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HEPATOPROTECTIVE ACTIVITY OF *ACACIA FERRUGINEA* DC. LEAVES AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS.

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ABSTRACT: The ethanol and aqueous extracts of *Acacia ferruginea* leaves were tested for their efficacy against carbon tetrachloride (CCL_4) induced hepatotoxicity in Wistar albino rats. The different groups of animals were administered with CCL_4 (1ml/kg, s.c.). The ethanol and aqueous extracts at the dose of 200mg/kg were administered to CCL_4 treated rats. The result of present study demonstrated that ethanol extract significantly decreases the level of alanine aminotransferase, aspartase aminotransferase, total bilirubin and direct bilirubin in blood, as compare to aqueous extract. The phytochemical screening revealed the presence of active phytoconstituents i.e. flavonoids and tannins, which may offer hepatoprotection. The present work support the traditional claim of plant in the treatment of liver injury, may provide a new drug against a war with liver diseases.

Keywords: *Acacia ferruginea,* Hepatoprotective effect, Carbon tetrachloride

INTRODUCTION

The diverse culture of our country is a rich source of traditional medicines, many of which are of plant origin. Scientific data on such plant derivative could be of clinical use[1]. Since the liver is an organ with diverse functional activity, the hepatoprotective activity of drug should be based on its ability to reduce the injurious effect or to preserve the architecture and physiological function of the liver disturb by hepatotoxin.

The experimental intoxication induced by carbon tetrachloride (CCL_4) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCL_4, which is cytochrome P-450 dependent. Free radicals initiate the process of lipid peroxidation, which is generally caused of inhibition of enzyme activity [2, 3]. It is now generally accepted that the hepatotoxicity of CCL_4 is the result of reductive dehalogenation, which is catalysed by P450, and which forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases [4].

The bark of *Acacia ferruginea* DC. is bitter and traditionally used as hot anthelmintic, cure itching, leucoderma, astringent, ulcers, stomatitis, and diseases of the blood. The extract of leaves is astringent, styptic, stops suppuration, enriches the blood, useful in liver complaints, disease of the eye, dysentery, gonorrhoea, gleet, burns and scalds, beneficial to the alimentary and urinary tracts. The gum is demulcent, emollient, and nutrient. The pods and the extract from them are astringent and demulcent. A decoction of the bark of this plant, together with the *Tamarindus Indica* and a few other trees is frequently resorted by the natives of this country, as a gargle in sore-mouth [5].

In the light of above information we carried out a study to assess the hepatoprotective activity of ethanol and aqueous extract of *Acacia ferruginea* DC. leaves in hepatic rats.

EXPERIMENTAL

PLANT MATERIAL AND PREPARATION OF EXTRACTS

The leaves of A*cacia ferruginea* DC were collected from the Kolli Hills, Tamilnadu, India. The plant material was taxonomically identified from Plant Anatomy Research Centre (PARC), Chennai and the voucher specimen was

retained in our laboratory for future reference. The dried powdered material of A*cacia ferruginea* leaves was defatted with petroleum ether (60-80^{o} C) and further separately extracted with ethanol and water by soxhlet extractor for 72 hour. The solvent was removed under reduced pressure and semisolid mass obtained dried in vacuum to yield solid residue (6.8%w/w and 5.2% w/w respectively). The chemical constituents of the extracts were identified by qualitative analysis followed by their confirmation by thin layer chromatography[6, 7].

ANIMALS

Male Wistar albino rats (150-200 gms.) were divided into five groups of six animals each. The animals were maintained under standard laboratory condition (temperature 25$\pm$2) with dark light cycle. They were allowed free access to standard dry pellet diet and water *ad libitum*, throughout the experimental period.

TOXICITY STUDIES

An acute toxicity study relating to the determination of LD_{50} was performed found that ethanol and aqueous extracts of *Acacia ferruginea* leaves (EEAF and AEAF respectively) did not show any sign of mortality up to the dose of 2000mg/kg. There was no change in general behaviour. Hence the biological dose was fixed 200mg/kg for ethanol and aqueous extract[8].

HEPATOPROTECTIVE ACTIVITY

Wistar albino rats were divided into five groups of six animals each. The carbon tetrachloride (1ml/kg) was administered to all groups of animals by subcutaneous injection. Group-I served as control received normal saline only (10 ml/kg i.p). Group-II received silymarin the reference drug (25mg/kg i.p). Group-III received CCL_4 (1ml/kgi.p). Group IV and Group V received aqueous and ethanolic extract respectively in a dose of 200mg/kg daily once for fifteen days after CCL_4 administration. All the animals were dissected at the end of 15th day after CCL_4 administration.

Blood was withdrawn from the carotid artery and serum was separated by centrifugation at 1000 rpm. The separated serum were used for the estimation of the following biochemical parameters

1) Alanine aminotrasferase (ALT/GPT)
2) Aspartase aminotrasferase (AST/GOT)
3) Alkaline phosphatase
4) Bilirubin (Direct and Total)

ALT and AST was assayed by using Serum diagnostic kits by Span Diagnostic Ltd.

ASSESSMENT OF LIVER FUNCTION

ALT, AST, alkaline phosphatase, total bilirubin and direct bilirubin were determined.

HISTOPATHOLOGICAL STUDY

The liver from each animal was removed after dissection; washed with ice cold saline, the liver sections were taken from each lobe of the liver and fixed with 10% neutral formalin solution; and embedded in paraffin by employing the standard technique, 5µ in thick section were cut and stained with hematoxylin-eosin for histological examination. The remaining liver was cut into approximately 50-100 mg portion on ice and stored and separately at 7^{0}C in plastic vials. The sections were taken by using microtome.

RESULT

Reports of preliminary phytochemical analysis indicated the presence of glycosides, carbohydrate, flavonoids, saponin, tannin and phenolic compound in both ethanolic and aqueous extracts. Where as alkaloid, steroid, triterpenoid, in ethanolic extract only.

Both extract of leaves of *Acacia ferruginea* DC. was found to be practically non-toxic since no mortality was observed even at the dose of 2000mg/kg body weight. Hence the biological dose was fixed 200mg/kg for EEAF and AEAF.

In the present studies, rats treated with CCl_4 , developed significant liver damage as observed from the elevated serum levels of hepato-specific enzymes as well as severe alteration in other biochemical parameters ($P<0.001$). Values of the biochemical parameters were observed to be increased in the CCl_4 intoxicated rats.

Treatment with EEAF and AEAF decreased the CCl_4 induced alterations in AST, ALT, alkaline phosphatase, total bilirubin and direct bilirubin in blood. It is found that both the extracts offer protection against toxin as evidenced by remarkable reduction in all biochemical parameter. Histopatholgical studies demonstrated that carbon tetrachloride causes focal necrosis, portal infiltration, fatty change, kupffer cell hyperplasia and hydropic change. In the treated groups, necrosis which is more severe form of injury is markedly prevented; milder form of injury like fatty change and reduced necrosis persisted in both the extracts.

DISCUSSION

In the assessment of liver damage by CCL_4 hepatotoxin, the determination of enzyme levels such as ALT (SGPT} and AST (SGOT) is largely used. Necrosis or membrane damage releases the enzyme in to circulation; therefore, it can be measured in serum. High levels of AST (SGOT) indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT (SGPT) catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT (SGPT) is more specific to the liver, and is thus a better parameter for detecting liver injury[20]. Our results using the model of CCL_4-induced hepatotoxicity in the rats demonstrated that ethanol and aqueous extracts caused significant inhibition of ALT (SGPT) and AST (SGOT) levels. Serum ALP and bilirubin levels on other hands are related to the function of hepatic cell. Increase in serum levels of ALP is due to the increased synthesis, in presence of increasing biliary pressure[21]. Our results using the model of CCL_4 - induced hepatotoxicity in rats demonstrated that EEAF and AEAF caused significant inhibition of serum ALP and bilirubin levels. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

It has been reported that *Acacia ferruginea* DC. contain tannins and related polyphenol[19]. A number of scientific report indicated certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties[22,23]. Presence of those compounds in EEAF and AEAF may be responsible for the protective effect on CCL_4 induced liver damage in rats.

In the present experimental conditions, EEAF and AEAF showed protection against toxin, as there is a significant reduction in all biochemical parameters on treatment. EEAF at a dose 200 mg/kg showed the better activity than AEAF in the CCL_4 induced rats. The observed protective effect of the plant extract against carbon tetrachloride may be attributed to the presence of phenolic compounds like flavonoids, tannins

TABLE NO. 1: DATA FOR EFFCT OF ETHANOLIC AND AQUEOUS LEAVES EXTRACTS OF *ACACIA FERRUGINEA* DC. ON CCl_4 INDUCED HEPATOTOXICITY IN RATS

Treatment	AST (U/I)	ALT (U/I)	ALP (IU/L)	ACP (U/L)	Bilirubin(mg/100ml of blood)	
					Dire ct	Total
Group-I Control 10ml/kg	45.3 ± 1.08	33.08 ± 0.3	16.92 ± 0.62	12.5 ± 0.074	0.24 ± 0.04	0.39 ± 0.05
Group-II Silymarin (25mg/kg)	90.4** ± 3.6	47.6** ± 2.5	25.7** ± 3.9	13.2* ± 2.2	0.17* ± 0.006	0.25* ± 0.03
Group-III CCl_4 1ml/kgi.p	174.3 ± 10.72	169.4 ± 10.84	96.4 ± 7.5	57.6 ± 3.4	0.57 ± 0.04	0.99 ± 0.86
Group-IV Aqueous Extract 200mg/kg	119.6** ± 5.78	78.5** ± 1.7	35.5** ± 2.8	18.6* ± 0.19	0.38 ± 0.018	0.57 ± 0.04
Group-V Ethanol extract 200mg/kg	96.8 ± 5.68	63.2 ± 1.9	29.2 ± 1.86	15.4 ± 0.16	0.29 ± 0.016	0.46 ± 0.03

Data are expressed as mean ± S.E., n =6
*P<0.01 Vs Control
**P<0.001 Vs Control

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