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Full Length Research Paper

Hepatoprotective activity of *Morus alba* (Linn.) leaves extract against carbon tetrachloride induced hepatotoxicity in rats

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The aim of the study is to investigate the hepatoprotective activity of *Morus alba* Linn. leaves extracts against carbon tetrachloride (CCl_4) induced hepatotoxicity. Leaves powder of *Morus alba* was successively extracted with petroleum ether extract (PEE), chloroform extract (CHE), alcoholic extract (ALE) and water extract (AQE) against carbon tetrachloride (CCl_4) induced hepatotoxicity using Standard drug Liv-52. Preliminary phytochemical tests were done. The ALE showed presence of alkaloids, flavonoides, carbohydrates, tannins and steroids, while carbohydrates, flavonoides, alkaloids were present with AQE. The PEE, CHE, ALE did not produce any mortality. Carbon tetrachloride produced significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP) and serum bilirubin.) and histological (damage to hepatocytes) using Standard drug Liv-52. Pretreatment with ALE and AQE extracts significantly prevented the biochemical and histological changes induced by CCl_4 in the liver. The present study shows that the ALE and AQE extracts possessed hepatoprotective activity.

Key words: Carbon tetrachloride, hepatoprotective, leaves extracts of *Morus alba* Linn.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward et al., 1999). The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang et al., 1992). Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (Ross et al., 1996).

Morus alba Linn (Moraceae) is also known as Tut in India. The white mulberry has a long history of medicinal use in Chinese medicine; almost all parts of the plant are used in one way or another. Recent research has shown improvements in elephantiasis when treated with leaf extract injections and in tetanus oral doses of the sap mixed with sugar (Bown et al., 1995). The fruit has a tonic effect on kidney (Duke et al., 1985). It is used in the treatment of urinary incontinence, dizziness, tinnitus, insomnia due to anemia, neurasthenia, hypertension, diabetes, premature graying of the hair and constipation in the elderly (Yeung et al., 1985). The leaves are showing analgesic and anti-inflammatory activity of hydroalcoholic extract of leaves (Vaghasiya et al., 2007). Phytochemical review shows the presence of tannins, Vitamin A,

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flavonoid, thiamine, protein and carbohydrates (Wealth of India, 1962). It has been used in the indigenous system of medicine for cooling, acrid, purgative, diuretic, laxative, anthelmintic, brain tonic, antibacterial and heap-topathy properties. They are useful in vitiated condition of *vata* and *pitta*, burning sensation (Arya, 1997).

Hence the present study was aimed at investigating the hepatoprotective activity of leaves extracts of *M. alba* L. against CCl_4 induced hepatotoxic model in rats.

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *M. alba* Linn. were collected from Ramling mudgad Dist.-Latur (Maharashtra) and authenticated by Dr. Harsha Hegade, research officer Indian Council of Medical Research, Belgaum. A voucher specimen has been deposited at the herbarium of RMRC-465.

Preparation of extracts

The plant materials (leaves) were dried for several days and powdered with the help of an electric grinder. The course material was extracted successively with petroleum ether, chloroform, alcohol (90%) and the plant mark was finally macerated with distilled water. The extracts were dried at 50°C in a water bath. The percentage yields obtained from the different successive extract were 4.51, 5.102, 8.231and 11.31% respectively.

Chemicals

Carbon tetrachloride (CCl_4) was obtained from Poona Chemical Laboratory while Pune and Liv-52 were obtained from Himalaya drug, Bangalore. All other chemicals used were of analytical grade.

Experimental animals

Swiss albino mice (18-20 g) and Wistar rats (150–200 g) of either sex were procured from Sri Venkateshwara Enterprises, Bangalore and were acclimatized for 10 days under standard housing condition maintained at a room temperature of 24±1°C; related humidity 45-55% with 12:12 h light/dark cycle. The animals were habituated to laboratory condition for 48 h prior to the experimental protocol to minimize any nonspecific stress.

LD_{50} determination

Acute oral toxicity (AOT) of PEE, CHE, ALE and AQE were determined using nulliparous non pregnant female mice. The animals were fasted for 3 h prior to experiment and were administered with single dose of extracts dissolved in 2% w/v Tween 80 and observed for mortality for upto 48 h. Based on the short term toxicity, the dose of next animal was determined as per OECD guidelines 425 (OECD, 2001). All the animals were also observed for long term toxicity. The LD50 of the test extracts were calculated using 'AOT 425' software provided by Environmental Protection Agency, USA.

Hepatoprotective activity

Hepatoprotective study was carried out as described by Brijesh et al. (2008). Albino rats of either sex (150-200 gm) were selected and divided into eleven groups of six animals each. The first group was fed 1 ml/kg p.o of sucrose solution (S.S.) for 4 days. the second group was fed 1 ml/kg p.o. of S.S. for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days. The third group was fed 5 ml/kg p.o. of Liv-52 for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days. Fourth and fifth groups were fed orally PEE- 125 and 250 mg/kg respectively for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days. Sixth and seventh groups were fed orally CHE- 150 and 300 mg/kg respectively for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days. Eight and ninth groups were fed orally ALE-150 and 300 mg/kg respectively for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days. Similarly, tenth and eleventh groups were fed orally AQE- 175 and 350 mg/kg respectively for 4 days along with 2 ml/kg of CCl_4 by s.c. on the second and third days (Brijesh et al., 2008). On the fifth day, all the animals were sacrified by mild ether anaesthesia.

Blood biochemistry

Blood samples were collected in glass tube from retro orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (Reitman and Frankel, 1957), ALP (Walter and Schutt, 1974) and bilirubin (Malloy and Evelyn, 1937) by standard method.

Histopathology

Histopathology of liver was carried out by a modified Luna (Luna LG., 1999) . In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 µ thickness microtone sections were made (Krajian, 1963). The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/protection.

Statistical analysis

The data obtained were analyzed by One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using computerized program. P-value <0.05 or was taken as the criterion of significance.

RESULTS

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, flavonoides, tannins, steroids in ALE, while alkaloids, carbohydrates, flavonoides and tannins were noticed in AQE. ALE was found to be nontoxic upto a dose of 3000 mg/kg and LD_{50} of AQE was found to be 3500 mg/kg.

Treatment of rats with CCl_4 produced an increase in the weight and volume of wet liver. Rats were pretreated with Liv-52, PEE, CHE, ALE and AQE. PEE and CHE showed non significant effect, that is, they did not show decrease in wet liver weight and volume compared to control (toxic) group. ALE and AQE showed significant effect. CCl_4

Table 1. Effect of *Morus alba* L. leaves extracts on CCl_4 induced hepatotoxicity in rats.

Treatment	Dose (ml/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)
Control	1	31.42 ± 0.7574	88.50 ± 2.766	91.17 ± 5.498	0.2733± 0.1258
Liv-52	4	47.17 ± 1.851	100.2 ± 3.260	107.5 ± 3.631	0.2367 ± 0.015
CCl_4	2	137.5 ± 4.595	308.3 ± 6.015	211.2 ±3.619	1.735± 0.045
PPE	125	141.2 ± 4.23	304.0 ± 4.719	218.3 ± 4.145	1.917± 0.026
	250	129.3 ± 3.201	295.3 ± 2.716	220.2 ± 2.120	1.778 ± 0.050
CHE	150	146.8 ± 5.44	307.1 ± 4.787	218.0 ± 4.830	1.943 ± 0.02
	300	137.5 ± 3.819	303.3 ± 6.627	220.0 ± 2.875	1.747 ± 0.033
ALE	150	121 ± 3.28*	285.8 ± 4.393*	191.7 ± 4.279*	1.463 ± 0.0542*
	300	118.5±2.172**	282.3 ± 1.820**	187.0 ±3.098**	1.388 ± 0.0379**
AQE	175	125.7 ± 2.319	293.3 ±1.820	207.7 ± 4.372	1.807 ± 0.0466
	350	120.0 ± 2.033*	287.2 ± 3.710*	190.8 ± 3.016*	1.428 ± 0.0427*

Results are expressed as mean ± SEM, n= 6, (P**< 0.01) Vs CCl_4 treated group using one –way ANOVA followed by Tukey Kramer's post hoc test.

administration resulted in significant elevation of SGOT, SGPT, ALP and serum bilirubin. Biochemical parameters were found to be decreased compared to normal control group due to pretreatment with Liv-52, ALE and AQE which significantly prevented the biochemical changes induced by CCl_4. The hepatoprotective effect offered by ALE was found to be significantly greater than AQE treatment (Table 1).

Hepatocytes of the normal control group showed normal lobular architecture of the liver. In the CCl_4 treated group the liver showed microvascular fatty changes and the hepatocytes were surrounded by large numbers of fat droplets, Liv-52, ALE and AQE pretreated groups showed minimal fatty changes (Figure 1) and their lobular architecture was normal indicating the hepatoprotective effect of these extracts.

However, ALE showed more microvascular fatty changes (Figure 2) than AQE. The hepatoprotective activity of the extracts were in the order of Liv 52 > ALE > AQE.

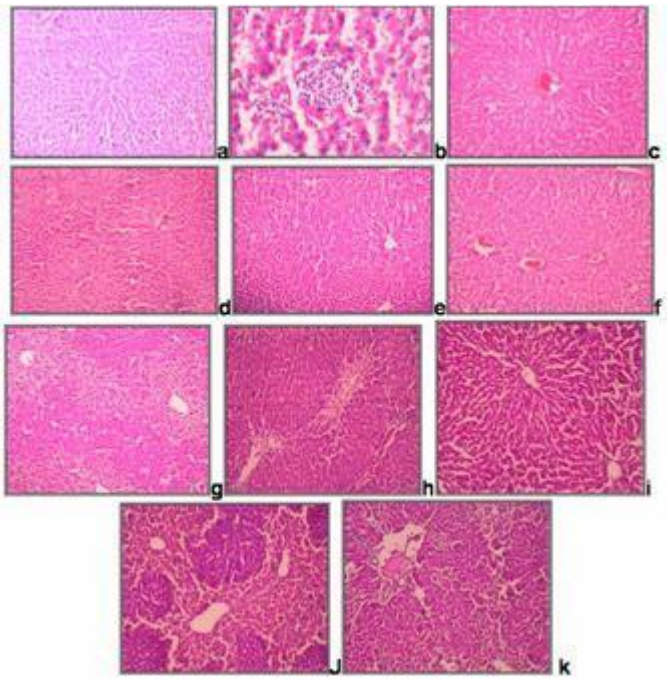

Figure 1. Histology of liver showing (a) normal hepatocyte (b) CCl_4 induced microvascular fatty changes surrounded by large number of small fatty droplets (c) hepatocytes in groups treated with Liv-52 (d and e) ALE (150 and 300 mg/kg) (f and g) AQE (175 and 350 mg/kg) (h and i) PEE (125 and 250 mg/kg), and (j and k) CHE (150 and 300 mg/kg) respectively, prior to administration of CCl_4 showing minimal fatty changes.

DISCUSSION

The liver can be injured by many chemicals and drugs. In the present study, CCl_4 was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. CCl_4 produces a constellation of dose related deleterious effects in the liver (Leo et al., 1982).

During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration (Deb et al., 1998).

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl_4 control group. Both extracts prevented these histological changes, further indicating their hepatoprotective

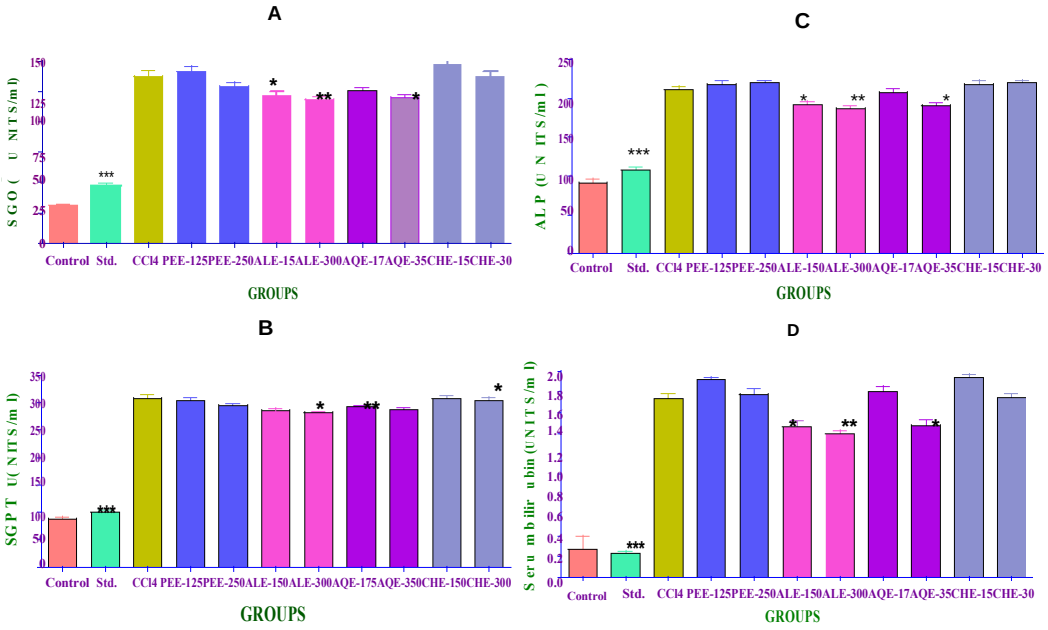

Figure 2. Effect of PEE, CHE, ALE and AQE on serum biochemical parameters against CCl_4 (1 ml/kg, po) induced liver damage. (A) Representation of alanine aminotrasferase (B) Representation of aspartate aminotrasferase (C) Representation of alkaline phosphatase (D) Representation of serum bilirubin.

activity. All the histological changes observed were in correlation with the biochemical and functional parameters of the liver.

It can be concluded that *M. alba* leaves extracts viz. ALE and AQE possess a protective effect against CCl_4 induced hepatotoxicity in rats but ALE shows more significant effect as evidenced by the biochemical, functional and histological parameters.

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