Evaluation of antihepatotoxic ability of *Cassia auriculata* (Linn.) Against antituberculosis drug rifampicin induced hepatotoxicity in rats

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**ABSTRACT**
To investigate the antihepatotoxic ability of aqueous leaf extract of *Cassia auriculata* on antituberculosis drug, rifampicin induced hepatotoxicity. In the present study, the aqueous leaf extract of *Cassia auriculata* (150, 300 and 600 mg/kg body weight) was examined for its antihepatotoxic ability against rifampicin induced liver injury. Fortytwo healthy male albino wistar rats (150-180 g weight) were chosen and divided in to seven groups. Rifampicin and aqueous leaf extract were given 28 days according to the experimental design. After 28 days of treatment, hepatic serum marker enzymes, antioxidant enzymes, lipid peroxidation and liver histology were analyzed. Rifampicin induced liver damage showed significantly elevated activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), cholesterol and bilirubin whereas protein level was decreased in serum. Moreover hepatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activities were decreased whereas lipid peroxidation level was increased and also necrosis, vacuolization, space formation and loss of cell boundries were observed in liver when compared with control group. Administration of aqueous leaf extract of *Cassia auriculata* or silymarin could significantly restored to near normal by decrease the activities of serum hepatic marker enzymes and lipid peroxidation level where as enhance the activities of antioxidant enzymes, serum protein and improving towards the normal liver histioarchitecture when compared with rifampicin alone treated rats. These present findings suggested that the aqueous leaf extract of *Cassia auriculata* exhibited antihepatotoxic ability against rifampicin induced hepatotoxicity compared to standard drug silymarin.

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**Introduction**
Tuberculosis (TB) is an infectious disease caused by the bacterial pathogen *Mycobacterium tuberculosis*. It remains as a major global health problem and significant cause of morbidity and mortality worldwide. Inefficient diagnostic assays, restricted vaccine efficacy and lack of effective drugs are the most important factors that aggravate the TB disease and pose a threat to global TB control. According to World Health Organization (WHO) global TB report, nearly 9 million new TB cases have emerged and 1.5 million deaths occurred in 2013(WHO, 2014; Pathakumar et al., 2015).

Tuberculosis is a hazardous disease, which gradually swallows the life span of human beings. It remains a major public health problem and most deadly infectious disease and also kills approximately two million people every year (Calleja et al., 2004). Nowadays, considerable efforts are being made to develop protective agents to be used therapeutically in cases of liver toxicity originating from diverse causes (Tsaduq et al.,2005). Most of these address chemical hepatotoxins and very few reports exist for frequently used drugs. Adverse drug reactions have become a major clinical concern with the long term administration of antituberculosis drugs such as rifampicin, isonizaid and pyrazinamide (Wong et al., 2000).

Rifampicin, a complex semisynthetic macrocyclic antibiotic derived from *Streptomyces mediteranei*, is a member of the rifamycin class of antibiotics (Maggi et al., 1966) used for the treatment of tuberculosis and other infectious diseases (Rees et al., 1970; Pahkla et al. 1999; Tsankov and Angelova 2003) It is categorized one of the first line antituberculosis agents, however various side effects such as hepatotoxicity, allergic rashes, lack of appetite, nausea or immunological disturbance have been reported associated with the administration of the drug (Deol and Khuller 1997; Gallieni et al. 1999; Tsankov and Angelova 2003).

Hepatotoxicity is one of the most important adverse drug reactions associated with antituberculosis chemotherapy (Lee 1995). Hepatitis has been reported to occur in 0.46% of patients receiving antituberculosis drug (Alexander et al. 1982) and the rate of hepatotoxic reaction was reported much higher in Indian patients (Ramachandran 1980). Hepatitis is a common disease in the world especially in the developing countries. Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Hence there are many researchers of traditional medicines attempting to develop new drugs for hepatitis (Liu 1989).

Existence of human beings on the earth is made possible because of the vital role played by plant kingdom. Besides providing basic requirements of man, the plants offer unique protection to mankind by providing innumerable drugs to
prevent and treat various disorders (Manjunatha et al., 2004). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, through relatively little knowledge about their mode of action. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine (Gupta et al., 2004).

*Cassia auriculata* belongs to the family Leguminosae and is mainly distributed in India, Sri Lanka, etc. This plant is used for the treatment of diabetes, rheumatism, asthma, and skin diseases in the Ayurvedic system of traditional Indian medicine (Yi et al., 2015). *Cassia auriculata* is a shrub with large bright yellow flowers found growing wild in India (Annie et al., 2005). It is a fast growing branched tall, evergreen shrub with reddish brown branches. The tribal people use this plant for the treatment of skin diseases, asthma, and conjunctivitis and in renal disorders (Vedavathi et al., 1997). Dried flower and leaf of the plants are being used for medical treatment (Sawhney et al., 1978; Joshi, 1986).

The extract made from the flower and seed has been shown to have anti diabetic activity ethno medicinally (Jain and Sharma, 1967). There are few experimental studies on the biological activity of the plant. *Cassia auriculata* has been shown to antiviral activity and antispasmodic activity (Dhar et al., 1968). The flower and leaf extract shown to antipyretic activity (Vedavaty and Rao, 1991). The leaf extracts also shows emollient effect (Nanba et al., 1994). The roots of *C. auriculata* are reported to contain flavonoids, polysaccharides, tannin and saponins, among other components (Rao et al., 2000; Rai and Dasundhi, 1990). Compounds present in *Cassia auriculata* include an alkaline – Non acosane – 6 – one (Lohar et al., 1981), Saponins (Gedeon and Kinel, 1956) and tannins (Balasooriya et al., 1982). Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant *Silybum marianum* (Shalan et al., 2005). It is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation and its associated cell damage in some experimental models (Soto et al., 1998). Silymarin was proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultrastructures of liver cells, and improving the performance of hepatic enzymes and bile production (Hagymasi et al., 2002, Lucena et al., 2002).

Silymarin, an extract from the medicinal plant, *Silybum marianum* (Milk thistle) containing various flavonolignans, has received a tremendous amount of attention over the last decade as a herbal remedy for liver diseases (Surai, 2015). Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant *Silybum marianum* (Shalan et al., 2005). It is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation and its associated cell damage in some experimental models (Soto et al., 1998). Silymarin was proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultrastructures of liver cells, and improving the performance of hepatic enzymes and bile production (Hagymasi et al., 2002, Lucena et al., 2002). There is no available report on the effect of *Cassia auriculata* on rifampicin induced liver damage. Therefore, the present investigation to evaluate the antihepatotoxic effect of aqueous leaf extract of *Cassia auriculata* on rifampicin induced liver injury in rats.

### Materials and Methods

#### Plant material

*Cassia auriculata* leaves were collected from Chidambaram in Cuddalore district of Tamil Nadu, India. The plant was identified and authenticated at the herbarium of Botany Directorate, Faculty of Science, Annamalai University. The leaves were shade dried and powdered. The powdered leaves were kept in airtight container in a deep freeze until the time of use.

#### Preparation of extract

100 g of *Cassia auriculata* leaves powder was mixed with 1000 ml of distilled water and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal saline and used in the study. The yield of the extract was approximately 15.8 g.

#### Animals

Male albino Wistar rats weighing 150-180 g were procured from the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and were maintained in polypropylene cages in an air conditioned cooling room (22 ± 1°C) under a 12/12 h light/dark cycle. A standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water were provided ad libitum. All studies involving animals were done according to NIH guidelines, after getting the approval of the Institute’s Animal Ethics Committee.

#### Experimental design

The rats were divided into six groups of six rats each. Group I received physiological saline (10 ml/kg body wt. orally) as normal control; group II received rifampicin (1 g/kg body wt. orally one day only) as treated group; group III received *Cassia auriculata* (150 mg/kg body wt. orally once daily for 28 days) to rifampicin treated group; group IV received *Cassia auriculata* (300 mg/kg body wt. orally once daily for 28 days) to rifampicin treated group; group V received *Cassia auriculata* (600 mg/kg body wt. orally once daily for 28 days) to rifampicin treated group; group VI received silymarin (25 mg/kg body wt. orally once daily for 28 days) to rifampicin treated group; group VII received *Cassia auriculata* (600 mg/kg body wt. orally once daily for 28 days) alone.

At the end of the experiment all the rats were sacrificed by decapitation. Blood samples were collected for evaluating the serum marker enzymes, bilirubin, cholesterol, protein, lipid peroxidation, antioxidant enzymes and liver sample for histology.

#### Biochemical analysis

Blood samples were taken into centrifuge tube with rubber caps, labelled and centrifuged at 3000 g for 15 minutes. Serum biochemical parameter such as ALT, AST, ALP, bilirubin, cholesterol, protein, lipid peroxidation, GSH, SOD, CAT activities were estimated and histology of liver according to standard methods (Reitman and Frankel 1957, King and Armstrong 1980, Malloy and Evelyn 1937, Zlatkis et al. 1953, Lowry et al. 1951, Niehaus and Samuelson, 1968, Ellman, 1959, Kakkar et al., 1984, Sinha, 1972, Gurr, 1959).

#### Statistical analysis

Data are expressed as mean ± SD. Statistical significance was analyzed by one way analysis of variance (ANOVA)
followed by Duncan Multiple Range Test (DMRT) using SPSS version 20.0.

Results

Table 1 shows the levels of body and organ weight changes in normal and experimental groups of rats. There was a significant decrease in the body weight and increase in the liver weight in antituberculosis drug rifampicin administered rats as compared to that of normal rats. Oral administration of Cassia auriculata (150, 300 and 600 mg/kg body wt.) and silymarin significantly increased the body weight and liver weight reduced in the antituberculosis drug, rifampicin induced decrease the body weight and increase the liver weight in group III, IV, V, VI and group VII rats as compared to those group II rats, indicating the antihepatotoxic role of Cassia auriculata. Oral administration of aqueous leaf extract of Cassia auriculata alone (group VII) was found to produce no significant body and organ weight in normal rats indicating the non-hepatotoxic nature.

Table 2 shows the levels of serum hepatic marker enzymes such as AST, ALT and ALP in normal and experimental groups of rats. There was a significant elevation noticed in the levels of serum hepatic marker enzymes in antituberculosis drug rifampicin administered rats as compared to that of normal rats. Oral administration of Cassia auriculata (150, 300 and 600 mg/kg body wt.) and silymarin significantly reduced the antituberculosis drug, rifampicin induced rise in the levels of serum hepatic marker enzymes in group III, IV, V and group VI rats as compared to those group II rats, indicating the antihepatotoxic role of Cassia auriculata. Oral administration of aqueous leaf extract of Cassia auriculata alone (group VII) was found to produce no significant elevation in serum hepatic marker enzymes in normal rats indicating the nonhepatotoxic nature.

Table 3 shows the levels of serum bilirubin, cholesterol and protein in normal and experimental groups of rats, respectively. Significant increases in the levels of bilirubin and cholesterol whereas protein level decreased in group II antituberculosis drug, rifampicin administered rats as compared to that of normal rats. The rats administered with Cassia auriculata (150, 300 and 600 mg/kg body wt.) and silymarin showed significantly near normal in group III, IV, V and group VI rats as compared to that of group II hepatotoxicity induced rats. The rats administered with Cassia auriculata alone (group VII) did not show any adverse effects indicating that Cassia auriculata is non-toxic.

Discussion

Liver diseases are still now a global serious health problem and it's classified as acute or chronic hepatitis (inflammatory liver diseases), hepatitis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis). Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects (Kumar et al., 2011; Asadi-Samanil et al., 2015).

Liver is the key organ in the metabolism, detoxification and secretory functions in the body and its disorders are numerous with no effective remedies and however, the search for new medicines is still ongoing (Jamshidzadeh et al. 2005). Many folk remedies from plant origin have been long used for treatment of liver diseases (Luper 1999). Liver injury in a patient on antituberculosis treatment often presents the clinician with a difficult problem of management (Dossing et al. 1996). Management of liver diseases is still a challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders (Praveen Reddy et al. 1992).

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP, which are enzymes originally present in higher concentration in cytoplasm (Wells 1988), when there is hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage (Plaa and Charbonneau 1994, Venukumar and Latha 2004). Indicators of hepatocellular integrity most commonly measured in clinical toxicology studies are the enzymes AST, ALT and bilirubin levels (Ballet 1997). ALT is frequently included in biochemical profiles for the purpose of assessing hepatic injury (Williamson et al. 1996) and is also regarded as indicative of liver effects in dogs, non-lumbar primates, rats, mice and hamsters (Smith et al. 2002, Lenaerts et al. 2005).

Liver-specific enzymes are considered to be very sensitive and reliable indices for measuring hepatotoxicity as well as hepatoprotective or hepatocurative effect of various compounds (Varley et al. 1988). The rise in serum levels of transaminases (AST and ALT) has been attributed to the damaged structural integrity of the liver (Chowwath and Hake, 1962).

An elevation in the levels of the serum marker enzymes in generally regarded as one of the most sensitive index of the hepatic damage (Kapil et al. 1995). ALP is a membrane bound glycoprotein enzyme, with high concentrations in sinusoids and endothelium. ALP reaches the liver mainly from bone. It is excreted into the bile so its elevation in serum occurs in hepatobiliary diseases (Burtis and Ashwood 1986). The elevation of alkaline phosphatase indicates the disturbed excretory function of liver (Kothavade et al. 1996). Assay of serum ALP activity has been recognized as a suitable marker of skeletal and hepatobiliary disorder. Moreover, an elevated serum level of ALP activity is frequently associated with
various pathological conditions (Simko 1991, Moss 1989). Alkaline phosphatase is a non-specific tissue enzyme widely spread, mainly in the bones, liver and biliary canaliculi (Poole and Lesile 1989; Ringler and Dubich 1979).

In the present study, administration of rifampicin treated rats showed an increase in the activities of AST, ALT and ALP when compared with control rats.

Oral administration of aqueous extract of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed an inhibition in the elevated activities of serum AST, ALT and ALP when compared with rifampicin alone treated rats. Similarly administration of garlic to isoniazid and rifampicin treated rats showed significantly decrease the elevated activities of AST, ALT and ALP (Pal et al. 2006). Lenaerts et al. (2005) have reported that elevated levels of serum hepatic marker enzymes were noticed in isoniazid, rifampicin and pyrazinamide treated mice. Administration of silymarin to rifampicin, isoniazid and pyrazinamide combination treated rats showed significantly inhibits the increased activities of AST, ALT and ALP (Tasduq et al. 2005). Administration of ethanolic root extract of *Ziziahus oenoplia* to antitubercular drugs treated rats showed significantly reduced theactivities of SGOT, SGPT, ALP and serum bilirubin levels when compared with antitubercular drugs alone treated rats (Rao et al., 2012).

Mujahid et al., (2013) reported that administration of *Adenanthera pavonina* to antitubercular drug induced rats showed decrease the activities of SGOT, SGPT, ALP, LDH and increase the protein and also albumin levels. Jaswal et al., (2013) explained that minimize the activities of AST, ALT, ALP and cholesterol level whereas albumin and protein levels were increased in thymoquinone administrer to antituberculosis drugs.. Administration of antituberculosis drug to *Hibiscus vitifolius* root treated rats 1 showed suppressed the activities of serum hepatic marker enzymes (Dineshkumar et al., 2012).

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function (Martin and Friedman 1992). In the present investigation, the rats treated with rifampicin showed significantly increased levels of bilirubin as compared to control rats. This result agreement induced hepatitis is characterised by increased levels of bilirubin in serum (Mitchell et al. with previous reports showed that rifampicin 1995, Rao and Mishra 1996, 1997, Lenaerts et al. 2005). Administration of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed decrease the increased bilirubin level when compared to rifampicin alone treated rats. The *Cassia auriculata* mediated reduction of the increased bilirubin level suggests the possibility of the extract being able to stabilise biliary dysfunction. Similarly administration of garlic to isoniazid and rifampicin treated rats showed significantly lowered bilirubin level (Pal et al. 2006). Rao and Mishra (1998) have reported that administration of monomethylfumarate isolated from *Fumaria indica* to CCl4, paracetamol and rifamicin treated rats showed significant inhibition of the elevated serum bilirubin. Administration of silymarin to rifampicin, isoniazid and pyrazinamide treated rats showed significant decline of the increased bilirubin level (Tasduq et al. 2005). Buzzelli et al. (1993) reported that silymarin improved liver function tests related to hepatocellular necrosis and/or increases membrane permeability. Ramadan et al. (2002) reported that the protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties. Results of Horvath et al. (2001) suggested that silybinin medulates the cellular immunoresponse and restores impaired liver function through its antioxidant capacity.

Lipids are the most important cellular entities which are not only the constituents of cell membrane but also involved in many cellular functions, metabolic processes and are vital for energy production. In the present study serum cholesterol was increased in rifampicin treated rats when compared to control rats. Any liver disease shows that an increased blood cholesterol level (McIntyre and Rosalki 1992). The significant increase of serum cholesterol may be due to the inability of the liver to remove cholesterol from circulation. The major disorder encountered in antituberculosis drugs induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism (Santhosh et al. 2006). The abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and the cell membranes (Brown and Goldstein 1986). Administration of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed that decrease the cholesterol content when compared to rifampicin alone treated rats. Similarly administration of chitosan (polysaccharide of marine origin is prepared from the shells of crustaceans) to antitubercular drugs treated rats showed decrease the elevated levels of cholesterol (Santhosh et al. 2006). It was found that feeding of animals on silymarin-phospholipid complex normalized lipid metabolism and inhibited atherosclerosis (Horvath et al. 2001).

Proteins are important organic constituents of the animal cells playing a vital role in the process of interactions between intra and extra cellular media. The depletion in the protein levels might be because of their metabolism to liberate energy during toxicity. The protein level was decreased due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localised in the endoplasmic reticulum which results in the loss of P450 loading to is functional failure with a decrease in protein synthesis (Sureshkumar and Mishra 2006). In the present study, serum protein level was decreased in rifampicin intoxicated rats when compared to control rats. Oral administration of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed increased the level of protein when compared to rifampicin alone treated rats.

Lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical induced cellular damages [Halliwell and Gutteridge, 1992]. Peroxidation reactions in biological systems are the underlying causes for a variety of pathological condition [Estuo and Hiroyuki, 1990]. Lipid peroxidation is a measurement of function of cellular membranes. The levels of TBARS are an indirect measurement of the lipid peroxidation [Halliwell,et al 1995]. The reactive free radicals initiate cell damage through two major mechanisms of covalent binding to cellular macromolecules and lipid peroxidation [Slater,1984 and rattin,1985]. The free radicals initiate lipid peroxidation and could produce a range of enzymatically damaging consequences and could result in membrane disorganization.
by peroxidizing mainly the highly unsaturated and polyunsaturated fatty acids by attacking the methylene bridge hydrogen [Slatet, 1972].

In the present study administration of carbon tetrachloride treated rats showed an increase in the level of lipid peroxidation when compared with control rats. Oral administration of aqueous extract of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to carbon tetrachloride treated rats showed an inhibition in the elevated levels of lipid peroxidation than carbon tetrachloride alone treated rats. Similarly administration of HD-03, a herbal formulation to paracetamol treated rats showed lipid peroxidation levels were decreased [Mitra et al., 1998]. Oral administration of extracts of *Astracantha longifolia* on carbonbterra toxin treated rats shows minimize the lipid peroxidation levels [Muthulingam, 2002]. Administration of *Cajanus indicus* to thioacetamide treated rats showed lipid peroxidation levels were decreased [Sarkar et al., 2005].

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiolis. Also it is substrate for glutathione peroxidase. Reduced glutathione (GSH) plays a key role in protecting cells against electrophiles and free radicals. This is due to the nucleophlicity of the SH group and to the high reaction rate of thiols with free radicals [Mukundan et al., 1999]. The role of extracellular GSH in detoxification of reactive oxygen intermediate has been well established. GSH as a co-substrate for glutathione peroxidase (GPX) plays an essential protective role against reactive oxygen species that may be generated under several stress conditions. It has been shown that glutathione ‘redox cycle’ dynamic balance between reduced glutathione represents one of the most effective endothelial cell antioxidant mechanisms [Suttorp, et al. 1986; Kuzuya 1986; Comporti, 1987]. Reduced glutathione is a cofactor for enzymes involved in protecting membrane against oxidative damage. GSH scavenges hydrogen peroxide in the reaction catalysed by glutathione peroxidase. A deficiency of glutathione and its antioxidant partners in the liver and an increase in toxic free radicals may contribute the progression of liver disease.

In the present study administration of rifampicin treated rats showed an decrease the level of reduced glutathione when compared with control rats. Oral administration of aqueous extract of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed an elevated levels of reduced glutathione than rifampicin alone treated rats. Paracetamol treated rats showed decreased GSH level. Oral administration of HD-03 to paracetamol treated rats showed enhanced the activity of GSH [Mitra et al., 1998]. Administration of *Swertia* species to paracetamol treated rats showed increased the activity of GSH [Reen et al., 2010]. Administration of *Astracantha longifolia* extract to alloxan treated rats shows enhanced the activity of reduced glutathione in pancreas [Muthulingam 2010]. Catalase catalyses the decomposition of H2O2 to water and oxygen and thus protecting the cell from oxidative damage by H2O2and OH [Tolbert, 1981].

In the present study administration of rifampicin treated rats showed decrease in the activities of superoxide dismutase and catalase when compared with control rats. Oral administration of aqueous extract of *Cassia auriculata* (150, 300 and 600 mg/kg body wt.) and silymarin to rifampicin treated rats showed an elevated levels of superoxide dismutase and catalase than rifampicin alone treated rats. Administration of *Asteracantha longifolia* extract and silymarin to CCl4 treated rats showed increased SOD and CAT activities [Muthulingam, 2002]. Thioacetamide treated rats showed decrease SOD and CAT activities. Oral administration of *Cajanus indicus* to thioacetamide treated rats showed an increase in SOD and CAT activity [Sarkar et al., 2005]. Administration of ethanolic root extract of *Ziziphus oenoplia* to antitubercular drugs treated rats showed significantly elevated the activities of SOD, CAT, GSH and GST whereas lipid peroxidation level was suppressed when compared with antitubercular drugs alone treated rats (Rao et al., 2012). Mujahid et al., (2013) noticed that administration of *Adenanthera pavonina* to antitubercular drug induced rats liver showed enhanced the activities of SOD, CAT, GSH and inhibited the elevated levels of lipid peroxidation. Administration of antituberculosis drug to *Hibiscus vitifolius* root treated rats showed elevated the activities of SOD, CAT and suppressed the TBARS levels (Dineshkumar et al., 2012).

In the present investigation, antituberculosis drug, rifampicin treated liver shows vacuolization, necrosis, aggregation of nucleus and lipid droplets were observed. Oral administration of aqueous leaf extract *Cassia auriculata* (150 and 300 mg/kg body wt.) shows minimized the above mentioned changes whereas administration of aqueous leaf extract *Cassia auriculata* (600 mg/kg body wt.) and silymarin shows completely minimized the above mentioned changes and apperred like normal histoarchitectural pattern. Moreover, administration of aqueous leaf extract *Cassia auriculata* (600 mg/kg body wt.) alone treated rats liver showed normal histoarchitectural pattern and absence of any changes in the liver. Similarly administration of ethanolic root extract of *Ziziphus oenoplia* to antitubercular drugs treated rats showed significantly minimized the changes caused by antituberculosis drugs when compared with antituberculosis drugs alone treated rats (Rao et al., 2012). Senthil kumar et al., (2003) addressed that administration with leaf extract of *Cassia auriculata* to alcohol treated liver shows that normal appearance of liver histology. Sabina et al., (2010) reported that degenerative changes and necrosis were observed in acetaminophen treated rats liver whereas administration with piperine an active ingredient of black pepper attenuates the acetaminophen induced liver damage in liver. Mujahid et al., (2013) addressed that administration of *Adenanthera pavonina* to antitubercular drug induced rats liver showed normal apperence of histology of liver. Jaswal et al., (2013) reported that necrosis, inflammation and cellular degeneration caused by antituberculosis drugs these changes were normalized with administration of thymoquinone. Administration of antituberculosis drug to *Hibiscus vitifolius* root treated rats liver showed absence of necrosis and inflammation (Dineshkumar et al., 2012).

It is concluded that treatment with aqueous leaf extract of *Cassia auriculata* decreases the rifampicin induced toxicity in biochemical parameters. These findings suggest that the aqueous leaf extract of *Cassia auriculata* was effective in bringing about functional improvement of hepatocytes. The enhancement of the antioxidant effect of this extract was also confirmed by minimize the lipid peroxidative activities were observed. This study demonstrates that, aqueous leaf extract of *Cassia auriculata* have a potential therapeutic approach to hepatoprotective properties.

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Table 1. Body, liver and kidney weight changes in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Kidney weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>173±12.75</td>
<td>215±9.84*</td>
<td>0.94±0.06*</td>
</tr>
<tr>
<td>Rifampicin (1g/kg)</td>
<td>186±10.24</td>
<td>157±7.32</td>
<td>1.21±0.09b</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (150 mg/kg)</td>
<td>182±14.52</td>
<td>198±11.28</td>
<td>0.81±0.05*</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (300 mg/kg)</td>
<td>179±9.78</td>
<td>206±14.55</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>Rifampicin + Silymarin (25 mg/kg)</td>
<td>185±15.36</td>
<td>224±13.72</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>Cassia auriculata (600 mg/kg) alone</td>
<td>175±11.98</td>
<td>219±12.67</td>
<td>0.96±0.04</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).

Duncan Multiple Range Test (DMRT).

Table 2. Serum hepatic marker enzyme activities in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.17±5.44*</td>
<td>47.32±2.41*</td>
<td>140.60±8.62*</td>
</tr>
<tr>
<td>Rifampicin (1g/kg)</td>
<td>235.70±12.80</td>
<td>152.13±8.83</td>
<td>584.33±42.51</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (150 mg/kg)</td>
<td>159.01±10.05</td>
<td>83.55±4.16</td>
<td>366.53±25.67</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (300 mg/kg)</td>
<td>128.05±4.98</td>
<td>71.74±3.59</td>
<td>225.67±13.39</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (600 mg/kg)</td>
<td>108.95±7.33</td>
<td>57.92±3.35</td>
<td>172.00±11.09</td>
</tr>
<tr>
<td>Rifampicin + Silymarin (25 mg/kg)</td>
<td>114.85±5.55</td>
<td>63.35±2.65</td>
<td>21.00±10.89</td>
</tr>
<tr>
<td>Cassia auriculata (600 mg/kg) alone</td>
<td>81.67±5.10</td>
<td>44.95±2.64</td>
<td>139.50±9.09</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).

Duncan Multiple Range Test (DMRT).

Table 3. Serum bilirubin, cholesterol and protein levels in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bilirubin (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>Protein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.78±0.04*</td>
<td>87.83±4.24</td>
<td>7.3±0.47</td>
</tr>
<tr>
<td>Rifampicin (1g/kg)</td>
<td>3.18±0.06*</td>
<td>160.00±11.05</td>
<td>4.8±0.33</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (150 mg/kg)</td>
<td>2.21±0.11*</td>
<td>128.33±5.86</td>
<td>5.8±0.36</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (300 mg/kg)</td>
<td>1.50±0.08*</td>
<td>109.00±5.27</td>
<td>6.5±0.27</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (600 mg/kg)</td>
<td>1.08±0.04*</td>
<td>95.00±5.14</td>
<td>6.9±0.48</td>
</tr>
<tr>
<td>Rifampicin + Silymarin (25 mg/kg)</td>
<td>1.28±0.06*</td>
<td>100.97±5.01</td>
<td>6.7±0.34</td>
</tr>
<tr>
<td>Cassia auriculata (600 mg/kg) alone</td>
<td>0.759±0.03*</td>
<td>85.17±4.46</td>
<td>7.5±0.60</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).

Duncan Multiple Range Test (DMRT).

Table 4. Levels of TBARS, GSH, SOD and CAT in liver of control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmole/mL)</th>
<th>GSH (mg/dL)</th>
<th>SOD (Units)</th>
<th>CAT (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.19±0.05*</td>
<td>9.25±0.46</td>
<td>6.21±0.36</td>
<td>63.32±3.52</td>
</tr>
<tr>
<td>Rifampicin (1g/kg)</td>
<td>3.50±0.21</td>
<td>4.81±0.22</td>
<td>4.32±0.24</td>
<td>28.90±1.38</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (150 mg/kg)</td>
<td>1.70±0.09*</td>
<td>6.45±0.37</td>
<td>4.85±0.22</td>
<td>40.32±2.28</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (300 mg/kg)</td>
<td>1.37±0.05</td>
<td>7.76±0.48</td>
<td>5.69±0.28</td>
<td>49.53±3.44</td>
</tr>
<tr>
<td>Rifampicin (1g/kg) + Cassia auriculata (600 mg/kg)</td>
<td>1.24±0.06</td>
<td>8.57±0.53</td>
<td>6.08±0.32</td>
<td>54.74±2.45</td>
</tr>
<tr>
<td>Rifampicin + Silymarin (25 mg/kg)</td>
<td>1.26±0.05*</td>
<td>8.01±0.60</td>
<td>6.05±0.27</td>
<td>53.04±2.25</td>
</tr>
<tr>
<td>Cassia auriculata (600 mg/kg) alone</td>
<td>1.17±0.05*</td>
<td>9.44±0.49</td>
<td>6.20±0.35</td>
<td>63.65±3.75</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).

Duncan Multiple Range Test (DMRT).

Units4 = one unit is as 50% inhibition of NBT/mg protein
Units5 = moles of H2O2 utilised/min/mg protein.

Fig 1. Liver section taken from control rat showing normal histoarchitectural pattern (XCa 100) CV-Central vein, H-Hepatocytes, N-Nucleus.
Fig 2. Liver section taken from rifampicin treated rats (XCa 100) FC-Fatty changes, NC-Necrosis, V-Vacuole, SF-Space formation, LCB – Loss of cell boundaries.

Fig 3. Liver section taken from rifampicin + acqueous extract of Cassia auriculata (150 mg/kg) treated rats showing normal histoarchitecture (XCa 100) FC-Fatty changes, V-Vacuole, SF-Space formation.

Fig 4. Liver section taken from rifampicin + acqueous extract of Cassia auriculata (300 mg/kg) treated rats showing normal histoarchitecture (XCa 100) CV-Central vein, H-Hepatocyte, N-Nucleus.

Fig 5. Liver section taken from rifampicin + acqueous extract of Cassia auriculata (600 mg/kg) treated rats showing normal histoarchitecture (XCa 100) CV-Central vein, H-Hepatocyte, N-Nucleus.

Fig 6. Liver section taken from rifampicin + Silymarin (25 mg/kg) treated rats showing normal histoarchitecture (XCa 100) CV-Central vein, H-Hepatocyte, N-Nucleus.

Fig 7. Liver section taken from Cassia auriculata (600 mg/kg) treated rats showing normal histoarchitecture (XCa 100) CV-Central vein, H-Hepatocyte, N-Nucleus.

References
Deo P., Khuller G.K.: Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal...