METHANOLIC EXTRACT
HEPATOPROTECTIVE EFFECT OF METHANOLIC EXTRACT

ORIGINAL
PROF-1993

DR. USMAN AHMED
Hamdard College of Medicine & Dentistry
Hamdard University, Karachi

DR. MUHAMMAD ASADULLAH
Hamdard College of Medicine & Dentistry
Hamdard University, Karachi

PROF. DR. S. I. AHMED
Institute of Pharmacology & Herbal Sciences,
Hamdard University, Karachi

Dr. Hafiz Muhammad Ilyas
Institute of Pharmacology & Herbal Sciences,
Hamdard University, Karachi

ABSTRACT… Purpose of Study: The aim of this study is to look at the plant based such compounds that are known to have hepatoprotective potential. With above perspective, the study has been planned to see the hepatoprotective potential. To see hepatoprotective effect on the enzyme levels and histopathology. With the help of this study, some new hepatoprotective compound may be discovered which will help in developing an effective medicine. **Material & Methods:** The extract of Melia azedarch's flower was screened for hepato protective effect. Albino rats were administered with carbon tetra chloride (CCl4) for inducing liver damage. **Results:** The effect of the extract was evaluated by measuring the liver enzymes like SGPT, Alkaline Phosphatase, Bilirubin and Gamma-GT in the serum. In the extract treated animals, there was significant decrease in liver enzyme levels. This observation leads to the conclusion that Melia flower extract possesses hepato protective activity. The hepato protective activity of the methanolic extract was compared with standard Poly herbal formulation named Jigrine CL. **Conclusion:** In our study, we found the extract of Melia Azedarach (Flowers) has strong hepatoprotective activity.

Key words: CCl4, SGPT, Gamma-GT.

INTRODUCTION

The use of medicinal plants to cure certain diseases has been practiced in many parts of the world for centuries. In addition to the direct use of concoctions, decoctions, infusions and plant extract as medicines, many conventional drugs have been isolated and now plants are their major source. Chinaberry, Umbrella tree and Persian lilac are the common names of Melia Azedarach. The increase in the use of plants in traditional medicine has been hampered by lack of adequate information, crudity of the traditional techniques and unravels the mysteries of philosophy embodied in the practice. However, there has been an attempt to investigate and unravel the mysteries of plants involved in folk medicine, and in many cases the bioactive ingredients have been identified. It is a medium stature tree belonging to the Meliaceae family, its leaves are compound and alternate which consists of leaflets around 2-8 cm. in length and having a dark green above its surface. The flowers of Melia Azedarach are five part sepals green and 1.5 – 2 meters long. Each flower has ten anthers. Fruit is stalked and is single-seeded and yellow to yellowish tan, and is one to one point five cm in diameter. It is differentiated from other members of the Meliaceae by the structure of its compound leaves, and its droppings[1,2]. A unique feature of its seeds is that it can tolerate desiccation and can remain alive for long period of time e.g. twenty six months[3]. This plant also reproduces vegetatively by forming root suckers[4]. Different compounds like Meliacine, from its leaves are isolated which has some against herpes simplex type 1 (HSV-1)[5]. Other uses of M. Azedarach are as antiseptic, abortifacient, larvicidal, pediculicidal, laxative, timber yielding etc[6-9].

Drug Induced hepatotoxicity is a common feature and one of the main reasons of drug withdrawal from the market such as Paracetamol (acetaminophen) which is widely used for the treatment of pain and fever has an ability to cause non repairable damage and acute hepatic failure if taken more than 2 gm/day especially in susceptible individuals[10]. Although the liver has a very high regenerative potential compared to other organs, acute liver failure has a high mortality rate. The drug-induced or other forms of liver damage such as viral hepatitis can also lead to long lasting chronic effects such as fibrosis resulting in cirrhosis or hepatocellular carcinoma. The mechanism of liver damage due to chemicals such as Paracetamol or other agents such as viruses is complex and only partly understood. It appears that free radical injury is involved in liver damage

although other mechanisms have also been described[11].

At the present moment there is no treatment for acute liver failure and only supportive treatment is available. There are many hepatoprotective natural products already known[12], several anti-oxidants appear to be useful in hepatic damage including Paracetamol-induced injury. In this study natural product has been investigated for their hepato-protective effect.

INTRODUCTION TO PRODUCT JIGRINE CL

Jigrine CL is one of the herbal medicines formulated by M/S Hamdard Laboratories (Waqf) Pakistan, used as a hepatoprotective in liver disorders. Jigrine CL is composed of following eight medicinal plants:

Ingredients of Jigrine CL	
Plant Name	**Family**
Achillea millifolium	Compositae
Tamarix dioica	Tamaricaceae
Melia azadirachta	Meliaceae
Damascene	Roasaceae
Sphaeranthus indicus	Compositae
Rheum emodi	Polygonacae
Allium sativum	Liliaceae

1. Achillea millefolium belongs to family compositae / Asteraceae.
   Active ingredients of Achillea like choline and achilleine are regarded as lipotropic and sudorific and effective in infectious hepatitis. Its specific pharmacological action is "Anti-inflammatory and carminative".

2. Artemisa Absinthium belongs to family Compositae / Asteraceae. Active ingredients are choline, santonin, artemisinine, etc. The herb is used in liver and spleen inflammation and debility of digestive system, and as anthelmintic.

3. Tamarix dioica belongs to family Tamaricaceae Tamarix species are effective for leucorrhoea, spleen troubles etc. It is also used in the treatment of infectious hepatitis.

4. Melia azadirachta vernacular name (Neem) belongs to family Meliaceae. Medicinally the different parts of Neem Tree are used independently to cure a variety of ailments. Active constituents are azadirachtin, Salnnin and resins, etc. Leaves are carminative; lessen Inflammation, useful in syphilitic scabies and in blood impurities.

5. Rosa damascena vernacular name Gulab belongs to family Roasaceae. Active constituents are volatile oil containing geranoil, citronellol. Rose flowers help in curing burning sensations, bad odor from mouth for improving appetite, relieving headache, Stomatitis, and intestinal affections buds are considered as astringent, Cardical, cephalic, tonic, removing biliousness and cold humors.

6. Rheum emodi vernacular name Rewand chini belongs to family polygonaceae. The root of Rheum emodi all useful in diarrhea, spleen, inflammation, dropsy and liver inflammation.

7. Sphaeranthus indicus vernacular name Mundi belongs to family Compositae / Asteraceae. All parts of the plant medicinally used. Stimulates the retentive powers of the intestine, thus helpful against diarrhoea. Its specific actions are blood purifier (immuno-Stimulant).

8. Allium sativum vernacular name Lehsan (Garlic) belongs to family liliaceae. Garlic preparations are considered as blood purifier, clean it of impurities, regulate the digestion and remove parasites in the intestine. Pharmacologically considered as appetite stimulant, carminative, and diuretic. It is also referred as valuable anti-diabetic[13].

PURPOSE OF STUDY

The aim of this study is to look at the plant based such compounds that are known to have hepatoprotective

potential. With above perspective, the study has been planned to see the hepatoprotective potential. To see hepatoprotective effect on the enzyme levels and histopathology. With the help of this study, some new hepatoprotective compound may be discovered which will help in developing an effective medicine.

MATERIALS AND METHODS

Plant Material
Methanolic extract of flowers (BFM) of Melia Azedarach was used in this study. This extract was water soluble.

Herbal Product
Jigrine CL was used as a reference drug obtained from Hamdard Laboratories (Waqf) Pakistan.

Chemicals
In order to produce hepatic injury CCl4 was used (BiOM Laboratories, Malaysia), enzyme estimation kits for Bilirubin, SGPT, Gamma-GT and Alkaline Phosphatase (Merck Germany), Formalin (Merck Germany), Xylene (Labscan Laboratories, Thailand), Chloroform (Labscan Laboratories, Thailand) and Hematoxylin & Eosin for staining slides.

Animals
Sprague Dawley rats of either sex weighing 220-275 gm obtained from the animal housing facility located in Dr. HMIIPHS. Animals were fed rat chow diet and H_2O was given ad libitum. Animals were kept in Poly propylene cages under suggested laboratory protocol by careful monitoring of temperature, humidity and day/night cycles (12-12hrs). Animals were acclimatized to laboratory conditions before experiment.

Study Design
Six rats three males and three females were used in each group and total six groups were studied.

Sample Description	
G1	Served as normal saline (0.9% NaCl) control.
G2	Served as Ccl4 only received Ccl4 (1.2 ml/kg) on 15th day of experiment.
G3	Served as BFM 100 mg/kg + Ccl4 (1.2 ml/kg)
G4	Served as BFM 200 mg/kg + Ccl4 (1.2 ml/kg)
G5	Served as BFM 400 mg/kg + Ccl4 (1.2 ml/kg)
G6	Served as Jigrine CL 20 ml/kg + Ccl4 (1.2 ml/kg)

STUDY PARAMETERS

Biochemical Study
At the end of study period, blood was drawn by direct heart puncture method. Blood was then allowed to stand and then centrifuged for serum at three thousand Rpm for 20 mins by a centrifuge machine (Model 80-2, No. 02561, Changzhou Gohua Electric Appliance Co, Ltd, China). Serum was then separated out and stored in eppendorf tubes. The serum Bilirubin, alkaline phosphatase, SGPT and Gamma-GT were estimated spectrophotometrically by Hitachi U-2000 spectrophotometer on the same day.

Autopsy
For liver tissue analysis, samples were obtained by freshly killed animals. The samples were immediately removed washed by 10 % Neutral formalin small pieces of samples were made and then fixed in the same solution.

Histopathological Study
For histopathological study, these fixed samples of liver were left over night in the 10 % neutral formalin solution for fixation, then these samples were processed (Dehydrated) in alcohol having strength of (80-100%), processed in Xylene, and fixed in Paraffin wax, four to five µm thick sections were prepared by Leica RM 2145-Rotary Microtome, then process of removing wax in xylene, gone through 80- 100% alcohol and stained with Hematoxylin (BDH Chemicals Ltd Poole England) and Eosin (E. Merck) (H & E). The tissues were studied and photographed using Nikon's Microscope with Nikon's Photography system.

STATISTICAL ANALYSIS
The results were expressed to compare the values of control and treated groups by using standard statistical analytical methods like Mean, Standard Error Mean and Standard Deviation. These changes in the values are

Table-I. Comparison of Effect of variable doses of Extract1 (BFM) on serum Enzymes (n=6)

Groups	Bilirubin (mg/dl)	Alkaline Phosphatase (IU/L)	Gamma-GT (L-GT) (IU/L)	ALAT (SGPT) (IU/L)
Control (0.9% NaCl)	0.85±0.096	1238.5±61.45	240.28±26.82	256.63±18.89
CCI4 Treated (1.2 ml/kg)	3.8±0.506	1521.01±167.20	383.91±51.46	417.58±71.98
BFM (100mg/Kg)	0.56±0.110	1247.25±71.03	239.68±8.98	253.01±22.15
BFM (200 mg/kg)	0.66±0.179	1079.16±78.22	231.7±30.89	246.4±19.79
BFM (400 mg/kg)	0.78±0.03	1192.6±85/60	240.98±6.79	255.73±12.34
Jigrine CL 20ml/kgCC14	0.76±0.094	1187.6±79.68	238.85±13.07	243.13±14.88

Table-II. Enzyme values for Control and CCl4 Only groups (n=6)

Enzymes	Control (0.9% NaCl)	Treated (CCl4 only) (1.2mg/kg)
Bilirubin (mg/dl)	0.85±0.096	3.8±0.506
Alkaline Phosphatase (IU/L)	1238.5±61.45	1521.01±167.20
Gamma GT (IU/L)	240.28±26.82	383.91±51.46
SGPT (IU/L)	256.63±18.89	417.58±71.98

Table-III. Enzyme values for Control and BFM 100 mg/kg+CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated (FBM100 mg/kg+CCl4)
Bilirubin (mg/dl)	0.85±0.096	0.56±0.110
Alkaline Phosphatase (IU/L)	1238.5±61.45	1247.25±71.03
Gamma GT (IU/L)	240.28±26.82	239.68±8.98
SGPT (IU/L)	256.63±18.89	253.01±22.15

Table-IV. Enzyme values for Control and BFM 200 mg/kg+CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated (FBM200 mg/kg+CCl4)
Bilirubin (mg/dl)	0.85±0.096	0.66±0.179
Alkaline Phosphatase (IU/L)	1238.5±61.45	1079.16±78.22
Gamma GT (IU/L)	240.28±26.82	231.7±30.89
SGPT (IU/L)	256.63±18.89	246.4±19.79

Table-V. Enzyme values for Control and BFM 400 mg/kg+CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated (FBM400 mg/kg+CCl4)
Bilirubin (mg/dl)	0.85±0.096	0.78±0.03
Alkaline Phosphatase (IU/L)	1238.5±61.45	1192.6±85.60
Gamma GT (IU/L)	240.28±26.82	240.98±6.79
SGPT (IU/L)	256.63±18.89	255.73±12.34

compared by using Student's t-test.

RESULTS AND DISCUSSION

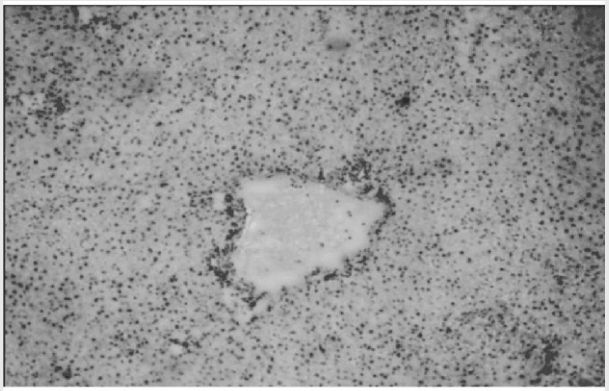
DISCUSSION

Methanolic extract of flowers (BFM) of Melia Azedarach was used in this study. This extract was water soluble. The Methanolic extracts from indigenous plants and a Poly herbal formulation was used in this study to look at the hepatoprotective potential of the extracts against the CCl4 treated animals. The hepatoprotective effect was evaluated by assessing Liver enzymes i.e., SGPT, ALP, bilirubin and gamma-GT and also by histopatho-logical study. These enzymes present in the cytoplasm are leaked into serum by hepatocellular damage by given CCl_4 and the serum levels are trustworthy parameters to

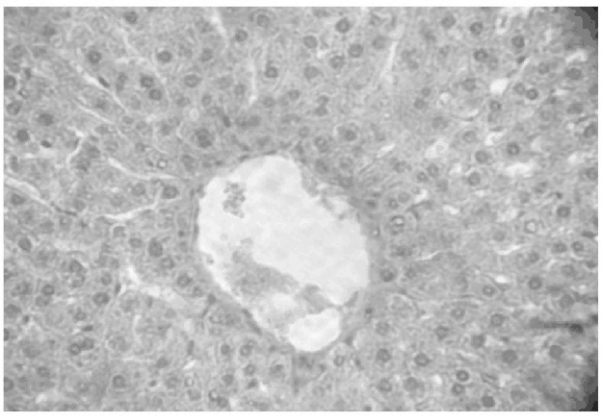
Table-VI. Enzyme values for Control and Jigrine CL (20ml/kg) +CCl4 group (n=6).

Enzymes	Control (0.9% NaCl)	Treated Jigrine CL (20ml/kg) +CCl4
Bilirubin (mg/dl)	0.85±0.096	0.76±0.094
Alkaline Phosphatase (IU/L)	1238.5±61.45	1187.6±79.68
Gamma GT (IU/L)	240.28±26.82	238.85±13.07
SGPT (IU/L)	256.63±18.89	243.13±14.88

HISTOPATHOLOGY



Rat liver after CCl4 (1.2 ml/kg) (10X)



Rat liver after extract BFM dose 100 mg/kg + CCl4 (10X)

assess the degree of hepatic damage. The present study revolves round the structural findings in the liver cells with respect to histological changes after Melia Azedarach extract treatment in CCl_4 induced liver damage.

Since CCl_4 caused hepatotoxicity through its transformation to reactive free radical trichloromethyl (CCl_3)[14], antioxidant treatment can be useful in treating the injury. However, the present study defines the hepatocytes changes to provide a better chance to view and to measure the extent of injury. Whereas, results obtained from this study are that Melia extract's most significant action is directly protecting the effects of CCl_4. In the nut shell, this study tells us about the pattern of damage in the shape and structure of hepatocyte after administering a hepatotoxin (CCl_4). Melia extract protects the cytoskeleton of hepatocytes. However, light microscopy is the magnification tool at present and it can be beneficial in protecting against the extent of damage and result of other medication scenario in treating chronic liver diseases.

CONCLUSIONS

In conclusion, the flower extract of M. Azedarach showed potent bioactivity against damaged hepatocytes, which is an important finding. Samples demonstrated the highest activity. This seems to be the first report of biological activities of M. Azedarach flower extract with respect to hepatocytes. However, a precise conclusion cannot be drawn on these findings.

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Correspondence Address:
Dr. Usman Ahmed
Hamdard College of Medicine & Dentistry,
Hamdard University, Karachi - 74600
hmiiphs@yahoo.com

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"He who fears being conquered is sure of defeat."

Napoleon bonaparte