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TO EVALUATE HEPATOPROTECTIVE ACTIVITY OF MELIA AZEDARACH (ROOTS)

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ABSTRACT

Purpose of study: To evaluate the hepato-protective activity of methanolic extract of Melia Azedarach (Root) in ccl4 induced liver damage in rats.
Material & Methods: Serum levels of Alanine amino-transferase, Alkaline Phosphatase, Bilirubin and Gamma-GT were analyzed in rats after treatment with extract of Melia Azedarch's root followed by administration of CCl4 in order to assess the preventive effect of extract on liver damage. In addition, the levels of the enzymes in rats administered with ccl4 followed by 14 days treatment with the plant extract were determined in order to assess the cure of liver damage. In the extract treated animals, there was significant decrease in liver enzyme levels. **Results:** 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. The extract of Melia Azedarch's root was screened for hepato protective effect. Albino rats were administered with carbon tetra chloride (CCl4) for inducing liver damage. **Conclusion:** It was concluded that the extract of Melia Azedarach found effective against CCl4 induced liver damage.

Key words: Rats, Liver enzyme, CCl4, Melia Azedarach, Jigrine CL.
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INTRODUCTION

Herbal medicine is the use of plants to restore or maintain health. Phytomedicine is a term often used to denote a more scientific approach to herbal medicine, where, for example, products are standardized and concentrated to contain specified amounts of the identified active substances in the herbal products. More rigorous research is also usually undertaken. Chinaberry, Umbrella tree and Persian lilac are the common names of Melia Azedarach which is a medium stature tree belonging to the Meliaceae family, its leaves are compound and alternate which consists of leaflets

RESULTS AND DISCUSSION

TABLE NO 1.
Comparison of Effect of variable doses of Extract2 (BKB) and Jigrine CL on serum Enzymes (n=6).

Groups	Bilirubin (mg/dl)	Alkaline Phosphatase (IU/L)	Gamma-GT (T-GT) (IU/L)	ALAT(SGPT) (IU/L)
Control (0.9% NaCl)	0.935±0.106	1362.34±67.61	264.31±29.50	282.29±20.78
CCl4 Treated (1.2ml/kg)	4.94±0.65	1977.32±217.37	499.09±66.90	491.79±97.41
BKB (100mg/kg)	0.866±0.268	1286.25±151.20	263.63±19.25	282.25±7.90
BKB (200mg/kg)	0.835±0.208	1280.40±93.90	260.45±19.62	277.25±46.90
BKB (400mg/kg)	0.95±0.081	1386.53±107.75	323.26±27.007	360.1±34.95
Jigrine CL (20ml/kg)	0.925±0.221	1276.81±82.54	262.18±20.21	280.05±11.97

TABLE NO 2.
Enzyme values for Control and CCl4 1.2 ml/kg groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated CCl4 Only(1.2ml/kg)
Bilirubin (mg/dl)	0.93±0.106	4.94±0.65
Alkaline Phosphatase(IU/L)	1362.34±67.61	1977.32±217.37
Gamma-GT (IU/L)	264.31±29.50	499.09±66.90
SGPT (IU/L)	282.29±20.78	491.79±97.41

TABLE NO 3.
Enzyme values for Control and BKB 100 mg/kg+ CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated BKB 100 mg/kg+CCl4
Bilirubin (mg/dl)	0.93±0.106	0.866±0.268
Alkaline Phosphatase(IU/L)	1362.34±67.61	1286.25±151.20
Gamma-GT (IU/L)	264.31±29.50	263.63±19.25
SGPT (IU/L)	282.29±20.78	282.25±7.90

TABLE NO 4.
Enzyme values for Control and BKB 200 mg/kg+ CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated BKB 200 mg/kg+CCl4
Bilirubin (mg/dl)	0.93±0.106	0.835±0.208
Alkaline Phosphatase(IU/L)	1362.34±67.61	1280.40±93.90
Gamma-GT (IU/L)	264.31±29.50	260.45±19.62
SGPT (IU/L)	282.29±20.78	277.25±46.90

TABLE NO 5.
Enzyme values for Control and BKB 400 mg/kg+ CCl4 groups (n=6).

Enzymes	Control (0.9% NaCl)	Treated BKB 400 mg/kg+CCl4
Bilirubin (mg/dl)	0.93±0.106	0.95±0.081
Alkaline Phosphatase(IU/L)	1362.34±67.61	1386.53±107.75
Gamma-GT (IU/L)	264.31±29.50	323.26±27.007
SGPT (IU/L)	282.29±20.78	360.1±34.95

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 H2O 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.

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 compared by using Student's t-test.

DISCUSSION

Many people believe that herbal medicine is similar to or part of other different traditional medicine, as both systems hold a holistic view and use natural herbal ingredients. However, there are fundamental differences between these two systems, For instance, herbal medicines are based on the understanding the movement of the active alkaloid according to its characteristic. It has its unique anatomy, physiology, and pharmacology, contained in a similar structure as western medicine and science. There has been an existing debate about the differences for identifying medicinal plants in the northern and other part of the country; which has been influenced the usage of medicinal plants in some specific cases. My purpose for research on these medicinal herbal plants is to make more widespread Pakistan herbal medical rejuvenation, help community to realize this chronic illness, and encourage the junior herbal medicine practioner to pay more attention on these diseases by treating with herbal medicines. Particularly, that will make a great contribution to more and more Pakistani health workers involved in the field of disease research. It will improve the people quality of life. Public interest in herbal medicine therapies is growing at a significant rate, easily outpacing the research conducted into their safety and effectiveness. People are often attracted to the 'natural' and safe & soft image of these therapies, particularly in treating chronic medical conditions, for which conventional treatments are often less than completely effective. There is variation in the quality and, therefore, the levels of the active constituents of herbal products. Herbal medicines are generally regulated as foodstuffs or dietary supplements in the UK. As such, there is the potential for self-medication, as they can be bought over the counter from most health food shops.

Methanolic extract of (BKB) of Melia Azedarach was used in this study. This extract was water soluble. The Methanolic extracts from

were left over night in the 10 % neutral formalin solution for fixation, then these samples were processed (Dehydrated) in alcohol of different grades (80-100%), cleared in Xylene, and fixed in Paraffin wax, four to five µm thick sections were cut by Leica RM 2145-Rotary Microtome, then removal of wax from tissue (Deparaffinization) in xylene, passed 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 Model X2T-21E equipped with Nikon's Photography system; Model UFX-DX-35. 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 compared by using Student's t-test. The enzyme levels were found to be high in CCl_4 treated animals, whereas, the blood enzyme levels in the animals who were treated with plant extract and Poly Herbal formulation were observed to be significantly lower in comparison to the CCl_4 only group and were almost similar to the normal values indicating hepatoprotective effect. The carbon tetrachloride (CCl4) and other halogenated alkanes (i.e., chloroform, dichloromethane, bromotrichloromethane, etc) undergo cytochrome P-450 catalyzed reductive dehalogenated and liberate trichloromethyl (CCl3). Several cellular macromolecules such as lipids, nucleic acids, proteins and polysaccharides are susceptible to CCl3 attack by hydrogen abstraction or addition reactions.

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) [24], 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.

AUTHORS INPUT

MA, Main Author of the Manuscript **MA** Work in Analysis and Methodology**, MS** Research Methodology

Conflict of interest: Author declare non conflact of interest

Ethical Issues: Author declare non Ethical Issues

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