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Anti-hyperglycemic and Anti-hyperlipidemic effect of combined plant extract of *Cassia auriculata* and *Aegle marmelos* in streptozotocin (STZ) induced diabetic *albino* rats.

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Abstract: *Cassia auriculata* 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 *C.auriculata* and *A.marmelos* on streptozotocin induced diabetic rats. The diabetes induced animals were fed with plant extracts at the increasing dosage of 250mg, 350mg and 450mg 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 β cells of islets of langerhans. The combined plant extracts treated animals revealed restoration of β-cells. The restoration of β cells was evident at higher dose level i.e. 450mg/by wt extracts fed groups.
Key words: *Cassia auriculata, Aegle marmelos,* streptozotocin (STZ), hyperglycemia, hyperlipidemia.

Introduction

Diabetes mellitus is a group of metabolic alterations 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 (Huang *et al*., 2005). It is a commonest endocrine disorder that affects more than 100 million people world wide (about 6% of population) and in the next 10 years, it may affect about 5 times more people than it does now (WHO1992, ADA, 1997). According to WHO report, India has 19.4 million diabetes patients (King *et al*., 1998). 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 diseases (Davis and Granner., 1996). Streptozotocin induction of diabetes is an experimental model widely used to study glycemic and lipedemic changes in plasma. There are more then 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.

C.auriculata is widely used in Indian traditional medicines (Shawney et al., 1978) and the root cures tumors, skin diseases and asthma; leaves are anthelmintic, good for ulcer, diarrhea and leprosy; the flowers are used in the treatment of urinary discharge, diabetes and dysentery (Mhaskar *et al.,* 2000); the aqueous extract of flower and seed have anti-diabetic activity (Jain and Sharma., 1967) and they have an emollient effect (Dhar *et al*., 1968). The alcoholic leaf extract of is effective in alcoholic liver injury (Rajagopala *et al*., 2003) and reported as a few anti-viral activity of the plant (Dhar *et al*., 1968).

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 (Mhaskar *et al.,* 2000); aqueous fruit extract reduced the blood glucose levels (Kamalakannan and Prince., 2003); the aqueous roots bark and leaf extract useful for hypoglycemic effect (Grover *et al*., 2002); aqueous leaf extract significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol (Ponnachan *et al*., 1993; Grover *et al*., 2002); 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 a effective used for hypoglycemic and antioxidant activity (Sabu and Kuttan, 2004, Upadhya *et al*., 2004); the fruit extract improved functional state of pancreatic β-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 *C. auriculata* and *A. marmelos* on serum insulin, hyperglycemia and serum lipid profile changes associated with diabetes.

Materials and Methods

Plant material:

The flowers of *Cassia auriculata* L. (*Caesalpineceae*) and leaves of *Aegle marmelos* L. (*Rutaceae*) were collected in and around Vellore District, Tamilnadu, India. The plant materials were cleaned with distilled water and shade dried at room temperature and authenticated by Dr.A.Annadurai, Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu., and voucher specimens (specimen No CAHC- Zoo -09/2008) were kept at the Department of Botany, C. Abdul Hakeem College, Melvisharam.

Plants extract preparation:

100gms of the dried powdered flowers and leaves of *C.auriculata* 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 7% (flowers), 10% (leaves).

Animals:

Adult male Wistar rats weighing around 180-200g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25 ± 2^0C 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 (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental induction of diabetes:

The diabetes was induced in the overnight fasted animals by a single intra peritoneal injection of freshly prepared solution of streptozotocin (Sigma,USA) 35mg/kg body weight in 0.1M cold citrate buffer pH 4.5 (Bursell *et al.,* 1997, Sun *et al.,* 2000, and Hemalatha *et al.,* 2004). The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia (Balasubramaian *et al.,* 2004). The control rats were injected with citrate buffer alone as placebo. The animals were considered diabetic if the blood glucose values were >250mg/dl on the third day after STZ injection.

Experimental design:

The studies conducted in the two groups of STZ induced diabetic animals

Group I: Normal rats.

Group II: Diabetic (STZ induced) control rats.

Group III: Diabetic induced animals were fed with plant extract for 7days.

(The plant extract were administered to animals in increasing dosages of 250mg, 350mg, and 450mg/kg body weight to assess therapeutic effect of the extracts. Separate groups were maintained for each dose levels).

Group IV: Diabetic induced animal were fed with plant extract for 25 days for histopathology studies.

Biochemical analysis:

The biochemical estimation was carried out in our lab by using the following methods. Serum TC (Parekh and Junk., 1970), TG (Rice. 1970), LDL, HDL, and VLDL (Burstein et *al*., 1972).

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. (Strate *et al.,* 2005,).

Statistical analysis:

Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

Results

The aqueous extract residue of *C.auriculata* and *A.marmelos* were combined (1:1) and administered orally in an aqueous solution at increased dose levels of 250mg, 350mg, and 450mg/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 450mg 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 450mg dose level, 26.4% in fasting rats, 67.27% 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. The combined plant extract was administered orally at increasing dose levels of 250mg, 350mg, and 450mg/kg body wt., to diabetic rats. The diabetic animals at 250mg/kg dosage recorded a non significant change in the TC, TG, HDL, LDL and VLDL levels. On the other hand when dosage levels were increased to 350mg and 450mg/kg body wt., a significant (P<0.001) depletion in the total cholesterol level was recorded in the diabetic animals. The depletion in the TC, TG, LDL, and VLDL was dose dependent and the highest reduction in the cholesterol recorded was 9.3%, TG was 13.26%, LDL was 25.2% and VLDL was 29.31% in 450mg/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 450mg/kg body wt., dosage level (23.07%).

Histological sections of endocrine regions of pancreas of STZ 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. These damage of the β-cells due to STZ induction. The reduction in β-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 β-cells repair. This recovery of the β-cells was recorded as dose dependant that is form 250mg to 450mg/kg body weight of the combined plant extract given animals. The plant extract fed animals revealed better restored β-cells of pancreas from the STZ induced damage. The restoration of β-cells was evident at higher dose level of 450mg/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 (Mohammad Ali, *et al.,* 2004). The reduction in the serum insulin levels in the STZ 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 STZ selectively destroys the pancreatic cells and induce hyperglycemia (Gilman *et el*., 1990, Kurup and Bhonde., 2000, Jarvenin 1995). Similar studies were recorded earlier in the STZ treated rats, the levels of serum insulin significantly reduced (Yoon and Ray 1985). Nitric oxide has been demonstrated to participate in the beta cell damage during STZ induced diabetes (Duran Reges *et al.,* 2004).

Diabetes affects both glucose and lipid metabolism (Sperling *et al*., 2000). In the post prandial state elevated serum insulin increases lipoprotein 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 STZ 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 (Ginsberg, 1991). 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) 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 (Coppack, 1994,). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atheroselerosis (Bopanna, 1997).

After the administration of the combined aqueous extract to the STZ 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 STZ.

The blood glucose level of combined plant extract fed animal was significantly (P<.001) reduced. The highest depletion was recorded in the 450mg/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 *C.auriculata* and *A.marmelos* to combat the diabetic condition in rats.

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

Parameters	Normal rats (N)	Diabetic Control rats (C)	Experimental groups			*P Values
			250mg/kg body wt	350mg/kg body wt	450mg/kg body wt	
Serum insulin	15.16±1.41	11.25±1.02 P<0.001	12.55±1.01 P<0.001	13.82±1.08 P<0.001	14.20±1.04 P<0.001	0.0001
Blood glucose						
Fasting rats (Control not induced diabetes)	77.0±2.09	68.0±1.37 P<0.001	64±3.23 N.S	55±2.13 P<0.001	50±2.09 P<0.001	0.0001
Diabetes induced rats	77.0±2.09	385±2.97 P<0.0001	241±10.48 P<0.0001	192±5.09 P<0.0001	126±9.45 P<0.0001	0.0001
Lipid profile						
TC	79±2.0	86±3.03 P<0.001	80.03±2.10 P<0.001	79.12±3.14 P<0.001	78.28±2.09 P<0.001	0.0001
TG	85.06±1.14	98.07±1.26 P<0.001	92.12±3.16 P<0.001	89.23±2.19 P<0.001	85.81±2.02 P<0.001	0.0001
HDL	30.03±1.14	26.10±1.31 P<0.001	27.31±1.26 P<0.001	29.37±1.79 P<0.001	32.07±3.1 P<0.001	0.0001
LDL	32.05±1.79	40.12±2.10 P<0.001	36.07±2.61 P<0.001	34.26±2.28 P<0.001	30.12±2.73 P<0.001	0.0001
VLDL	16.25±2.37	24.41±2.74 P<0.001	22.41±2.74 P<0.001	20.14±2.16 P<0.001	16.56±2.64 P<0.001	0.0001

Each value represents six individual observations. Mean±SD, '+', '-' 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. NS-Non significance, TC- Total cholesterol, TG- Triglyceride, HDL- High density lipoprotein, LDL- Low density lipoprotein, VLDL- Very low density lipoprotein.

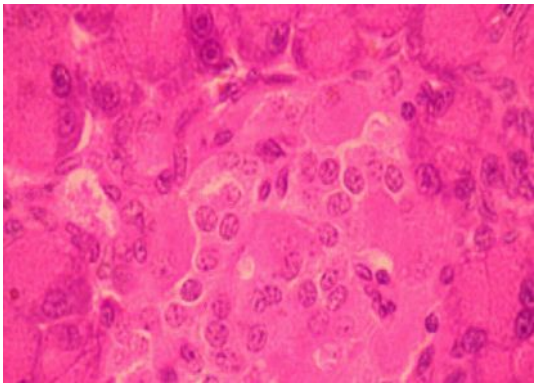

Fig-1. The Pancreatic islets of langerhans of normal rat showing alpha cells and beta cells.

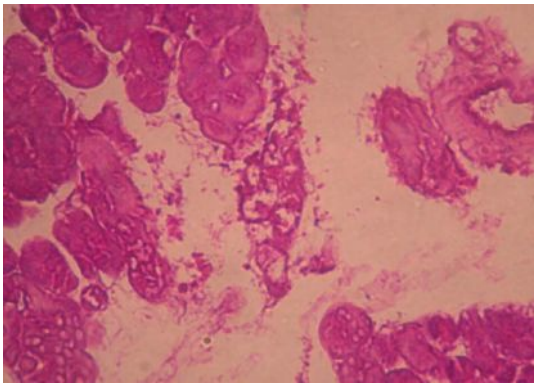

Fig-2: STZ induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells.

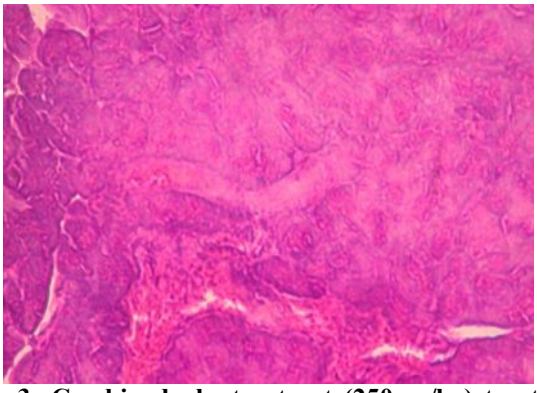

Fig-3: Combined plant extract (250mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the STZ induced diabetic control rats.

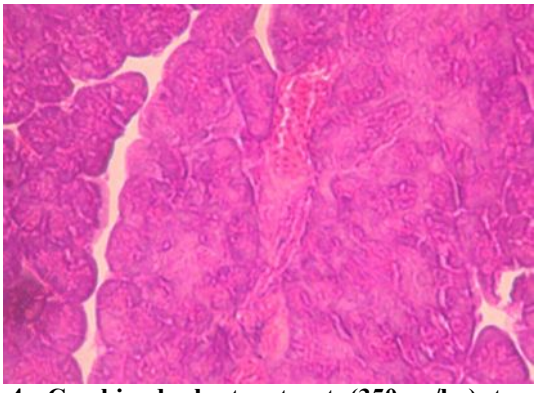

Fig-4: Combined plant extract (350mg/kg) treated pancreatic islets show partial revealed better restoration, when compared to the STZ induced diabetic and also 250 mg/kg treated rats.

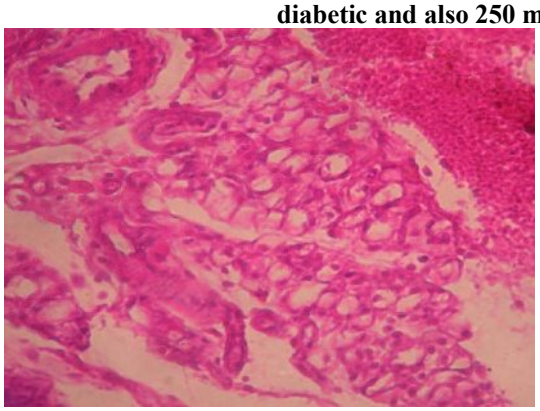

Fig 5: Combined plant extract (450mg/kg) treated pancreatic islets shows partial proliferation of beta cells. The animals revealed better restoration / proliferation from the STZ induced damage when compared to control as well as 350 mg/kg treated animal.

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