



Antihyperglycemic and antihyperlipidemic effects of extracts of the pulp of *Syzygium cumini* and bark of *Cinnamomum zeylanicum* in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: The antidiabetic effect of the aqueous extract of the pulp of Jamun (*Syzygium cumini*) and the bark of Cinnamon (*Cinnamomum zeylanicum*) in single and composite way was studied for managing streptozotocin-induced diabetes mellitus in rats.

Methodology and results: Hyperglycemia was induced by a single intraperitoneal injection of streptozotocin, which significantly elevated the blood glucose levels, decreased the serum insulin levels and decreased the body weight of diabetic rats when compared to that of normal control. Administration of the composite extract in diabetic rats resulted in a significant reduction on blood glucose levels. In addition, it significantly recovered serum insulin levels and prevented the decrease in body weight than a single administration of the extract. Hyperlipidemia, marked increase in lipid peroxide levels and concomitant decrease in antioxidant enzymes was observed in untreated diabetic rats. Treatment with composite extracts significantly reversed these conditions to near normal levels than single administration of extracts.

Conclusions and application of findings: These above results justify the use of a combination of aqueous extracts of pulp of *Syzygium cumini* and bark of *Cinnamomum zeylanicum* for the remedial effects against streptozotocin induced diabetic state.

Key words: Hyperglycemia, Composite, Insulin, Hyperlipidemia, lipid peroxides.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which results from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2009). Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus in which diabetic patients experience various vascular complications, such as atherosclerosis, coronary heart disease, diabetic nephropathy and neuropathy (Sheetz, 2002). Diabetes mellitus is associated with profound alterations in plasma lipid and lipoprotein profile which are considered as risk factors for coronary heart

disease (Betteridge J, 2002). The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes (Wild *et al.*, 2004). The major mode of controlling diabetes can be achieved by diet, exercise, insulin replacement therapy (Pankaj Modi, 2007; Sean F Dinneen, 2007). In modern medical system, managing diabetes without side effects is still a challenge. The drawback of insulin and oral hypoglycemics has increased the popularity of traditional and complimentary medicines. Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world (Huang *et al.*, 2005). Many



pharmaceuticals used in modern medicine are also of natural, plant origin (Grover *et al.*, 2002). In Indian system of folk medicine, more than 100 medicinal plants are mentioned, for managing diabetes in which more than one plant in combined way are used for correcting the health disorders and this composite plant extract either in the form of tonic or mixture exhibits a better results than single plant extract treatment (Mallick *et al.*, 2007; Eshrat Halim *et al.*, 2002; Borchers *et al.*, 1999; Wu *et al.*, 1998). Based on this background the composite extract of two plant materials have been used for this study.

In India, *Syzygium cumini* (*S.cumini*) of the family Myrtaceae has been widely used to treat diabetes by the traditional practitioners over many centuries (Nadkarni, 1954). *S.cumini* is commonly called Jamun, Black plum or Indian Black berry. It is a large tree found in all forests over the greater part of India from the sub-Himalayan tract to extreme south. It is also found in Thailand and Philippines. The fruits of *S.cumini* are oval to elliptical, 1.5–3.5 cm long, dark purple or nearly black, luscious, fleshy and edible (Chopra *et al.*, 1958). The fruits are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidin glucoside and other components. So far the antihyperglycemic activity of seeds of *S.cumini* has been well established (Shrotri *et al.*, 1963; Bansal *et al.*, 1981; Kohli *et al.*, 1983; Achrekar *et al.*, 1991; Grover *et al.*, 2000; Vikrant *et al.*, 2001; Sharma *et al.*, 2003) but there are only very few reports regarding the antihyperglycemic activity of fruit-pulp of *S.cumini* (Shrotri *et al.*, 1963; Achrekar *et al.*, 1991; Suman *et al.*, 2006; Rekha *et al.*, 2008). Here the fruit pulp of *S.cumini* was used for the current investigation.

Cinnamon zeylanicum (*C.zeylanicum*), commonly Cinnamon known as belonging to the family Lauraceae, is a small evergreen tree, approximately 10-15 m tall, native to srilanka and southern India (Jellin, 2006). *C.*

zeylanicum bark is one of the widely used cooking spice possessing potentially significant hypoglycemic effects (Jayaprakasha *et al.*, 2003). In addition to its flavouring application, *C. zeylanicum* has exhibited health beneficial properties, such as antimicrobial activity, inhibiting the proliferation of various cancer cell lines, and treating common cold (Anderson *et al.*, 2004; Murcia *et al.*, 2004). The bark contains dimeric, trimeric, and higher oligomeric proanthocyanidins with doubly linked bis-flavan-3-ol units in the molecule (Liwei *et al.*, 2004). In addition, the aqueous extracts of *C. zeylanicum* have also been shown to improve insulin receptor function by activating insulin receptor kinase and inhibiting insulin receptor phosphatase, leading to increased insulin sensitivity (Imparl *et al.*, 1998). Qin *et al.* (2003) have reported that triglyceride and total cholesterol were decreased by administration of cinnamon extract in rats treated with streptozotocin for 3 weeks. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel has been studied on streptozotocin-induced diabetes in rats (Ravi *et al.*, 2005). However there is no report about hyperlipidemic potency of these plants in composite manner.

C.zeylanicum has shown to have good antioxidant activity *invitro*, and studies in humans have demonstrated that it posses good antioxidant activity when consumed as tea (Ranjbar *et al.*, 2006). Antioxidant activity of *S.cumini* fruit skins have been reported by Archana Banerjee *et al.*, 2005. Also our previous study demonstrated the effect of pulp of *S.cumini* on the *invivo* antioxidant defense system (Rekha *et al.*, 2008).

Traditionally in Indian medicinal system it is always found that composite extract is most effective than the separate one. The present work attempts to study the antihyperglycemic and antihyperlipidemic potencies of *S.cumini* and *C.zeylanicum* in single as well as composite manner in diabetic state.

MATERIALS AND METHODS

Chemical: Streptozotocin (STZ) was obtained from Sigma Chemical Co (St Louis, MO-USA). Biochemical kits and all other chemicals utilized were of analytical grade.

Plant Materials: The plant material of *S.cumini* fruit and *C.zeylanicum* bark were procured from local market during the month of June. This was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. The voucher sample for *S.cumini* fruit (No.PARC/2006/11)

and *C.zeylanicum* bark (No. PARC/2006/12) were kept in our laboratory for reference.

Preparation of *S.cumini* Extract: The ripe fruits of *S.cumini* (1 Kg) were first washed well and the pulp was separated from the seeds. The pulp was ground for 10 min in a mixer along with the distilled water (500 ml). It was allowed to stand overnight and then filtered through several layers of muslin cloth. The whole procedure was carried out in the cold condition at 4°C. The filtrate was centrifuged in a refrigerated centrifuge at 10,000 rpm for 10 min. The supernatant was



lyophilized to get a thick paste of water extract. The yield of lyophilized water extract was about 8.9 g from 650g of pulp, obtained from 1 kg fruits of *S.cumini*.

Preparation of *C.zeylanicum* Extract: *C.zeylanicum* sticks were grinded with a plant tissue grinder. The powder (500 g) was extracted in double distilled water (5:1) overnight for 14 h with continuous stirring. The extract obtained was centrifuged at 400xg for 30 min to remove particulates and fiber. The supernatant was then filtered through Whatman #1 paper. The filtrate collected was then lyophilized and the yield obtained was about 13.1 g.

Animals: Thirty Female Wistar rats weighing 170 ± 10 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were maintained on standard rat feed supplied by Hindustan Lever Ltd., Bangalore, India. The animals were housed in stainless steel cages and kept in a room where a 12 h light/dark cycle was maintained. Rats had free access to water and standard feed throughout the period of the experiment. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

Induction of Diabetes in Rats: After one week of acclimatization, the rats were subjected to overnight fasting. Diabetes was induced with a single intraperitoneal injection of STZ, freshly dissolved in citrate buffer 0.01M, pH 4.5 (Rakieten *et al.*, 1963) at a dose of 55 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After 3 days, blood glucose levels were measured and the animals with a glucose concentration of more than 230 mg/dL were classified as diabetic (Cetto *et al.*, 2000) and taken for the experiment. Administration of the single and composite plant extracts was started on 4th day after STZ injection and this was considered the 1st day of treatment, which was continued for 15 days.

Experimental Design

Group I: Normal control rats, received citrate buffer (0.01M, pH 4.5).

Group II: Diabetic controls, received STZ (55 mg/kg body weight, Intraperitoneal once).

Group III: Diabetic rats, receiving aqueous extract of *S.cumini* (200 mg/kg body weight) orally for 15 days.

Group IV: Diabetic rats, receiving aqueous extract of *C.zeylanicum* (200 mg/kg body weight) orally for 15 days.

Group V: Diabetic rats receiving aqueous extract of *S.cumini* and *C.zeylanicum* in composite ratio (1:1) at the dose of (200 mg/kg body weight) for 15 days.

At the end of the experiment, all the rats were decapitated after fasting for 16 hours. Blood was collected without anticoagulant to separate serum for various biochemical estimations. The liver was dissected out and cleared off blood. This was immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1N Tris-HCl buffer (pH 7.4). The tissue homogenates were used for the following estimations: Thiobarbituric acid reactive substances (TBARS) and hydroperoxides were estimated according to method of Ohkawa *et al.* (1979) and Jiang *et al.* (1992) respectively. GSH was estimated by the method of Ellman (1959). The activity of SOD was assayed by the method of Misra *et al.* (1972). CAT activity was assayed by the method of Sinha *et al.* (1972).

Measurement of Body weight & Blood Glucose

Level: The body weight and blood glucose level were measured at about every 5 days interval. Blood samples were obtained by tail vein puncture of both the normal and STZ induced diabetic rats. Blood glucose level was measured by single touch glucometer.

Measurement of Serum lipid profile: Serum total cholesterol (TC) was quantified by spectrophotometric method (Allain *et al.*, 1974). Serum LDL-c and Serum HDL-c was measured according to protocol of Friendswald *et al.*, 1972 and Waenic *et al.*, 1978 respectively. Serum triglyceride was measured by using kit (McGowan *et al.*, 1983).

Serum Insulin levels : Serum insulin levels were estimated in each sample of blood using enzyme linked immunosorbant assay Kits by Brugi *et al.*, 1988 (Boehringer Mannheim, Germany).

Statistical analysis: Statistical evaluation of data was performed by Graph pad prism version.5 using one-way analysis of variance (ANOVA) followed by Dunnet's t-test. P-values < 0.05 were considered as significant.



RESULTS

Body weight changes: The body weight changes in diabetic group was significantly decreased ($p < 0.001$) when compared with the normal control, which was restored to near normal in diabetic rats treated with aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum*. The recovery of this parameter was better in composite extract treated group than separate administration. The results were shown in (Table 1).

Blood Glucose Level: The results of blood glucose level changes in normal, STZ induced diabetic rats and extract treated diabetic rats (both single and composite extract) were shown in (Table 2). There was a significant ($p < 0.001$) increase in blood glucose levels in STZ induced diabetic rats when compared with normal rats. Administration of aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* in separate manner decreased the blood glucose level to near normal but treatment with composite extract showed better decrease in blood glucose level when compared with separate administration.

Serum Lipids: The changes in the level of serum lipids in control and experimental rats are illustrated in (Fig 1 & 2). The total-cholesterol, LDL-c and triacylglycerol significantly increased and HDL-c significantly decreased in STZ induced diabetic rats ($p < 0.001$) when compared with the normal control rats. Administration of the extracts offered significant

protection against alteration in the serum lipid profile. However, the recovery was better in the composite extract treated groups than the individual extract treated group.

Serum insulin: Serum insulin levels decreased significantly in diabetic groups when compared to normal control groups. After treatment with aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* in single way the insulin levels increased to near normal. However, there was a better increase in serum insulin levels in composite extract administered group than individual extract treated groups (Fig 3).

Lipid peroxidation and Hydroperoxides: The levels of MDA in liver were significantly ($p < 0.001$) increased in STZ induced diabetic rats when compared to normal rats. Coadministration of *S.cumini* and *C.zeylanicum* extracts in composite manner offered significant reduction in the level of lipid peroxidation products (MDA) and hydroperoxides in diabetic rats than single administration of extracts (Fig 4 & 5).

Antioxidant enzymes: A significant decrease ($p < 0.001$) in the activities of antioxidant enzymes such as SOD, CAT and reduced glutathione were observed in the liver of STZ induced diabetic rats when compared with that of normal rats. Treatment with composite extracts significantly reversed these conditions to near normal levels than a single administration (Table 3)

Table 1: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on body weight in STZ induced diabetic rats.

Treatment	Body weight (g)			
	0 day	5 th day	10 th day	15 th day
Normal Control (Group I)	171.21 ± 7.3	182.79 ± 8.1	190.39 ± 7.8	204.62 ± 9.2
Diabetic Control (Group II)	173.14 ± 6.2	144.29 ± 7.4a*	136.72 ± 6.8a*	130.93 ± 8.4a*
Diabetic + <i>S.cumini</i> 200mg/kg, p.o (Group III)	171.92 ± 8.1	151.32 ± 7.2b ^{NS}	162.48 ± 7.9b*	169.12 ± 6.4b*
Diabetic + <i>C.zeylanicum</i> 200mg/kg, p.o (Group IV)	174.19 ± 8.5	146.29 ± 6.8c ^{NS}	159.24 ± 7.2c*	166.18 ± 6.4c*
Diabetic + <i>S.cumini</i> and <i>C.zeylanicum</i> coadministered group 200mg/kg, p.o (Group V)	173.18 ± 7.4	158.46 ± 7.9d [#]	167.96 ± 8.2d*	171.68 ± 6.3d*

Values are given as Mean ± SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c, d Comparison of Group III, Group IV & Group V vs Group II.

$p < 0.01$, * $p < 0.001$ statistically significant (ANOVA followed by Dunnett's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control

NS - Non significant



Table 2: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on blood glucose in STZ induced diabetic rats.

Treatment	Fasting blood glucose level (mg/dL)				
	Initial	1 st day	5 th day	10 th day	15 th day
Normal Control (Group I)	85.61 ± 7.1	88.25 ± 6.8	86.52 ± 6.2	88.92 ± 5.3	87.42 ± 5.6
Diabetic Control (Group II)	86.29 ± 5.4	236.14 ± 9.2a*	254.38 ± 7.4a*	286.48 ± 8.2a*	317.56 ± 10.2a*
Diabetic + <i>S.cumini</i> 200mg/kg, p.o (Group III)	85.12 ± 6.3	237.76 ± 7.1b ^{NS}	189.29 ± 8.6b*	156.32 ± 7.4b*	119.54 ± 6.8b*
Diabetic + <i>C.zeylanicum</i> 200 mg/kg, p.o (Group IV)	85.71 ± 7.2	235.63 ± 0.8c ^{NS}	216.39 ± 7.9c*	163.18 ± 6.2c*	131.62 ± 6.4c*
Diabetic + <i>S.cumini</i> and <i>C.zeylanicum</i> coadministered group 200mg/kg, p.o (Group V)	86.29 ± 6.2	234.54 ± 8.3d ^{NS}	161.25 ± 6.9d*	114.42 ± 5.4d*	98.29 ± 5.2d*

Values are given as Mean ± SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c, d Comparison of Group III, Group IV & Group V vs Group II.

* $p < 0.001$ statistically significant (ANOVA followed by Dunnet's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control.

Table 3: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on antioxidant enzyme levels in STZ induced diabetic rats

Treatment	SOD	CAT	GSH
Normal Control (Group I)	7.12 ± 0.52	49.17 ± 4.2	1.28 ± 0.16
Diabetic control (Group II)	3.29 ± 0.08a*	19.23 ± 3.2a*	0.486 ± 0.06a*
Diabetic + <i>S.cumini</i> 200mg/kg, p.o (Group III)	5.62 ± 0.28b*	37.94 ± 4.8b*	0.752 ± 0.04b*
Diabetic + <i>C.zeylanicum</i> 200 mg/kg, p.o (Group IV)	5.09 ± 0.18c*	32.19 ± 3.6c*	0.691 ± 0.04c*
Diabetic + <i>S.cumini</i> and <i>C.zeylanicum</i> coadministered group 200mg/kg, p.o (Group V)	6.83 ± 0.21d*	43.26 ± 5.1d*	0.931 ± 0.03d*

Values are given as Mean ± SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c, d Comparison of Group III, Group IV & Group V vs Group II.

* $p < 0.001$ statistically significant (ANOVA followed by Dunnet's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control

Units

SOD - Units/mg protein. 1 unit - The amount of enzyme required bring about 50% of inhibition of autooxidation of epinephrine.

CAT - n moles of hydrogen peroxide decomposed/min/mg protein.

GSH - n moles of DTNB conjugated /mg protein.



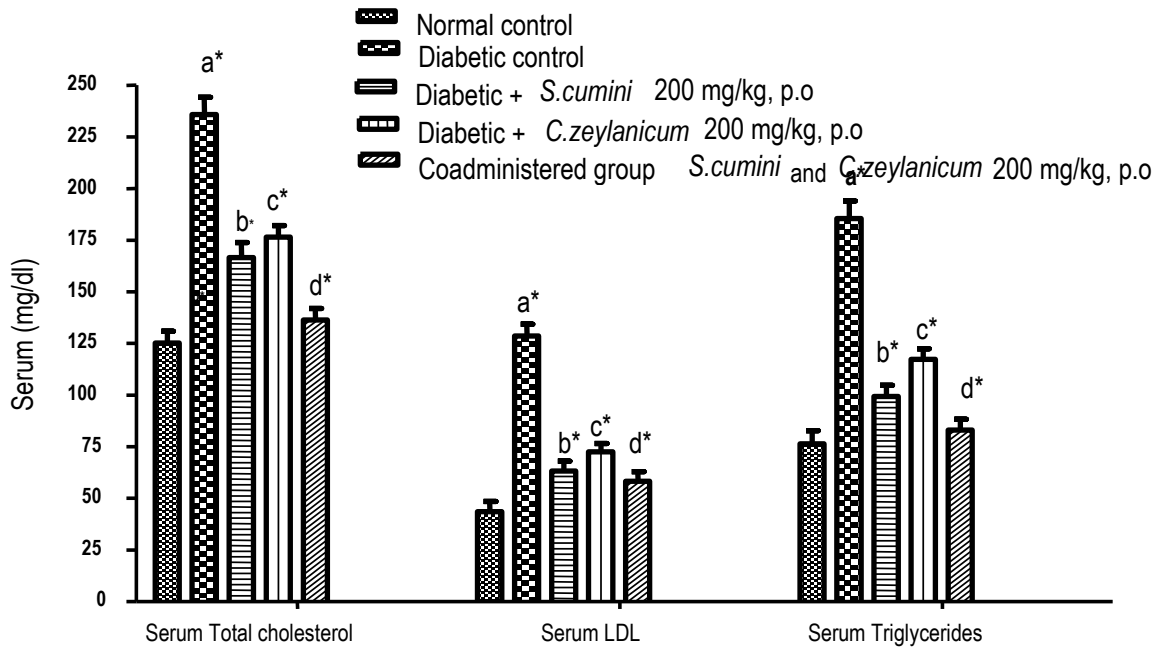


Figure 1: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on serum lipid profiles in STZ induced diabetic rats.

Values are given as: Mean ± SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c,d Comparison of Group III, Group IV & Group V vs Group II.

* $p < 0.001$ statistically significant (ANOVA followed by Dunnet's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control.

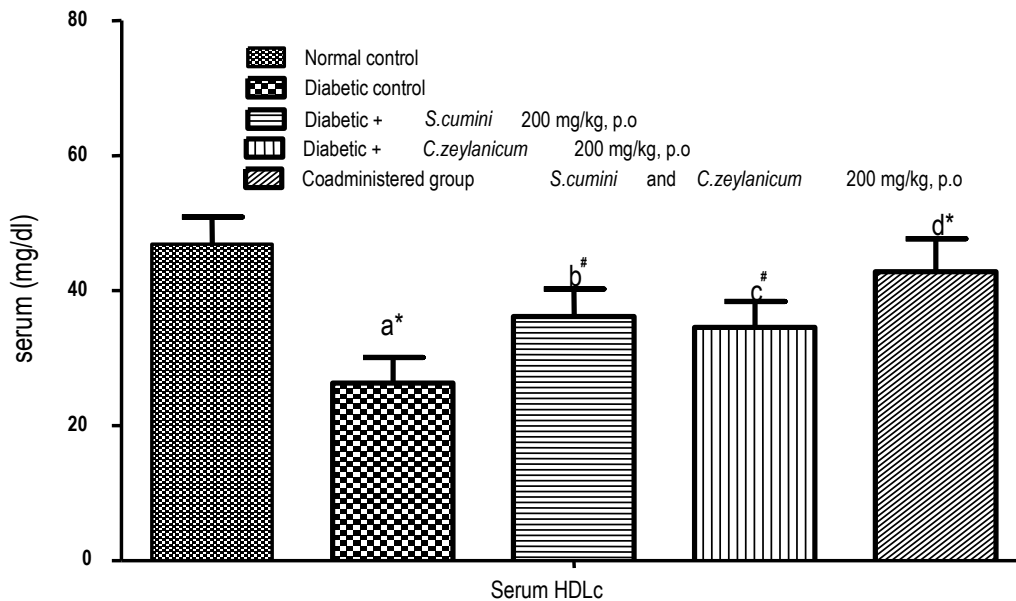


Figure 2: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on serum HDLc in STZ induced diabetic rats.



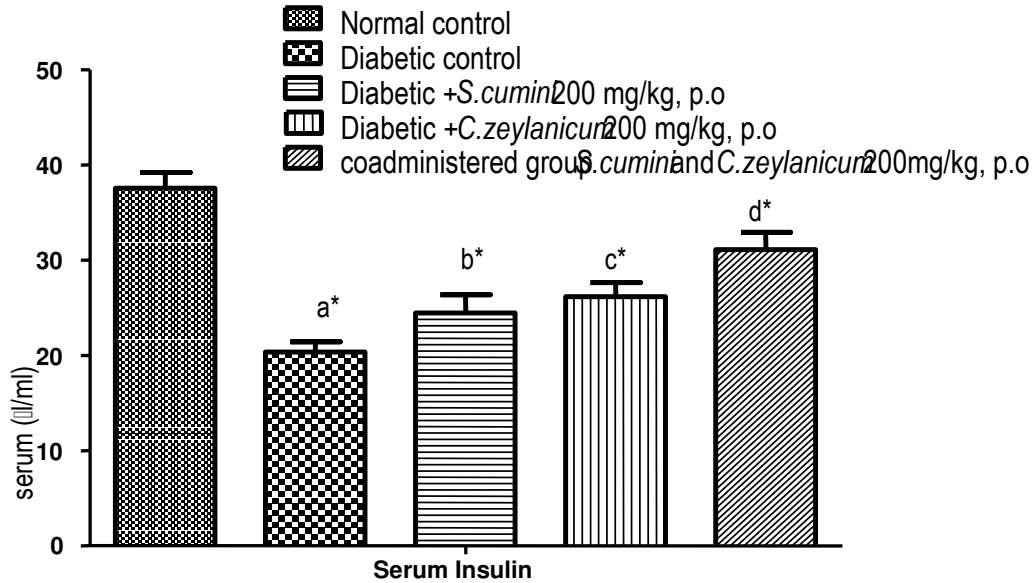


Figure3: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on serum insulin in STZ induced diabetic rats.

Values are given as :Mean ± SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c, d Comparison of Group III , Group IV & Group V vs Group II.

* $p < 0.001$ statistically significant (ANOVA followed by Dunnet's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control.

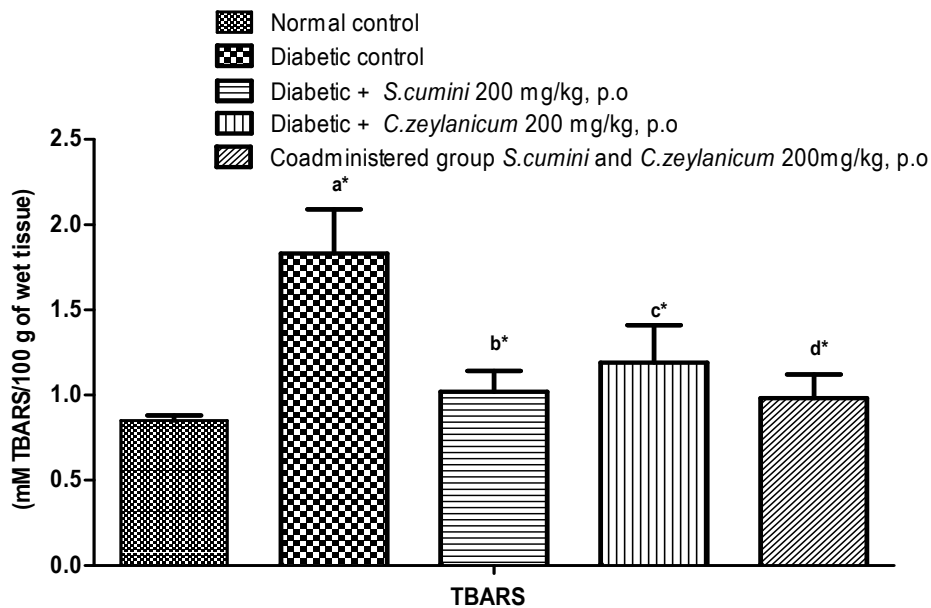


Figure 4: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on lipid peroxidation in STZ induced diabetic rats



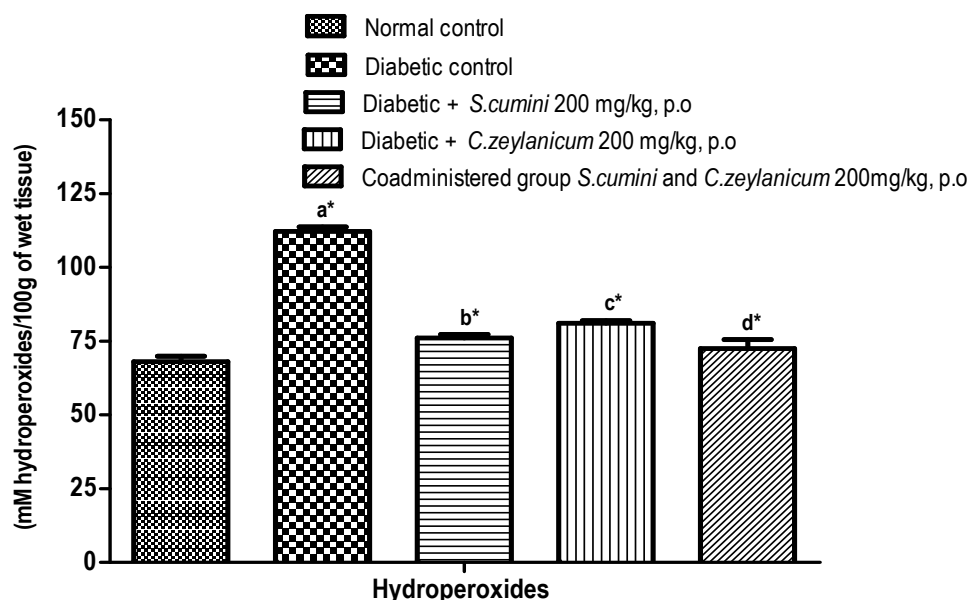


Figure 5: Effect of separate and composite aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* on hydroperoxides in STZ induced diabetic rats

Values are given as: Mean \pm SEM of six animals in each group.

a Comparison of Group I vs Group II.

b,c, d Comparison of Group III, Group IV & Group V vs Group II.

* $p < 0.001$ statistically significant (ANOVA followed by Dunnet's t-test), when diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control.

DISCUSSION AND CONCLUSION

The present investigation deals with the antidiabetogenic activity of individual and combinational effect of aqueous extract of pulp of *S.cumini* and bark of *C.zeylanicum* in single and composite way. Experimental diabetes mellitus was induced by injecting streptozotocin, which is probably due to the destruction of β cells of islets of Langerhans (Maiti *et al.*, 2004; Beppu *et al.*, 2006) leading to high levels of blood glucose in animals. Administration of the plant extracts in single as well as in composite way significantly decreased the blood glucose level in treated groups when compared to the STZ induced diabetic rats. However, the recovery was more effective in the composite extract in duration dependent manner than the single administration of extracts suggesting the hypoglycemic property of the plant products. The composite extract was more effective than single administration in significantly increasing the serum insulin levels, which could correct other essential metabolic alterations. The significant increase in serum insulin levels may be by potentiating the effect of insulin

in serum or by increasing the pancreatic secretion of insulin from the existing beta cells or its release from the bound form suggesting the insulinotropic property of these plants. From the current observation, it is evident that these findings closely correlate with some previous workers who used the above plants separately. Achrekar *et al.* (1991) reported that water extract of pulp of *Eugenia jambolana* stimulates release of insulin both in *invivo* and *in vitro*. The inhibition of insulinase activity from liver and kidney by extract of *Eugenia jambolana*, points to an extrapancreatic mechanism of action. The aqueous extract of *C.zeylanicum* was also reported to exhibit direct insulinotropic effect (Eugen *et al.*, 2005).

The characteristic loss of bodyweight in streptozotocin-induced diabetes may be due to increased muscle wasting and due to loss of tissue proteins (Shirwaikar *et al.*, 2004). The composite extract treated diabetic rats showed significant recovery in body weight gain when compared to single administration extract. This may be due to controlling muscle wasting and improvement in

insulin secretion as well as glycaemic control by the extracts.

Diabetes is associated with hyperlipidemia (Maiti *et al.*, 2005). It is well documented that there is elevation of serum lipid concentration in diabetics (Chase & Glasgow, 1976). The extract treated groups showed hypocholesteremic effect, when compared to untreated diabetic groups. The composite extract treated diabetic rats showed significant recovery in serum lipid profile when compared to single administration extract. This may be due to the presence of hypocholesterolemic compounds that may act as inhibitors for some enzymes such as hydroxyl methyl glutaryl CoA reductase, which participates in cholesterol synthesis or reduces the absorption of cholesterol from intestine (Sharma *et al.*, 2003)

There was also considerable restoration in HDL-c and LDL-c levels to near normal suggesting the hypolipidemic effect of the extracts. In IDDM patients, the HDL-c levels correlate with lipoprotein lipase (LPL) levels (Nikkila *et al.*, 1977). Also increased LDL-cholesterol may arise from glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the LDL receptor and hence, decreased metabolism (Golay *et al.*, 1986). Hence it is evident that the plant extracts may be helpful in controlling the metabolism of certain lipoproteins which results in significant attenuation of serum HDL and LDL towards normal levels thereby supporting the hypolipidemic effect of the extracts.

The deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. Bruan and Severson (1992), Lopes-Virella *et al.* (1983) reported that treatment of diabetes with insulin served to lower plasma triglyceride levels by returning lipoprotein lipase levels to normal. Administration of plant extracts to STZ induced diabetic rats improved the serum triglyceride levels suggesting its insulin like activity.

Exposure of liver to elevated glucose level reduces the activities of SOD and CAT which leads to an excess availability of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation. (Searle & Wilson, 1980). SOD is a major defense for aerobic cells combating the toxic effects of superoxide radicals (Mc Crord *et al.*, 1976). CAT protects tissues from highly reactive hydroxyl radicals (Chance *et al.*, 1952). Any compound, natural or synthetic, with antioxidant properties, might contribute

towards the partial or total alleviation of this damage. Therefore, removing O_2^- and OH^* is probably one of the most effective defenses of a living body against diseases (Lin *et al.*, 1995). Single and combinational administration of *S.cumini* and *C.zeylanicum* increased the activity of SOD and CAT enzymes in STZ induced diabetic rats suggesting the free radical scavenging activity and its protective effect against tissue damage. Our studies showed the decreased level of GSH in diabetic rats. Decreased activity of GSH levels in liver during diabetes may be due to its increased utilization by the hepatic cells in order to counteract the increased formation of lipid peroxides (Gregus *et al.*, 1996). Our study showed that administration of extracts helps in restoring these levels to near normal.

Streptozotocin generated lipidperoxidation and DNA breaks in pancreatic islet cells have been demonstrated (Rakieten *et al.*, 1963). Prakasam, (2003) have reported an elevated lipid peroxidation and lowered antioxidants in streptozotocin induced diabetes mellitus. Also it has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides (Metz, 1984; Walsh & Pek, 1984). Increase in lipid peroxidation (TBARS), is an indirect evidence of intensified free radical production (Maritim *et al.*, 2003). In the present study, the concentrations of lipid peroxides and hydroperoxides were significantly increased in liver of diabetic rats, indicating an increase in the generation of free radicals. An observed increase in the level of TBARS in liver may be due to increased susceptibility of the tissue of diabetic rats to lipid peroxidation (Matkovics *et al.*, 1998). Administration with extracts protects the cells through attenuation of lipid peroxidation and decreased the production of free radical derivatives, as evident from the decreased levels of liver MDA and hydroperoxides.

Thus this study showed that administration of aqueous extract of pulp of *S.cumini* and bark *C.zeylanicum* in composite manner exhibited better antidiabetogenic activity than when compared to the individual extract of the plants. It can be suggested that the active antihyperglycemic agents present in the composite extract helps in overcoming the diabetic complications by increasing the insulin secretion or by scavenging free radicals and preventing the depletion of endogenous antioxidants. However the exact mechanism is not clear and further biochemical and pharmacological investigations are needed to isolate and identify the active ingredient(s) in the composite extract.



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