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RESEARCH ARTICLE

PROTECTIVE ROLE OF ALBIZZIA LEBBECK AND SYZYGIUM CUMINI EXTRACT IN HEPATIC INJURIES: CAN NATURAL PLANT BE AN ALTERNATIVE?

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

[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

Free radical injury can cause a wide spectrum of tissue damage. An experimental study was performed to see the beneficial effect of secondary plant extract with organo-protective effect. Experimented animals (rats) were exposed to oxidative injury with Streptozotocin (STZ) and d-Galactosamine hydrochloride (GAL), for the generation of free radicals. The administration of these toxic agents like STZ or GAL induced various patho-physiological changes in the liver and generated disease condition due to free radical damage and oxidative stress. The experiment was followed with co-administration of aqueous, methanolic and methanolic fraction of aqueous extract of *Albizzia lebbeck and Syzygium cumini*. These secondary plant extracts significantly increased antioxidant enzymes and reduced the elevated serum levels of malondialdehyde in the experimental animals. The established hepato-protective 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, either alone or in conjunction with other herbal molecules with proven hepato-protective action. Future efforts should be taken to establish such isolated compounds as potential drug in clinical practice.

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INTRODUCTION

Liver is the most metabolically active organ in human body with wide range of functions. It is essentially a controlling hub for metabolism. Although liver has a very high regenerative capacity, there are many toxins with variety of hepato-toxic potential and activity. Free radical damage is one of the major pathological damage seen in liver leading to serious complications. The pioneering studies on the role of free radical reactions in the genesis and the expression of cellular and tissue damage and protective role of natural plant extract have been carried out mainly in the liver. This has been conducted in particular, during the last 25 years, by using acute rat poisoning with carbon tetrachloride (CCl_4) as a model system. Different groups of people (Ozturk, 2009; Slater, 1984 and Recknagle, 1983) have demonstrated the different mechanisms by which CCl_4activation to free radical metabolisms leads to liver fatty degeneration and necrosis.

Corresponding author: Raghavendra Rao, M. V.
Avalon University School of Medicine, Curacao, Netherlands Antilles

Albizzialebbeck and Syzygiumcumini

In the present study we have studied the protective action of Albizzia and Syzygium in experimental albino rats by inducing hepatic damage with agents like streptozotocin (STZ) and galactosamine (GAL). Albizzialebbeck belongs to family leguminosae and is represented throughout the tropics. Syzygiumcumini belongs in a member of the family, Myrtaceae. Syzygium has been used traditionally in vinegar preparation and is believed to have diuretic, stomachic and carminative properties.

Role of free radicals in Hepato-toxicity

There are several reports present in the literature with known hepatic toxicity, like carbon tetra chloride, streptozotocin, D-

galactosamine, paracetamol etc. The results obtained over the years by various scientists with different prooxidanthepatotoxins outlined the paradigmatic role of experimental CCl_4 poisoning in the research field of free radical mechanisms of liver injury. Briefly, the hemolytic cleavage of the halo alkane, occurring mainly at the hepatic microsomal cytochrome P450 site, gives rise to the trichloromethyl radical ($CCl_3^{\cdot}$) (Slater, 1984) which, in turn, rapidly reacts with molecular oxygen yielding the trichloromethylperoxyl radical (CCl_3O_2) (Packer, 1978). These free radicals initiate the damaging process through covalent binding to cell macromolecules ($CCl_3^{\cdot}$), enhancement of membrane lipid peroxidation ($CCl_3O_2^{\cdot}$) and in a second step, rearrangement of intracellular free calcium homeostasis (Manibusan, 2007). A complex interaction among these primary and various secondary molecular mechanisms of injury itself with its eventual expression at the cellular level in terms of abnormal fatty accumulation and death. Through the investigations of CCl_4 induced liver damage, (Dianzani, 1995 and Recknagel, 1983) made an outstanding and essential contribution both to the biochemical characterization and to the toxicological consideration of lipid peroxidation. Other essential approaches to the oxidative damage of membrane phospholipids have been carried out by Hochstein in 1964 and by the groups of McCay, 1971 and Aust, 1990 mainly with iron complexed with adenosine diphosphate (ADP).More recently, a combination of studies designed have made possible the biochemical and toxicological characterization of the main aldehyde compounds which are formed during lipid peroxidation of biological membranes stimulated either by CCl_4 or ADP-iron (Benedetti, 1980 and Esterbauer, 1992).
In particular, CCl_4 and DBE simultaneously administered to rat hepatocytes or to the whole animal show a synergistic effect both on the simulation of lipid peroxidation and on hepatocytedeath (Danni, 1988; Danni, 1991). Cytolysis is the main liver damage with haloalkanes. The liver shows fibrosis only in the CCl_4 exposure models.

Experimental Evidence: Free Radical – Mediated Hepatic Injury and Protection

It is now generally accepted that reactive free radicals can exert cellular damage through a variety of mechanisms e.g. lipid peroxidation, covalent binding, depletion of glutathione and protein thiols, derangement of intracellular free calcium homeostasis, DNA fragmentation etc., with different relevance in the various conditions (Freeman, 1982). We carried different experiments in animal models to elicit the hepatotoxic behavior of free radicals and beneficial effect of natural plants products with active secondary metabolites.

Serum Malondialdehyde (MDA) Determination

Serum MDA is considered to be a very important marker for oxidative stress and there are evidences of its role in metabolic diseases (Ranabir Pal, 2011). The stimulation of Lipid Hydroperoxide (LPO) as a consequence of tissue injury, a relationship between lipid peroxidation and hepatotoxicity has been suggested and the elevated levels of LPO was reported in GAL treated mitochondrial, microsomal, and cytosol factors. Stimulation of lipid peroxidation by GAL has been demonstrated in liver homogenates in liver microsomal and in isolated hepatocytes (Sreedevi, 2009). Lipid peroxidation is destructive process for biological membranes. In the present study, the lipid peroxidation levels were found to be significantly increased in D-galactosamine treated rats. We found increased serum MDA levels in animals ($p<0.01$) exposed to GAL(group 2) as compared to Control(group 1) in our experiment. However, pretreatment with ASW, ASM, & ASWM extracts are found to inhibit the lipid peroxidation in D-Galactosamine treated animals. The serum MDA levels of animals were decreased significantly ($P<0.05$) in group 4 (ASW+GAL), group 6 (ASM+GAL) and group 8 (ASWM+GAL) as compared to group 2 (STZ) (Table-18) in this experiment. The effectiveness of aqueous extract of *Albizzialebbeck* and *Syzygiumcumini* have also been documented in the past through inhibition of free radical induced lipid peroxidation (Manoj *et al.,* 1992). Many of the compounds are cited in the literature as being protective agents against GAL induced liver injury. The protection against GAL mediated lipid peroxidation can be achieved (i) through decreased production of free radical derivatives, and (ii) due to the antioxidant activity of the protective agent itself. Other substances like sulfhydryl compounds, N-acetyl cysteine and Dithiothreitol, and Adenosine and Inosine (Singh*,* 1992) have shown to suppress the GAL induced hepatotoxicity. It has been shown (Jayathilaka *et al.,* 1990) that the plant extract Melotheria extract significantly inhibited the GAL mediated increase in MDA.

Table 18: Effects of administration of *Albizzialebbeck*and *Syzygiumcumini*extracts 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	GAL	4.06 ± 0.08[b]
3	ASW	1.92 ± 0.08
4	ASW+GAL	3.36 ± 0.11[a]
5	ASM	1.89 ± 0.65
6	ASM+GAL	2.99 ± 0.13[a]
7	ASWM	1.91 ± 0.08
8	ASWM+GAL	2.73 ± 0.05[a]

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

GAL is G-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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 Estimation

Administration of GAL to rats on normal diet produced a marked change in body weight. The body weight of animals was decreased significantly ($p<0.05$) in group 2 (GAL) as

compared to group 1 (Control), and increased significantly (P<0.05) in group 4 (ASW+GAL), group 6 (ASM+GAL) and group 8 (ASWM+GAL) as compared to group 2 (STZ) (Table-12). Reversible block of GH secretion and imparted drug metabolism was observed in adult rats exposed to GAL. GAL exposed rats were hypophagic due to: hypothalamic syndrome and decrease in plasma insulin growth factor-I. This contributed to the reduction of body weight on rats exposed to GAL with normal diet; and increase in body weight in rats on control diet.The extracts ASW, ASM & ASWM produced marked increase in body weight in GAL exposed animal groups. This may be due to presence of vital proteins and carbohydrates, polyphenolic compounds in them (Gokhale AB, 2002).

Table 12. Effects of *Albizzialebbeck* and *Syzygiumcumini* 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	GAL	139.00 ± 3.9[a]
3	ASW	210.00 ± 11.0
4	ASW+GAL	178.00 ± 4.6[a]
5	ASM	212.00 ± 6.0
6	ASM+GAL	185.00 ± 4.3[a]
7	ASWM	210.00 ± 11.0
8	ASWM+GAL	198.00 ± 4.6[a]

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

GAL is D-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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 Liver Enzymes and Bilirubin estimation

Serum level of liver enzymes: serum glutamate-pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (ALKp), serum acid phosphatase(ACIDp) and serum total bilirubin (TBil)were determined in experimental rats after exposure of GAL, and plant extracts. The results obtained were subsequently compared to elicit the hepato-toxic effect of GAL and hepato-protective effect of target plant extracts. The SGPT/SGOT, ALKp/ACIDp and TBil levels of animals were increased significantly (p<0.05) in group 2 (GAL) as compared to group 1 (Control). In contrast to it, the serum levels of SGPT(Table-13), SGOT(Table-14), ALKp (Table-15), ACIDp(Table-16) and TBil(Table-17) of animals were decreased significantly (P<0.05) in group 4 (ASW+GAL), group 6 (ASM+GAL) and group 8 (ASWM+GAL) as compared to group 2 (STZ). Transamination plays a key role in intermediary metabolism as it is required for the synthesis & degradation of amino-acids in living cells. Aspartate transaminase activity is widely distributed in human tissues, heart, skeletal muscle and kidney. Smaller amounts are also found in the pancreas, spleen and lungs (Itoh, 2009). Alanine transaminase enzyme is widely distributed in human tissues, but the richest source is the liver and is used primarily as a specific marker for hepatic damage. Elevation in the serum SGPT and SGOT activities has been reported previously after GAL administration (Akashi, 2009).The serum enzyme increase is attributed in injury and synthesis from hepatocytes. The newly synthesized enzyme may more easily cross the damaged membranes and appear in serum.A number of indigenous formulations have been reported to reduce the SGPT and SGOT levels in GAL challenged animal (Akashi, 2009).The present study confirms that the extracts of Albizzia and Syzygium significantly reduced the elevated levels of transaminases in experimental animals when challenged with GAL.

Table 13. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* extracts on the levels of Serum Glutamate Pyruvate Transaminase (SGPT), of rats after 30 days of various treatments

Group No	Groups	SGPT (U/dl)
1	Control	112.08 ± 9.8
2	GAL	376.30 ± 3.9[a]
3	ASW	113.30 ± 8.4
4	ASW+GAL	121.00 ± 13.5 [a]
5	ASM	112.90 ± 8.9
6	ASM+GAL	121.00 ± 13.6[a]
7	ASWM	112.80 ± 9.2
8	ASWM+GAL	118.00 ± 11.2[a]

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

GAL is D-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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 14. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* extracts on the levels of Serum Glutamate Oxaloacetate transaminase (SGOT), of rats after 30 days of various treatments

Group No	Groups	SGOT (U/dl)
1	Control	87.20 ± 5.4
2	GAL	418.80 ± 31.1[a]
3	ASW	88.10 ± 5.3
4	ASW+GAL	128.00 ± 10.9[a]
5	ASM	87.80 ± 6.2
6	ASM+GAL	108.30 ± 11.2[a]
7	ASWM	86.90 ± 8.2
8	ASWM+GAL	89.10 ± 4.8[a]

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

GAL is D-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.),

ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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. Phosphatases are group of relatively non-specific enzymes, which hydrolyses a variety of esters of orthophosphates under alkaline condition (ALKp) or acidic conditions (ACIDp).Acid phosphatase is lysosomal enzyme and ALKp is a membrane bound enzyme. The AKP and ACIDp are referred as markers of liver function test (Rahman MF, 2004). Elevated ALKp&ACIDp levels of serum have been reported in GAL exposed animals (Pushpavalli, 2008). (Sreedevi, 2009) also showed the significantly increased levels of ALKp&ACIDp in GAL and paracetamol treated animals. Increased levels of ACIDp in lysosomal suspension have been reported in GAL induced hepatotoxicity (Pushpavalli, 2008). In the present study the levels of ALKp of ACIDp were significantly increased in animals exposed to GAL.Similarly the aqueous *Albizzialebbeck* and *Syzygiumcumini* extracts ASW, ASM, & ASWM have significantly reduced the elevated levels of ALKp and ACIDp in serum of experimental animals. This may be attributed to the membrane and cellular stabilizing activity of extracts.

Table 15. Effects of administration of *Albizzialebbeck* and Syzygium*cumini* extracts on the levels of Serum Alkaline Phosphatase (ALKp), of rats after 30 days of various treatments

Group No	Groups	ALKp (U/dl)
1	Control	108.00 ± 9.8
2	GAL	257.80 ± 13.2[a]
3	ASW	107.00 ± 8.4
4	ASW+GAL	132.00 ± 13.2[a]
5	ASM	108.30 ± 6.8
6	ASM+GAL	125.00 ± 11.5[a]
7	ASWM	107.90 ± 5.3
8	ASWM+GAL	107.60 ± 6.1[a]

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

GAL is d-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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 16. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* extracts on the levels of Serum Acid Phosphatase (ACIDp) of rats after 30 days of various treatments

Group No	Groups	ACIDp (U/dl)
1	Control	12.8 ± 1.1
2	GAL	37.6 ± 1.6[a]
3	ASW	21.3 ± 0.3
4	ASW+GAL	12.6 ± 1.3[a]
5	ASM	20.5 ± 1.1
6	ASM+GAL	12.7 ± 1.4[a]
7	ASWM	11.9 ± 0.9
8	ASWM+GAL	11.9 ± 1.6[a]

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

GAL is D-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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. Bilirubin is a bile pigment stored in the gall bladder of the living animals. The compounds which are showing damaging reaction of the liver are known to increase the bilirubin levels in the serum. The levels of bilirubin in the serum are direct marker of liver function. The TBil levels are known as the Liver Function Test in the literature.

Many herbs and their extracts have an important protective action on the liver. In this present study, it has been found that the hepato protection is made by the extracts of Albizzialebbeck and syzygiumcumini sweet extracts in all the experimental animals. It is indication of hepato protection by the plant extracts.

Table 17. Effects of administration of *Albizzialebbeck* and Syzygium *cumini* extracts on the levels of Serum Total Bilirubin (TBil), of rats after 30 days of various treatments

Group No	Groups	TBil (U/dl)
1	Control	0.403 ± 0.01
2	GAL	1.480 ± 0.14[a]
3	ASW	0.394 ± 0.03
4	ASW+GAL	0.520 ± 0.01[a]
5	ASM	0.402 ± 0.04
6	ASM+GAL	0.484 ± 0.01[a]
7	ASWM	0.448 ± 0.03
8	ASWM+GAL	0.467 ± 0.03[a]

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

GAL is D-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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 estimation of antioxidant

The major antioxidant enzymes are super oxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). All these enzymes are significantly decreased in D-Galactosamine exposed animals. When the D-Galactosamine intoxicated animals were pretreated with various extracts of *Albizzialebbeck* and *Syzygiumcumini* the antioxidant enzymes levels were brought back to near normal. The SOD, CAT and GSH levels of animals were decreased significantly ($p<0.01$) in group 2 (GAL) as compared to group 1 (Control).The serum SOD (Table-19), CAT (Table-20) and GSH (Table-21) levels

of animals were increased significantly (P<0.05) in group 4 (ASW+GAL), group 6 (ASM+GAL) and group 8 (ASWM+GAL) as compared to group 2 (STZ). Superoxide dismutase metabolizes superoxide anion radicals. It is an effective defense of the cells against endogenous and exogenous generation of O^{2-} (Brawn, 1980). In the present observation the SOD levels were decreased in Galactosamine treated animals. Pretreatment extracts of Albizzialebbeck and syzygiumcumini extracts restored the SOD levels to near normal. This might be either because of the boosting production of endogenous antioxidant enzyme like superoxide dismutase or scavenging released superoxide radicals due to exposure to GAL.

Table 19. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* 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	GAL	1.35 ± 0.08[b]
3	ASW	3.36 ± 0.16
4	ASW+GAL	2.10 ± 0.09[a]
5	ASM	3.32 ± 0.18
6	ASM+GAL	2.65 ± 0.13[a]
7	ASWM	3.29 ± 0.18
8	ASWM+GAL	2.93 ± 0.16[a]

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

GAL is d-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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.

Catalase has been shown to be responsible for the detoxification of significant amounts of H_2O_2 (Brenner, 1953). Catalase may function to protect cells against the onslaught of horrendous amounts of exogenous H_2O_2Catalase deficient organisms are more readily killed by H_2O_2 (MamEation, 1990).

Table 20. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* 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	GAL	2.93 ± 0.12[b]
3	ASW	6.38 ± 0.43
4	ASW+GAL	4.83 ± 0.83[a]
5	ASM	6.15 ± 0.18
6	ASM+GAL	5.76 ± 0.36[a]
7	ASWM	6.32 ± 0.19
8	ASWM+GAL	5.97 ± 0.82[a]

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

GAL is d-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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. Reduced glutathione (GSH) is a protective molecule against chemical induced cytotoxicity (Orrenious, 1984).Depletion of cellular reduced glutathione (GSH) has been reported to play an important role in tissue injury (Comporti, 1987).The GSH levels were maintained by GSH generating enzymes glutathione reductase and GSH utilizing enzymes GPX and GST. GSH metabolism plays a vital role in many biological processes, in detoxication of xenobiotics, reaction of oxygen species and free radicals (Meister, 1984).The major function of GSH is reducing H_2O_2 and organic hydroperoxides. Hepatic glutathione levels were declined in $CCl_4$and in D-Galactosamine treated animals.It has been reported that animals pretreated for 7 days with extracts of Melothriamaderaspatana increased the liver and blood GSH (Jayathilaka, *et al.*, 1990). Galactosamine fed animals, clearly showed the protective nature of Albizzialebbeck and syzygiumcumini. The level of reduced glutathione was maintained to normal in Albizzialebbeck and syzygiumcumini pretreated group of animals. Glutathione peroxidase is selenocysteine containing 4 atoms of Se in each molecule at its active site. It detoxifies various hydroperoxides in the cell.GPx alone has little protection against CCl_4 –dependent lipid peroxidation, (Slater, 1984).Some loss of activity has occurred in the absence of reduced glutathione.Present study showed decreased level of GPx in galactosamine and CCl_4 treated animals, Albizzialebbeck and syzygiumcumini pretreatment restored the level of GPx to near normal. Glutathione-s-transferase plays a physiological role in initiating the detoxification of potential alkylating agents (Booth *et al*., 1961; Wood 1970).The enzyme activity was significantly reduced in galactosamine and CCl_4 treated animals. Chemicals like chloroform, CCl_4 etc. alter hepatic glutathione-s-transferase activity.

Table 21. Effects of administration of *Albizzialebbeck* and *Syzygiumcumini* 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	GAL	1.89 ± 0.12[b]
3	ASW	4.95 ± 0.23
4	ASW+GAL	3.73 ± 0.19[a]
5	ASM	4.87 ± 0.12
6	ASM+GAL	3.78 ± 0.22[a]
7	ASWM	4.96 ± 0.16
8	ASWM+GAL	4.62 ± 0.36[a]

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

GAL is d-Galactosamine hydrochloride administered at the dose of (400mg/kg, body weight, i.p.), ASW is aqueous extract of Albizzialebbeck and syzygiumcumini, sweet (1 ml/kg body weight, i.p.), ASM is Methanolic extract of Albizzialebbeck and syzygiumcumini, sweet (1ml/kg body weight, i.p.), ASWM is Methanolic Fraction of aqueous extract of Albizzialebbeck and syzygiumcumini, 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.

Toxicological changes: Histopathological study

Figure: Photomicrographs showing liver of rats after the following treatments for 30 days: (Magnification 40X)

I	Control
II	GAL
III	ASW+ GAL
IV	ASM+ GAL
V	ASWM+ GAL

Hydropic Changes

Pyknotic Nuclei

Fatty Infiltration

Plate-I: Group-II: GAL

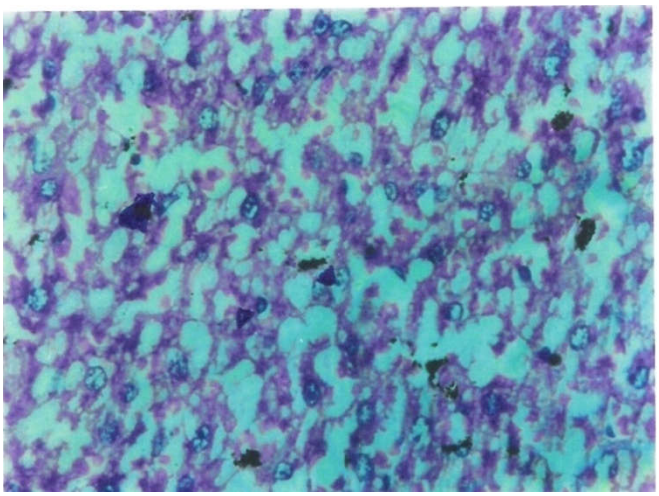


Plate-II: Group-III: ASW+GAL

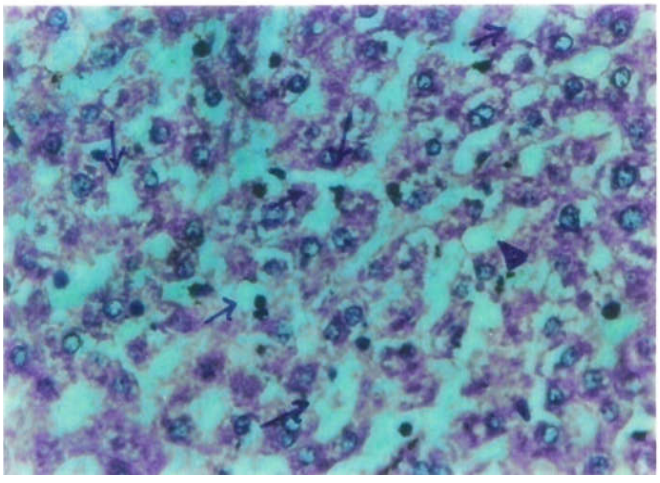


Plate-III: Group-IV: ASM+ GAL

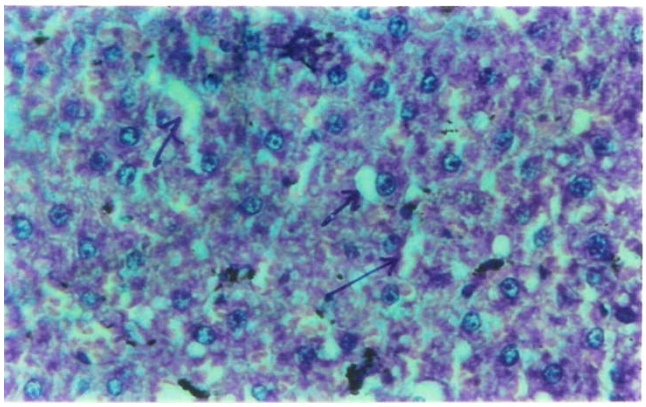


Plate-IV: Group-V: ASWM+GAL

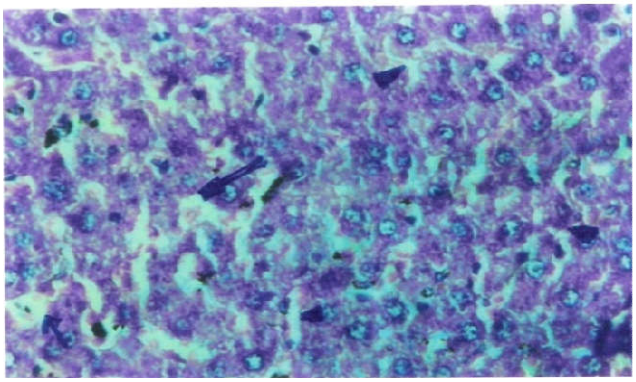


Figure : Photomicrographs showing liver of rats after the following treatments for 30 days: (Magnification 40X)

I	Control
II	STZ
III	ASW+ STZ
IV	ASM+ STZ
V	ASWM+ STZ

Hydropic Changes

Pyknotic Nuclei

Fatty Infiltration

Plate – I Group-I Control

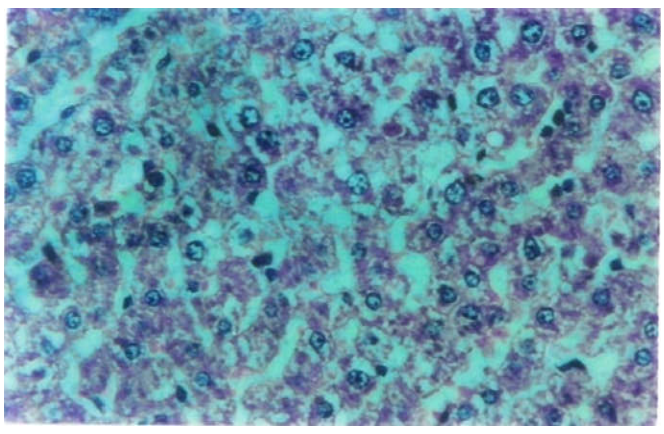


Plate-II: Group-II: STZ

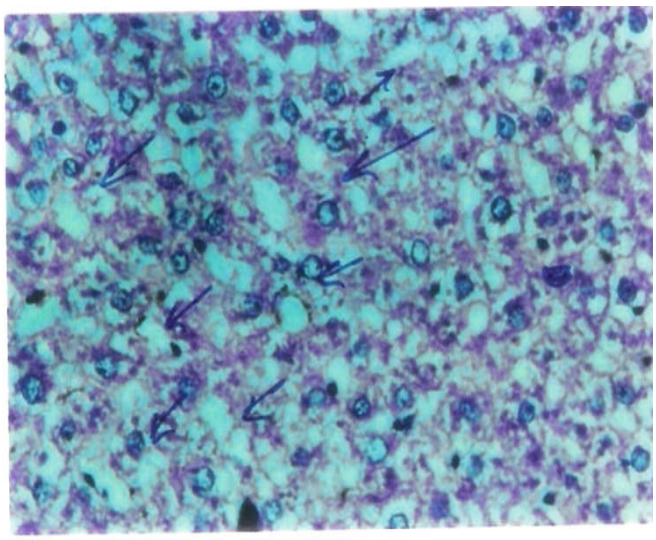


Plate-III: Group-IV: ASW+ STZ

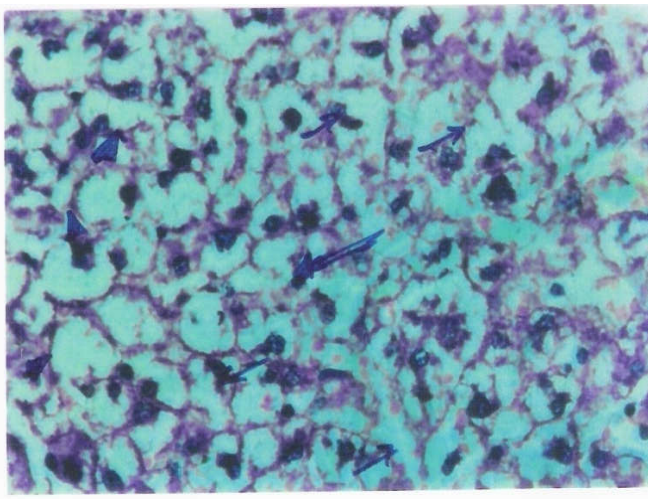


Plate-IV: Group-IV: ASM+ STZ

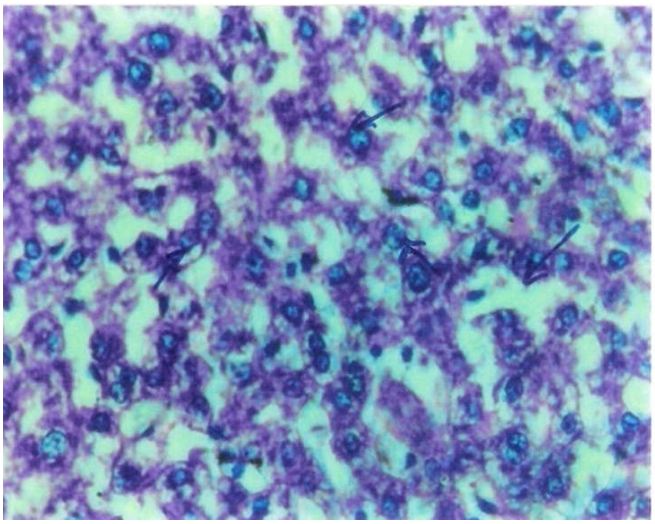
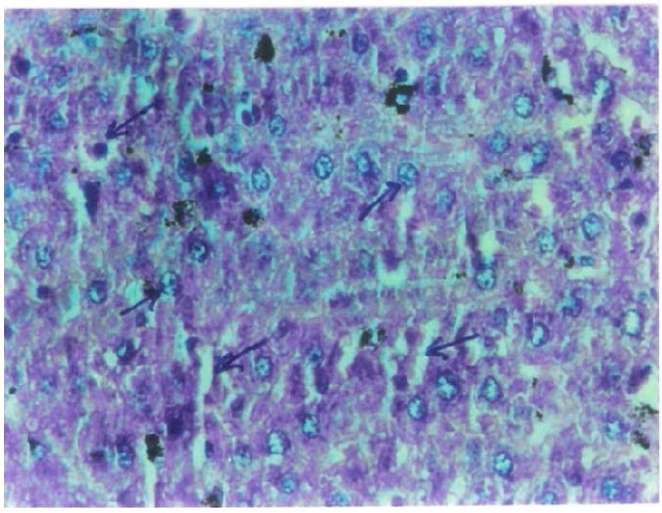


Plate-V: Group- V: ASWM+ STZ



Toxicological Studies

The major findings in liver histopathology of GAL treated animals are diffused liver cell injury with lobular disarray, necrosis of random liver cells and evidence of hepatocyte degeneration. Liver cell injury is manifested by swelling, sometimes with partial clearing of cytoplasm secondary to hydropic distension of endoplasmic reticulum named as ballooning degeneration. At the outset in GAL treated rats, lipid accumulates in a micro vesicular form within the cytoplasm of the liver cells, predominantly in periventricular (centrilobular) and in periportal region. There was sign of cellular enlargement, rupture, and coalescence of adjacent expanded cells which altogether shown a formation of fatty cyst. In GAL exposed rats lobular disarray was also seen in the periportal region producing loss of normal structure, less radial array. The cytoplasm was intensely eosinophilic, cytoplasmic inclusions that look form of "Candle dripping" or haphazard coalescent perinuclear skeins (Mallory bodies).Most neutrophils with scattered mononuclear leukocytes were found within and about degenerating liver inflammatory mediator's infilterring the portal tracts with spillover into the adjacent parenchyma. Another prominent feature in liver of GAL treated rats was marked hypertrophy and probable hyperplasia of kupfer cells and sinusoidal cells, both of which might have added with lipofusion pigments. Administration of various extracts ASW, ASM and ASWM drastically protected the liver of animals form above mentioned changes. The protection of disturbed cytoskeletal structure of liver is a sign of hepatoprotective property of the plant extracts. The liver of animals exposed to GAL and treated with ASWM extract was protected completely as compare to other two extracts.

DISCUSSION

Liver, although an organ known to normalizes very fast, the nature of damaging compound limits this process. It has been revealed that, many herbs and their extracts have an important protective action on the liver. The present study has suggested that the administration of some toxic agents like STZ or GAL induces various patho-physiological changes in the liver and

generates disease condition due to free radical damage and oxidative stress. The lipid peroxidation process initiated is indicated by the significant increase in the serum levels of malodialdehyde in animals exposed to streptozotocine and d-galactosamine. This damage was potentiated with reduction in the levels of endogenous antioxidant enzymes like superoxide dismutase, catalase and endogenous antioxidant compounds like reduced glutathione after 30 days of experimental period suggesting the generation of free radicals like superoxide anion and hydrogen peroxide like molecules in animals. The levels of serum total bilirubine, serum glutamate pyruvate transminase, serum glutamate oxaloacetate transaminase, in serum alkaline phosphatase and serum acid phosphatase enzyme levels in animals intoxicated with d-galactosamine were increased. The co-administration of aqueous, methanolic and methanolic fraction of aqueous extract of Albizzialebbeck and Syzygium cumin significantly increased the reduced serum levels of these antioxidant and hepatic enzymes in experimental animals on thirty days of experimental schedule indicating protective effect of the plant extracts against generation of superoxide anion radical, hydrogen peroxide and other xenobiotics initiated by the compounds like streptozotocine and d-galactosamine. It is indication of hepato protection by the plant extracts. All these findings were further substantiated by the results of histopathological studies done in liver and of the animals used in hepatoprotective models of experiments. This protection is consequence of the prevention of damage to the ultra-structure of liver of experimental animals rather that regeneration of these organs.

Conclusion

Hepatic injury can be due to oxidative stress in animals through the generation of free radicals. Plants with secondary metabolites possess antioxidant properties and protect us against the ROS insult. Albizzialebbeck and Syzygium cumin are natural plants with hepato-protective properties because of their proven antioxidant property. Therefore, it is pertinent to isolate and characterize the active chemical constituent of this plant extracts and efforts should be taken to establish such isolated compounds as potential drug having such a wide variety of action.

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