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Effect of Ethanolic Extract of Fruits of *Eriobotrya japonica* on Lipid Profile and Body weight in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Eriobotrya japonica locally called as loquat in Kashmir has been studied in various parts of the world but little work has been reported on Kashmiri loquat. The chemical nature of fruits and vegetables offers a great diversity of biological properties and plays an important role in the field of pharmacology. There is a quest for newer drugs with few adverse effects and this poses a challenge for the development of new drugs. The study was undertaken to study the activities of ethanolic extract of *Eriobotrya japonica* fruits in streptozotocin induced diabetic rats. The phytochemical screening of the plant was also done. The animals were divided into five groups. Normal Control group received only the vehicle. Toxic group included those animals in which diabetes was induced by streptozotocin. The 3rd group was those animals which received streptozotocin and standard antidiabetic drug-glibenclamide. 4th group included those diabetic animals which received 50 mg/kg b.w dose of fruits of *Eriobotrya japonica.* 5th group animals included those diabetic animals which received 100 mg/kg b.w of the plant extract. The biochemical parameters that were evaluated were blood glucose levels and lipid profile tests. The body weight was also checked. Histopathology of pancreas was also done. The results showed significant decrease in blood glucose levels, lipid profile tests in animals treated with different doses of the plant extracts. Histopathology of pancreas also showed positive results.

Keywords: *Eriobotrya japonica fruits,* blood glucose levels, lipid profile, histopathology.

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INTRODUCTION

Diabetes mellitus has been defined as metabolic disease with hyperglycaemia which leads to many complications like diabetic neuropathy, diabetic nephropathy, diabetic retinopathy and many other complications. WHO defines the disease as the 7th cause of death in 2030 (WHO 2016). About 180 million people across the globe have type 2 DM characterized by hyperglycaemia that affects eyes, nerves, kidney and may lead to risk linked with cardiovascular disease. Diabetes mellitus is a major degenerative disease in the world today affecting at least 15 million people. [1] 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. [2]

Many 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. Studies have shown that more than 800 plant species have anti- diabetic activity. These days 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 and for other complications related to diabetes. [3-10]

One of the plants is *Eriobotrya japonica* locally known as loquat, has been used since olden medicinal uses. The leaves are of great importance and have been used to treat nausea, vomiting times in the ethno medicine for treating diseases. It is an evergreen tree having many, belching, hiccups and gastro-intestinal disorders. The flowering period of this plant is from April to June. Although it is native to China and Japan, it grows in many parts of the world including India. One variety is found in Kashmir also. The reported bioactive compounds include flavonoids, triterpenic acids, carotenoids, volatile compounds which attribute to aroma, oleanolic acid and unsolid acid. The reported pharmacological activities include anti-oxidant, anti-mutagenic, anti-viral, hypolipidemic, anti-inflammatory and other activities. [11-22] The present study focused on the fruits of *Eriobotrya japonica* on which very little work has been done. The present study was aimed to investigate its effect on body weight in diabetes and hypolipidemic activity of ethanolic extract of *Eriobotrya japonica* fruit in streptozotocin induced diabetic rats, because diabetes mellitus affects many parts of the human body.

MATERIALS AND METHODS

Plant Material

The ripe fruits of *Eriobotrya japonica* (family Rosacea) 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 plant material was dried in a well-ventilated room with outside temperature ranging between 18 to 32°C.

Preparation of the extract

The fruits were coarsely powdered and 500 g of the material was allowed to macerate for 48 hours with 50% ethanol, with occasional shaking. After 48 hours, the ethanolic extract was filtered through Whatman 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 extract was refrigerated at 4°C for future use in experimental studies.

Phytochemical Screening [23-25]

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

Pharmacological Study [26]

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°C, relative humidity (70%) and 12 hours 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].

Induction of Diabetes

Hyperglycaemia was induced by administering a single dose of streptozotocin (STZ) 50 mg/kg b.w. It was freshly dissolved in 0.1 M citrate buffer (pH 4.5) and injected intraperitoneally within 15 minutes of dissolution in a vehicle volume of 0.4 ml with 1 ml of tuberculin syringe fitted with 24-gauge needle. Diabetes is confirmed on 3rd day post administration of streptozotocin by estimating the fasting blood glucose concentration. During this period the animals are given free access to water. Fasting blood glucose concentration is checked by glucostrips. The rats having blood glucose levels > 250 mg/dl are separated and selected for further studies. The animals are given the following treatment in the study.

Group I. Normal Control received 2% of gum acacia.

Group II. Diabetic Control received STZ 50 mg/kg b.w single dose *i.p.*

Group III. STZ + Glibenclamide (3 mg/kg)
Group IV. STZ + *Eriobotrya japonica* fruits (EBJF) [50 mg/kg b.w]
Group V. STZ + EBJF [100 mg/kg b.w]
This treatment was started on the same day except in normal control and diabetic control rats which lasted for a period of 15 days orally. These rats were given free access to standard diet and water during this period. Fasting blood glucose levels were estimated on 1st, 4th, 9th and 15th day of the treatment. On the 16th day, blood samples from these animals were collected from overnight fasting animals by cardiac puncture. The diabetic rats were anaesthesized by mild ether anesthesia before cardiac puncture. The blood sample taken was kept aside for 30 minutes for clotting. By centrifuging the sample at 6000 r.p.m for 20 minutes, the serum was separated and analyzed for various biochemical parameters. At the end of the experiment, the animals were sacrificed and pancreas was taken out. Histopathology of pancreas was also done.

Sample Collection

The blood sample of these rats were collected by pricking the tail from overnight fasted animals and blood glucose levels were estimated using One Touch Ultra strips (Johnson & Johnson Ltd) on day 1st, 4th, and 9th day. On the 15th day, blood was collected from overnight fasted animals under ether anesthesia by cardiac puncture, which was kept aside for 30 min for clotting. By centrifuging the sample at 6000 rpm for 20mins, the serum was separated and analyzed for various biochemical parameters.

Statistical Analysis

The data which was obtained from the biochemical estimations was expressed as Mean ± SEM for each group. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by student t test. Values $p>0.05$ were considered non-significant, $p<0.05$ as significant, $p< 0.01$ as highly significant and $p<0.001$ as very highly significant respectively.
The biochemical parameters were estimated as per the following methods [27-33]

i. Serum glucose levels (Recorded on day 1, day 4, day 9 and day 15) [28-29]
ii. Serum Lipid profile (Recorded on day 15th)

Besides this Average Body Weight of the animals was also checked Histopathology of Pancreas was also done [34-35]

Table 1: Phytochemical Results of *Eriobotrya japonica fruits*

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

RESULTS AND DISCUSSION

Physical Characteristics and Percentage Yield of the Ethanolic extract of *Eriobotrya japonica* fruits

Weight of the dried whole plant taken= 500 grams
Weight of the extract obtained= 150 grams
% yield = Weight of the extract obtained ×100
Weight of the dried whole plant taken
% age yield of the ethanolic extract = 30 %

Extract	Colour	Odour	% Extractive value
50% Ethanolic	Dark Brown	Characteristic	30%

Streptozotocin has been the usual substance used for the induction of diabetes mellitus apart from alloxan. It has shown a destructive effect of the beta cells of the pancreas. It causes a massive reduction in insulin release by the destruction of beta-cells of the islets of Langerhans thereby inducing hyperglycemia· Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose. Diabetes mellitus has been classified as metabolic disorder characterized by resistance in the action of insulin, insufficient insulin recreation or both. It is one of the most common diseases of the world. Nowadays Type II diabetes in young has increased 30-fold over the last 20 years concomitant with increase in obesity. Recent studies have revealed that all incidences of diabetes in this young age group are 2.5% and alarmingly 25% of their young adults have abnormalities of blood glucose. [37-38]
Herbal plants have received greater attention as an alternative to conventional therapy. The demand for these remedies has currently increased. Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products. The Indian indigenous drugs have great importance both from professional and economic point of view. A large number of plants have been reported to possess anti-diabetic activity e.g., *Aconitum napeilus, Aloe vera, Carum carvi, Cichorium intybus, Allium cepa, Aralia cachemirica, Allium sativum, Momordia charantia.*

Table 2: Effect of ethanolic extract of *Eriobotrya japonica* fruits (EBJF) on Blood Glucose Levels (mg/dl) against Streptozotocin induced diabetes mellitus in rats.

Groups	Treatment	Blood Glucose Levels(mg/dl)			
		DAY 1	DAY 4	DAY 9	DAY15
I	Normal control 0.2 ml of 2% gum acacia	80.83 ± 3.63	79.58 ± 3.37	80.26 ± 3.96 (NS)	77.62 ± 4.96 (NS)
II	Diabetic control 0.2 ml of 2% gum acacia	200.48 ± 2.89	200.24 ± 3.67	206.76 ± 3.23 (NS)	207.50 ± 2.97 (NS)
III	STZ+ Std drug Glibenclamide (3 mg/kg. b.w)	220.85 ± 2.37	201.98 ± 6.58	158.71 ± 4.04**	129.56 ± 12.97**
IV	STZ + EBJF (50 mg/kg b.w)	215.50 ± 2.09	184.83 ± 1.91	167.98 ± 3.06**	152.06 ± 4.36***
V	STZ + EBJF (100 mg/kg b.w)	225.27 ± 1.98	202.59 ± 4.19	176.92 ± 3.82**	136.65 ± 2.37***

Table 3: Effect of ethanolic extract of *Eriobotrya japonica* fruits on lipid profile in streptozotocin induced diabetic rats.

Group	Treatment	Serum total Cholesterol mg/dl	Serum triglyceride mg/dl	Serum HDL Cholesterol mg/dl	Serum LDL Cholesterol mg/dl
I	Normal Control (0.2 ml of 2% gum acacia)	88.22 ± 2.01	76.71 ± 3.45	33.07 ± 2.15	42.51 ± 2.35
II	Diabetic control (STZ)	195.18 ± 3.54***	193.01 ± 4.84***	18.88 ± 2.62***	87.02 ± 3.07***
III	STZ + Std Antidiabetic drug Glibenclamide (3 mg/kg)	193.34 ± 5.69	186.96 ± 4.31	19.75 ± 1.95	86.26 ± 3.02
IV	STZ +EBJF (50 mg/kg)	161.46 ± 2.66	159.90 ± 5.68	22.65 ± 0.77	92.56 ± 2.52
V	STZ+EBJF (100 mg/kg)	132.92 ± 4.63	124.95 ± 5.68	30.37 ± 3.95	75.01 ± 2.58

STZ Dissolved in 0.1M citrate buffer at a. dose of 50 mg/kg b.w and injected *i.p* single dose. Diabetes confirmed on third day post administration of streptozotocin. Standard drug Glibenclamide & plant given as ethanolic extracts were administered orally for 15 days, in a single dose daily after confirmation of hyperglycaemia
n = 6 (Number of animals in each group)
Group II is compared with Group I and all other groups are compared with group II. *** p<0.001 Very highly significant; ** p< 0.01; Highly significant; *p<0.05 significant p>0.05 Non-significant.

Table 4: Effect of ethanolic extract of *Eriobotrya japonica* fruits (EBJF) on Average Body Weight (grams) against Streptozotocin induced diabetes mellitus in rats

Groups	Treatment	Average Body weight (in grams)			
		DAY 1	DAY 4	DAY 9	DAY 15
I	Normal control 0.2 ml of 2% gum acacia	250.58 ± 8.14	253.90 ± 9.93	262.12 ± 10.47 (NS)	267.23 ± 13.09 (NS)
II	Diabetic control 0.2 ml of 2% gum acacia	205.83 ± 7.64	202.16 ± 6.61	175.56 ± 6.77**	158.8 ± 7.51**
III	STZ + Std drug Glibenclamide (3 mg/kg. b.w)	203.17 ± 4.91	200.67 ± 4.94	180.92 ± 7.63*	160.00 ± 8.69**
VI	STZ + EBJF (50 mg/kg b.w)	240.13 ± 9.15	237.13 ± 9.17	228.80 ± 12.58*	222.12 ± 13.83**
VII	STZ + EBJF (100 mg/kg b.w)	223.87 ± 6.71	230.42 ± 5.76	238.80 ± 4.76*	245.50 ± 4.27**

STZ Dissolved in 0.1M citrate buffer at a. dose of 50 mg/kg b.w and injected *i.p.* single dose Diabetes confirmed on third day post administration of streptozotocin. Standard drug Glibenclamide & three plants given as 50% ethanolic extracts were administered orally for 15 days, in a single dose daily after confirmation of hyperglycaemia. n = 6 (Number of animals in each group)
DAY 1 compared with DAY 15 *p< 0.05 significant; **p<0.01 highly significant; ***p< 0.001 very highly significant; p> 0.05 non-significant (NS)

Rats weighing in the range of 180-210 g were procured from IIIM Jammu and kept in polypropylene cages under uniform conditions of food, water, temperature and degree of nursing care. It was ensured that the animals were in good health. These animals were free from diseases. Male and female animals were kept in separate cages so that there was no interference in evaluation of biochemical parameters during the period of study. The temperature and the humidity were in the range of 15-25°C and 70-75% respectively.

The phytochemical investigation of ethanolic extract of fruits of *Eriobotrya japonica* carried out by standard procedures revealed the presence of alkaloids, flavanoids, glycosides and carbohydrates (Table 1).

Histopathology of Pancreas in Rats Diabetes induced by Streptozotocin (STZ)

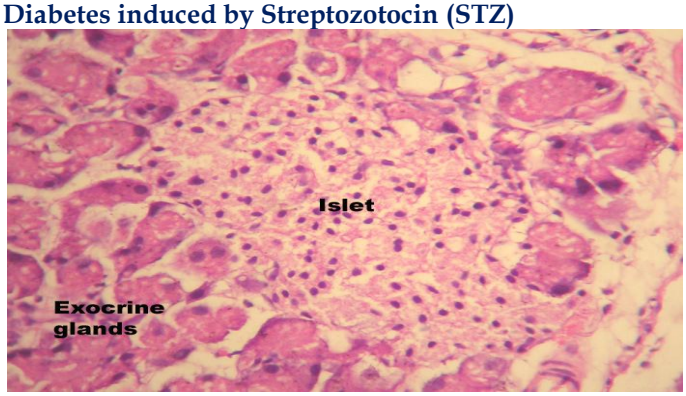

Fig. 1: **Group –I Normal Control**
Pancreas of rats showing a large islet structure surrounded by exocrine gland tissue. No inflammatory cells are seen in the islet (H&E x 40X)

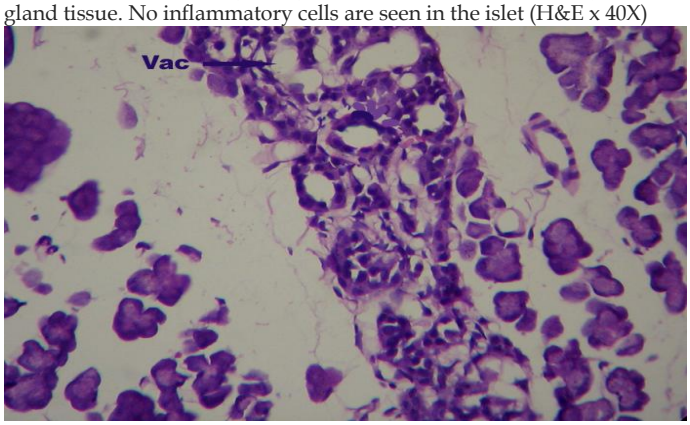

Fig. 2 (a): Group-II – Diabetic Control
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X)

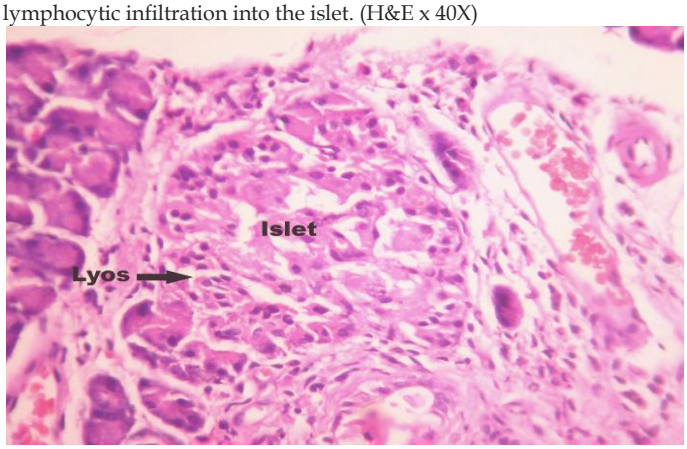

Fig. 2 (b): Group-II – Diabetic Control
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X)

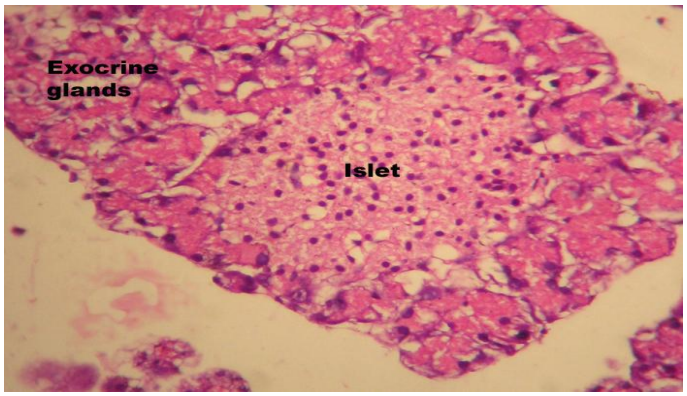

Fig. 3: Group III–STZ*+Standard anti diabetic drug Glibenclamide (3 mg/kg b.w)
Pancreas from diabetic rats showing a large islet structure surrounded by exocrine gland tissue with no vacuolation. No inflammatory cells are seen in the islet (H&E x 40X)

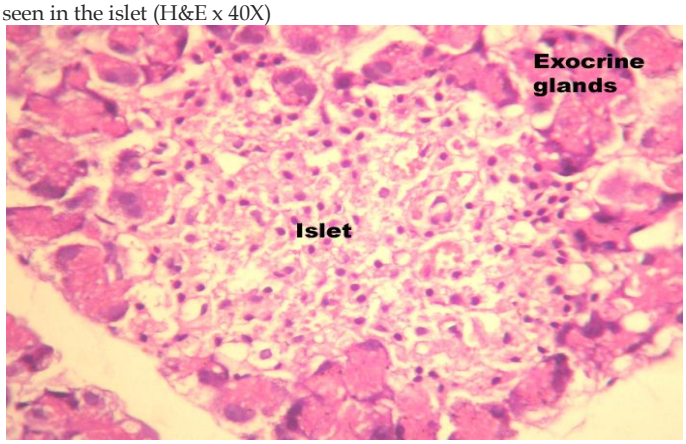

Fig. 4: Group IV STZ*+ *Eriobotrya japonica* fruit 50 mg/kgb.w)
Pancreas from diabetic rats showing an islet structure surrounded by exocrine gland tissue. Few inflammatory cells are seen at the margins of the islet. (H&E x 40X)

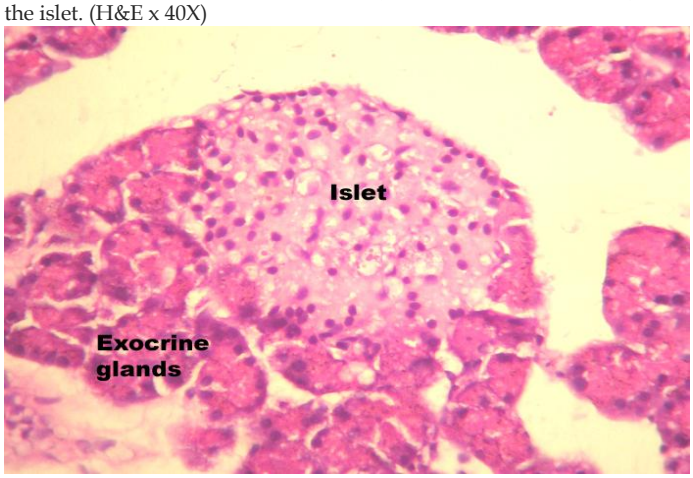

Fig. 5: Group V STZ*+ *Eriobotrya japonica* fruit (100 mg/kg b.w)
Pancreas from diabetic rats showing a comparatively smaller islet structure with exocrine gland tissue seen at the lower and left edges. No inflammatory cells are seen in the islet. (H&E x 40X)
*Streptozotocin (STZ) (50 mg/kg) b.w. given once *i.p*

The results of the present study found that ethanolic extract of *Eriobotrya japonica* reduced the glucose level in animals made diabetic with streptozotocin. Streptozotocin has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of streptozotocin induced free radical damage.

In the present investigation, ethanolic extract of *Eriobotrya japonica* demonstrated the significant anti-diabetic and hypolipidemic activity (Table 2-4). The histopathological studies also showed positive results (Fig. 1-5). The antidiabetic 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. [36-37]

The literature reports reveal that flavonoids present in the plant extract known to possess antidiabetic and hypolipidemic activity. Since many antidiabetic drugs do not correct lipidemic disorders, the observed effects of the plant extract in diabetic rats makes *Eriobotrya japonica* 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.

The ethanolic extract of *Eriobotrya japonica* fruits has beneficial effects on blood glucose levels. Further studies on pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will help in projecting this plant as a therapeutic target in diabetic research. The level of morbidity and mortality because of this disease and its potential complications which are enormous, pose significant healthcare burdens on the families and society in India. It has shown tremendous increase in younger people than in an elderly people. There is an urgent need to change the lifestyle of people and inclusion of fruits and vegetables that will reduce the frequency of taking medicines in near future.

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