ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *HIBISCUS ROSA SINENSIS* ROOTS IN EXPERIMENTAL ANIMALS

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Received on 08-01-2013 *Accepted on 26-01-2013*

Abstract

Ethanolic extract of *Hibiscus rosa sinensis roots* was investigated for their Hepatoprotective potentials against liver injury induced by CCl_4 and paracetamol in rats. The liver damage was induced in male albino rats (150 – 220 g) byadministering CCl_4 (1ml/kg) for 6 days and the extent of damage was studied by assessing the biochemical parameters such as total bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin (TBL), albumin and globulin. Histopathological changes of liver sample were compared with respective control. The oral treatment of rats with ethanolic extract of *Hibiscus rosa sinensis* at the dose 200 mg/kg for 6 days resulted in significant hepatoprotection for total bilirubin only, while at 400 mg/kg, the restoration was significant for enzyme markers, viz. SGOT, SGPT, ALP in CCl_4 and paracetamol treated rats. The maximum tolerated dose of the extract was found to be greater than 2000 mg/kg, p.o. Thus from the present study it was concluded that ethanolic extracts of *Hibiscus rosa sinensis roots* is having hepatoprotective effect against CCl_4 and paracetamol induced liver injury.

Keywords: CCl_4, Hibiscus rosa sinensis roots; Hepatoprotective; Serum glutamate pyruvate transaminase; Serum glutamate oxaloacetate transaminase.

Introduction

The liver is the largest organ in the body weighing 1400-1600 gm in the males and 1200-1400 gm in the female and this organ also regulating homeostasis in the body .Thus liver is expected not only to perform physiological function , but it has to protect itself against the hazards of harmful study was aimed to investigate the hepatoprotective activity of ethanolic extract of *Hibiscus rosa sinensis* root(s) which was separated in to different fractions against paracetamol medicines and chemicals activity. Inspite of the tremendous scientific advancement in the field of

hepatology in recent years. Some other plant of this genus have been reported to possess other activity like anti oxidant, anti inflammatory. The liver injury may take several forms and involve the hepatocytes, likes vascular cells or bile ducts. The most important diseases are: biliary obstruction, metabolic lesions caused by genetic disorder or exogenous substance, such as alcohol, inflammation, especially caused by cirrhosis, neoplasia. Presently only a few hepatoprotective drugs and that too from natural sources, are available for the treatment of liver disorders. [1]

Hepatitis is one of the major health problems, throughout the world more than 350 million peoples were affected with chronic hepatitis infection. It is well known that free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals. Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the human diet. Plant phenolics comprise a great diversity of compounds, such as flavonoids (anthocyanins, flavanols, flavonols, flavones, among others) and several classes of non-flavonoids (phenolic acids, lignins, stilbenes). Several works have shown that phenolic compounds play a role as antioxidants through different mechanisms of action, such as: scavenging of free radicals, quenching of reactive oxygen species, inhibiting of oxidative enzymes chelating of transition metals or through interaction with biomembranes. [1]

These properties make those types of compounds good candidates as potential protectors against food oxidation and biological aging of tissues. Thus, phenolic compounds could be considered as natural antioxidants with potential applications in protecting the liver. The liver is among the most complex and important organs in the human body. Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system. A total loss of liver function leads to death within minutes demonstrating the liver's importance.[2]

Material and Method

1. Plant material (Roots).
2. Chemicals.
3. Soxhlet Apparatus.
4. Water-bath.

Plant Material

The roots of *Hibiscus rosa sinensis* were collected from the plantation in the Vanmandal Garden Dewas. The plant material was identified by Dr. R.B. Upadhyay, Reader, Govt. of Horticulture, Sanjay Nikunj, Harnauda Tonkkhurd, District Dewas M.P., their voucher specimen were deposited in their Department of Govt. of Horticulture, Sanjay Nikunj, Harnauda Tonkkhurd, District Dewas M.P.

Preparation of Ethanolic Extract of *Hibiscus rosa sinensis root(s):*

The shade dried roots of *Hibiscus rosa sinensis* were powdered and passed through 10 mesh sieve. The coarse powder was soxhalated with 70% ethanol for about twenty seven hours [yield 12.88%]. Then the extracts were filtered and concentrated on rotary vacuum evaporator. The extract was resuspended in water and used for hepatoprotective studies The extract at different doses (200 and 400 mg/kg) were given to the animals through oral gavage.[4]

Animal Selection for Study

Male Wistar rats (150-200g) were obtained from the animal house, RKDF College of Pharmacy, Bhopal M.P. They were maintained under standard housing condition. The animals were maintained given free assess to food and water. The animals were maintained under standardized environmental condition, 27 ± 2^{o} C. All the animals received humane care according to the guidelines of **CPCSEA / IAEC Code: 780September 2006** for the care and use of laboratory animals prepared by the national academy of the sciences and published by National Institute of Health [3]

Qualitative Phytochemical Analysis of *Hibiscus rosa sinensis root(s):*

Table No.1- describe the various qualitative test for ethanolic extract confirm the presence of carbohydrate, glycosides, alkaloids, flavanoids, terpenes, saponins along with other phytochemicals.[5]

Hepatoprotective Study

Preparation of the formulations

(i) The semisolid extract was suspended in normal saline & administered orally to specific groups of animals.

(ii) Silymarin (Silybon-70, MICRO LABS Ltd.) was obtained as a gift sample from RKDF College of Pharmacy, Bhopal (M.P.) for research.

Research Methodology

Hepatoprotective Effect Against Acute Dose of PCM in Rats :

The male Wistar albino rats were kept at polypropylene cage and further the animals were separated into five groups. Each group contains five no. of animals. Treatment is given for 6 days. Group 1 served as normal (Distilled water 10ml/kg p.o. for 6 days), Group 2 served as control (Paracetamol 3gm/kg p.o. for 6 days), Group 3was treated as Standard (Silymarin20 mg/kg + Paracetamol 3gm/kg p.o. for 6 days) Group 4 & 5 received the ethanolic extract of *H. rosa sinensis root(s)* 200 & 400 mg/kg + Paracetamol 3gm/kg p.o. respectively, for 6days).The drug solution was prepared in Tween 20 solution and administered orally according to body weight of the animal. 200 & 400 mg/ml of ethanol extract was prepared respectively and 1ml /100gm of body weight was administered to each animal for 6 days. After treatment, blood samples were removed from all animals by retro orbital puncture method. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min and used for measurement of various biochemical markers like aspartate and alanine aminotransferase (AST and ALT) activities, alkaline phosphatase (ALP) activity using commercially available kits. All the biochemical parameters were estimated. Finally the animals were sacrificed 24h after last dose of Paracetamol administration on the 6^{th} day and liver were dissected. Weight of each liver was taken and then histopathology of the liver samples was carried out.[6,7]

Hepatoprotective Effect Against Acute Dose of CCl_4 in Rats :

Animals were randomized into five groups of five rats each. Group 1 served as control, received only the normal saline, group 2 intoxicated by CCl_4 , group 3 was treated with silymarin (20 mg/kg, p.o for 6 days) as standard, groups 4 and 5 received the extract (200 and 400 mg/kg p.o., respectively, for six days). On the day four, 2 h after treatment, groups 2-5 rats received CCl_4 (0.37ml/kg,i.p.) in liquid paraffin (1:1). The animals were killed, 48h after the acute dose of CCl_4 . The blood was collected by heart puncture and serum was separated by centrifugation (3000 rpm at 4^o C for 10 min). The liver was immediately removed. Tissue and serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities were measured. [8,9] alkaline phosphatase (ALP) activity.[10]

Histopathological Studies

After draining the blood, liver samples were excised, washed with normal saline & processed seperatly for histological observations. Initially, the liver was excised quickly and fixed in 10% formalin and stained with

haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any. Results of the histopathological studies of respected Figures enclosed in the result section.[11,12] Gross anatomy is that level of organization that can be viewed by simply cutting up (Greek ana=up + tomia = cutting) the body of the organism. Histology is the study of tissue which is the second order of organization of the body of a multicellular organism. The tissues are composed of similar type of cell either structurally or functionally or both. Since the cell in general cannot be viewed by naked eyes, the histology may be defined in other words as to be microscopic anatomy.[13]

Estimation of Hepatoprotective Parameters

Liver Function Test (LFT or LF_S):

Liver function tests are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a liver. The parameters measured include SGPT /ALP, SGOT /AST, ALP, Albumin, Globulin, Total protein; Total Bilirubin are NOT liver function tests but are biomarkers of liver injury with some degree of intact liver function. It mainly include-

(a) Alanine Transaminase (ALT):

The aminotransferases (transaminases) are the most frequently utilized and specific indicators of hepato cellular necrosis. These enzymes Aspartate Aminotransferase (AST) or serum glutamate oxaloacetic trans aminase (SGOT) and Alanine Amino Transferase (ALT), formerly serum glutamic pyruvate transaminase (SGPT) catalyze the transfer of the amino acids of aspartate and alanine respectively to the keto group of ketoglutaric acid. ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver. Whereas the AST is present in both the mitochondria and cytosol of hepatocytes, ALT is localized to the cytosol. Large increases in mitochondrial AST occur in serum after extensive tissue necrosis. Because of this, assay of mitochondrial AST tissue necrosis. Because of this, assay of mitochondrial AST have been advocated in myocardial infarction. Mitochondrial AST is also increased in chronic liver disease.

(b) Alkaline Phosphate (ALP):

Alkaline phosphatases are a family of zinc metaloenzymes, with a serine at the active center; they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues. Highest levels of alkaline phosphatase occur in cholestatic disorders. Elevations occur as a result of both intrahepatic and extrahepatic

obstruction. Elevated serum levels of intestinal alkaline phosphatase have been found in cirrhosis and hepatitis. [14,15,16]

(c) Total Bilirubin (TBIL): The source of 70 to 90% of bilirubin is decomposed hemoglobin of senescent erythrocytes.Hemoglobin is decomposed into globin and heme in the reticuloendothelial system, and the heme molecule loses its iron and ring-shaped structure to form bilirubin. The produced indirect (unconjugated) bilirubin undergoes glucuronide conjugation in the liver, and the formed direct (conjugated) bilirubin is secreted from the liver into the bile. The proportion of indirect bilirubin gradually increases in cases of liver cirrhosis or hepatic failure.

Serum Proteins:

The liver is the major source of most the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors. [14,15,16]

Result & Discussion

Table no. 1- Qualitative Chemical test of ethanolic extract of *Hibiscus rosa sinensis* root(s).

S. No.	Component name	Name of the test	Ethanolic Extracts
1.	Carbohydrates	Molisch's test	+
2.	Monosaccharide	Barfoed's test	+
3.	Tannins	Ferric chloride	+
4.	Saponnins	Frothing test	-
5.	Flavonoids	1.Shinoda's Test	+
		2.Conc. H_2SO_4 test	
6.	Terpenes/steroids	1.Liebermann Burchard's Test	+
		2.Salkowski test	
7.	Alkaloids	1.Mayer's test	-
		2.Wagner's test	
8.	Proteins	Biuret test	-

Table no. 2- Acute Toxicity Studies.

Extract	No. of animals used / Sex	Limit dose (mg/kg)	Sign of toxicity	Duration of effect	Mortality
Ethanolic extract of *H. rosa sinensis* root(s)	Five rats(male)	50	No	No	No
	Five rats(male)	300	No	No	No
	Five rats(male)	500	No	No	No
	Five rats(male)	2000	No	No	No

Acute oral toxicity study shows all the doses 50, 300, 500 and 2000 mg/kg body weight of ethanolic extract of Hibiscus rosasinensis roots used for acute oral toxicity study were found non- toxic and safe. No mortality and behavioural changes were observed even in highest dose (2000 mg/kg) employed to animals. So, doses of 200 and 400 mg/kg body weight of the extract were selected for the pharmacological investigation of hepatoprotective activity.

Table No-3: Effect of ethanolic Extract of *Hibiscus rosa sinensis* root(s) on Paracetamol induced hepatotoxicity.

Groups	SGOT (units/ml)	SGPT (units/m	ALP (KA units)	TBL (mg/dl)
Normal (Distilled water 10ml/kg)	63.67±1.5	67.00 ± 2.3	10.35 ± 0.070	0.88±0.2
Control (Paracetamol 3gm/kg)	166.17 ±1.3	111.83 ±2.16	16.448 ± 0.61	3.42 ± 0.17
Standard (Silymarin 20 mg/kg + paracetamol 3gm/kg)	76.67 ±2.05*	78.71±1.84*	11.038±0.2*	1.56±0.02
Test [Ethanolic extract of *H. rosa sinensis* root(s) 200mg/kg + paracetamol 3gm/kg)]	93.67±0.95*	95.58±1.94*	13.50±0.06*	1.12±0.03
Test [Ethanolic extract of *H. rosa sinensis* root(s) 400mg/kg + paracetamol 3gm/kg)]	81.65 ±0.85*	83.41 ± 1.92*	12.26±0.06*	1.25±0.03*

Significance evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t test control verses all. *P<0.01 is considered as criterion for significance. Values are mean ±SEM, (n=5) SGOT-Serum Glutamate Oxaloacetate Transaminase, SGPT- Serum Glutamate Pyruvate Transaminase, ALP- Alkaline Phosphatase.

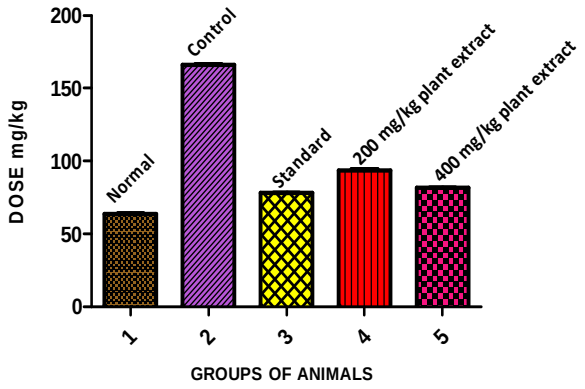

Fig. no. 3.1 Histogram showing SGOT profile of PCM induced hepatotoxicity.

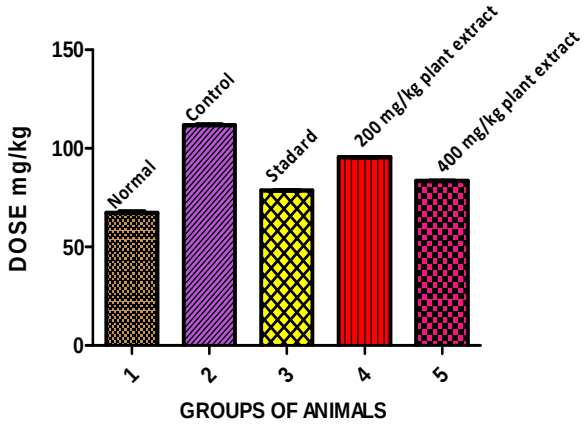

Fig. no. 3.2 Histogram showing SGPT profile of PCM induced hepatotoxicity.

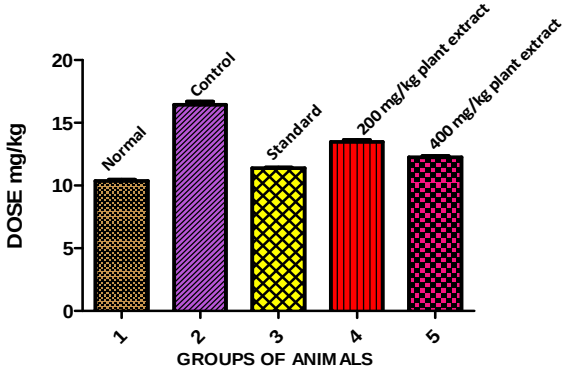

Fig. no. 3.3 Histogram showing ALP profile of PCM induced hepatotoxicity.

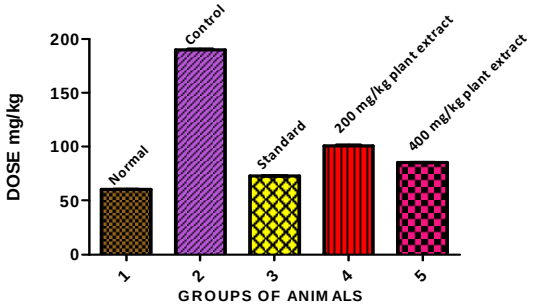

Fig. no. 3.4 Histogram showing TBL profile of PCM induced hepatotoxicity.

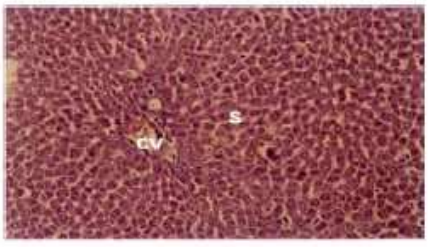

Fig. no. 3.5 Normal group (Distilled water).

Observation: Liver section of the rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoid spaces and central vein.

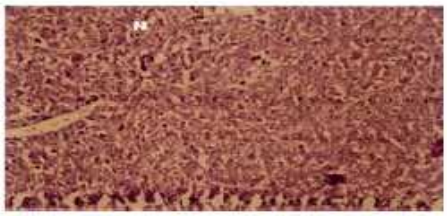

Fig. No-3.6: Control (Paracetamol (3gm/kg).

Observation: Liver section of the rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone sinusoidal hemorrhages and dilation.

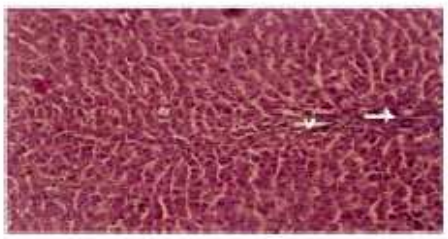

Fig. no. 3.7 Standard silymarin 20mg/kg + PCM

Observation: Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less disarrangement and degeneration of hepatocytes.

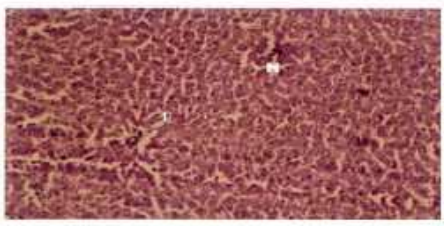

Fig. no. 3.8 Ethanolic extract of *H. rosa sinensis* root (s) 200 mg/kg

Observation: Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less disarrangement and degeneration of hepatocytes.

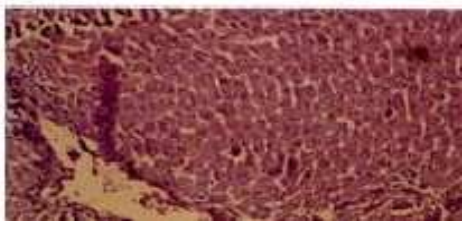

Fig. no. 3.9 Ethanolic extract of *H. rosa sinensis* root (s) 400mg/kg

Observation: Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less disarrangement and degeneration of hepatocytes.

Table No-4: Effect of the ethanolic extract of *H. rosa sinensis* root(s) (p.o. for 6 days) on serum enzymatic changes in CCl_4 - induced hepatotoxicity in rats.

Treatment	SGOT (µl)	SGPT (µl)	ALP (µl)	TBL (mg/dl)
Normal (Distilled water 10ml/kg)	60.0 ± 3.3*	58.5 ± 5.7*	141.0 ± 1.2*	0.79 ± 0.52
CCl_4 0.25 ml/kg	190.0 ± 25	268.3 ± 2.6	250.1 ± 1.91	2.28 ± 0.57
Silymarin + CCl_4 20 mg/kg	73.0 ± 9.1*	62.70 ± 3.5*	155.6 ± 5.4*	0.86 ± 0.25*
***H. rosa sinesis* root(s) + CCl_4 200 mg/kg**	100.86 ± 7.2*	160.58 ± 2.3*	190.5 ± 1.53*	1.31 ± 0.91*
***H. rosa sinesis* root(s) + CCl_4 400 mg/kg**	85.14 ± 3.3*	90.48 ± 6.4*	170.52 ± 1.81*	0.95 ± 0.62*

Values are mean ± SEM (n = 5); *P< 0.001 vs control, student's t-test. CCl_4 (0.25 ml/ kg. i.p) was administered to all groups except normal group on day 6.

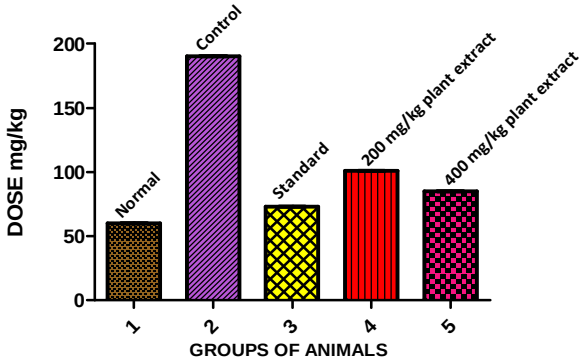

Fig. no. 4.1 Histogram showing SGOT profile of CCl_4 induced hepatotoxicity.

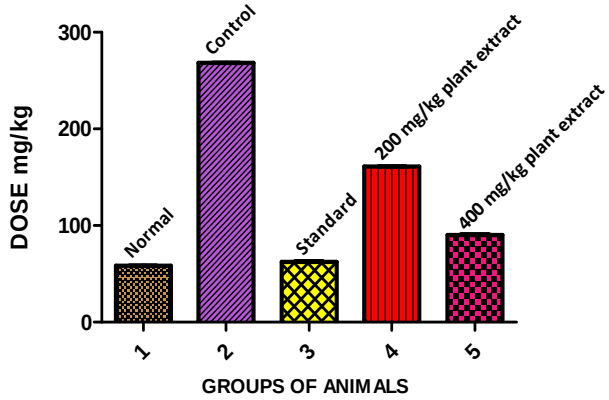

Fig. no. 4.2 Histogram showing SGPT profile of CCl_4 induced hepatotoxicity.

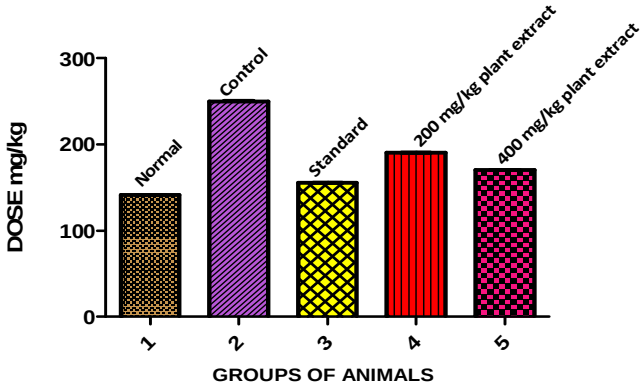

Fig. no. 4.3 Histogram showing ALP profile of CCl_4 induced hepatotoxicity.

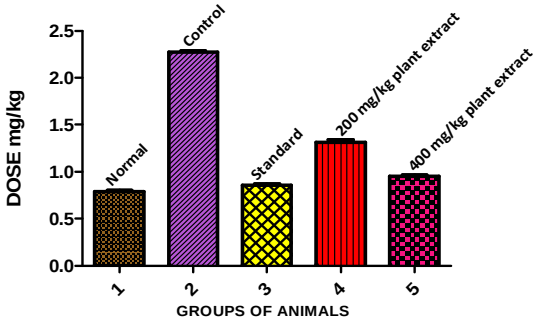

Fig. no. 4.4 Histogram showing TBL profile of CCl_4 induced hepatotoxicity

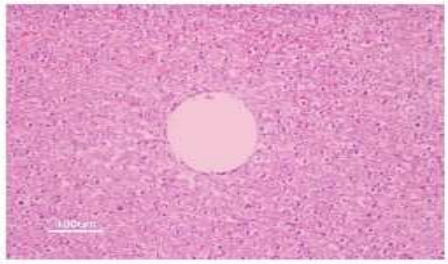

Fig. no. 4.5 Normal group (Distilled water)

Observation: Liver section of the rat showing normal (cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein).

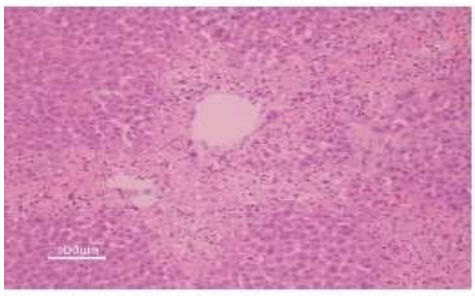

Fig. no. 4.6 Control [CCl_4 (0.25 ml/kg)]

Observation: Liver section of the rat showing necrosis and periportal round cell collection.

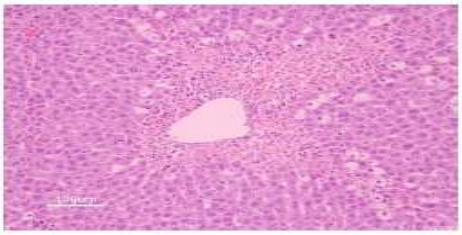

Fig. no. 4.7 Standard silymarin (20mg/kg)

Observation: Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less arrangement of hepatocytes with minimal granuloma.

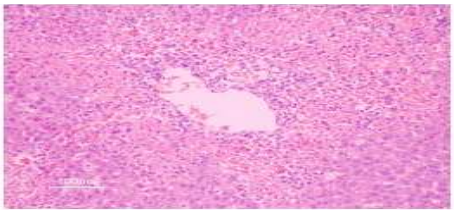

Fig. no. 4.8 Ethanolic extract of *H. rosa sinensis* root(s) (200 mg/kg + CCl_4)

Observation: Liver section of the rat showing normal disarrangement and degeneration of hepatocytes.

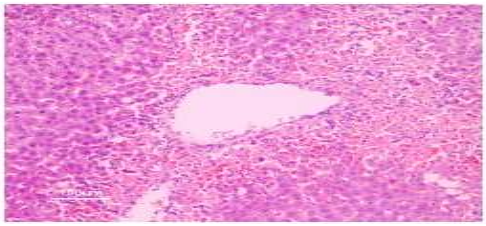

Fig. no. 4.9 Ethanolic extract of *H. rosa* root(s) 400 mg/kg + CCl_4

Observation: Liver section of the rat showing normal arrangement of hepatocytes with minima granuloma.

Stastical Analysis

Result of the biochemical estimations are reported as mean ± SE (Standard Error mean). Total variation present in a set of data was estimated by one way analysis of variance (ANOVA) using the graph pad software followed by Dunnet's test. The level of significance was set at $P<0.05$.

Results

Hepatic damage induced by CCl_4 & PCM caused significant rise in marker enzymes SGPT, SGOT, and ALP and also serum bilirubin **(Table no. 3 and 4)**.Oral administration of *H. rosa sinensis root(s)* is seen to lower significantly the levels of marker enzymes namely SGPT, SGOT and ALP. It also lowered serum bilirubin. The level of serum proteins was significantly ($P <0.05$) increased in rats which received *H. rosa sinensis root(s)* as compared to standard group. **(Table no. 3 and 4)**. The effect of *H. rosa sinensis root(s)* seems dosed dependent. However, the protection offered by Silymarin seemed relatively greater.

Discussion and Conclusion

It is well established that hepatotoxicity by CCl_4 & PCM is due to enzymatic activation to release CCl_3 radical in free state & PCM is activated by hepatic cytochrome P-450 to a highly intracellular GSH, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles respectively.

The increased level of SGPT, SGOT, ALP, and bilirubin is conventional indicator of liver injury. In the present study, also it was seen that administration of CCl_4 & PCM elevates the levels of serum marker enzymes SGPT, SGOT, ALP, and serum bilirubin. Level of total protein is lowered. *H. rosa sinensis root(s)* and silymarin-treated groups exhibited lower levels of SGPT, SGOT, ALP, and bilirubin as compared to CCl_4 & PCM treated groups. The treatment with *H. rosa sinensis root(s)* also significantly elevated total protein levels. The stabilization of serum bilirubin, SGPT, SGOT, and ALP levels by *H. rosa sinensis root(s)* is a clear indication of the improvement of the functional status of the liver cells.

The characteristics feature of experimental hepatic damage observed is significant decrease in protein level. The rats in a group which received *H. rosa sinensis root(s)* showed rectification of lowered protein levels.These findings can be further corroborated with histopathological studies. The histopathological examination clearly reveals that the hepatic cells, central vein, and portal triad are almost normal in *H. rosa sinensis root(s)* (200 mg/kg & 400 mg/kg, p.o.) group in contrast to group which received CCl_4 & PCM. Thus, *H. rosa sinensis root(s)* can be considered to be an effective hepatoprotective in nature, as it normalize the damage caused by CCl_4 & PCM to hepatic function. Thus, the constituents of *H. rosa sinensis root(s)* seems to possess hepatoprotective activity.

Conclusion

Hepatotoxicity, a clinical entity is as old as mankind, and often posse's problems in clinical practice, Liver is a versatile organ in the body concerned with regulation of internal chemical environment. Research on hepatoprotective drugs is a developing area in modern biomedical science. Some plants have screened scientifically for the evaluation of their hepatoprotective activity in different pharmacological models.

In conclusion, it can be said that the ethanolic extract of the *H. rosa sinensis root(s)* have shown significant hepatoprotective activity. Both at the dose of the drug 200 mg/kg and 400 mg/kg have significant hepatoprotective activity but the later dose i.e. 400 mg/kg have better activity than that of 200 mg/kg. The increased level of SGPT, SGOT, ALP, and bilirubin is conventional indicator of liver injury. In the present study, also it was seen that

administration of CCl_4 & PCM elevates the levels of serum marker enzymes SGPT, SGOT, ALP, and serum bilirubin, level of total protein is also lowered. *H. rosa sinensis root(s)* and silymarin-treated groups exhibited lower levels of SGPT, SGOT, ALP, and bilirubin as compared to CCl_4 & PCM treated groups. The treatment with *H. rosa sinensis root(s)* also significantly elevated total protein levels. The stabilization of serum bilirubin, SGPT, SGOT, and ALP levels by *H. rosa sinensis root(s)* is a clear indication of the improvement of the functional status of the liver cells. The rats in a group which received *H. rosa sinensis root(s)* showed rectification of lowered protein levels. These findings can be further corroborated with histopathological studies. The histopathological examination clearly reveals that the hepatic cells, central vein, and portal triad are almost normal in *H. rosa sinensis root(s)* (400 mg/kg, p.o.) group in contrast to group which received CCl_4 & PCM. Thus, *H. rosa sinensis root(s)* can be considered to be an effective hepatoprotective in nature, as it normalizes the damage caused by CCl_4 & PCM to hepatic function. Thus, the constituents of *H. rosa sinensis root(s)* are seem to possess hepatoprotective activity. Further the toxicity studies and appropriate formulation and development for this herbal extract have to be emphasized and the evaluation parameters of the prepared formulation are to be studied. To conclude, in most developing countries the incidence of liver damage are very high as they are caused by many agents and thus investigation for effective hepatoprotective agents from natural resources have to be emphasized as they are much safer and economical than the other system of therapies.

Acknowledgement

It is a moment of gratification and pride to look back with a deep sense of contentment and gratitude at the long travelled path, to be able to capture some of the finest moments, to be able to thank the infinite number of people, some who were with me from the beginning and some who joined me later, whose love and blessing have made this day possible for me.

It is said that ‘accomplishments must be credited to those who have put up the foundations of the particular chore’. Here, I pay tributes to **my parents, elder brother and my family members** for lifting me up till this phase of life. I thank them for their love, trust, patience and support, and of course, for bearing all kind of stresses, they could, to make me what I am. I owe everything to them.

As said “Where there is a will there is a way”, I had the will but the way to accomplish my goal was shown only and only by my esteemed research guide. I express my deep sense of gratitude and indebtedness towards my

research guide **Mr. Sudhir kumar Chauhan** for his excellent guidance and moral support during the project work. I consider myself privileged to have worked with him, as he shared his vast experience so generously and patiently. His keen interest and encouragement served as a constant inspiration during the entire course of study. He has always given me freedom to work and added new dimension to my thinking in scientific way. Time to time encouragement and excellent guidance by him always kept me on the right track. His caring and parental attitude always enlightened the way during my research work.I am also thankful to **Mr. Rakesh Punekar** and **Mr. Amol Chandekar** of RKDF College of Pharmacy, Bhopal where I carried out my experimental work.

I would like to acknowledge and express my obligation to our Principal, **Mr. R. V. Sheorey** and **Mr. Rakesh K. Jatav and all other faculty members** for providing the necessary infrastructure and all the facilities required for carrying out my research work.

I wish to express my sincere and respectful thanks to our Head of department, **Mr. Sudhir Chauhan .**

Besides this, there are several other people who have knowingly or unknowingly helped me in the successful completion of this project. I am thank full to **Prakash Ojha, Pooja Sharma, Ishan Dubey** and **all my M. Pharma classmates** for every ounce of efforts they contributed.

I hope that the precious life of the animals used during this project would not be invain; it would contribute to the development of the science in one way or another.

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