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Diabeto-protective studies of albizzia lebbeck and syzygium cumini (l-syzygium gambolana)

G V Balakrishna[1], Krishna Sowmya J[2],Vijay Raj B[3], Yogesh Acharya[4] and M.V. Raghavendra Rao[4]

[1]Department of biotechnology, Acharya Nagarjuna University, Guntur, AP, India.

[2]Burjil Hospital, Abu Dhabi, UAE.

[3]Loyola college, Vijayawada, Andhra Pradesh, India.

[4]Avalon University School of Medicine, Curacao, Netherlands Antilles.

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ABSTRACT

Albizzia lebbeck and Syzygium cumini are two natural plants with secondary metabolites and antioxidant properties, which was shown to have a diabeto-protective effect in animal experiments. There were major reductions in the levels of endogenous antioxidant enzymes like superoxide dismutase, catalase and reduced glutathione after 30 days of experimental period with STZ suggesting the generation of free radicals in animals. The co-administration of aqueous, methanolic and methanolic fraction of aqueous extract of Albizzia lebbeck and Syzygium cumini significantly increased these antioxidant enzymes and reduced the elevated serum levels of malondialdehyde in the experimental animals. The established diabetoprotective actions of various extracts of Albizzia lebbeck and Syzygium cumini in experimental hepatic injury in rats widens the scope for further investigations in the field of research on other models either alone or in combination with other herbal molecules with proven diabeto-hepato-protective action in clinical practice.

Introduction

Plants are capable of synthesizing a unique and complex variety of low molecular weight organic compounds, called as secondary metabolites. These secondary metabolites include phenols, steroids, alkaloids, terpenoids and glucosinolates etc. They have an increased implication in organic utility productions including pharmaceuticals, insecticides, fragrance and dyes. The pharmaceutical values of medicinal plants reside on the quantity and quality of secondary metabolites present in them. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds (Briskin, 2000).

Some of these plants may also have a potential effect in Diabetes Mellitus (DM). Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis and lipid metabolism. DM is typically divided into Insulin Dependent DM (IDDM) or type I, more common in younger age groups and Non Insulin Dependent DM (NIDDM) or type II, more commonly seen in adults or increasing age.

Diabetes is a major determinant of health in modern society with rapidly increasing disease burden. Diabetes has the capability to affect different organs in the long run and acutely. This complication can result in acute conditions like Diabetic Ketoacidosis(DKA), Hyperosmolar nonketotic coma and predilection of different infections. Similarly the long term complication can be due to diabetic microangiopathy; leading to retinopathy, nephropathy, neuropathy, diabetic foot and adverse cardiovascular events. Because of these potential complications and increased morbidity and mortality related with it, diabetes has become the major determinant of health and wellness in the modern society.

Role of Oxidation in Diabetes

Much of the evidence concerning a role for oxidation in the induction of diabetes mellitus comes from the study of two drugs which induce diabetes in experimental animals; alloxan and streptozotocin (STZ). Both of these chemicals appear to selectively destroy the islets of Langerhans by oxidant production. Desferrioxamine blocks diabetes inducted by multiple low doses of STZ (Wolff, 1993), suggesting that transition metal catalyzed free radical reactions may be contributed to STZ toxicity, but the situation is clouded by conflicting effects of other free radical modifiers as well as the very recent invocation of nitric oxide both in insulin secretion as well as islet killing during insulitis (Malaisse, 1982).

Many studies have compared lipid peroxidation products in normal and age-matched plasma and tissues with those from diabetic individuals, using the non-specific thiobarbituric acid (TBA) assay. Lipid peroxide levels in diabetic plasma have been found to be significantly higher than in healthy individuals (Wolf, 1993). Increased levels of plasma TBA-reactivity have been found in NIDDM patients with retinopathy (Armstrong, 1991) and angiopathy (Higuichi, 1982; Barbosa, 1984).

These human data are consistent with work on diabetic animals. In streptozotocin-diabetic rats, the level of kidney and retinal lipid peroxides increases about 2-fold, which appears to correlate with the severity of retinopathy (Milani, 2005).

Tele: 5999-691-0461
E-mail address: reachdrmvrrao@gmail.com

The location of peroxide in plasma is not well-defined by these studies but low density lipoprotein (LDL) has been reported to be more prone to oxidation in diabetes (Milani, 2005).

Role of Anti-oxidant in Diabetes

Extreme caution has to be observed in interpretations of classical indicators of lipid peroxidation in disease processes. Further evidence of oxidative stress can be gathered from examination of antioxidant status. Most importantly, plasma levels of ascorbic acid are decreased in both human and animal diabetes, including white blood cells. Subsequently the level of dehydroascorbate, its primary oxidant product is increased (Rahimi, 2005). Platelet vitamin E level has been observed to be depressed in rats with chemically induced diabetes in IDDM (Dornan, 1982) and in NIDDM (Steams, 1978). Total plasma copper levels are higher in diabetic individuals than normal, and are highest in diabetics with angiopathy and alterations in lipid metabolism (Barbosa, 1984). It is unknown whether this copper is present in the form of ceruloplasmin or as a fraction able to catalyze the oxidation of reducing agents. The evidence that there are abnormalities in transition metal metabolism in diabetes mellitus is derived, in particular, from investigation of the long-term protein modification reactions which accompany diabetes.

Blood Glucose Determination

STZ treated rats are catabolic in nature and tissues exhibit insulin resistance despite increased in insulin binding (Meyerovithch, 2002). STZ is a B-Cell cytotoxic agent which can result in a deficiency of insulin in the experimental animals and significant increase in the blood glucose level. We found that the blood sugar levels (BSL) were increased significantly ($p<0.001$) in group 2 (STZ) as compared to group 1 (Control). The STZ also affects lipid levels in animals. These animals exhibit low endogenous production of insulin with high levels of circulating glucose.

The BSL levels were decreased significantly ($P<0.001$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ) (Table-2). This may be because of ability of extracts to improve either the insulin reaction from B-cells or the carbohydrate absorption in experimental animals.

Table 1. Effects of administration of Albizzia lebbeck and Syzygium cumini on the levels of blood sugar (Glucose) levels of rats after 30 days of various treatments.

Group No	Groups	BSL (mg/dl)
1	Control	102.5 ± 6.8
2	STZ	384.6 ± 9.3[C]
3	ASW	101.2 ± 5.6
4	ASW+STZ	198.6 ± 7.8[C]
5	ASM	102.9 ± 4.8
6	ASM+STZ	165.6 ± 6.2[C]
7	ASWM	105.2 ± 3.6
8	ASWM+STZ	178.6 ± 7.2[C]

Values are expressed as mean ± SEM, n= 6.

STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

Degrees of freedom (6, 30); NS means not significant.

Body Weight of Experimental Animals

There is a decrease in body weight of animals after exposure to STZ as compared to control. The body weight of animals was decreased significantly ($p<0.05$) in group 2 (STZ) as compared to group 1 (Control) in our experiment. However, no significant change was observed in body weight of animals treated with various extracts of Albizzia lebbeck. It has been reported that STZ decreases the body weight of animals probably due to changes in the metabolic process of the body (Mohamamad, 2009). The body weights of animals were increased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and group 8 (ASWM+STZ) as compared to group 2 (STZ). It is also indicative that treatment with ASW, ASM and ASWM extracts to experimental animals has shown protection from the deleterious effects of STZ and subsequent loss in body weight (Table-1).

Table 2. Effects of administration of *Albizzia lebbeck* and Syzygium *cumini* administration on body weight and organ weight in rats after 30 days of various treatments.

Group No	Groups	Body Weight (gm)
1	Control	216.00 ± 9.0
2	STZ	145.00 ± 7.6[a]
3	ASW	210.00 ± 11.0
4	ASW+STZ	183.00 ± 5.6[a]
5	ASM	212.00 ± 6.0
6	ASM+STZ	176.00 ± 6.3[a]
7	ASWM	210.00 ± 11.0
8	ASWM+STZ	183.00 ± 5.6[a]

Values are expressed as mean ± SEM, n = 6.

STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b :<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

Degrees of freedom (6, 30); NS means not significant.

Serum Lipids Determination

The earlier reports of (Jaiswal, 2009) (Eliza J, 2009) have shown prominent increase in serum and tissue lipid levels in rats treated with STZ. In our experiment , the serum triglyceride levels (TGLY) (Table-3), total cholesterol levels (CHOL) (Table-4) and low density lipoprotein level (LDL) (Table-5) were increased significantly ($p<0.01$) in group 2 (STZ) as compared to group 1 (Control). Similarly they were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ). STZ had an inverse effect in serum high density lipoprotein cholesterol level (HDL), which was decreased by STZ and increased by plant extract (Table-6).

It was observed in our study that, the Albizzia lebbeck and Syzygium cumini extracts i.e. ASW; ASM & ASWM have hypolipidiomic property. The extracts produced

conspicuous effects by antagonizing the elevated levels of serum lipids in animals. This might be because of either enhancing cholesterol metabolism via HDL-cholesterol which constitutes 50% of total cholesterol.

Macrophages do not form foam cells with native LDL but with oxidatively modified LDL (OLDL) (Goldstein, 1979). When the influx of LDL particles and their subsequent oxidative modification exceeds the removal capacity of the macrophage scavenger receptor system, the oxidized LDL particles accumulate within the arterial intima and cause injury to endothelian cells, smooth muscles and macrophages, forming atherosclerotic plaque. There is considerable direct and indirect evidence that oxidatively modified LDL component is demonstrated within the lesions on the line with the above STZ to produced prominent increase in lipid levels in experimental animals. The ASW, ASM & ASWM extracts produced a conspicuous effect by antagonizing the elevated levels of serum lipids in animals. Reduction in plasma LDL levels reduces the risks of clinical events related to CAD, in both patients with preexisting CAD and with hypercholesterolemia who are free of CAD at the beginning of the intervention (Nagai R, 2008).

Treatment with all the extracts has significantly reduced the levels at triglycerides in serum of animal exposed to STZ. Hypertriglyceremia is also associated in metabolic consequences of hypercoagulability insulinemia, insulin resistance & glucose tolerance (Ginsherg, 1994). This may prevent the progression of atherosclerosis and complications of hypertriglyceridemia (Anstin, 1994). All the extracts under evaluation increased the HDL cholesterol levels thus it may be useful in diseases like DM & CHD because of their inverse relationship.

Table 3. Effects of administration of *Albizzia lebbeck* and *Syzygium cumini* on the levels of Serum Triglycerides (TGLY) of rats after 30 days of various treatments.

Group No	Groups	TGLY mg/dl)
1	Control	12.19 ± 0.61
2	STZ	34.79 ± 0.52[b]
3	ASW	12.01 ± 0.54
4	ASW+STZ	26.41 ± 0.35[a]
5	ASM	12.65 ± 0.33
6	ASM+STZ	24.25 ± 0.32[a]
7	ASWM	11.95 ± 0.33
8	ASWM+STZ	19.25 ± 0.32[a]

Values are expressed as mean ± SEM, n = 6.

STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

Degrees of freedom (6, 30); NS means not significant.

Table 4. Effects of administration of Albizzia lebbeck and Syzygium cumini extracts on the levels of serum Cholesterol (CHOL), of rats after 30 days of varioustreatments.

Group No	Groups	CHOL (mg/dl)
1	Control	67.52 ± 0.97
2	STZ	88.42 ± 0.73[b]
3	ASW	68.12 ± 0.92
4	ASW+STZ	80.02 ± 0.62[a]
5	ASM	68.23 ± 0.84
6	ASM+STZ	76.89 ± 0.52[a]
7	ASWM	67.23 ± 0.84
8	ASWM+STZ	71.89 ± 0.52[a]

Values are expressed as mean ± SEM, n = 6.

STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

Degrees of freedom (6, 30); NS means not significant.

Table 5. Effects of administration of Albizzia lebbeck and Syzygium cumini, sweet extracts on the levels of serum Low Density Lipoprotein Cholesterol (LDL), of rats after 30 days of various treatments.

Group No	Groups	LDL (mg/dl)
1	Control	35.3 ± 1.84
2	STZ	83.6 ± 2.35[b]
3	ASW	34.9 ± 2.61
4	ASW+STZ	56.3 ± 3.20[a]
5	ASM	35.5 ± 3.21
6	ASM+STZ	55.3 ± 2.32[a]
7	ASWM	35.2 ± 2.35
8	ASWM+STZ	38.2 ± 3.31[a]

Values are expressed as mean ± SEM, n = 6.

STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).

P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.

Degrees of freedom (6, 30); NS means not significant.

Table 6. Effects of administration of *Albizzia lebbeck* and *Syzygium cumini* on the levels of serum High Density Lipoprotein Cholesterol (HDL), of rats after 30 days of various treatments.

Group No	Groups	HDL (mg/dl)
1	Control	33.6 ± 1.93
2	STZ	16.4 ± 1.62[b]
3	ASW	33.40 ± 1.81
4	ASW+STZ	25.60 ± 2.36[a]
5	ASM	32.90 ± 1.35
6	ASM+STZ	21.35 ± 2.52[a]
7	ASWM	32.80 ± 1.81
8	ASWM+STZ	20.81 ± 1.56[a]

Values are expressed as mean ± SEM, n = 6.
STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).
P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.
Degrees of freedom (6, 30); NS means not significant.

Serum Malondialdehyde (MDA) Determination

Toxic oxygen metabolite has emerged as a major final common pathway of tissue injury in a wide variety of desperate disease process (Hiraishi H, 1992). Consequently, free radical ablation offers a substantial for the treatment of human disease. This is because many constituent of the cell are potentially subject to free radical attack (Slater, 1972). Toxic metabolites are generated normally by aerobic metabolism in cells and this generation can significantly increase in certain pathological conditions. When endogenous antioxidant defense capabilities are exceeded by this oxidative flux, tissue injury occurs.

MDA is an indicator of free radical oxidative stress which is produced by peroxidation of polyunsaturated lipids (Pryor WA, 1975). Changes observed in lipid peroxidation product, endogenous antioxidants, other enzymes and lipids after 30 days' treatment schedule were observed as per experimental design. A frequent cellular target is deactivation, base hydroxylation of nucleic acids, cross linking or strand scission, mutation or even in cell death. Extracellular tissue components, including hyaluronic acid and collagen are also vulnerable to tissue injury by toxic oxidants. The progression from free radical generation to tissue injury yields many levels for potential intervention (Ji, 2001).

The serum MDA levels were increased significantly ($p<0.05$) in group 2 (STZ) as compared to group 1 (Control). Lipid peroxidation is a free radical mediated process which has been implicated in a variety of disease sates. Biological membranes lipids are susceptible to peroxidative attack. Lipid peroxidation products were increased in animals exposed to STZ. This may be due to high lipogenic activity of STZ itself. Thus lipid hydroperoxides and conjugated diene measurement has an advantage over thiobarbituric acid assay.

Increase in lipid peroxidation in STZ treated rats suggests increased phospholipase activities during peroxide deposition of different sub organelle and plasma membrane lipids (Rhee SJ, 2005). A relationship has been suggested by previous researchers (Adibhatla, 2008 and Battacharya, 2009). Activation of PLA^2 causes production of variety of eicosanoids which are responsible for different metabolic disorder and cell injury.

The serum MDA levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ) (Table-7). Antioxidant herbal Extracts may protect hepatocytes by direct action by thiol restoration and under oxidant insult a number of enzymes important for the release and metabolism of AA might undergo S-thiolation.

Table 7. Effects of administration of Albizzia lebbeck and Syzygium cumini on the levels of serum Malondialdehyde (MDA), of rats after 30 days of various treatments.

Group No	Groups	MDA (nMol/ml)
1	Control	1.89 ± 0.09
2	STZ	5.06 ± 0.09[b]
3	ASW	1.92 ± 0.08
4	ASW+STZ	3.32 ± 0.11[a]
5	ASM	1.89 ± 0.65
6	ASM+STZ	2.73 ± 0.19[a]
7	ASWM	1.91 ± 0.08
8	ASWM+STZ	2.63 ± 0.11[a]

Values are expressed as mean ± SEM, n = 6.
STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).
P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.
Degrees of freedom (6, 30); NS means not significant.

Serum Super oxide dismutase (SOD) Determination

Superoxide dismutase metabolizes superoxide anion radical. It is an effective defense of the cell against endogenous generation of superoxide radical (Halliwell, 1989).

The serum SOD levels were increased significantly ($p<0.01$) in group 2 (STZ) as compared to group 1 (Control). The MDA levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ) (Table-8).

In this present study, the superoxide dismutase levels were decreased significantly in serum of STZ treated animals. This may be attributed to the release of superoxide radical in the body of the animals due to exposure to STZ. On the other hand, the superoxide dismutase levels were restored significantly to normal in animals treated with extracts of the plant; this is a major indication of protective effect of the plant extracts against oxidative damage.

Table 8. Effects of administration of *Albizzia lebbeck* and Syzygium *cumini* extracts on the levels of serum Super Oxide Dismutase (SOD), of rats after 30 days of various treatments.

Group No	Groups	SOD (U/ml)
1	Control	3.32 ± 0.12
2	STZ	1.62 ± 0.09[b]
3	ASW	3.36 ± 0.16
4	ASW+STZ	2.89 ± 0.13[a]
5	ASM	3.32 ± 0.18
6	ASM+STZ	3.11 ± 0.16[a]
7	ASWM	3.29 ± 0.18
8	ASWM+STZ	3.25 ± 0.16[a]

Values are expressed as mean ± SEM, n = 6.
STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and

syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).
P values: a: <0.05, b: 0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.
Degrees of freedom (6, 30); NS means not significant.

Serum Catalase (CAT) Determination

Catalase has been reported to be responsible for detoxification of hydrogen peroxide (Kaliakos, 2008). Catalases may function to protect the cells against hydrogen peroxide and its toxic effects in cells.

The superoxide dismutase, catalase and reduced glutathione levels were also reported to be decreased in rats exposed to STZ with an increase in lipid peroxidation in the earlier study (Rhee, 2005). In the present study, the catalase levels were decreased significantly in animals exposed to STZ. The serum SOD levels were increased significantly ($p<0.01$) in group 2 (STZ) as compared to group 1 (Control). The MDA levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ) (Table-9).
This is again an indication that the STZ is generating the hydrogen peroxide like molecules upon exposure. Administration of various extracts of Albizzia lebbeck and Syzygium cumini significantly effective in bringing back the levels of catalase to normal in all the experimental animals exposed to STZ.

Table 9. Effects of administration of *Albizzia lebbeck* and *Syzygium cumini* extracts on the levels of serum Catalase (CAT), of rats after 30 days of various treatments.

Group No	Groups	CAT (Kat f/ml)
1	Control	6.35 ± 0.12
2	STZ	2.92 ± 0.12[b]
3	ASW	6.38 ± 0.43
4	ASW+STZ	4.93 ± 0.83[a]
5	ASM	6.15 ± 0.18
6	ASM+STZ	5.66 ± 0.85[a]
7	ASWM	6.32 ± 0.19
8	ASWM+STZ	5.93 ± 0.81[a]

Values are expressed as mean ± SEM, n = 6.
STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).
P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.
Degrees of freedom (6, 30); NS means not significant.

Serum Reduced Glutathione (GSH) Determination

Decrease in the concentration of GSH brings about impairment of the cellular constituents. Glutathione protects the tissue by combining with the reactive metabolites of STZ and thereby preventing their covalent bonding to tissue protein. It is found in our study GSH levels decreased consistently in STZ treated rats.

In cells, oxidation of glutathione (GSH) leads to the formation of glutathione disulphide (GSSG) by disulphide interchange catalyzed by thiol transferases, changes in the level of reduced glutathione were observed in drug toxicity.

The serum GSH levels were increased significantly ($p<0.01$) in group 2 (STZ) as compared to group 1 (Control). The serum GSH levels were decreased significantly ($P<0.05$) in group 4 (ASW+STZ), group 6 (ASM+STZ) and in group 8 (ASWM+STZ) as compared to group 2 (STZ) (Table-10). It implicates that under thiol depleted conditions one could expect uninhibited as well as accelerated uptake of ROS by STZ due to loss of GSH with concurrent increase in GSSG content.

Table 10. Effects of administration of *Albizzia lebbeck* and *Syzygium cumini* extracts on the levels of serum Reduced Glutathione (GSH), of rats after 30 days of various treatments.

Group No	Groups	GSH (nMol/ml)
1	Control	4.82 ± 0.61
2	STZ	1.83 ± 0.12[b]
3	ASW	4.95 ± 0.23
4	ASW+STZ	3.83 ± 0.19[a]
5	ASM	4.87 ± 0.12
6	ASM+STZ	3.98 ± 0.22[a]
7	ASWM	4.96 ± 0.16
8	ASWM+STZ	4.12 ± 0.34[a]

Values are expressed as mean ± SEM, n = 6.
STZ is Streptozotocin administered at the dose of (45mg/kg, body weight, i.v.), ASW is aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzia lebbeck and syzygium cumini, sweet (1ml/kg body weight, i.p.).
P values: a: <0.05, b:<0.01, c:<0.001 When group 2, 3, 5 and 7 are compared with group 1; Group 4, 6 and 8 are compared to group 2.
Degrees of freedom (6, 30); NS means not significant.
There is good evidence that superoxide dismutase (SOD) and catalase are the enzymes that scavenge the free radicals during lipid peroxidation. SOD is an enzyme which scavenges superoxide radical and catalases (CAT) catalytically decompose hydrogen peroxide. It is clear that the cytotoxicity of molecular oxygen is held in check by the delicate balance between the rate of generation of the partially reduced oxygen species and the rate of their removal by the different defense mechanisms, and shift in this delicate balance can lead to cellular damage. STZ treated rats in normal and atherogenic diet shows a decreased activity of these enzymes suggesting partially reduced oxygen species. Such changes are observed in variety of disorders mediated by free radicals. All our extracts ameliorate the depleted levels of CAT and SOD in rats treated with STZ. These along with a correction of the lipid peroxidation by the drugs strongly express their antiperoxide, antioxidant effect in various pathological for heart, liver and kidney.

Histopathological Changes.

1. Liver

The inflammatory response in liver and its repair (liver cell regeneration) following exposure to STZ have been known to be mediated by cytokines (Talbot, 2009). The histopathological examination of liver and kidney showed the pathological changes after exposure to STZ during the experimental period. The lesions include early changes like

dilation of rough endoplasmic reticulum, increase in hydropic changes (swelling) with loss of cristae, pyknotic nuclei and clumps of coagulated chromatin leading to shrinking of nucleolus and nucleus. There was overall fatty infiltration (swelling) in hepatic cells along with the hydropic changes.

There were few signs of inflammatory mediator's infiltration in the postal tracts with spillover into the adjacent parenchyma. The infiltration of polymorphs was prominent during the study. Treatment with our extracts to normal animals did not alter any ultrastructure of liver. But administration of these compounds to the experimental animals significantly decreased the severity of damage to liver and kidney. There was partial lipid deposition and mild degree of swelling (seen by less number of vacuoles in the cells) in animals treated with extract ASM & ASWM along with STZ. There was reduction in hydropic changes and fatty infiltration in animals exposed to STZ and treated with ASW, ASM & ASWM extracts. There was less degree of damage to the liver and kidney cells observed in animals treated with ASM & ASWM extracts where shrinkage of nucleolus and nucleus were found along with mild architectural damage.

2. Kidney

In present study, there are prominent histopathological changes in renal cells, which might be attributed to increased renal burden by STZ. There are also evidences that STZ may be associated with renal neoplasia in experimental animals (Miyao, 1991).

The histopathological changes in kidney- indicated the swelling in renal tubular epithelial cells of animals exposed to STZ. In the present study there was an enlargement of kidney with infiltration in the renal cells. In addition to these lesions, the kidney showed marked cystic dilation of the proximal convoluted tubules. Granular debris was seen in tubular Lumina along with fatty infiltration.

Treatment with various extracts of *Albizzia lebbeck* and *Syzygium cumini* i.e; ASM, ASW & ASWM did not showed any changes when administered alone. But administration of these extracts to the animals exposed to STZ showed protective changes in kidneys of experimental animals in terms of decreased fatty deposition and decrease in tubular epithelial swelling (hydropic changes).

Figures: Photomicrographs showing kidney of rats after the following treatments for 30 days :(Magnification 10X)

Plate I. Group I. Control.

► Hydropic Changes

Dilated Proximal Tubule

Fatty Infiltration

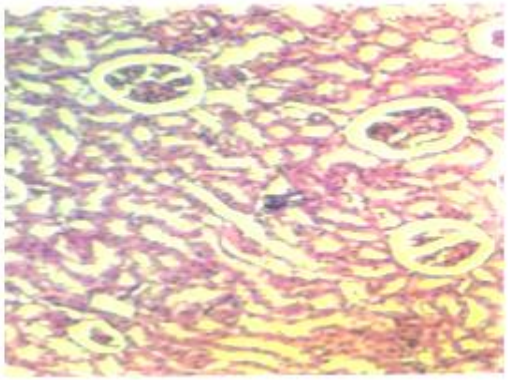


Plate-II. Group-II. STZ.

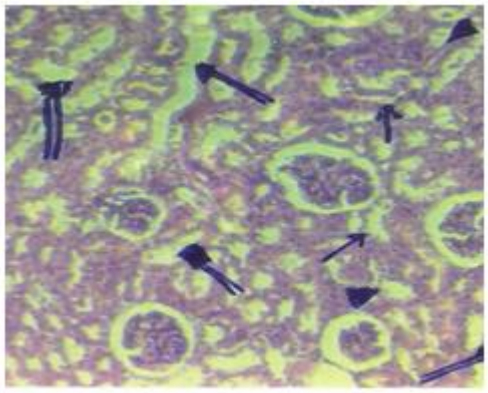


Plate-III.Group-III. ASW+STZ.

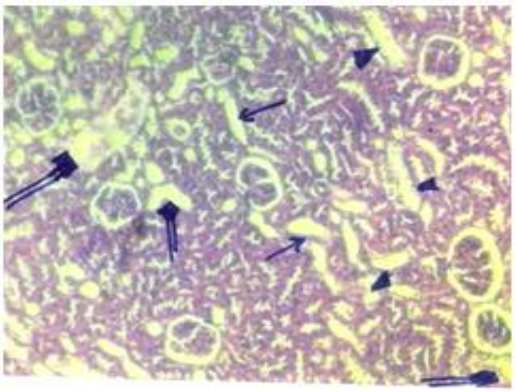


Plate-IV. Group-IV. ASM+STZ.

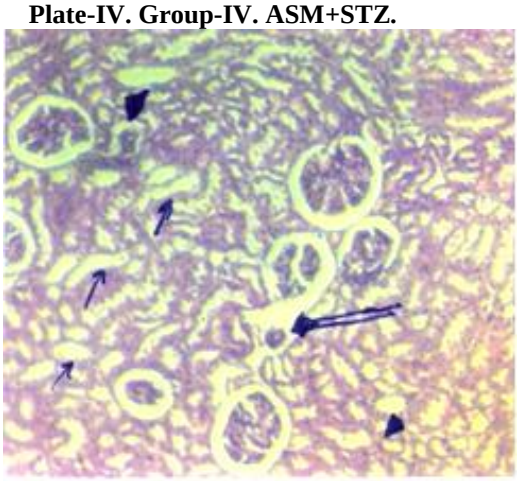
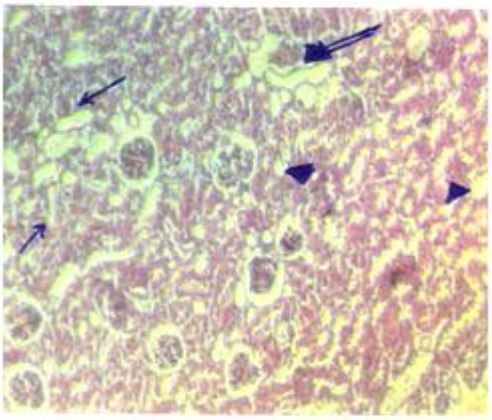


Plate-V. Group-V. ASWM+STZ.



Discussion

Various herbs do possess a potent antioxidant, anti-stress and protective properties by generation of various active endogenous antioxidants like SOD, CAT & GSH or modulates the levels of exogenous antioxidants like vitamin C, vitamin E etc. that forms an active metabolite that protects different organs from oxidative injury. In many pathophysiological conditions, local endogenous antioxidant capabilities can be exceeded and tissue injury may result. In these cases, the administration of exogenous antioxidants to counteract the proportionate magnitude of the cell injury plays a pivotal role in the treatment of free radical mediated diseases. Optimization of antioxidant status by exogenous antioxidants should offer a preventive for 'optimum health' defined by WHO.

Thus the present study has shown that the oxidative stress in animals is through the generation of free radicals, and is an important consequence of intoxication with streptozotocin and d-galactosamine. This study also documented the antidiabetic action of aqueous extract of Albizzia lebbeck and Syzygium cumini, exhibited by virtue of their organoprotective and antioxidant property. This findings in experimental rats widens the scope for further investigations in the field of research on other models like obesity, type-II diabetes, alcoholic hepatitis, viral hepatitis, cirrhosis and carcinoma of the liver, on varieties of allergies either alone or in combination with other herbal molecules with proven diabeto-protective action in clinical practice

Conclusion

Plants have the natural properties to produce the secondary metabolites which can have added benefit in treatment of many metabolic diseases. One of the most studied metabolic diseases is diabetes mellitus. Diabetes Mellitus is a muti-factorial multisystem disease with a high morbidity and mortality. There are evidences, where the progression of disease process in DM can be halted or controlled with natural products. We have discussed about the extracts from the two plants *Albizzia lebbeck* and *Syzygium cumini* here. These plants extract have shown significant beneficial effect in experimental study in diabetes in animals. The possibility of the use of these plant extracts and its potential to control the disease process should be studied extensively and explored in humans.

References

- Adibhatla, RM., Hatcher, JF. (2008). Phospholipase A2, reactive oxygen species, and lipid peroxidation in CNS pathologies. *BMB Rep*., 41(8), 560-567.
- Anstin, NA., Mokanson, JE. (1994). Epidemiology of triglycerides small dense low density lipoprotein metabolism. New York: H Greten, Springer-verlag, 119-124.
- Armstrong, D., Al-Awadi, F. (1991). Lipid peroxidation and retinopathy in streptozotocin induced diabetes. *Free Radic Biol Med*., 11, 433-436.
- Barbosa, J., Saner, B. Do genetic factors play a role in the pathogenesis of diabetic microangiopathy? *Diabetologia*. 27, 487-492.
- Battacharya, A et al. (2009). Denervation induces cytosolic phospholipase A2 mediated fatty acid hydroperoxide generation Albano, E., Lott, K., Slater. TF,. Stier. A., Symons, M., Tomasi. (1982). A. Spin-trapping studies on the free radical formed by metabolic activation of carbon tetrachloride in rat liver microsomal fractions isolated hepatocytes and in vivo in the rat. *Biochem J*., 204, 593-603.
- Briskin, D P. (2000), Medicinal plants and phytomedicines linking plant biochemistry and physiology to Human health, Plant Physiology. 124:507–514.
- Doran, TL., Ting, A., McPherson, CK., et al. (1982). Genetic susceptibility to the development of retinopathy in insulin dependent diabetics. *Diabetes*., 31, 226-231.
- Eliza, J., Daisy, P., Ignacimuthu, S., Duraipandiyan, V. (2009). Normo-glycemic and hypolipidemic effect of costunolide isolated from Cosus specious Sm. in streptozotocin-induced diabetic rats. *Chem Boil Interact*. 179(2-3), 329-334.
- Ginsberg, HN. (1994). Lipoprotein metabolism and its relationship to atherosclerosis med. *Cli North Am*., 78, 1-20.
- Goldstein, JL., Schrott, HG., Haggard, WR., Bierman, EL., Motnlsky, AG. (1973). Hyperlipidemia in coronary heart disease II. Genetic analysis of lipid levels in 176 families and delineation of new inherited disorder, combined hyperlipidemia. *J chin. Invest*., 52, 1544-1568.
- Halliwell, B. and Gutteridge, J. M. C. (1989). Free radicals in biology and medicine. II edition, 130-131. Clarendon Press, Oxford.
- Higuichi, Y. (1982). Lipid peroxides and α-tocopherol in rat streptozotocin induced diabetes mellitus. *Acta Med Okayama*., 36, 165-175.
- Hirashi, H., Terano, A., Razandi, M., Sugimoto, T., Harada, T., Ivey, KJ. (1992). Role of cellular superoxide dismutase against reactive oxygen metabolite injury in cultured bovine aortic endothelial cells. *J Biol Chem*., 267(21), 14812-14817.
- Inada, A., Kanamori, H., Arai, H., Akashi, T., Araki, M., Weir, GC. Fukatsu, A. (2008). A model for diabetic nephropathy: advantages of the inducible cAMP early repressor transgenic mouse over the streptozotocin-induced diabetic mouse. *J Cell Physiol*., 215(2), 383-391.
- Jaiswal, D., Kumar, Rai P., Kumar, A., Mehta, S., Watal, G. (2009). Effect of Moringa oleifera Lam. Leaves aqueous extract therapy on hypoglycemic rats. *J Ethnophamacol*., 125 (3), 392-396.
- Ji LL. (2001). Exercise at old age: does it increase or alleviate oxidative stress? *Ann N Y Acad Sci*., 928, 236-247.
- Koliakos, GG. Befani, CD., Mikropoulos, D., Ziakas, NG. Konstas, AG. (2008). Prooxidant- antioxidant balance, peroxide and catalase in the aqueous humor and serum of patients with exfoliation syndrome or exfoliative glaucoma. *Graefes Arch Clin Exp Opthalmol*., 246(10), 1477-1483.
- Malaisse, WJ. (1982). Alloxan toxicity of the pancreatic B-cells- a new hypothesis. *Biochem Pharmacol*., 22, 3527-3534.

- Meyerovithch J, Farfelz, Sack J, Schechter Y. (2002). Oral and – of vanadate normalize BSL in STZ-traeted rats. *J Biol-Chem., 202*, 6658.
- Milani, E., Nikfar, S., Khorasani, R., Zamani, MJ., Abdollahi, M. (2005). Reduction of diabetes induced oxidative stress by phosphodiestrase inhibitors in rats. *Comp Biochem Physiol C Toxicol Phamacol*., 140, 251-255.
- Miyao, N., Kumamoto, Y., Tsukamoto, T. (1991). Histopathological analysis of chemical carcinogenic process by streptozotocin in the mouse kidney. *Nippon Hinyokika Gakkai Zasshi*., 82(9), 1399-1407.
- Mohammad, AA., Shibli, JA., Razia, K., Mohammad, A. (2009). Pharmacological investigation of protective effects of *Nigella Sativa* oil in experimental diabetic neuropathy in rats. *Indian J Pharm Educ Res*., 43(2), 166-176.
- Nagai, R etal. (2008). Rationale and design of a study to examine lower targets for low density lipoprotein-cholesterol and blood pressure in coronary artery disease patients. *Circ J*., 72(4), 515-520.
- Pryor WA, Stanley JP (1975). "Letter: A suggested mechanism for the production of malondialdehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation". J. Org. Chem. 40 (24): 3615–7. doi:10.1021/jo00912a038. PMID 1185332
- Rahimi, R., Nikfar, S., Larijani, B., Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother*., 59, 302-306.
- Rhee, SJ., Jeong, YC., Choi, JH. (2005). by muscle mitochondria. J Biol Chem., 284(1), 46-55.
- Slater, TF. (1972). Free radical mechanisms in tissue injury. *British Medical Bulletin*., 49(3). 482.
- Steams, SB., Benzo, CA. (1978). Glucagon and insulin relationship in genetically diabetic (db/db) and in streptozotocin induced diabetic mice. *Horm Metab Res*., 10, 20-23.
- Talbot, S., Theberge-Turmel, P., Liazoghli, D., Senecal, J., Gaudreau, P., Couture, R. (2009). Cellular localization of kinin B1 receptor in the spinal cord of streptozotocin diabetic rats with a fluorescent [Nalpha-Bodipy] - des- Arg9-bradykinin. *J Neuroinflammation*., 26, 6-11.
- Wolff, S. P. (1993). Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Br. Med. Bull*., 49, 642-648.