Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Zoology

Elixir Appl. Zoology 96 (2016) 41296-41304

ISSN: 2229-712X

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

Minnady Muthulingam

Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar-608002, Chidambaram, Tamilnadu,

ARTICLE INFO

Article history:

Received: 21 May 2016;

Received in revised form:

22 June 2016;

Accepted: 28 June 2016;

Keywords

Cassia auriculata,

Bilirubin,

Hepatotoxicity,

Histology,

Rifampicin,

Silymarin.

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. Fourty two 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 hsitoarchitecture 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.

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 occured in 2013(WHO, 2014; Pathakumari 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 (Tasduq 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

Tele:

E-mail address: muthuau@rediffmail.com

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 anti diabetic activity ethno medically (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 alkane – 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 the body weight and increase 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 levels of bilirubin, cholesterol and protein 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.

Table 4 shows the levels of lipid peroxidation, superoxide dismutase, catalase and reduced glutathione in normal and experimental groups of rats, respectively. Significant increases in the levels of lipid peroxidation whereas SOD, CAT and GSH activities were 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 levels of lipid peroxidation, superoxide dismutase, catalase and reduced glutathione 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.

Histological photographs 1 shows the histoarchitecture of liver in normal and experimental groups of rats, respectively. Significant changes of vacuolization, necrosis, aggregation of nucleus and lipid droplets were observed 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), hepatosis (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 confirmity 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 (Willianson et al. 1996) and is also regarded as indicative of liver effects in dogs, non-luman 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 hepatotoxic 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 (Chenoweth 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 execratory 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 phosphate is a non-specific tissue enzyme widely spread, mainly in the bones, liver and biliary canaliculi (Poole and Lesile 1989; Ringler and Dabich 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 *Ziziphus 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 antituberculor 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 administerd to antituberculosis drugs.. Administration of antituberculosis drug to *Hibiscus vitifolius* root treated rats l 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 CCl_4, paracetamol and rifampicin 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 silibinin 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 anitubercular 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 hapatotoxin intoxication. The reduction is attributed to the damage produced and localised in the endoplasmic reticulum which results in the loss of P_{450} 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 increase 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 [Slater, 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 reveals of lipid peroxidation than carbon tetrachloride alone treated rats. Similarly administration of HD-03, a herbal formulation to paracetamol treated rats showed lipid peoxidation levels were decreased [Mitra,et al., 1998]. Oral administration of extracts of *Astracantha longifolia* on carbontetra chloride 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 thiols. 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 nucleophilicity 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 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 leveals of superoxide dismutase and catalase than rifampicin alone treated rats. Administration of *Astercantha longifolia* extract and silymarin to CCl_4 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 antituberculor drug induced rats liver showed enhanced the activities of SOD, CAT, GSH and inhibited the levels of lipid peroxidation. Administration of antituberculosis drug to *Hibiscus vitifolius* root treated rats liver 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 appered 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. Simillarly 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 antituberculor 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.

Acknowledgements

The author thankful to Dr. N. Indra, Professor and Head, Department of Zoology and Dr. S. Sethupathy, Professor and Head, Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University for providing facilities.

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

Groups	Body weight (g)		Liver weight (g)	Kidney weight (g)
	Initial	Final		
Control	173±12.725	215±9.84[a]	6.12±0.28[a]	0.94±0.06[a]
Rifampicin (1g /kg)	186±10.24	157±7.32[b]	7.65±0.54[b]	1.21±0.09[b]
Rifampicin (1g /kg) + *Cassia auriculata* (150 mg/kg)	182±14.52	198±11.28[e]	5.73±0.42[e]	0.81±0.05[d]
Rifampicin (1g /kg) + *Cassia auriculata* (300 mg/kg)	179±9.78	206±14.55[c]	5.61±0.37[c]	0.74±0.04[c]
Rifampicin (1g /kg) + *Cassia auriculata* (600 mg/kg)	185±15.36	224±13.72[d]	5.28±0.34[d]	0.73±0.06[c]
Rifampicin + Silymarin (25 mg/kg)	178±8.24	209±15.98[c]	5.34±0.42[d]	0.79±0.05[d]
Cassia auriculata (600 mg/kg) alone	175±11.98	219±12.67[a]	6.10±0.31[a]	0.96±0.04[a]

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.

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

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.

Groups	Bilirubin (mg/dL)	Cholesterol (mg/dL)	Protein (mg/dL)
Control	0.783±0.04[a]	87.83±4.24[ab]	7.3±0.47[de]
Rifampicin (1g /kg)	3.183±0.06[f]	160.00±11.05[f]	4.8±0.33[a]
Rifampicin (1g /kg) + *Cassia auriculata* (150 mg/kg)	2.217±0.11[e]	128.33±5.86[e]	5.8±0.36[b]
Rifampicin (1g /kg) + *Cassia auriculata* (300 mg/kg)	1.500±0.08[d]	109.00±5.27[d]	6.5±0.27[c]
Rifampicin (1g /kg) + *Cassia auriculata* (600 mg/kg)	1.083±0.04[b]	95.00±5.14[c]	6.9±0.48[cd]
Rifampicin + Silymarin (25 mg/kg)	1.283±0.06[c]	100.97±5.01[a]	6.7±0.34[c]
Cassia auriculata (600 mg/kg) alone	0.759±0.03[a]	85.17±4.46	7.5±0.60[e]

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.

Groups	TBARS (nmoles/mL)	GSH (mg/dL)	SOD (Units[A])	CAT (Units[B])
Control	1.19±0.05[a]	9.25±0.46[e]	6.21±0.36[d]	63.32±3.52[e]
Rifampicin (1g /kg)	3.50±0.21[d]	4.81±0.22[a]	4.32±0.24[a]	28.90±1.38[a]
Rifampicin (1g /kg) + *Cassia auriculata* (150 mg/kg)	1.70±0.09[c]	6.45±0.37[b]	4.85±0.22[b]	40.32±2.28[b]
Rifampicin (1g /kg) + *Cassia auriculata* (300 mg/kg)	1.37±0.05[b]	7.76±0.48[c]	5.69±0.28[c]	49.53±3.44[c]
Rifampicin (1g /kg) + *Cassia auriculata* (600 mg/kg)	1.24±0.06[a]	8.57±0.53[d]	6.08±0.32[cd]	54.74±2.45[d]
Rifampicin + Silymarin (25 mg/kg)	1.26±0.05[ab]	8.01±0.60[c]	6.05±0.27[cd]	53.04±2.25[d]
Cassia auriculata (600 mg/kg) alone	1.17±0.05[a]	9.44±0.49[e]	6.20±0.35[d]	63.65±3.75[e]

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)

Units[A] = one unit is as 50% inhibition of NBT/mg protein

Units[B] = □moles of H_2O_2 utilised/min/mg protein.

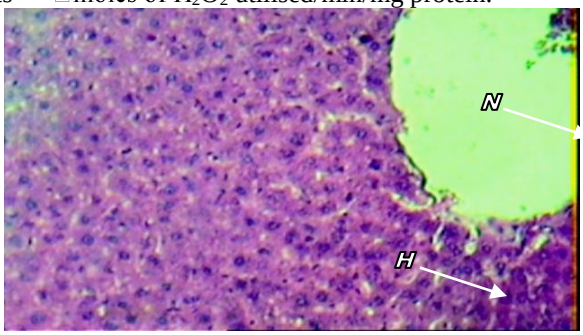

Fig 1. Liver section taken from control rat showing normal histoarchitectural pattern (XCa 100) CV-Central vein, H-Hepatocytes, N-Nucleus.

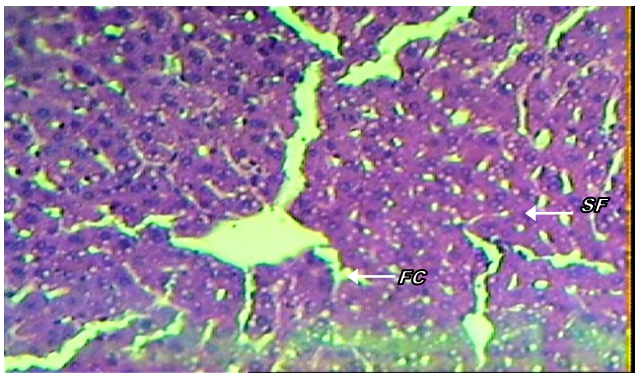

Fig 2. Liver section taken from rifampicin treated rats (XCa 100) FC-Fatty changes, NC-Necrosis, V-Vacuole, SF-Space formation, LCB – Loss of cell boundaries.

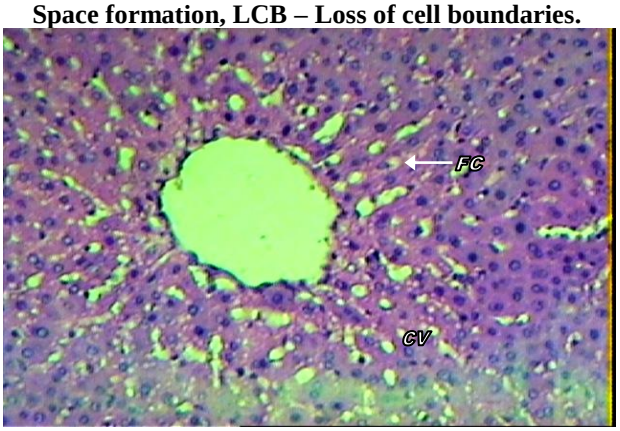

Fig 3. Liver section taken from rifampicin + acqueous extract of *Cassia auriculata* (150 mg/kg) treated rats showing normal histoarcitecture (XCa 100) FC-Fatty changes, V-Vacuole, SF-Space formation.

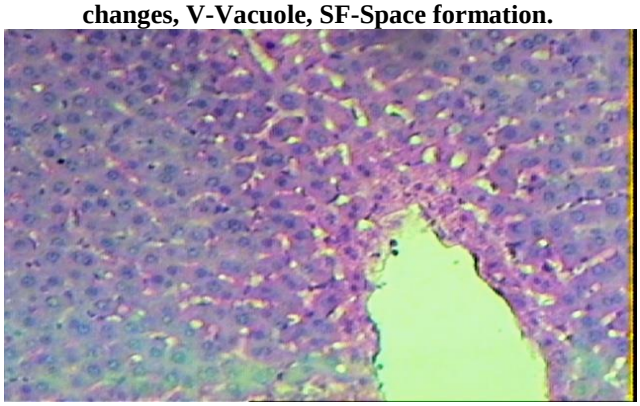

Fig 4. Liver section taken from rifampicin + acqueous extract of *Cassia auriculata* (300 mg/kg) treated rats showing normal histoarcitecture (XCa 100) CV-Cenral vein, H-Hepatocyte, N-Nucleus.

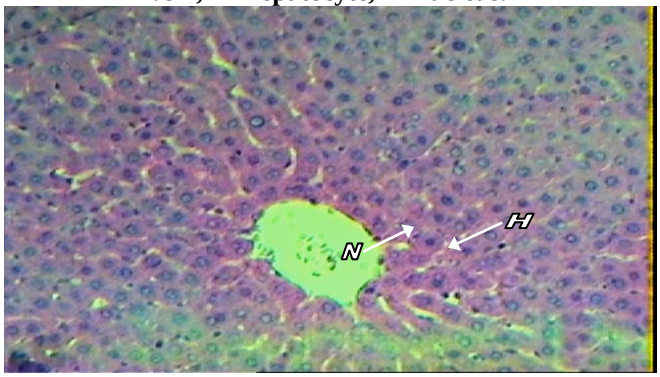

Fig 5. Liver section taken from rifampicin + acqueous extract of *Cassia auriculata* (600 mg/kg) treated rats showing normal histoarcitecture (XCa 100) CV-Cenral vein, H-Hepatocyte, N-Nucleus

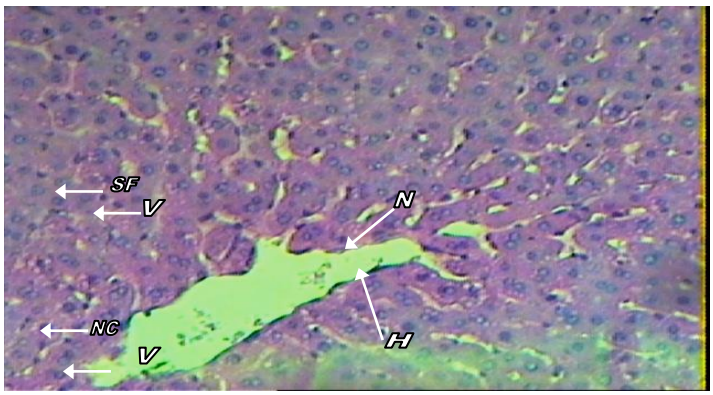

Fig 6. Liver section taken from rifampicin + Silymarin (25 mg/kg) treated rats showing normal histoarcitecture (XCa 100) CV-Cenral vein, H-Hepatocyte, N-Nucleus.

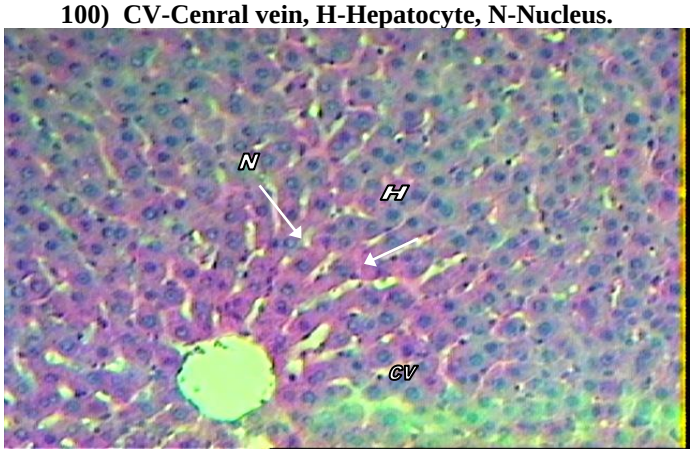

Fig 7. Liver section taken from *Cassia auriculata* (600 mg/kg) treated rats showing normal histoarcitecture (XCa 100) CV-Cenral vein, H-Hepatocyte, N-Nucleus.

References

Alexander M.R., Louie S.G., Guernsey B.G.: Isoniazid associated hepatitis. Clin. Pharmacol. 1: 148-153, 1982.

Ballet F.: Hepatotoxicity in drug development: detection, significance and solutions. J. Hepatol. 26: 26-36, 1997.

Boericke W.: New manual of homoeopathic *Materia medica* with repertory, 1103, 2005.

Brattin, W.J., lende E and Recknaged R. O. Pathological mechanisms in CCl_4 hepatotoxicity. *J. Free. Rad. Biol. Med.*, 1985; 1: 27-28.

Brown M.S., Goldstein J.L.: A receptor mediated pathway for cholesterol homeostasis. Science 232: 34-47, 1986.

Burtis C.A., Ashwood E.R.: Textbook for clinical chemistry, W.B. Saunders Company, Philadelphia, Pennsylvania, 1986.

Buzzelli G., Mosarella S., Giusti A., Duchini A., Marena C., Lampertico M.: A pilot study on the liver protective effect of silybin-phospholipid complex (IdB 1016) in chronic active hepatitis. Int. J. Clin. Pharmacol. Ther. Toxicol. 31: 456-460, 1993.

Calleja I., Blanco-Prieto M.J., Ruz N., Renedo M.J., Dios-Vieitez M.C.: 2004. High performance liquid chromatographic determination of rifampicin in plasma and tissues. J. Chromatogr. A 1031: 289-294, 2004.

Chenoweth M.B., Hake C.L.: The smaller halogenated aliphatic hydrocarbons. Annu. Rev. Pharmacol. 2: 363-389,1962.

Comporti, M. Glutathione depleting agents and lipid peroxidation. *Chem. Phys. Lipids*, 1987; 45: 143-149.

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

antituberculosis drugs in mice. Biochem. Biophys. Acta 1334: 161-172, 1997.
Dossing M., Wilcke J.T.R., Askgaard D.S., Nybo B., 1996. Liver injury during antituberculosis treatment: an 11 year study. Tuber. Lung Dis. 77: 335-340.
Estuo, K.N. and Hiroyuki, S. *Arch. Biochem. Biophys.,* 1990; 279: 40-405.
Gallieni M., Braidotti P., Cozzolino M., Romagnoli S., Carpani P.: Acute tubulo-interstitial nephritis requiring dialysis associated with intermittent rifampicin use: case report. Int. J. Artif. Organs 22: 477-481, 1999.
Gupta M., Mazumder U., Sivakumar T., Gomathi P., Sambathkumar R.: Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian J. Pharmacol Ther. 3: 12-20, 2004.
Hagymasi K., Kocsis I., Lugasi A., Fesher J., Blazovics A.: Extrahepatic biliary obstruction: Can silymarin protect liver function? Phytother. Res. 16: S78-S80, 2002.
Halliwell, B., Aeschbach, R Loliger J and Aruoma O. I The characterization of antioxidants. *Food Chem. Toxic.,* 1995; 33(7): 601-617.
Halliwell, B. and Gutteridge J.M.C. *FEBS Lett.,* 1992; 307: 108.
Horvath M.E., Gonzalez C.R., Blazovics A., Van der Looij M., Barta I., Muzes G., Gergely P., Feher J.: Effect of silibinin and vitamin E on retardation of cellular immune response after partial hepatoctomy. J. Ethnopharmacol. 77: 227-232, 2001.
Jamshidzadeh A., Fereidooni F., Salehi Z., Niknahad H.: Hepatoprotective activity of *Gundelia tourenfortii*. J. Ethnopharmacol. 101: 233-237, 2005.
Kapil A., Suri O.P., Koul I.B.: Antihepatotoxic effects of chlorogenic acid from *Anthrocephalus cadamba*. Phytother. Res. 9: 189-193, 1995.
King E.J., Armstrong A.R.: Calcium, magnesium, phosphorus and phosphatase. In: Varley B., Gowenlock A.H., Bell M. (eds.). Practical Clinical Biochemistry, vol. 1, Heinemann, London, 850, 1980.
Kothavade R.J., Joylekar S.N., Barodavalla S.A.: Protective effect of indigenous drug livomyn on ketoconazole induced hepatotoxicity. Indian J. Pharm. Sci. 58: 142-146, 1996.
Kuzuya, M., Naito, M., Funaki, C., Hayashi, T., Aasai, K and Kuzuya, F. *Biochem. Biophys. Res. Comun.,* 1986; 163: 1466.
Lee W.M.: Drug induced hepatotoxicity. N. Engl. J. Med. 333: 1118-1127, 1995.
Lenaerts A.J., Johnson C.M., Marrieta K.S., Gruppo V., Orme I.M.: Significant increases in the levels of liver enzymes in mice treated with antituberculosis drugs. Int. J. Antimicrobial Agents 26: 152-158, 2005.
Liu G.T.: Pharmacological actions and clinical use of *Fructus schizandrae*. Chinese Med. J. 102: 740-749, 1989.
Lowry O.H., Rosebrough N.J., Farr A.L, Randall R.J.: Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265, 1951.
Lucena M.I., Andrade R.J., de la Cruz J.P., Rodriguez-Mendizabal M., Blanco R., Sanchez de la Cuesta F.: Effect of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo controlled clinical study. Int. J. Clin. Pharmacol. Ther. 40: 2-8, 2002.
Luper S.: A review of plants used in the treatment of liver disease. Alter. Med. Rev. 4: 178-189, 1999.
Maggi N., Pasqualucci C.R., Ballota R., Sensi P.: Rifampicin: a new orally active rifamycin. Chemotherapia 11: 285-292, 1966.
Malloy H.T., Evelyn K.A.: The determination of bilirubin with the photometric colorimeter. J. Biol. Chem. 119: 481-490, 1937.
Manjunatha B.K., Vidya S.M., Narayanamurthy G., Krishna V.: Preliminary phytochemical and antibacterial studies on crude extracts of *Solanum stramoenifolium* JACQ., *S. seaforthianum* ANDR. and *S. violaceum* ORTG. Asian J. Microbial. Biotech. Env. Sci. 6: 587-589, 2004.
Martin, P., Friedman, L.S.: Assessment of liver function and diagnostic studies. In: Friedmann L.S., Keeffe E.B. (eds.): Hand book of liver disease, Churchill Livingstone, Philadelphia 1992, pp.1-14.
McIntyre N., Rosalki S.: Biochemical investigation in the management of liver disease. In: Prieto J., Rodes J., Shafritz D.A. (eds.): Hepatobiliary diseases, Springer-Verlag, Berlin 1992, pp. 39-71.
Mitchell I., Wendon J., Fitt S., Williams R.: Antituberculous therapy and acute liver failure. Lancet 345: 555-556, 1995.
Moss D.W.: The nature and origin of alkaline phosphatase in hepatotoxicity disease. Z. Med. Lab. Diagnost. 30: 335-363, 1989.
Mukundan, H., Bahadur, A.K., Kumar A., Sardana, S and Naik, S.L.D., Ray, A and Sharma, B.K. Glutathione level and is relation to radiation therapy in patients with cancer of uterins cervix. *Indian J. Exp. Biol.,* 1999; 37: 859-864.
Muthulingam, M. Studies on the curative efficacy of *Asteracantha longifolia* on carbontetra chloride induced hepatotoxicity in rats. *Ph.D. Thesis*, Annamalai University, 2002.
Muthulingam, M. Antidiabetic efficacy of leaf extracts of *Asteracantha longifolia* (Linn.) Nees. on alloxan induced diabetics in male albino wister rats. *Int. J. Pharm. Biomed. Res.*, 2010; 1 (2), 28 -34.
Orisakwe O.E., Afonne O.J., Chude M.A., Obi E., Dioka C.E.: Subchronic toxicity studies of the aqueous extract of *Boerhavia diffusa* leaves. J. Health Sci. 46: 444-447, 2003.
Pahkla R., Lambert J., Ansko P., Winstanley P., Davis P.D., Kiivet R.A.: Comparative bioavailability of three different preparations of rifampicin. J. Clin. Pharm. Ther. 24: 219-225, 1999.
Pal R., Vaiphei K., Sikander A., Singh K., Rana S.V.: Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. World J. Gastroenterol. 12: 636-639, 2006.
Plaa G., Charbonneau M.: Detection and evaluation of chemically induced liver injury. In: Hayes A.W. (ed.): Principles and methods of toxicology, Raven Press, New York 1994, pp. 841-846.
Poole A., Lesile G.B.: A practical approach to toxicological investigation, Cambridge, 1989, pp. 44-86.
Prajapathi N.D., Purohit S.S., Sharma A., Kumar T.: A handbook of medicinal plants: A complete source book. Agrobios, India, 2004.
Praveen Reddy B., Kokate C.K., Rambhau D., Venkateshwarlu V., Murthy V.N.: Antihepatotoxic activity of some ayurvedic preparations. Indian J. Pharm. Sci. 55: 137-140, 1992.
Ramachandran P.C.: Chemotherapy of tubercular meningitis with isoniazid plus rifampicin. Kinterim findings in a trial in children. Ind. J. Tuberc. 27: 54-56, 1980.

Ramadan L.A., Roushdy H.M., Abu Senna G.M., Amin N.E., El-Deshw O.A.: Radioprotective effect of silymarin against radiation induced hepatotoxicty. Pharmacol. Res. 45: 447-454, 2002.

Rao K.S., Mishra S.H.: Antihepatotoxic activity of monomethyl fumarate isolated from *Fumaria indica*. J. Ethnopharmacol. 60: 207-213, 1998.

Rao K.S., Mishra S.H.: Anti-inflammatory and antihepatotoxic activities of the roots of *Moringa pterygosperma* Gaertn. Indian J. Pharm. Sci. 60: 12-16, 1997.

Rao K.S., Mishra S.H.: Hepatoprotective activity of the whole plants of *Fumaria indica.* Indian J. Pharm. Sci. 59: 165-170, 1996.

Reen, R.K., Karan, M Singh, K., Karan, V., Johri R.K and Singh, J. Screening of various *Swertia* species extracts in primary monolayer cultures of rat hepatocytes against carbon tetrachloride and paracetamol- induced toxicity. *J. Ethanopharmacol.,* 2001; 75: 239-247.

Rees R.J., Pearson J.M., Waters M.F.: Experimental and clinical studies on rifampicin in treatment of leprosy. Br. Med. J. 10: 89-92, 1970.

Reitman S., Frankel S.: Determination of serum glutamate oxaloacetate and glutamic pyruvic acid transaminase. Am. J. Clin. Pathol. 28: 56-66, 1957.

Ringler D.H., Dabich L.: Haematology and clinical biochemistry, In: Baker H.J., Lindsey J.R., Weisbroth S.H. (eds.): The laboratory rat, vol. 1, Academic Press, London 1979, pp. 105-118.

Santhosh S., Sini T.K., Anandan R., Mathew P.T.: Effect of chitosan supplementation on antituberculosis drugs-induced hepatotoxicity in rats. Toxicology 219: 53-59, 2006.

Sarkar, K., Ghosh A and Sil P. C. Preventive and curative role of a 43 KD protein from the leaves of the herb *Cajanus indicus* L. on thioacetamide-induced hepatotoxicity *in vivo*. *Hepatol. Res.*, 2005; 33: 39-49.

Shalan M.G., Mostafa M.S., Hassouna M.M., Hassab El-Nabi S.E., El-Refaie A.: Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology 206: 1-15, 2005.

Simko V.: Alkaline phosphatase in biology and medicine. Dig. Dis. Sci. 9: 189-209, 1991.

Slater, T.F. Free radical mechanisms in tissue injury. *Biochem. J.* 1984; 222, 1–15.

Slater, T. F. Free Radical Mechanisms in Tissue injury, Pion Ltd., London. 1972.

Smith G.S., Hall R.L., Walker R.M.: In: Haschek W.M., Rousseaux C.G., Wallig M.A. (eds.): Handbook of toxicologic pathology, vol. 2. San Diego, CA: Academic Press, 2002.

Soto C.P., Perez B.L., Favari L.P., Reyes J.L.: Prevention of alloxan induced diabetes mellitus in the rat by silymarin. Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol. 119: 125-129, 1998.

Suttorp, N., Toepher W and Roka, L. *Am. J. Physiol.*, 1986; 252: 671.

Sureshkumar S.V., Mishra S.H.: Hepatoprotective effect of extracts from *Pergularia daemia* Forsk. J. Ethnopharmacol. 2006, in press.

Tasduq S.A., Peerzada K., Koul S., Bhat R., Johri R.K.: Biochemical manifestations of antituberculosis drugs induced hepatotoxicity and the effect of silymarin. Hepatol. Res. 31:132-135, 2005.

Tolbert, N.E.: Metabolic pathways in peroxisomes and glyoxysomes. Annu. Rev. Biochem., 50, 133 (1981).

Tsankov N., Angelova I.: Rifampicin in dermatology. Clin. Dermatol. 21: 50-55, 2003.

Varley H., Gowenlock A.H., Bell M.: Practical clinical biochemistry, 5th ed., William Hanemann Medical Book Ltd., London, 1988.

Venukumar M.R., Latha M.S.: Effect of *Coscinium fenestratum* on hepatotoxicity in rats. Indian J. Exp. Biol. 42: 792-797, 2004.

Wells F.E.: Tests in liver and biliary tract disease, in Varley's Practical Clinical Biochemistry 1988, pp. 744.

Willianson E.M., Okpako D.T., Evans F.J.: Selection, preparation and pharmacological evaluation of plant material, John Wiley, England, 1996.

Wong W.M., Wu P.C., Yuen M.F., Chen C.C., Yew W.W., Poon C.W.: Antituberculosis drug-related liver dysfunction in chronic hepatitis B infection. Hepatol. Res. 31: 201-206, 2000.

Zlatkis A., Zak B., Boyle G.J.: A method for the determination of serum cholesterol. J. Clin. Med. 41: 486, 1953.

Kumar CH, Ramesh A, Kumar JNS, Ishaq BM., 2011. A review on hepatoprotective activity of medicinal plants. Int J Pharm Sci Res, 2: 501-515.

Asadi-Samani, M., Kafash-Farkhad, N., Azimi, N., Fasihi, A., Alinia-Ahandani, E., Rafieian-Kopaei, M., 2015. Medicinal plants with hepatoprotective activity in Iranian folk medicine Asian Pac J Trop Biomed 5(2): 146-157.

Rao, Ch. V., Rawat, A.K.S., Singh, A.P., Singh, A and Verma, N., 2012. Hepatoprotective potential of ethanolic extract of Ziziphus oenoplia (L.) Mill roots against antitubercular drugs induced hepatotoxicity in experimental models. Asian Pacific Journal of Tropical Medicine, 283-288.

Senthil Kumar, R., Ponmozhi, M., Viswanathan, P and Nalini, N., 2003. Activity of *Cassia auriculata* leaf extract in rats with alcoholic liver injury. Journal of Nutritional Biochemistry. 14: 452–458.

Sabina, E.P., Souriyan, A.D.H., Jackline, D and Rasool, M. K., 2010. Piperine, an active ingredient of black pepper attenuates acetaminophen-induced hepatotoxicity in mice. Asian Pacific Journal of Tropical Medicine, 971-976.

Pathakumari, B., Prabhavathi, M and Raja, A., 2015. Evaluation of cytokine and chemokine response elicited by Rv2204c and Rv0753c to detect latent tuberculosis infection, Cytokine, 76 : 496–504.

World Health Organization,2014. Global tuberculosis control 2014. Available From: , 2014.

Yi, Z., Seikou, N., Souichi, N., Tao, W., Masayuki, Y., and Hisashi, M., 2015. Chemical structures of constituents from the seeds of *Cassia auriculata*, Tetrahedron, 71: 6727 – 6732.

Surai, P.F., 2015. Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. Antioxidants, 4: 204 – 247.

Mujahid, M., Siddiqui, H.M., Hussain, A and Hussain, Md.S., 2013. Hepatoprotective effects of Adenanthera pavonina (Linn.) against antituberculor drugs induced hepatotoxicity in rats. Pharmacognosy Journal, 5: 285 – 290.

Jaswal, A., Sinha, N., Bhadauria, M., Shrivastava, S and Shukla, S., 2013. Therapeutic potential of Thymoquinone against antituberculosis drugs induced liver damage. Environmental Toxicology and Pharmacology, 36: 779 – 786.

Dineshkumar, C., Anandarajagopal, K and Saraswathi, A., 2012. Hibiscus vitifolius (Linn.) root extracts shows potent protective action against antitubercular drug induced hepatotoxicity. Journal of Ethnopharmacology, 141: 396 – 402.