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RESEARCH ARTICLE

AMELIORATIVE ROLE OF *CARALLUMA ATTENUATA* (WIGHT) AGAINST ANTITUBERCULOSIS DRUG, RIFAMPICIN INDUCED HEPATOTOXICITY IN MALE ALBINO WISTAR RATS

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ABSTRACT

Tuberculosis is one of the most common diseases in India and has attained epidemic proportions. With 3.4 million cases, India carries more than 20% of the world's TB burden. However TB is not just a public health problem but also a socioeconomic challenge. Rifampicin is an antituberculosis drug effectively cures tuberculosis unfortunately liver get damage. The present study was undertaken to scientifically prove the traditional use of the plant, *Caralluma attenuata* aqueous extract against liver disorders. The ameliorative potential of aqueous *Caralluma attenuata* on liver damage was evaluated by rifampicin induced hepatotoxicity in rats. Male albino Wistar rats were orally treated with aqueous *Caralluma attenuata* (125, 250 and 500 mg/kg body weight) or silymarin (25 mg/kg) daily to rifampicin (1 g/kg, one day only) treated rats. Rifampicin induced liver damage and significantly increased the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamaglutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH) and bilirubin and decreased the levels of protein in serum as compared with control group. Treatment with aqueous *Caralluma attenuata* or silymarin could significantly decrease the ALT, AST, ALP, GGT and bilirubin whereas protein level in serum was increased when compared with rifampicin alone treated rats. The result revealed that aqueous extracts of *Caralluma attenuata* could be useful in ameliorating antituberculosis drug rifampicin induced liver injury.

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INTRODUCTION

Tuberculosis is one of the fatal communicative diseases and is spread easily amongst people. Over one third of the world population is estimated to be infected with Mycobacterium tuberculosis and over 2 million people a year will die of the disease (Shishoo *et al.,* 2001; Ravi *et al.,* 2010). WHO declared that the tuberculosis as "Global health emergency" (Anon, 1997). Tuberculosis is a leading public health problem worldwide, particularly in developing countries. About one third of world's population has latent tuberculosis and approximately 9 million cases of active tuberculosis emerge annually resulting in 2-3 million deaths (Adhvaryu *et al.,* 2007). Out of 1.86 billion people were living in developing countries, such as India and China (Santhosh *et al.,* 2006). In the past decade, there has been a great increase in the use of complimentary treatments such as herbal remedies in the treatment of disease (Bolkent *et al.,* 2005). Liver is the largest and most complex internal organ in the body. It plays an important role in maintenance of internal environment with the help of its multifarious and several functions. It is connected to inter mediatory metabolism of proteins, fats, and carbohydrates as well as in the synthesis of number plasma proteins such as albumin, fibrinogen and in the production of various enzymes, formation and excretion of bile (Somnath *et al.,* 2017). Drugs such as paracetamol, carbon tetrachloride, thioacetamide and isoniazid catabolize the radicals, bring on lipid peroxidation, damage the membranes of liver cells and organelles, cause the inflammation and necrosis of hepatocytes and leads to the liberation of cytosolic enzymes into the systemic transmission (Singh *et al.,* 1998; Saleem and Naseer, 2014). The most common disease of the liver is jaundice can be presented as yellow coloration of eye sclera, skin and mucous membrane due to increase amount of bilirubin in body, having prehepatic, hepatic or post-hepatic causes (Tortora and Grabowski, 2002; Saleem and Naseer, 2014). Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties. Several Indian plants are also being used in Ayurvedic and Siddha medicines. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases (Handa *et al.,* 1996; Sailaja and Setty, 2006). The plant, *Caralluma attenuata* belongs to the family Asclepiadaceae, is a thick, succulent perennial herb growing wild in dry hill slope regions of southern India. It is

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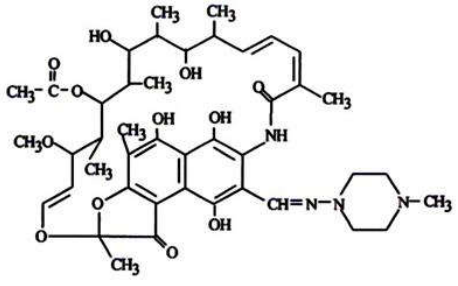
known as Kallimulaiyan indigenously and is commonly used in traditional medicine as decoction for the treatment of many diseases and also used as a vegetable. Certain species of *Caralluma* are used in folk medicine as antipyretic, antirheumatic and reported to possess significant anti-inflammatory (Ahmad *et al.,* 1983) antihyperglycemic (Venkatesh *et al.,* 2003) diabetic (Kalaivani and Mary Violet Hristy, 2011) ulcer healing activity (Garg *et al.,* 2016). Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant *Silybum marianum* (Shalan *et al.,* 2005). Silymarin is the extract of silybum marianum, or milk thistle, and consists of seven flavonoglignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) (Kim *et al.,* 2003). Among these substances, silybin is mainly prevalent and has the most important biological effect. It makes up about 70% of the total composition of silymarin in the form of two diastereoisomeric compounds: silybin a and silybin b (Loguercio and Festi, 2011; Crocenzi and Roma, 2006; Alessandro *et al.,* 2017). 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). The purpose of this study was to evaluate the ameliorative efficacy of *Caralluma attenuata* extract on antituberculosis drug rifampicin induced hepatotoxicity.

MATERIALS AND METHODS

Procurement and rearing of experimental animals

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (27±2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 150-180 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water *ad libitum* and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. The study was approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College and Hospital (160/1999/CPCSEA), Annamalai University, Annamalainagar, Chidambaram.

Chemical structure of rifampicin



Preparation of aqueous extract

The collected plant, *Caralluma attenuata* were air dried and powdered. The powdered *Caralluma attenuata* were kept in airtight containers in a deep freeze until the time of use. A sample containing 250 g of *Caralluma attenuata* 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 physiological saline and used in the study. The yield of the extracts was approximately 28.5 g. The suitable optimum dosage schedule were identified by administering the aqueous extract of *Caralluma attenuata* extracts at different dosages (125, 250, 500 and 1000 mg/kg body weight) in a day daily for twenty eight days. The optimum doses were selected as 125, 250 and 500 mg/kg body weight of the animals for twenty eight days respectively.

Experimental design

The animals were divided into 7 groups of 6 rats each.

Group 1: Control rats given physiological saline solution 10 mL/kg body wt.
Group 2: Rats given rifampicin (1 g/kg body wt./p o) for one day only.
Group 3: Rats given rifampicin + *Caralluma attenuata (*125 mg/kg body wt / p o)
Group 4: Rats given rifampicin + *Caralluma attenuata (*250 mg/kg body wt / p o)
Group 5: Rats given rifampicin + *Caralluma attenuata* (500 mg/kg body wt / p o)
Group 6: Rats given rifampicin + silymarin (25 mg/kg bodywt / p o)
Group 7: Rats given *Caralluma attenuata* (500 mg/kg body wt / p o) alone

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. Blood was collected without anticoagulant for the separation of serum and liver tissues were preserved in 10% formalin for histological examinations.

Biochemical analysis

Blood samples were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Serum biochemical parameter such as Transaminases (AST and ALT), ALP, GGT, LDH, Bilirubin and protein levels were estimated according to standard methods (Reitman and Frankel, 1957; King and Armstrong, 1980; Rosalki and Rau, 1972; King, 1965; Malloy and Evelyn 1937, Lowry *et al.,* 1951).

Histopathological studies

All rats were sacrificed by cervical dislocation and then midline laparotomy was performed. Dissected liver specimens of each rat in all groups were fixed in 10% buffered formaldehyde for 24 hours and embedded in paraffin after 16 h of alcohol process. Five µm thick sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. Each slide was examined under a light microscope (Gurr, 1959).

Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test

(DMRT). The level of statistical significance was set at $p \leq 0.05$ (Duncan, 1957).

RESULTS

Hepatic serum marker enzymes

The activities of serum AST, ALT, ALP, GGT, LDH, level of bilirubin and protein were estimated in normal and experimental rats. Significant elevation in serum AST, ALT, ALP, GGT, LDH and bilirubin whereas protein level was reduced in rats treated with rifampicin when compared with the corresponding control rats. Oral administration of aqueous extract of *Caralluma attenuata* (125, 250 and 500 mg/kg body wt.) and silymarin to rifampicin induced hepatic damage rats caused a marked reduction in the activities of these enzymes and protein level was enhanced. Extract alone administered rats did not shows any significant change (Fig. 1 and 2).

Histology

In the present study, the histological examination of the liver sections revealed that the normal liver architecture (Plate 1a) was disturbed by the administration of antituberculosis drug, rifampicin which showed necrosis, ruptured hepatocytes, space formation, vacuolization, fatty accumulation, loss of cell boundaries and enlargement of hepatocytes (Plate 1b). In the sections obtained from the rats treated with aqueous extracts of *Caralluma attenuata* and standared drug, silymarin showed gradually minimized aforementioned damage (Plate 1 c,d,e,f) and normal cellular architecture was retained to some extent, thereby confirming the antihepatotoxic efficacy of the *Caralluma attenuata* extract. administration of *Caralluma attenuata* extract alone (500 mg/ kg body weight) showed normal histoarchitecture of liver (Plate 1g). administration of *Caralluma attenuata* extract (500 mg/ kg body weight) to rifampicin treated rats revealed maximum ameliorative effect.

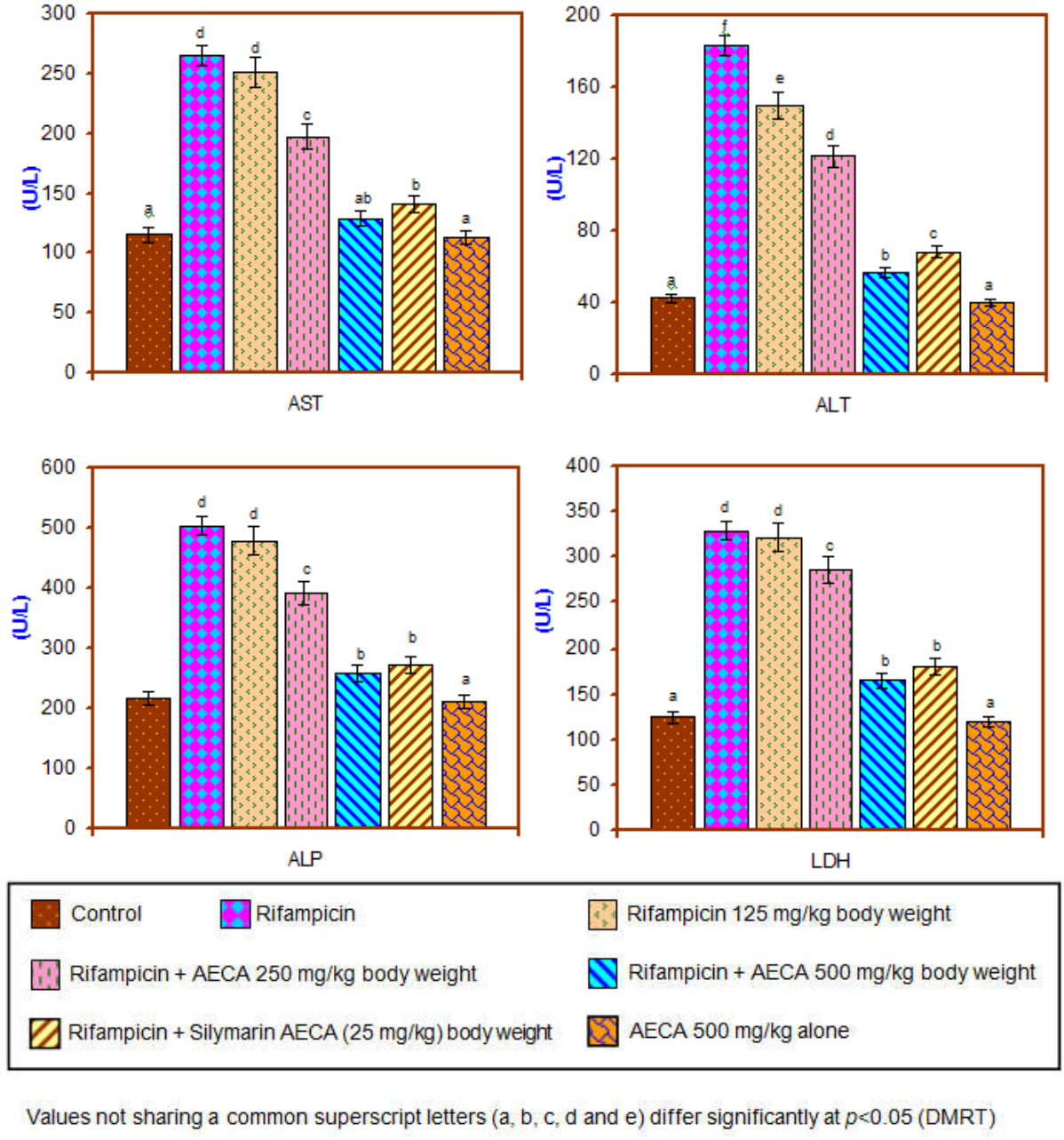
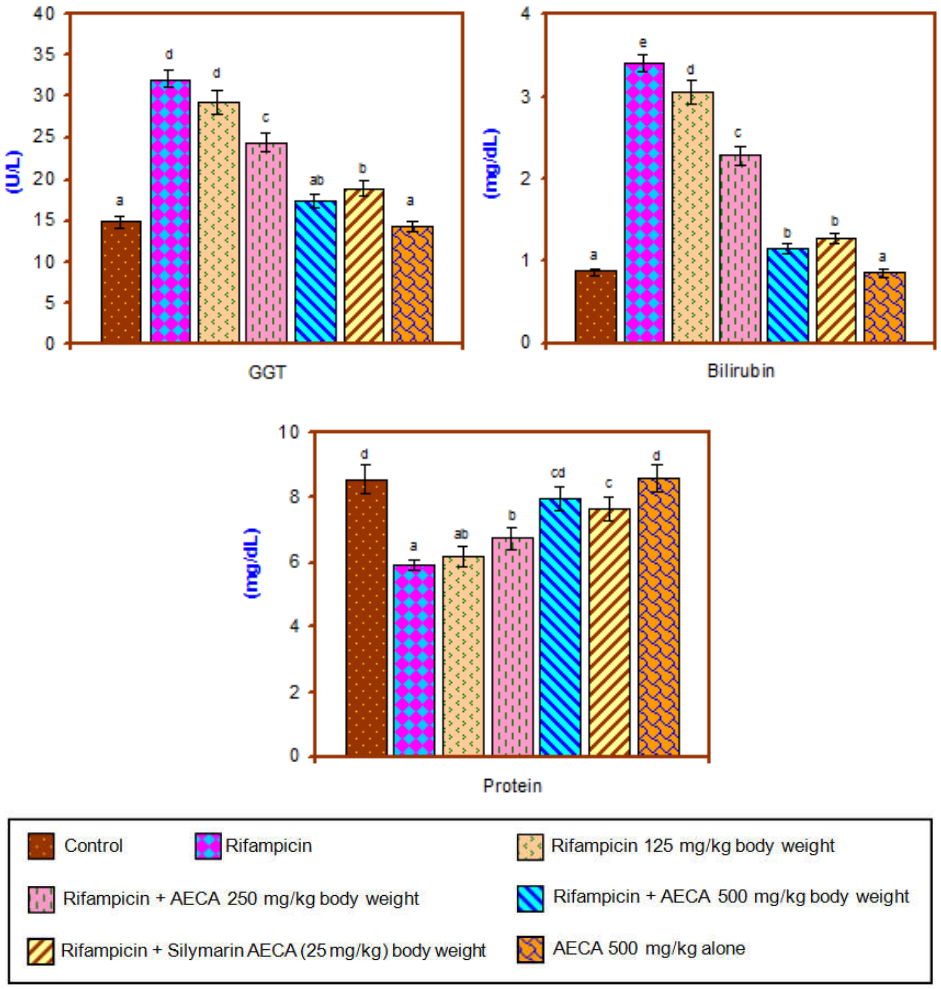


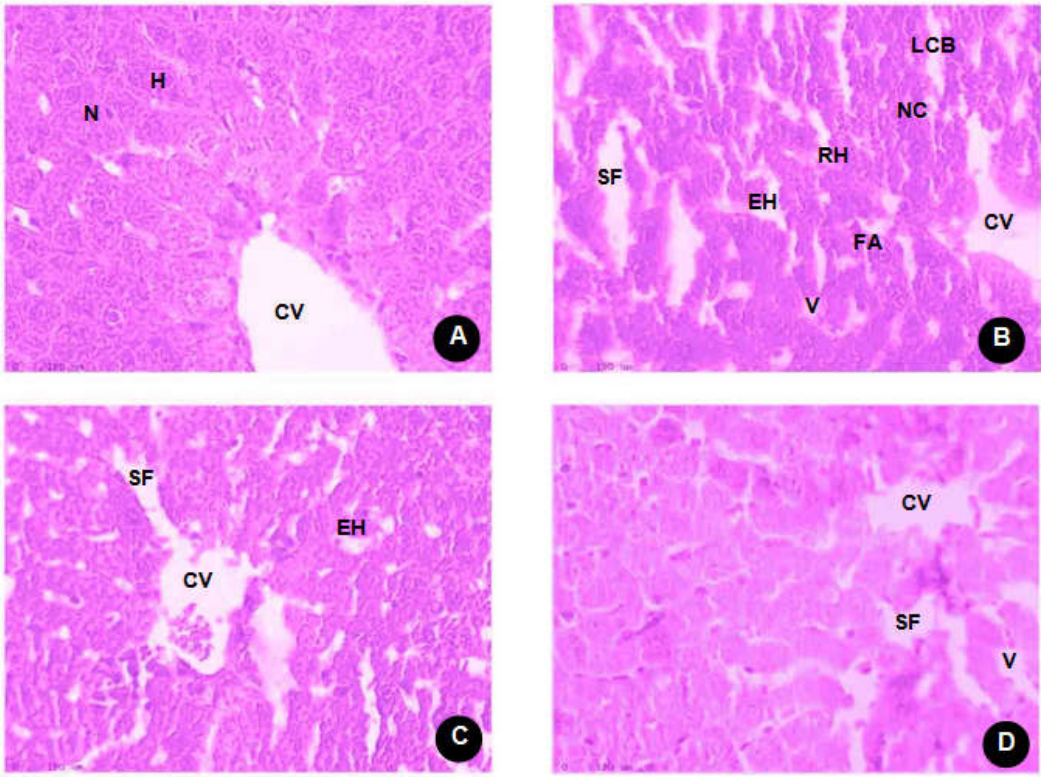
Fig. 1. Effect of aqueous extracts of *Caralluma attenuata* on serum marker enzymes

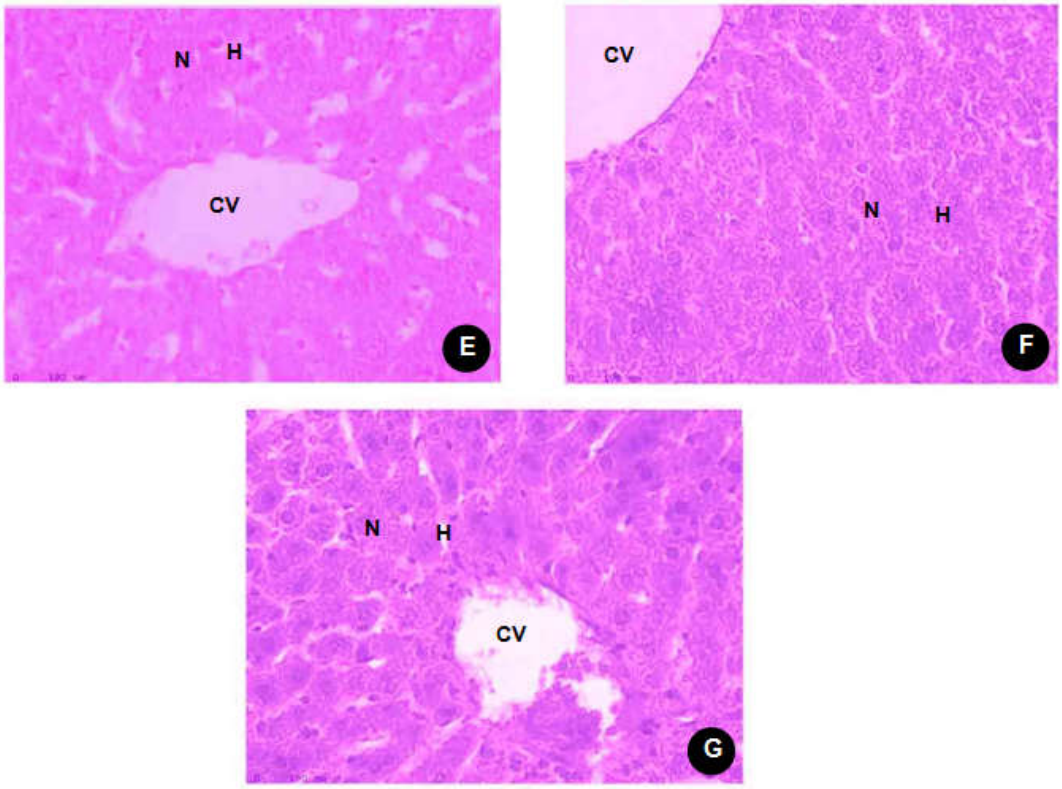


Values not sharing a common superscript letters (a, b, c, d and e) differ significantly at $p<0.05$ (DMRT)

Fig. 2. Effect of aqueous extracts of *Caralluma attenuata* on GGT, bilirubin and protein

Plate 1. Histology of Liver





DISCUSSION

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appear to be the most common contributing factor (Nadeem *et al.,* 1997). Among the many diseases that can affect the liver the most commonis viral hepatitis. Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. About 20,000 deaths found every year due to liver disorder. The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world. Scientifically evaluation of plants has often shown that active principles are responsible for therapeutic success (Sharma *et al.,* 2009). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are the source of great economic value all over the world. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993; Joji Reddy *et al.,* 2012). 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). Serum AST, ALT, ALP and bilirubin are the most sensitive tests which are considered as the index for diagnosis of liver diseases (Mahendale *et al.,* 1985). GGT and ALP are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. The elevation of serum GGT concentrations is regarded as one of the most sensitive indices of hepatic damage (Szczeklik *et al.,* 1961). LDH catalases the conversion of lactate to pyruvate using NAD^+ as coenzyme of NAD (Burtis and Ashwood, 1986). The increase in LDH activity in serum may be due to leakage of the enzyme from the tissues into the blood on account of cellular injury. 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). The reduction in the total protein is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of CYP 450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Husne, 2008).

In the present study, administration of rifampicin treated rats showed an increase in the activities of AST, ALT, ALP, LDH, GGT, bilirubin and decrease the level of protein when compared with control rats. Oral administration of aqueous extract of *Caralluma attenuata* (125, 250 and 500 mg/kg body wt.) and silymarin to rifampicin treated rats showed an inhibition in the elevated activities of serum AST, ALT, ALP, LDH, GGT, bilirubin and protein level was increased 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). Enhanced the synthesis of total protein which may be by accelerating the regeneration process and protecting the liver cells. The increased levels of total protein in serum are indicator of the hepatoprotective activity (Husne, 2008). 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). Iwo *et al.* (2017) reported that combination of isoniazid and rifampicin treated rats serum marker enzymes ALT activity was increased. Administration with ethanolic extracts of *Vernonia amygdalina* (50 and 100 mg/kg) to antituberculosis drug treated rats showed decreased the elevated activity of ALT. Administration of metanolic extract of *Vigna mungo* to rifampicin treated rats showed suppressed the elevated activities of AST, ALT, ALP and level of bilirubin (Nitin *et al.,* 2013). Administration of *Luffa acutangula* to CCl_4 and rifampicin treated rats showed decreased the elevated levels of transaminases, ALP, LDH whereas protein level was increased (Jadhav *et al.,* 2010). Pretreatment with methanolic extract of *Sphaeranthus amaranthoides* and *Oldenlandia umbellate* (50 and 100 mg/kg) to carbontetra chloride treated rats showed reduction in serum marker enzymes such as SGOT, SGPT, ALP and bilirubin (Somnath *et al.,* 2017). Xin *et al.* (2017) reported that administration of bicyclol to antituberculosis drug treated rats showed reduction of elevated serum marker enzymes and bilirubin near normal levels and histological changes in the liver also remarkably improved.

Histology is the study of their microscopic structures of cells and tissues of plants and animals. It is often carried out by examining a thin slice of tissue under a light microscope or an electron microscope. In order to distinguish different biological structures more easily and accurately histological stains are often used to add colours to enhance the colours of certain types of biological structures differently from other types of structures that may be located next to and/or in contact with each other. In the present investigation, antituberculosis drug, rifampicin treated rats liver showed necrosis, ruptured hepatocytes, space formation, vacuolization, fatty accumulation, loss of cell boundaries and enlargement of hepatocytes. Oral administration of aqueous extracts of *Caralluma attenuatta* (125, 250 and 500 mg/kg body wt.) and reference drug silymarin to rifampicin treated rats showed gradually reduced aforementioned histopathological changes in the liver. The maximum ameliorate action found in 500 mg/kg body wt of aqueous extract of *Caralluma attenuatta.* The results obtained indicate that the plant *Caralluma attenuata* extract is useful in the treatment of drug induced liver related complications. Similarly **A**dministration of *Cichroium intybus* root extract against carbon tetrachloride treated rats showed that regeneration of hepatocytes conform the hepatoprotective activity (Nallamilli *et al.,* 2013). The rats treated with ethanolic extract of *Ixora pavetta* against isonizid and rifampicin showed a sign of protection has it was a evident for the absence of necrosis with regeneration changes at central vein in the liver tissue (Jyothi Reddy *et al.,* 2013). In the rifampicin treated group the liver showed loss of lobular architecture, extensive central vein dilation, focal hepatocytes drop out, focal necrosis and extensive inflammation. Administration of metanolic extract of *Vigna mungo* and silymarin to rifampicin treated rats showed normal architecture of the liver was maintained and hepatocytes showed regeneration (Nitin *et al.,* 2013). Pretreatment with methanolic extract of *Sphaeranthus amaranthoides* and *Oldenlandia umbellate* (50 and 100 mg/kg) to carbontetra chloride treated rats showed reduction in fatty changes, focal necrosis and hydrophine changes were observed in histology of liver (Somnath *et al.,* 2017). Iwo et al., (2017) reported that combination of isoniazid and rifampicin treated liver showed inflammation, hydropic degeneration and necrosis. Administration with ethanolic extracts of *Vernonia amygdalina* (50 and 100 mg/kg) to antituberculosis drug treated liver showed absence of inflammation, hydrophic degeneration and necrosis.

Conclusion

The results of the present study indicate that the administration of *Caralluma attenuata* extracts minimize the rifampicin induced hepatotoxicity in rats. Biochemically the high dose of *Caralluma attenuata* leaf extract showed better results as compared to low dose. The overall antihepatotoxic efficacy of *Caralluma attenuata* is probably due to counteraction of 25-desacetyl rifampin formed from rifampicin which is responsible for liver damage. Further pharmacological and isolation of active principles were underway to find out antihepatotoxic role of *Caralluma attenuata*.

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