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TREATMENT OF DIABETES AND HYPERLIPIDEMIA WITH EXTRACTS OF *EUGENIA JAMBOLANA* SEED AND *AEGLE MARMELOS* LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

SHIVANAND PANDEY

MCOPS, Manipal Udupi, karnataka, India

Corresponding Author dot.shivanand@gmail.com

ABSTRACT

The present study was carried out in rats to test the efficacy of aqueous combined extract of *E.jambolana* and *A .marmelos* on serum insulin, hyperglycemia and serum lipid profile changes associated with diabetes. *Eugenia jambolana* and *Aegle marmelos* are used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focuses on the serum insulin augmentation, anti-hyperglycemic and anti-hyperlipidemic property of a combined aqueous extracts of *E.jambolana* and *A.marmelos* (EA) on alloxan induced diabetic rats. The diabetes induced animals were fed with plant extracts at the increasing dosage of 200mg, 300mg and 400mg of body wt. The combined plant extracts administrated animals revealed a significant ($P<0.001$) increment of serum insulin levels, higher reduction in hyperglycemia and hyperlipidemia when compared to the diabetic control rats ($P<0.001$).

KEY WORDS

Eugenia jambolana, Aegle marmelos, alloxan, hyperglycemia, hyperlipidemia.

INTRODUCTION

Diabetes mellitus is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed in this century, medical science cannot claim that it knows all that needs to be known about this disease, including its management. This is the main reason for the persistent interest all over the world to explore alternative remedies from the so-called "alternative systems" of medicine. The disease was well known to the ancient Indian medical experts. All the renowned classic texts of Ayurveda like Charaka Samhita (1000 B.C.), Sushruta Samhita (600 B.C.) and subsequent works refer to this disease under the term *Madhumeha* or *Ikshumeha* (literally meaning sugar in the urine). Apart from detailed description of its etiopathogenesis (according to Ayurvedic concepts), the two types of diabetic patients (obese and lean) and a definite familial prediction to the disease are referred to in Ayurveda, besides the importance given to dietary regulations, physical exercises and baths, in addition to the use of a number

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of plant drugs in the management of the disease [1]. Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, action or both. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels.[2] It is a commonest endocrine disorder that affects more than 100 million people worldwide (about 6% of population) and in the next 10 years, it may affect about 5 times more people than it does now [4]. According to WHO report, India has 19.4 million diabetes patients[5]. It is the fourth leading cause of death in the most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations. Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipid, carbohydrates and proteins, in addition to damaged β-cells of pancreas and increased risk of complications of vascular disease[6]. Alloxan induction of diabetes is an experimental model widely used to study glycemic and lipedemic changes in plasma. There are more than 1200 plants species worldwide that are used in the treatment of diabetes mellitus and a substantial number of plants have shown effective hypoglycemic activity after laboratory testi[7]. A multitude of herbs spices and other plant materials have been described of the treatment of diabetes throughout the world [8, 9.] The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies[10]. India has about 45,000 plant species and many of them have medicinal properties. Out of a large number of herbal drugs stated to possess anti-diabetic activity in the Ayurvedic system of medicine of India. *E.jambolana* which belongs to the family Myrtaceae is a large evergreen tree growing up to 30 m height, found widely in India and the Asian subcontinent. The seeds of this plant have been reported to possess many medicinal properties in the Ayurveda system of medicine.

The fresh seeds are most effective in diabetes as they quickly reduce sugar in urine[11, 12]. Reported the hypoglycemic response of seed and pulp extract on diabetic mice. Although *E.Jambolana* is established for its antidiabetic potential in ayurveda, as well as in the modern scientific community. *Eugenia jambolana* seeds have hypoglycemic, anti-inflammatory; neuropsychopharmacological, anti-bacterial, anti-HIV and antidiarrheal effects[15]have reported that the Eugenia jambolana seed contains several biologically active constituents such as flavonoids, gallic acid, ellagic acid, glycosides, triteripenoids and saponins. Recently, we have reported the anti-diabetic and anti-oxidant property of *E.jambolana* seed kernels on streptozotocin-induced diabetic rats[16]

A.marmelos is being widely used to treat diabetes by the traditional practitioners over many centuries. The root is sweet; cures fever due to 'tridosha' pain in the abdomen, palpitation of the heart, urinary problems. The leaves are astringent, digestive; laxative and febrifuge when fresh; the flowers allay thirst and vomiting; useful in dysentery. The ripe fruit is tonic restorative, astringent, laxative; good for the heart and brain [17] aqueous fruit extract reduced the blood glucose levels [18]. The aqueous roots bark and leaf extract useful for hypoglycemic effect [19]. Aqueous leaf extract significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol [19, 20] .Showed histo-pathological alterations in the pancreatic, liver and the kidney tissues indicating the potential of hypoglycemic nature of the extract [21]. The methanolic leaf extract

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elucidated as an effective used for hypoglycemic and antioxidant activity [22, 23] the fruit extract improved functional state of pancreatic β-cells and partially reversed the damage[24].

MATERIALS AND METHODS

Plant material

The fresh seeds of *E.jambolana* L. (Myrtaceae) and leaves of *Aegle marmelos* L. (*Rutaceae*) were collected in and around Amreli District, Gujarat, India. The plant materials were cleaned with distilled water and shade dried at room temperature and authenticated by Dr.H.B.Singh, Head of Raw materials herbarium and museum, NISCAIR,New Delhi and voucher specimens (specimen No-NISCAIR/RHMD/Consult/ 2008 -09/1077/108 were kept at the – NISCAIR, New Delhi.

Plants extract preparation

100gms of the dried powdered fresh seeds and leaves of *E.jambolana* and *A.marmelos* were taken separately and mixed with 500ml of distilled water and magnetically stirred in a separate container for overnight at room temperature. The residue was removed by filtration and the aqueous extracts were lipholization and concentrated under vacuum to get solid yield of 10% (leaves). Aqueous extract was administered orally to animals after suspending it in 1% w/v Carboxy methyl cellulose aqueous solution.

Animals

Adult male Wistar rats weighing around 180-200g were obtained from zydus healthcare, Ahmadabad, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±20C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines. The study protocol was approved by institutional animal ethical commit, MCOPS, Manipal India

Induction of diabetes

Adult wistar rat with an initial body weight of 180 to 200g were taken, and divided into six groups each containing six animals. Except normal control rat (NC) all rat were treated by Alloxan monohydrate (80 mg/kg, Sigma Chemicals, USA). Alloxan dissolved in citrate buffer (pH 4.0) was injected intravenously to the overnight fasted rats through tail vein. Food was provided to them 2 h after the injection. After 1 month, the rats showing stabilized diabetes having fasting blood glucose (FBG) values 250 mg/dl or above was considered as diabetic animals consider it as zero days. Dosing with the aqueous extracts was started on the first day and continued for 25 days according to the following schedule:

Group I: Normal control (Distilled water),

Group II: Disease control (suspension of 1% CMC),

Group III: Aqueous extract of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts (200 mg/kg, p.o.),

Group IV: Aqeous extract of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts (300 mg/kg, p.o.),

Group V: Aqueous extract of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts (400 mg/kg, p.o.).

On day 7th blood was collected for hemolytic parameter and at 25th day of experiment animal was sacrificed for histopathological studies[25].

Biochemical analysis

The biochemical estimation was carried out in our lab by using the following methods. Serum TC[26], TG,

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LDL, HDL, and VLDL [27].

Histopathology

The pancreatic tissues were dissected out and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5µm and the sections were stained with haematoxylin and eosin [28].

Statistical analysis

Statistical analysis was carried out by using one way ANOVA as in Graph pad prism software.

RESULTS

The aqueous extract residue of *E.jambolana* and *A.marmelos* were combined (1:1) and administered orally in an aqueous solution at increased dose levels of 200mg, 300mg, and 400mg/kg body wt. to diabetic rats to assess the synergetic impact of the plant extracts. The decreased insulin levels in the diabetic animals were enhanced significantly ($P<0.001$) in the combined extracts treated animals. The highest increment was recorded at 400mg dose level (23.48%). The combined plant extract were fed with fasting and diabetes induced rats. The blood glucose levels was significantly ($P<0.001$) reduced when compared to the specific control animals. The highest depletion was recorded at 400mg dose level, 24.73% in fasting rats, 65.87% in diabetic induced rats.

The lipid profile such as TC, TG, LDL and VLDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats. When treated with different extract of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts. The depletion in the TC, TG, LDL, and VLDL was dose dependent and the highest reduction in the cholesterol recorded was 9.4%, TG was 12.88%, LDL was 19.06% and VLDL was 30.85% in 400mg/kg body wt., when compare to the diabetic control animals. The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P<0.001$) after the administration of the plant extract. The highest increment was recorded at 400mg/kg body wt., dosage level (25.55%).

Histological sections of endocrine regions of pancreas of Alloxan induced diabetic rats revealed a significant reduction in the size of the islets when compared to that of normal groups.

Further the study revealed the presence of damaged β-cell population. This damage of the β-cells due to alloxan induction. The reduction in β-cell number can be as low as 50% during diabetes. On the other hand, studies on the supplementation of combined plant extracts the diabetic rats revealed restoration of size of the islets along with β-cells repair. This recovery of the β-cells was recorded as dose dependant that is form 200mg to 400mg/kg body weight of the combined plant extract given animals. The plant extract fed animals revealed better restored β-cells of pancreas from the alloxan induced damage. The restoration of β-cells was evident at higher dose level of 400mg/by wt extract fed groups.

DISCUSSION

In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels. The reduction in the serum insulin levels in the Alloxan treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The Alloxan selectively destroys the pancreatic cells and induce hyperglycemia. Similar studies were recorded earlier

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in the Alloxan treated rats, the levels of serum insulin significantly reduced. Nitric oxide has been demonstrated to participate in the beta cell damage during Alloxan induced diabetes. Diabetes affects both glucose and lipid metabolism. In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism. The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes. The lipoprotein levels in the Alloxan induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver. The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx. The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atheroselerosis.

CONCLUSION

After the administration of the combined aqueous extract to the Alloxan induced diabetic rats revealed augmented serum insulin levels. The increment of serum insulin levels might be due to increased secretion of the hormone, which might reflect the probable 'repair' of the damaged beta cells of the endocrine of the pancreas due to Alloxan.

The blood glucose level of combined plant extract fed animal was significantly (P<.001) reduced. The highest depletion was recorded in the 400mg/kg body wt., dosage rats. The levels of serum TC, TG, LDL, and VLDL were found to be significantly reduced in the plant extracts treated diabetic animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk.

The histological studies of the endocrine region of pancreas of the diabetic and combined plant extract treated animals revealed that shrinkage of β-cells of islets of langerhans in the diabetic animals. The combined plant extracts treated animals' revealed restoration of β-cells. The restorations of the β-cells in diabetic treated (extract fed) animals corroborate the increased serum insulin levels in treated animals.

The present study suggests that the combined extract had synergetic hypoglycemic effect revealed by increased serum insulin levels, decreased serum lipid levels and therefore attribute to therapeutic value of the combined plant extracts of *E.jambolana* and *A.marmelos* to combat the diabetic condition in rats.

Table 1.

TREATMENT OF DIABETES AND HYPERLIPIDEMIA WITH EXTRACTS OF *EUGENIA JAMBOLANA* SEED AND *AEGLE MARMELOS* LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

Effect of the aqueous extract of combined plant extract on Serum insulin (µu/ml), Blood glucose (mg/dl), Lipid profile (mg/dl), in Alloxan induced diabetic animals

PARAMETER	Normal rats (NC)	Diabetic Control rats (DC)	EA200	EA300	EA400
Serum Insulin	17.99±0.41	10.92±0.48 @	12.55±0.37 #	14.33±.33 #	15.03±.17#
Blood Glucose Fasting rats (Control not induced diabetes)	78.14±0.48	69.05±0.3 @	65.52±0.74 #	56.33±0.62 #	52.18±0.79 #
Diabetes Induce	78.46±0.49	385.38±1.02@	246.27±1.47#	194.73±1.0 #	127.87±0.56#
VLDL	17.63±0.49	25.88±0.65 @	25.44±0.69 #	22.56±0.55 #	17.87±0.56 #
TC	77.72±0.73	88.88±0.36 @	85.04±0.45 #	80.24±0.67 #	78.45±0.34 #

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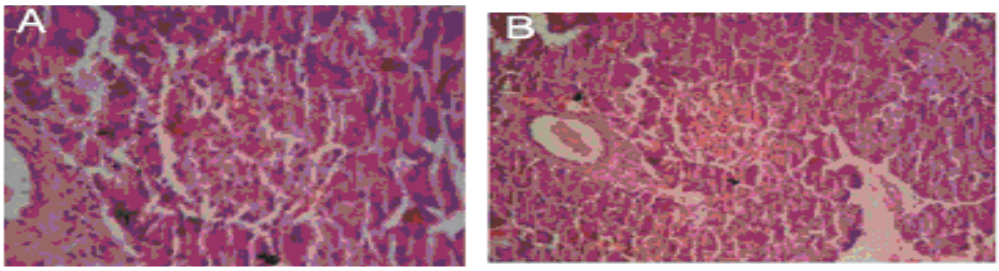
HDL	31.33±0.37	26.88±0.25 @	27.94±0.25 #	30.23±0.31 #	33.85±0.45 #
TG	84.39±1.41	98.88±0.64 @	94.13±0.64 #	91.11±0.42 #	86.13±0.31 #
LDL	33.72±0.42	41.21±0.39 @	37.44±0.38 #	35.23±0.32 #	33.35±0.28 #
VLDL	17.67±0.49	25.88±0.65 @	25.44±0.69 #	22.56±0.55 #	17.85±0.56 #

Each value represents six individual observations. Mean±SEM, '+', '-' indicate percent increase or decrease over control 'P' denotes the statistical significance and '*****P' denotes statistical significance of ANOVA, to test the difference between the experimental groups. TC- Total cholesterol, TG- Triglyceride, HDL High density lipoprotein, LDL- Low density lipoprotein, VLDL- Very low density lipoprotein. @- indicate significant difference (P<0.001) then normal control while #-indicate significant difference (P<0.001) then disease control



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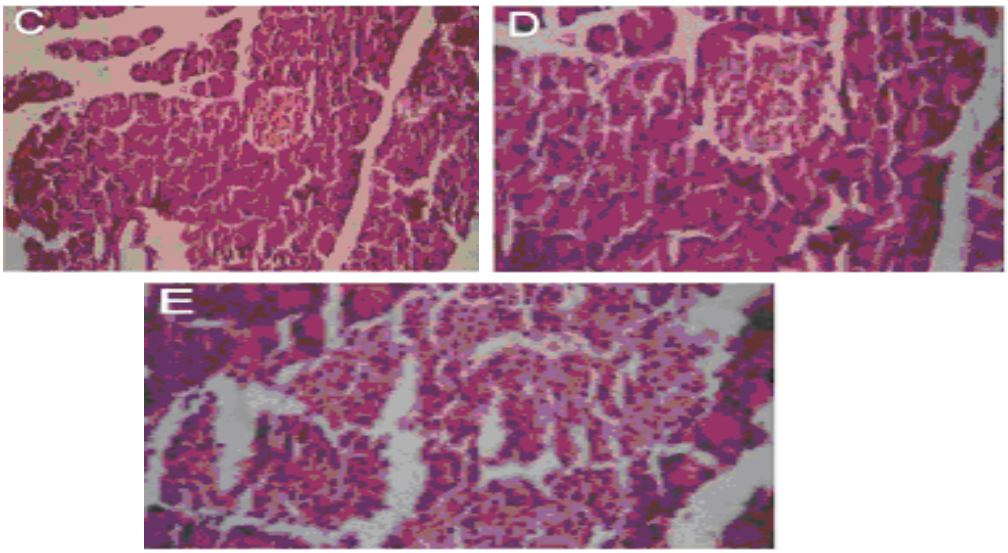

Fig-A: The Pancreatic islets of langerhans of normal rat showing alpha cells and beta cells.
Fig-B: Alloxan induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells.
Fig-C: Combined plant extract (200mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic control rats.
Fig-D: Combined plant extract (300mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic and also 200 mg/kg treated rats.
Fig-E: Combined plant extract (400mg/kg) treated pancreatic islets shows partial proliferation of beta cells. The animals revealed better restoration / proliferation from the Alloxan induced damage when compared to control as well as 300 mg/kg treated animal

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