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Evaluation of *In-vitro & In-vivo* Hepatoprotective Potential of *Ficus benjamina* Linn. against CCl_4 Induced Toxicity

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Abstract: The present study evaluated the Ficusbenjamina /pukar plant (Moraceae) for its possible hepatoprotective potential. Powdered crude drug 100 g were successively extracted in a soxhlet apparatus with petroleum ether (60-80ºC), chloroform and methanol. After successive solvents extraction chloroform and the methanolic extract was used for testing of hepatoprotective potential using carbon tetrachloride (CCl_4) induced liver injury in mice. Phytochemical analysis showed the presence of glycosides, alkaloids flavonoids, triterpenoids, saponins and Phenolic compounds in a high level. Silymarin was used as a standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity in rats. Treatment of mice with methanolic extract of leaves of *Ficusbenjamina* Linn at a dose of 400mg/kg body weight, exhibited a significant reduction in CCl_4 induced elevation of SGPT (150.0±1.61U/L), SGOT (126.5±1.37U/L), ALT (63.86±1.58U/L), ALP (389.0±1.543U/L) and Total Bilirubin (0.53±0.02mg/dl). Thus it can be concluded that the leaves of *Ficus benjamina* L contain significant hepatoprotective properties, which is possibly due to the presence of flavonoids or phenolic compounds.

Keywords: Ficus benjamina Linn. Pukar, Hepatoprotective activity, Carbon tetrachloride.

INTRODUCTION

The liver regulates various important metabolic functions. It plays a major role in the detoxification and excretion of many endogenous and exogenous compounds and injury to it or impairment of its functions may lead to many implications on one health. The liver filters harmful substances from the blood and regulates hormones, vitamins, and sugar[1].

Ficus benjamina is an annual herb in the family of Moraceae. The plant is known locally as "bringing, waringin and jejawi"[2].The leaves, roots, bark, and fruits of Ficus are said to be widely used in folk medicine[3]. It is traditionally used as a stomachic, hypotensive and anti-dysentery agent. Figs were traditionally used to treat constipation and bronchitis.[4]. Traditionally,*Ficusbenjamina*isused for bronchitis, wound healing, and analgesic and in liver diseases. (materia) The literature revealed that plants containing flavonoids and Phenolic compounds were responsible for the hepatoprotective activity and the selected plant *(Ficusbenjamina)* also had these constituents so it was hypothesized that it may also be able to protect the liver[5]. However, there is no scientific basis or reports in the modern literature regarding its usefulness as a hepatoprotective agent. Thus, the present study was conducted to evaluate the hepatoprotective activity of the methanolic extract of the FB by using CCl4-induced hepatic injury in mice. So, I worked on the hepatoprotective activity of *Ficusbenjamina*[6].

MATERIALS AND METHODS

Animals

The animal study was performed in VNS institute of pharmacy Bhopal. Adult mice (50-80 g) ofeither sex used in the experiment were allowed to acclimatize to the laboratory conditions for 7 days in plastic bottom hygiene cages prior to the commencement of the experiment at a temperature of 30±2° C the animals were maintained with a balanced diet and water *ad libitum*.

Drugs and chemicals

Silymarin used was a triturated tablet (siliban-70) available in the open market. CCl_4 was purchased from Central Drug House (P) ltd, New Delhi. Diagnostic kits used for the estimation of SGOT, SGPT and total bilirubin were obtained from Star diagnostics. All chemicals used for the experiments were of analytical grade.

Procurement of diagnostic kit and chemicals

Diagnostic kits used for the estimation of SGOT, SGPT, AST, ALP and total bilirubin were obtained from Star diagnostics. All chemicals used for the experiments were of analytical grade.

Plant Material

The fresh leaves of *Ficus benjamina* Linn were collected in November 2019 from the ParyavaasBhavan, Bhopal (M.P.).The plant material were authenticated by professor Dr. (Mr.) Zia-ulHussen, Assistant Professor, Department of Botany, Saifia College of Science, Peer Gate, and Bhopal (M.P.). Voucher specimen No.: 132/Bot/Safia/2019.

Preparation of extracts

The shade-dried, coarse-powdered plants were successively extracted in a Soxhlet apparatus with petroleum ether, chloroform, and methanol. (Harborne)[7]. The extracts were concentrated to dryness in a vacuum or rotary evaporator to give petroleum ether extract (yield: 2.28), chloroform extract (yield: 4.37) and methanol extract (yield: 12.33).

Phytochemical studies

Freshly prepared extracts were subjected to a phytochemical screening test for the detection of various constituents using the conventional protocol.[8]

EXPERIMENTAL DESIGN

Hepatoprotective activity

Preparation of Extract

The dosage form was made in 0.3% carboxy methyl cellulose. 1gm of, chloroform and methanolic extracts were taken in the mortar and pestle and triturated with 10 ml of 0.3% CMC solution continuously to get homogenous suspensions being a concentration of 100 mg/ml of drug in each case. Suspensions were stored in airtight bottles in a cool place. Silymarin suspension (**Silybon**) was also take-up for study[9].

1.1 Animals

The animal study was performed in VNS institute of pharmacy Bhopal. Adult mice (50-80 g) of either sex used in the experiment were allowed to acclimatize to the laboratory conditions for 7 days in plastic bottom hygiene cages prior to the commencement of the experiment at a temperature of 30±2° C the animals were maintained with a balanced diet and water *ad libitum*. Animals were divided into 5 groups of six rats each and all the testing drugs were administered intraperitoneally at the calculated dose[10]. The procedures were reviewed and approved by the Institutional Animal Ethics Committee (Registration No.778/03/c/CPCSEA).

Animals were divided into 5 groups of six rats each and all the testing drugs were administered intraperitoneally at the calculated dose. Group, I served as normal control and received 0.3% Carboxy methyl cellulose (1ml/kg p.o.) once daily for 7 days. Group II served as the treated negative control group receiving CCl_4 and olive oil (50%v/v, 0.5ml/kg) once daily for 7 days. Group III served as standard reference and received a mixture of CCl_4 and olive oil and the standard drug Silymarin (200mg/kg p.o.)

simultaneously for 7 days. Group IV received a mixture of CCl_4 and olive oil and Chloroform extract of *Ficusbenjamina* (400mg/kg)[11]. Group V received a mixture of CCl_4 and olive oil and methanolic extract of *Ficus benjamina* (400mg/kg) simultaneously for 7 days. On the 8th day blood was collected directly from the cardiac puncture of all mice and serum was separated by centrifugation at 3000 rpm at 30^0C for 15 min. and analyzed for different biochemical analysis[12].

Statistical Analysis

The activities of serum hepatic marker enzymes namely alanine aminotransferase (ALT), Serum Glutamic Pyruvate Transaminase(SGPT), and Serum Glutamic Oxaloacetic Transaminase (SGOT) were assayed in serum using a standard kit. The results were expressed as units/liter (U/L).[Friedman]Results of biochemical parameters are reported as mean ±S.E.M. Statistical significance was determined by one-way analysis of variance followed by Student's t-test[13]

RESULTS

CCl_4 damaged the hepatocytes too much, which results in necrosis. In CCl_4 control (group II) showed a significant (*P <0.001) increase in liver enzyme parameters as compared to control animals (group I, normal)(Table1). Serum Glutamic Pyruvate Transaminase (SGPT) (399.2±1.75U/L), Serum Glutamic Oxaloacetic Transaminase (SGOT) (391.2±1.14 U/L), Alanine aminotransferase (ALT) (254.0±1.68 U/L), ALP (941.4±1.116) and Total Bilirubin (2.017±0.015mg/dl) concentration as compared to the normal control group (110.1±0.964, 96.29±1.33U/L, 57.90±1.097 U/L, 389.8±2.04 U/L, 0.44±0.015mg/dl respectably). Treatment of mice with chloroform and methanolic extracts of leaves of *Ficusbenjamina* Linn at a dose of 400mg/kg body weight, exhibited a significant reduction in CCl4-induced elevation of SGPT (171.9±1.39U/L, 150.0±1.61U/L), SGOT (211.3±1.67U/L, 126.5±1.37U/L), ALT (55.69±2.05U/L, 63.86±1.58U/L),ALP (408.5±1.74U/L, 389.0±1.543U/L) and Total Bilirubin (0.61±0.28mg/dl, 0.53±0.02mg/dl) (Table 2).Administration of silymarin suspension, methanolic and aqueous extracts of leaves of *Ficusbenjamina* at the respective dose remarkably prevented CCl4-induced elevation of serum SGOT, SGPT, ALP, ALT and T. Bilirubin levels(Fig 1-4). The aqueous portion was shown to possess good hepatoprotective activity. Reduction in levels of serum SGOT, SGPT, ALP, ALT and T. Bilirubin close to normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. Lowering of all enzyme level i.e. SGPT, SGOT, Bilirubin and ALP confirm the hepatoprotective activity of *Ficusbenjamina*(Fig 1-4)**.** In histopathological changes of CCl_4 control (group II) reflects the areas of hydropic changes and degeneration of hepatocytes and congestion of the sinusoids, necrosis and central veins are present (Fig.7) when compared with control group (I) (reveals normal liver structure with normal central vein, sinusoids are normal, the hepatocytes are healthy, shown regular under surface without any evidence of hemorrhage and necrosis). (Fig. 6)

In histopathological changes of Silymarin and CCl_4 group (III) showed recovery of hepatocytes, lesser necrosis, and well-identified nucleus also as compared to control (group I) (Fig. 8).In histopathological studies of methanolic extract, the hepatocytes are radially arranged. The vacuolation is present but is very much similar to that of normal(Fig. 9).. The hepatocytes are mostly normal but have few vacuoles and some damaged cells, but the extent of the area of necrotic cells located in this region was considerably reduced. There seems to be an appreciable recovery. (Fig.10)

Table: 1 Effect of methanolic Extract of FB on Liver Enzyme Parameters (On 8th Day)

Groups/ Parameter	Control(I)	CCl_4 (0.5ml/ kg body wt.)(II)	Silymarin+ CCl_4 (III)	methanol extract+ CCl_4 (400mg) (IV)

SGOT (U/L)	96.29±1.33	391.2±1.14*	124.6±2.07**	126.5±1.37**
SGPT (U/L)	110.1±0.964	399.2±1.75*	146.0±1.89**	150.0±1.61**
ALP (U/L)	389.8±2.04	941.4±1.116*	293.1±2.43**	389.0±1.543**
ALT (U/L)	57.90±1.097	254.0±1.68*	65.37±1.99**	63.86±1.58**
Total Bilirubin	0.44±0.015	2.017±0.015	0.513±0.17	0.53±0.02

N = 6 albino rats per group, tabular value represents mean ±SEM, *p<0.001,
*p<0.001, (Comparison of group I with II)
**p<0.001, (Comparison of group II with Group III and IV)

Table: 2 Effect of chloroform Extract of FB on Liver Enzyme Parameters (On 8th Day)

Groups/ Parameters	**Control(I)**	**CCl_4 (0.5ml/ kg body wt.)(II)**	**Silymarin+ CCl_4 (III)**	**Chloroform extract+ CCl_4 (400mg) V**
U/L)	96.29±1.33	391.2±1.14*	124.6±2.07**	211.3±1.67**
U/L)	110.1±0.964	399.2±1.75*	146.0±1.89**	171.9±1.39**
/L)	389.8±2.04	941.4±1.116*	293.1±2.43**	408.5±1.74**
ALT (U/L)	57.90±1.097	254.0±1.68*	65.37±1.99**	55.69±2.05
Total Bilirubin	0.44±0.015	2.017±0.015	0.513±0.17	0.61±0.28

N = 6 albino rats per group, tabular value represents mean ±SEM, *p<0.001,
*p<0.001, (Comparison of group I with II);
**p<0.001, (Comparison of group II with Group III and V)

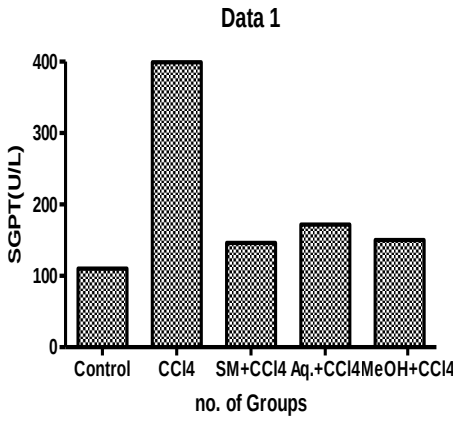


Fig.1 *In-vivo* studies for SGPT

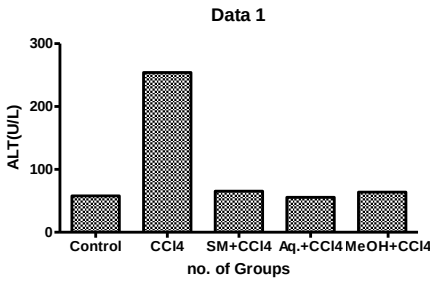


Fig.2 *In-vivo* studies for ALT

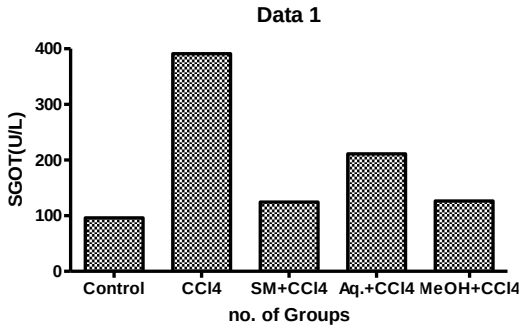


Fig.3 *In-vivo* studies for SGOT

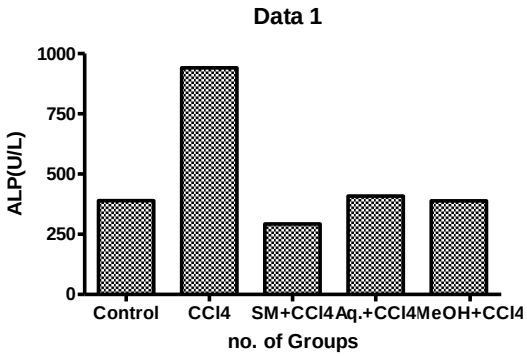


Fig.4 *In-vivo* studies for ALP

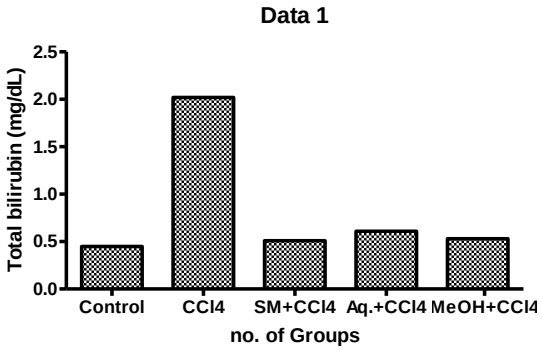
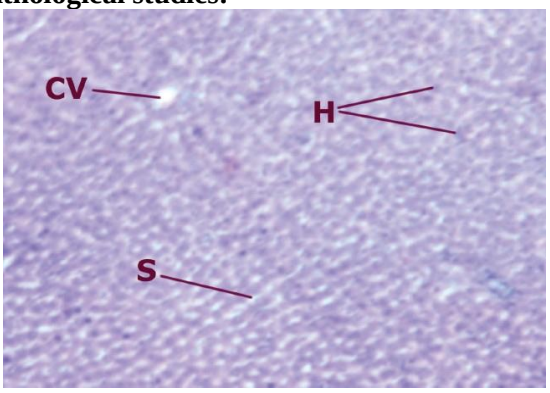


Fig.5 In-*vivo* studies for Total Bilirubin

Histopathological studies:



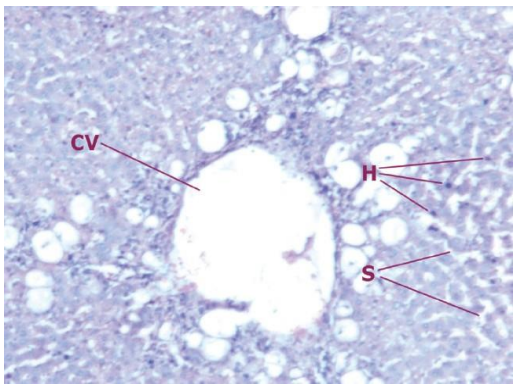
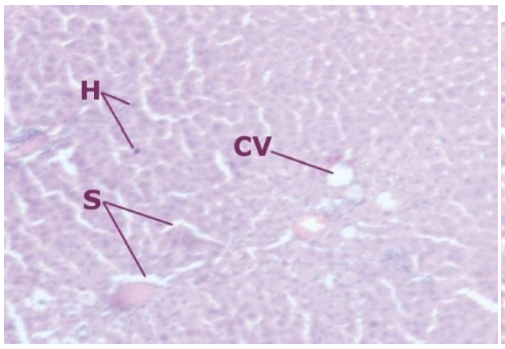


Fig.6 Control(Without any drug treatment)Fig 7 Carbon Tetrachloride control (CCl_4)



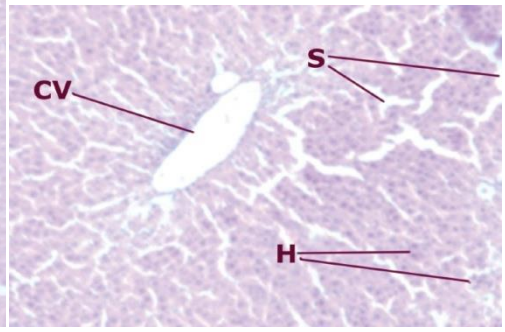


Fig 8 Silymarin+ CCl_4 (200 mg/kg + 0.50 ml/kg)Fig. 9Chloroform Extract of FB (400 mg) + CCl_4 treated

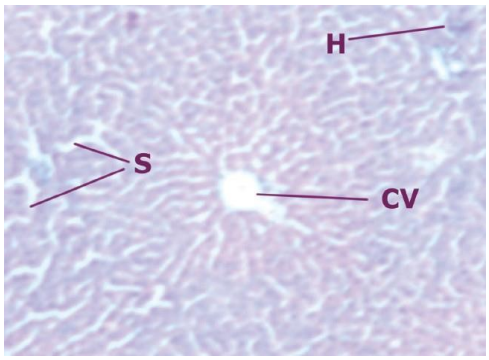


Fig. 10Methanol Extract (400 mg) + CCl_4 treated

H=Hepatocytes **CP=Cytoplasm**
N=Nucleus **CV=Central vein**
NC=Necrosis **B=Ballooning**

DISCUSSION

The CCl_4 is one of the most commonly used hepatotoxins in experimental study of liver disease [Johnson]. CCl_4 toxicity is dependent on one of its highly reactive products, the trichloromethyl radical (CCl_3). This radical binds covalently to the neighboring proteins and lipids and initiates lipid peroxidation that causes severe membrane alterations. Transaminase leaks out through the damaged membrane, elevating the serum level. Inhibition of CCl_4 bioactivation could reduce this toxic effect. Many compounds exhibit liver protection against CCl_4 either by decreasing the production of CCl_3 free radicals or by impairmentof CCl4-induced lipid peroxidation. A decrease in the concentration of these enzymes after treatment by various extracts of FB indicates the possibility of induced accelerated regeneration of liver cells by reducing the leakage of GPT, GOT and Bilirubin into the blood and thereby lowering their values in the serum. Serum transaminase returns to normal with the healing of liver parenchyma and regeneration of liver cells.

CONCLUSION:

These findings suggested that methanolic extract administration has significantly neutralized the toxic effect of CCl_4.Since, the preliminary phytochemical analysis of the extracts has shown the presence of flavonoids and phenolic compounds, which have been known for their hepatoprotective activity. Thus, it

can be concluded that the possible mechanism of hepatoprotective activity of *Ficus benjamina* leaves may be due to the presence of flavonoids and phenolic compounds.

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