanolic extract (EBJS,100 mg/kg)	170.55±9.03**	127.49±12.66**	25.04±3.21*	68.91±13.34**
V	Alloxan monohydrate +Ethanolicextract (EBJS,200 mg/kg)	121.68±14.57**	124.29±10.67***	31.29±6.12*	50.47±2.63**

Animal: Albino Rats Alloxan: 120 mg/kg.i.p
Extract: p.o.

Value are Mean ±S.E.M: n=6 except in Group V where n =5
* p> 0.05 non significant
**p< 0.01 significant
***P< 0.001 highly significant;
Groups III,IV,V vs Diabetic Control (Group II) and Group I vs Group II on 10th day

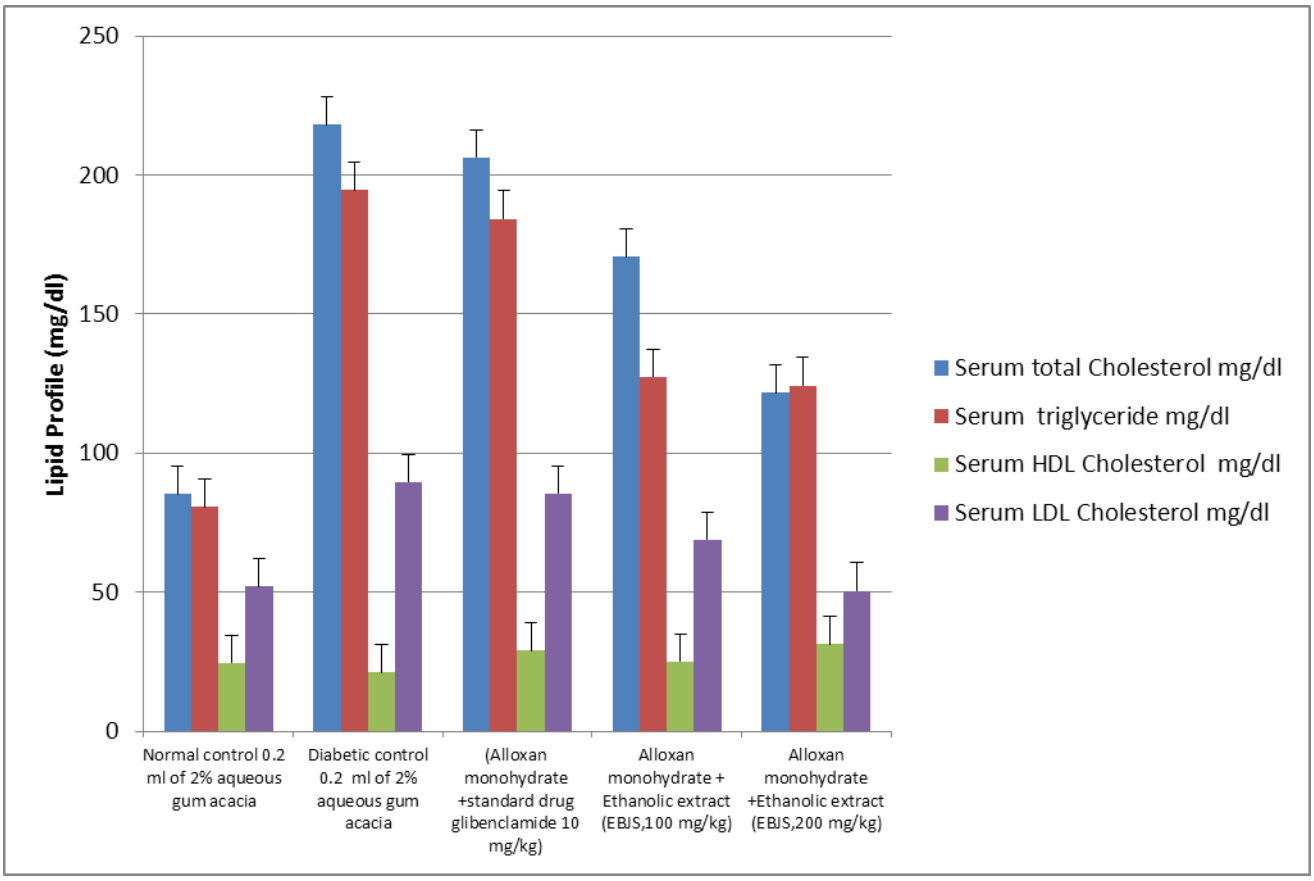

Fig 2: Effect of 50% ethanolic extract of *Eriobotrya japonica* seeds on lipid profile in alloxan induced diabetic rats after 10 days of dosing. Each data column represents the mean ± SEM.

Table 4: Effect of 50% ethanolic extract of *Eriobotrya japonica* (EBJS) seeds on average body weight
(g) in alloxan induced diabetic rats

Table 4

Group	Treatment	Average Body weight of the animal (g)			
		1st day	4th day	7th day	10th day
I	Normal control 0.2 ml of 2% aqueous gum acacia	228.85±6.03	229.88±6.66	233.00±7.07	222.05±4.75
II	Diabetic control 0.2 ml of 2% aqueous gum acacia	180.58±3.66***	163.86±2.08***	152.21±3.12***	124.76±2.35***
III	Alloxan monohydrate+ standard drug glibenclamide (10 mg/kg)	183.83±3.34	173.85±3.37	152.98±4.44	137.50±3.54***
IV	Alloxan monohydrate + Ethanolic extract (EBJS,100 mg/kg)	183.87±2.83	174.70±1.64	165.52±2.73	162.37±2.74***
V	Alloxan monohydrate + Ethanolic extract (EBJS,200 mg/kg)	185.56±3.74	178.11±3.24***	171.43±3.41***	167.20±4.05***

Animal: Albino Rats Alloxan: 120 mg/kg.i.p
Extract: p.o.

Value are Mean ±S.E.M: n=6 except in Group V where n =5
* p> 0.05 non significant
**p< 0.01 significant
***P< 0.001 highly significant;
Groups III,IV,V vs Diabetic Control (Group II) and Group I vs Group II on 10th day

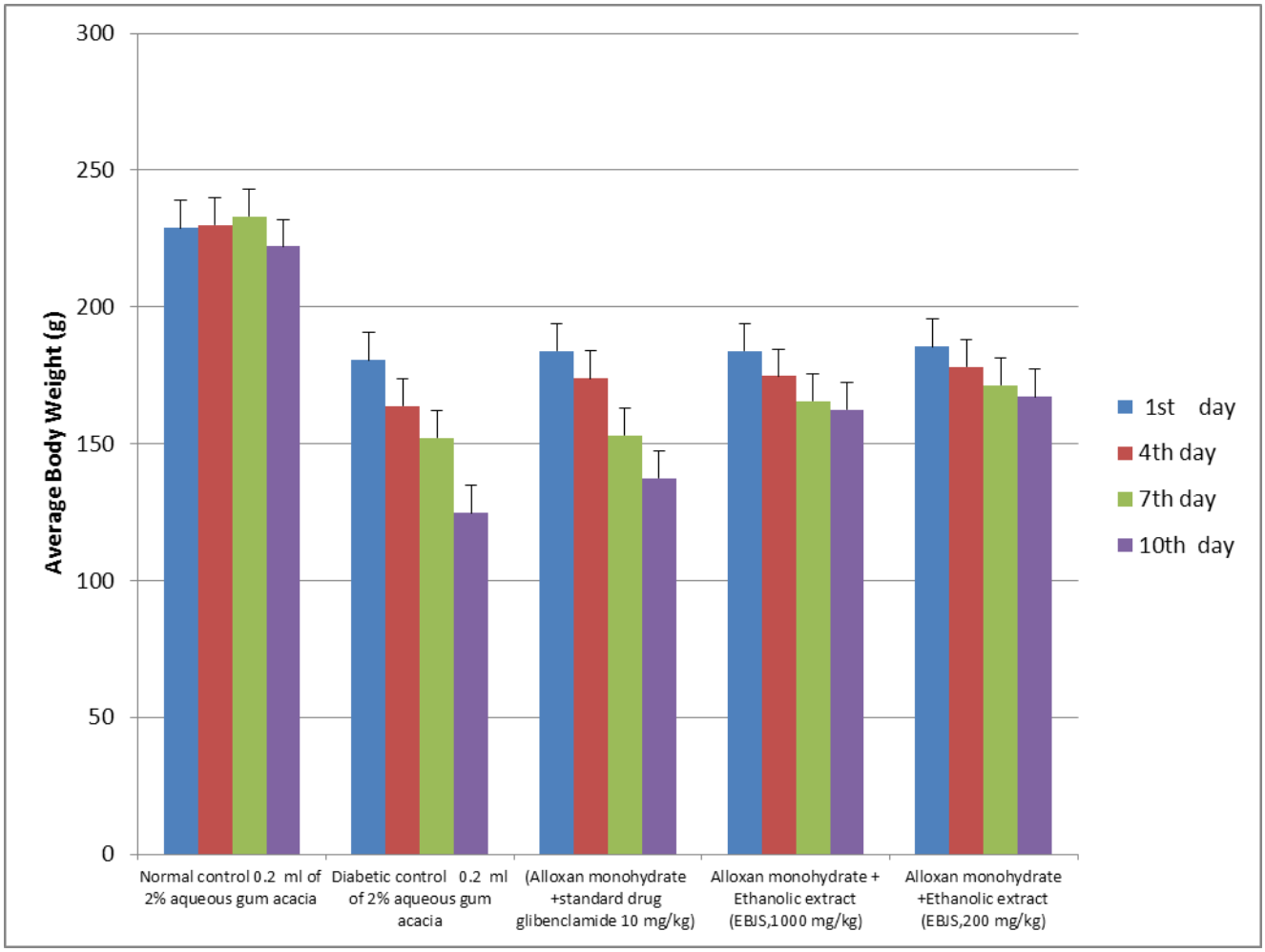

Fig 3: . Effect of 50% ethanolic extract of *Eriobotrya japonica* seeds on Average Body Weight (g) in alloxan induced diabetic rats after 10 days of dosing. Each data column represents the mean ± SEM.

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