

HEPATOPROTECTIVE ACTIVITY OF TAMARIND INDICA AND HOMALOMENA AROMATICA IN RATS

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ABSTRACT

The study was performed to assess and compare the hepatoprotective activities of Tamarind *indica* and Homalomena *aromatica* in acute and chronic animal models. In this study, animals were divided into 5 groups containing 6 rats in each group. Carbon tetrachloride was used to induce liver injury in rats and Silymarin was used as the standard drug. The estimation of liver weight and blood parameters consist of serum protein, total bilirubin, serum glutamic oxalate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphates and albumin to globulin ratio were performed. SGOT in (91.3±2.1) acute and (94.6±1.9) chronic models and SGPT in (101.3±2.8) acute and (111.3±2.8) chronic models was high in homalomena aromatic treated group when compared to tamarind indica treated group. However, serum protein, SGPT, ALP and albumin: globulin ratio of homalomena aromatic and tamarind indica treated groups in both acute and chronic models were found to be (P<0.05) significant. The finding of this study suggest that both the extracts i.e., tamarind indica and homalomena aromatica in acute and chronic animal models have similar hepatoprotective activities.

Keywords: Tamarind *indica*, Homalomena *aromatica*, Carbon tetrachloride, Hepatoprotective

INTRODUCTION

The liver is an organ of paramount importance, which plays an essential role in the metabolism of xenobiotics entering the body. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. Conventional drugs used in the treatment of liver diseases are often inadequate and thus human beings suffered from hepatic diseases and complications. An indigenous system of medicine in India has a long tradition of treating liver diseases with plant drugs. During the recent times, the study of indigenous drug has undergone numerous changes. The research work on indigenous drug has received considerable encouragement and has made satisfactory progress.

In view of all these aspects, the present study was carried out to assess and compare the hepatoprotective activity of tamarind indica and homalomena aromatica which are most commonly found indigenous plants in north-east part of India. The tamarind indica (tatli, imli, Indian date, etc) is a tree found in family of fabaceae and having monotypic genus tamarindus [1] while homalomena aromatica (sugandhmantri) is a rhizomatous aromatic perennial herb found in Assam [2]. This herb found in family of Araceae and having genus homalomena. To compare the hepatoprotective activity of above two indigenous plants we have considered silybum marianum, as a standard hepatoprotective agent. Silybum marianum (Family: Asteraceae/Compositae) commonly known as 'milk thistle' is one of the oldest and thoroughly researched plants in the treatment of liver diseases [3].

METHODS AND MATERIALS

The present study was performed in the department of pharmacology, Gauhati medical College and Hospital, Guwahati.

Preparation of aqueous extract of Tamarind indica

Dry fruits of tamarind indica were collected from the Guwahati. After removing the outer skin as well as the seeds, the fruit pulp of tamarind indica was mashed to get a soft mass. The mass was then macerated with water for seven days at room temperature with occasional stirring daily. On 8th day, the pulp was filtered and the filtrate was heated below 55°C and evaporated under reduced pressure till a strong brownish mass was obtained. This aqueous

extract was dissolved in distilled water and given orally in the experiment.

Preparation of ethanolic extract of Homalomena aromatica

Dry rhizome of Homalomena aromatica were collected from khetri, Kamrup district and was grinded to powder. The dry powder was soxhlated with (99.9%) absolute alcohol, filtered using Whatman filter paper no.1, and evaporated using vacuum rotary evaporator (Buchi). A dark brown gummy extract was obtained and stored at -20° C in a deep freezer. The extract was dissolved in normal saline and given orally for the study.

Drugs and chemicals

1. Silymarin: powder of silymarin tablet which was obtained from the Microlaboratory Pvt Ltd and dissolved in distilled water (100mg/2ml).
2. Carbon tetrachloride: It was used to induce hepatic injury and was procured from Central drug house (P) Ltd, New Delhi and dissolved in olive oil to make 50% v/v of CCl₄.

Animals

The study was approved by Institutional Ethical Committee, Guwahati Medical College. Healthy intact, pathogen free, colony bred albino rats of both sexes weighing 200-250 gm were collected from Central Drug Research Institute, Lucknow. They were allowed to acclimatize to the laboratory environment for 2 weeks and were provided water ad libitum and standard diet. After the animals were acclimatized, they were divided into two models (acute and chronic) and five groups, each group containing 6 animals.

GROUP A(n=6)- Control group

GROUP B(n=6)- carbon tetrachloride induced hepatic injury. This group was divided into 2 subgroups for acute and chronic model containing 3 animals each.

GROUP C(n=6)- (standard) carbon tetrachloride induced hepatic injury and silymarin (25mg/kg, p.o) treated group. This group was divided into 2 subgroups for acute and chronic model containing 3 animals each.

GROUP D(n=6)- carbon tetrachloride induced hepatic injury and Tamarind indica (350 mg/kg, p.o) treated group. This group was

divided into 2 subgroups for acute and chronic model containing 3 animals each.

GROUP E(n=6)- carbon tetrachloride induced hepatic injury and Homalomena aromatica (200 mg/kg, p.o) treated group. This group was divided into 2 subgroups for acute and chronic model containing 3 animals each.

Induction of hepatic injury

Carbon tetrachloride was used to produce acute and chronic liver injury. Acute liver damage was induced by a single substance administration of 4ml/kg dose of 50% v/v carbon tetrachloride in olive oil. Chronic liver damage was induced by subcutaneous injection 50% v/v CCl₄ in olive oil at the dose of 2 ml/kg twice a week for 14 days [4].

Procedure

The animals were sacrificed and blood samples and livers were collected after 24 hrs of intoxication in case of acute liver damage model whereas in case of chronic liver damage model blood samples and livers were collected on 15th day. Blood was collected by intra cardiac puncture and kept for 30 minutes without disturbing. The clot was dispersed with glass rod and centrifuged for 15-20 minutes at 200 rpm to separate serum. The serum from blood were collected and estimated for serum protein, total bilirubin, serum glutamate oxalate transaminase (SGOT), serum

glutamic pyruvic transaminase (SGPT), alkaline phosphates and albumin to globulin ratio. The isolated liver was weighed in electronic weighing machine. All the biochemical estimations were done by a computer-assisted semi automatic Boehringer 4020 Photometer as well as auto-analyzer.

The statistical significance between acute and chronic models was analyzed by student t test. All the analysis was performed in MS Excel 2003. The value p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results obtained from the study have been summarized in the tables. Results have been reported as Mean ± Standard Error Mean (SEM) of 5 groups containing 6 animals in each group.

It was observed from table no.1 that in group B there was increased weight of liver in both (7.16±0.03) acute and (7.06±0.02) chronic studies after 14 days of treatment as compared to group C. The serum protein level was high in (8.20 ± 0.03) acute and (8.22 ± 0.02) chronic models of group C and albumin-globulin ratio was also high in (0.38 ± 0.01) acute and (0.38 ± 0.01) chronic models of same group as compared to group B. In contrast to this finding, bilirubin level in (0.93 ± 0.03) acute and (0.96 ± 0.03) chronic models; SGOT level in (79.3 ± 2.9) acute and (83 ± 2.8) chronic models; SGPT level in (68.66 ± 2.51) acute and (71.83 ± 1.92) chronic models and alkaline phosphate in (166.6 ± 4.24) acute and (171.83 ± 4.09) chronic models were low in group C as compared to group B.

Table 1: Comparison of liver weight and various blood parameters among Group A, Group B and Group C

Parameters	Group A (Control)		Group B (Carbon tetrachloride treated group)		Group C (Carbon tetrachloride and Silymarin treated group)	
	Acute	Chronic	Acute	chronic	Acute	Chronic
Liver weight (gm)	6.29±.13	6.29±.13	7.16±.01t=6.1	7.06±.01t=4.1	6.91±.01t=5.61	6.94±.01t=2.41
Serum protein	6.74 ± 0.07	7.73 ± 0.08	5.29 ± 0.03* t = 17.1	6.66 ± 0.05* t = 10.1	8.20 ± 0.03* t = 62.1	8.22 ± 0.02* t = 24.1
Total bilirubin	0.71 ± 0.04	0.74 ± 0.05	1.77 ± 0.04* t = 15.1	1.80 ± 0.04* t = 16.1	0.93 ± 0.03* t = 14.1	0.96 ± 0.03* t = 15.1
SGOT	58.55± 1.17	57.6 ± 0.88	211.6 ± 2.8* t = 49.1	214.6 ± 1.5* t = 89.1	79.3 ± 2.9* t = 32.1	83 ± 2.8* t = 41.1
SGPT	58.55 ± 1.76	58.01 ± 1.63	159.66 ± 6.2* t = 15.61	186.66 ± 6.1* t = 20.61	68.66 ± 2.51* t = 13.1	71.83 ± 1.92* t = 18.1
ALP	150 ± 3.03	154.3 ± 2.27	250.1 ± 1.53* t = 34.1	250.1 ± 1.53* t = 34.1	166.6 ± 4.24* t = 14.1	171.83 ± 4.09* t = 17.1
Albumin: Globulin	0.24 ± 0.01	0.24 ± 0.01	0.18 ± 0.01* t = 12.1	0.23 ± 0.01* t = 3.1	0.38 ± 0.01* t = 29.1	0.38 ± 0.01* t = 22.1

Values are expressed as mean±SEM. ns= not significant, *P<0.05 compared to respective Carbon tetrachloride treated group.

SGOT in (91.3±2.1) acute and (94.6±1.9) chronic models and SGPT in (101.3±2.8) acute and (111.3±2.8) chronic models was quiet high in group E when compared to group D. However, the values were found to be significant for serum protein, SGPT, ALP and albumin:

globulin ratio of group D and group E in both acute and chronic models. Liver weight was not having any significant difference in both models. Both the groups have shown more or less similar values in acute and chronic models as shown in table no.2.

Table 2: Comparison of liver weight and various blood parameters between Group D and Group E

Parameters	Group D (Carbon tetrachloride and Tamarind indica treated group)		Group E (Carbon tetrachloride and Homalomena aromatica treated group)	
	Acute	Chronic	Acute	Chronic
Liver weight (gm)	6.97±.03 ns t=1.6	6.90±.02 ns t=0.70	6.89±.02 ns t=0.30	6.85±.02 ns t=1.6
Serum protein	7.39±.07* t=9.5	7.73±.05* t=7.7	7.53±.08* t=18.4	7.75±.03* t=12.4
Total bilirubin	0.99±.01 ns t=1.7	1.02±.01 ns t=1.8	1.17±.02* t=5.3	1.21±.02* t=11.7
SGOT	76.1±1.6 ns t=0.9	79.1±1.64 ns t=1.2	91.3±2.1* t=3.2	94.6±1.9* t=3.4
SGPT	81.5±2.4* t=3.7	83.3±1.4* t=4.8	101.3±2.8* t=8.6	111.3±2.8* t=11.5
ALP	178.5±3.1* t=2.2	182.8±3.1* t=2.1	182.6±2.5* t=3.2	185.3±2.3* t=2.85
Albumin:Globulin	0.23±.01* t=23	0.64±.01* t=14	0.24±.01* t=21.1	0.67±.01* t=36.6

Values are expressed as mean±SEM. ns= not significant, *P<0.05 compared to respective Carbon tetrachloride treated group.

In this study, we have compared the Tamarind indica and Homalomena aromatica by observing the biochemical parameters in serum. We have considered silymarin as a standard hepatoprotective agent which has been used in several previous studies [5][6]. When we compare the serum bilirubin level among different groups, it was seen that serum bilirubin level was increased in group B which was only carbon tetrachloride treated but in drug treated groups in group C, group D and group E have shown significant improvement in reducing blood levels of bilirubin. It signifies that the extracts of Tamarind indica and Homalomena aromatica can prevent or reduce hepatic damage which is more or less equipotent to Silymarin.

The degree of rise in SGOT and SGPT activity reflects the extent of hepatic damage. The SGOT and SGPT levels which shows significant rise in carbon tetrachloride treated in group B whereas these levels were considerably reduced in group C (Silymarin treated), group D (Tamarind indica treated) and group E (Homalomena aromatica treated).

The serum alkaline phosphatase was significantly increased in both acute and chronic model of carbon tetrachloride treated B group. But after the administration of Silymarin, aqueous extract of Tamarind indica and ethanolic extract of Homalomena aromatica, the levels of alkaline phosphatase has been considerably reduced. Likewise the serum protein levels have been decreased in acute as well as chronic models of carbon tetrachloride treated B group but in drug treated groups C, D and E, there were significant rise in serum protein level whereas albumin: globulin ratio was significantly altered in drug treated groups of both acute and chronic models. The liver weights have not shown any significant difference in any groups.

Carbon tetrachloride is widely used as hepatotoxin in experimental studies. The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal hepatic physiology which is disturbed by hepatotoxin. Carbon tetrachloride causes hepatotoxicity due to its active metabolite, the free radical which covalently bound to macromolecule and causes peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This lipid peroxidative degradation of macromolecule is one of the major explanations of hepatotoxicity. This eventually leads to hepatocellular necrosis and is reflected by marked change in various enzymatic parameters [7]. Schmist et.al have reported that, when liver cell plasma is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their stimulation in serum is a useful quantitative marker in hepatocellular damage [8].

It has been reported that T. indica contains flavonoids, ascorbic acid and β carotene and have protective effect on liver due to their antioxidant properties[9][10][11]. Presence of those compounds in T. indica may be responsible for its protective effect on carbon tetrachloride induced liver damage in rats. Likewise simultaneous treatment of ethanolic extract of Homalomena aromatica with carbon tetrachloride produces lesser degree of damage to the hepatocytes cells as compared to the animals treated with carbon tetrachloride alone. The chemical components of the extract responsible for this effect were however not investigated. Further investigations are needed for identification of the active compounds responsible for the hepatoprotective activity. The present finding provides scientific evidence to the ethnomedical use of this plant by the tribal people of north east India in treating jaundice and other hepatic disorder.

CONCLUSION

In our study, we have not considered the histopathology examinations even though, the present investigations indicated that both the extracts of Tamarind indica and Homalomena aromatica provide significant protection against carbon tetrachloride induced hepatotoxicity in rats. Considering all the results from the study it has been concluded that both the extracts has equal hepatoprotective activity. So, taking into the account all aspects of the both plants, their regular use in diet as different dishes in developing countries like India can be used as alternative to Silymarin in preventing as well as treatment of liver disorders as they are safe and cost effective.

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