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Full Length Research Paper

Treatment with extracts of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts prevents hyperglycemia and hyperlipidemia in alloxan induced diabetic rats

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***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 200, 300 and 400 mg 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). The histological studies of the endocrine region of pancreas of diabetic animals revealed that shrinkage of cells of islets of langerhans. The combined plant extracts treated animals revealed restoration of -cells. The restoration of cells was evident at higher dose level that is, 400 mg/by wt extracts fed groups.**

Key words: *Eugenia jambolana*, *Aegle marmelos*, alloxan, hyperglycemia, hyperlipidemia.

INTRODUCTION

Diabetes mellitus is a syndrome characterized chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jaykar and suresh, 2003). According to WHO report, 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. 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 testing (Eddouks et al., 2005) . A multitude of herbs spices and other plant materials have been described of the treatment of diabetes throughout the world (Marles and Fransworth, 1995; kesari et al., 2005, 2006) . 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 (Bailey and Day, 1989) . 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.

Eugenia 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 (Achrekar et al., 1991) 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. *E. jambolana* seeds have hypoglycemic, anti-inflammatory,

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neuropsychopharmacological; anti-bacterial, anti-HIV and antidiarrheal effects (Bhatia and Bajaj, 1974) have been reported. The *E. 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 (Ravi et al., 2003, 2004a, b, c).

Aegle 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. The fresh flowers allay thirst and vomiting; useful in dysentery. The ripe fruit is tonic restorative, astringent, laxative; good for the heart and brain (Mhaskar et al., 2000). The Aqueous fruit extract reduced the blood glucose level (Kamalakannan and Prince, 2003). The aqueous roots bark and leaf extract useful for hypoglycemic effect (Grover et al., 2002) . The aqueous leaf extract significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol (Ponnachan et al., 1993; Grover et al., 2002). It showed histo-pathological alterations in the pancreatic, liver and the kidney tissues indicating the potential of hypoglycemic nature of the extract (Das et al., 1996). The methanolic leaf extract elucidated as an effective used for hypoglycemic and antioxidant activity (Sabu and Kuttan, 2004; Upadhya et al., 2004). The fruit extract improved functional state of pancreatic -cells and partially reversed the damage (Kamalakannan and Mainzen, 2005).

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.

MATERIALS AND METHODS

Plant material

The fresh seeds of *E. jambolana* L. (Myrtaceae) and leaves of *A. 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

100 g of the dried powdered fresh seeds of *E. jambolana* and 100 g of the dried powdered fresh leaves of *A. marmelos* were taken separately and mixed with 500 ml 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 lipholized and concentrated under vacuum to get solid yield of 8% (seeds) 10% (leaves). Both Aqueous extracts were mix in 1:1 ratio at time of administration aqueous extract was administered orally to animals after suspending it in 1% w/v carboxyl methyl cellulose aqueous solution.

Animals

Adult male Wistar rats weighing around 180 - 200 g were obtained from zydus healthcare, Ahmedabad, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25 ± 20°C and 55 - 65% relative humidity 12 ± 1 h 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, RBPMPC, Atkot, India

EXPERIMENTAL DESIGN

Induction of diabetes

Adult wistar rat with an initial body weight of 180 to 200 g were taken and divided into eight groups each containing six animals. Except normal control rat (NC) all rat were treated by alloxan. Alloxan monohydrate (80 mg/kg, Sigma Chemicals, USA) 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 day. 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 *E. jambolana* seed and *Aegle marmelos* leaf extracts (200 mg/kg, p.o.),
Group IV: Aqueous extract of *E. jambolana* seed and *Aegle marmelos* leaf extracts (300 mg/kg, p.o.),
Group V: Aqueous extract of *E. jambolana* seed and *Aegle marmelos* leaf extracts (400 mg/kg, p.o.).
Group VI: Aqueous extract of *E. jambolana* seed leaf extracts (400 mg/kg, p.o.).
Group VI: Aqueous extract of *A. 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 (Watal et al., 2005).

Biochemical analysis

After the 7th day of treatment, blood was collected retro-orbitally from the inner cantus of the eye (under light ether anesthesia) using capillary tubes (micro hematocrit capillaries, mucaps) in fresh vials containing sodium fluoride and sodium oxalate as anti-coagulants agents. Blood glucose level measured by accu chek active glucostrips (Roche Diagnostic India Pvt. Ltd, Mumbai). The serum was separated was separated in a T8 electric centrifuge (remi udyog, New Delhi) at 2000 rpm for 2 min and Serum TC (Parekh and Junk, 1970), TG (Rice, 1970), LDL and HDL (Burstein et al., 1972) were determined by using triglycerides test kit (Triglyceride LG061, Span diagnostic Ltd, Surat Gujarat India) and cholesterol test kit (cholesterol MB924A, Span diagnostic Ltd, Surat Gujarat India)

respectively using colorimeter (Janaki impex pvt ltd Ahmedabad, Gujarat, India) while serum insulin (Savita,2008) measured by Insulin kit, (Mercodia Insulin ELISA) using spectrophotometer (thermo-electronics India). The procedure involved an equilibrium assay carried out in BSA-borate buffer.

Histopathology

On 25th day animal were sacrificed. 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 5 m and the sections were stained with haematoxylin and eosin (Strate et al., 2005).

Statistical analysis

All the grouped data was statistically evaluated via the graph pad prism version 5 included one-way analysis of various (ANOVA) followed by least significant difference test. P-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.E.M for 6 animals in each group.

RESULTS

The aqueous extract residue of *E. jambolana* and *A. marmelos* (EA) were combined (1:1) and administered orally in an aqueous solution at increased dose levels of 200 (EA200), 300 (EA300) and 400 mg/kg body wt (EA400) to diabetic rats to assess the synergetic impact of the plant extracts. This synergistic effect was compare with individual dose of aqueous extract of *E. jambolana (E400)* and *A. marmelos* (A400) (Table 1). The decreased insulin levels in the diabetic animals were enhanced significantly ($P < 0.001$) in all group of treated animals (Table 1). The highest increment was 37.64% recorded with EA400.

While with individual dose of E400 and A400 enhancement of insulin level were 31.41 and 30.49% respectively. The blood glucose levels was significantly ($P < 0.001$) reduced when compared to the specific diabetic control animals (Table 1). The highest depletion of blood glucose was recorded 65.87% with EA400 in diabetic induced rats. While depletion of blood glucose were recorded 59.03 and 60.77%.with individual dose E400 and A400 respectively.

The lipid profile such as TC, TG and LDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats (Daisy et al., 2009). When treated with different extract of *E. jambolana* seed and *A. marmelos* leaf extracts.

The depletion in the TC, TG and LDL was dose dependent and the highest reduction in the choles-terol recorded was 10.4, TG was 12.89 and LDL was 19.06% in 400 mg/kg body wt (EA400), when compare to the diabetic control animals (Table 1). The individual treatment with E400 and A400 show reduction in cholesterol were 9.7 and 9.1% respectively. Reduction in TG with E400 was 9.57% while reduction in TG with A400 was10.25%. Reduction in LDL with E400 was 25.32 and A400 was 23.81%.

The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P < 0.001$) after the administration of the plant extract (Table 1). The highest increment was recorded 22.00% with EA400.The incra-ment in HDL level were 15.7 and 16.2%. With E400 and A400 respectively. 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 (Figures 1 and 2).

Further the study revealed the presence of damaged -cell population. This damage of the -cells due to alloxan induction. The reduction in -cell number can be as low as 50% during diabetes (Hayashida et al., 1983). On the other hand, studies on the supplementation of combined plant extracts the diabetic rats revealed restoration of size of the islets along with -cells repair.

This recovery of the -cells was recorded as dose dependant that is form 200 to 400 mg/kg body weight of the combined plant extract given animals (Figures 3, 4 and 5). The plant extract fed animals revealed better restored -cells of pancreas from the alloxan induced damage. The restoration of -cells was evident at higher dose level of 400 mg/by wt extract fed groups (EA400) as seen in Figure 5 compare to higher dose level of 400mg/kg of *E. jambolana* (E400) and higher dose level of 400mg/kg of *A. marmelos* (A400) as see in Figures 6 and 7.

The levels of serum lipids are usually elevated in DM and such an elevation represents a risk factor for coro-nary heart disease. This abnormal high level of serum lipids is mainly due to the uninhitbited actins of iipolytic hormone on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However in a diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceimdemia (Pushparaj et al., 2007).

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 (Ali et al., 2004). An Alloxan cause a massive reduction in insulin release by the destruction of -cells of the islets of langerhans and results in to reduction of serum insulin level (Yoon and Ray, 1985).

Thereby induces hyperglycemia (Kurup and Bhonde, 2000; Siyem et al., 2002). Diabetes affects both glucose and lipid metabolism (Sperling et al., 2000). In the post prandial state elevated serum insulin increases lipoprotein

Table 1. Effect of the aqueous extract of combined plant extract on Serum insulin (ng/dl), Blood glucose (u/ml), Lipid profile (mg/dl), in Alloxan induced diabetic animals.

Parameter	Normal rats (NC)	Diabetic control rats (DC)	EA200	EA300	EA400	E400	A400
Serum Insulin (ng/dl)	17.99±0.41	10.92±0.48 *	12.55±0.37**	14.33±.33**	15.03±.17**	14.35±1.28**	14.25±1.59**
Blood Glucose (u/ml)	78.46±0.49	385.38±1.02*	246.27±1.47**	194.73±1.0**	127.87±0.56**	151.36±14.3**	157.78±18.1**

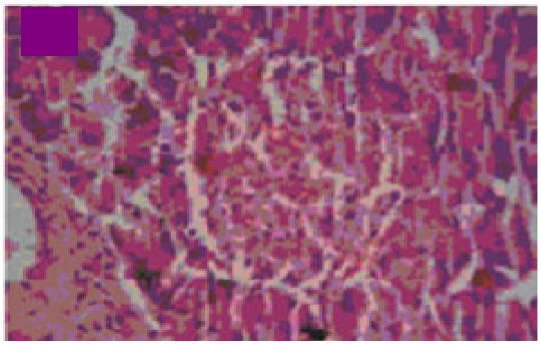

Figure 1. The Pancreatic islets of langerhans of normal rat showing alpha cells and beta cells.

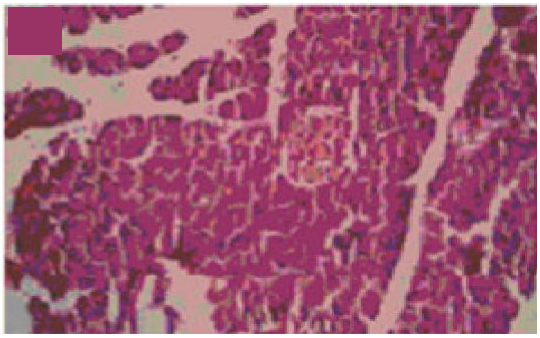

Figure 3. EA200 treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic control rats.

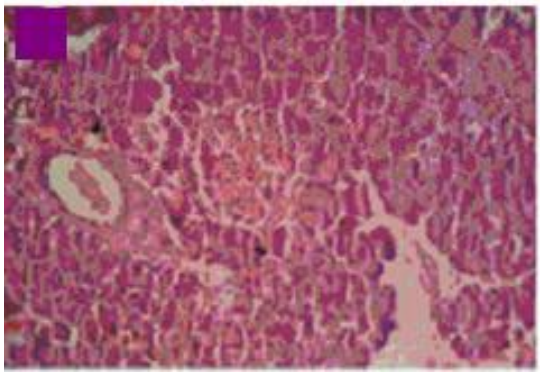

Figure 2. Alloxan induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells.

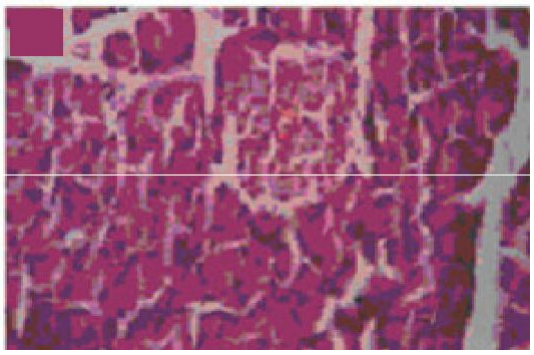

Figure 4. EA300 treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic and also 200 mg/kg treated rats.

lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (Bhagavan, 2002). The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (Ranganathan et al., 2000). 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. 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 (Coppack, 1994; Ohno, 2000). The increased levels of low-density lipoprotein (LDL) in the diabetic animals might be due to over production of LDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Coppack, 1994).

After the administration of the combined aqueous extract to the Alloxan induced diabetic rats revealed

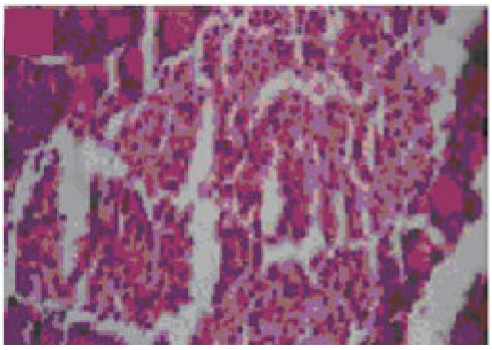

Figure 5. EA400 treated pancreatic islets shows partial proliferation of beta cells. The animals revealed better restoration / proliferation from the Alloxan induced damage when compared to control as well as EA200, EA300, E400 and A400 treated animal.

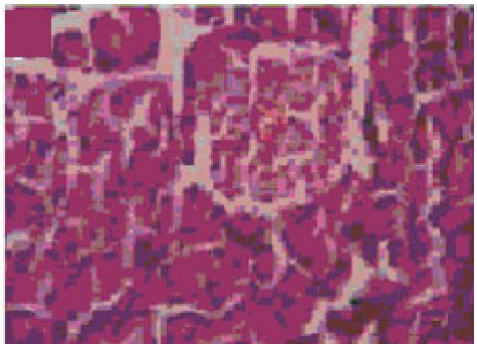

Figure 6. Aqueous extract of *E. jambolana* (E400) treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic control rats.

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 -cells of the endocrine of the pancreas due to Alloxan. The blood glucose level of combined plant extract fed animal was significantly ($P < 0.001$) reduced. The highest depletion was recorded in the 400 mg/kg body wt; dosage rats (EA400) which is also higher as compare to 400 mg dose of E400 and A400. The levels of serum TC, TG and LDL 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 histological studies of the endocrine region of pancreas of the diabetic and combined plant extract treated animals revealed that shrinkage of -cells of islets of langerhans in the diabetic animals. The combined plant extracts treated animals' revealed restoration of -cells.

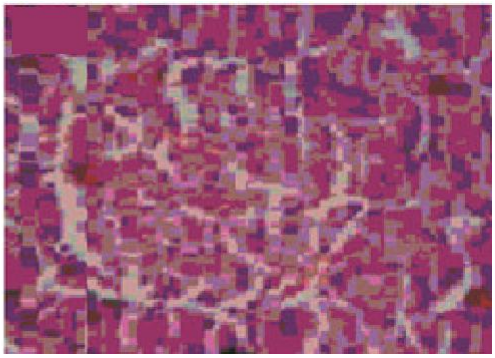

Figure 7. Aqueous extract of *A. marmelos* (A400) treated pancreatic islets show partial revealed better restoration, when compared to the Alloxan induced diabetic control rats.

The restorations of the -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. As compared to individual dose of *E. jambolana* and *A. marmelos* at 400 mg/kg body weight.

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