International Journal of Biomedical Research

HEPATO-PROTECTIVE ACTIVITY OF STEM BARK EXTRACTS OF *FICUS RELIGIOSA* LINN IN RATS

Kavita Suryawanshi[1]*, Sanjay Khakre[2], Ashish Chourasia[1], P.K. Chaurasiya[1], R.S. Pawar[1], Deenanath Jhade[3]

1. Department of Pharmacology, VNS Institute of Pharmacy, Bhopal (M.P.) India
2. Truba college of Pharmacy, Bhopal (M.P.) India
3. School of Pharmacy, Chouksey Engg. College, Bilaspur (C.G.) India

Corresponding author*: Khakrempy@gmail.com

This article is available online at **www.ssjournals.com**

ABSTRACT

Hepatoprotective activity of stem bark extracts of *Ficus religiosa* Linn. (Moraceae) investigated against Paracetamol (2g/kg) induced hepatotoxicity in rats. Stem bark powder of *Ficus religiosa* was successively extracted with different solvent by soxhlet apparatus. The extracts of *F. religiosa* stem bark were administered in a dose of 200 mg/kg orally. Silymarin (100mg/kg) was used as standard drug. The hepatoprotective effect of these extracts was evaluated by the assessment of biochemical parameter such as SGOT, SGPT, ALP, Total bilirubin, and histopathological studies of the liver. The preliminary Phytochemical screening of *F. religiosa* revealed the presence of Triterpenoids, Flavonoids, Saponins, Steroids, Tannins and Phenolic compounds, Carbohydrate, Protein. The treatment with extract of *F. religiosa* showed significant reduction of Paracetamol induced elevated serum enzyme levels. The present study shows that extract of *Ficus religiosa* L. has significant hepatoprotective activity and methanolic extract was found to exhibit greater hepatoprotective activity than the other extract.

KEY WORDS: Hepatoprotective activity, Paracetamol, *Ficus religiosa*, Histopathology.

1. INTRODUCTION

Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major worldwide health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Liver disease is an important cause of morbidity and mortality in the United States, affecting persons of all ages, but most frequently individuals in the productive years of life, between the ages of 40 and 60 years[1].

They are mainly caused by chemicals and some drugs when taken in very high doses. Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells.

The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver. There is urgent need, therefore, for effective drugs to replace/supplement those in current use. The plant kingdom is undoubtedly

valuable as a source of new medicinal agents[2].

Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and liver damage[3].

Ficus religiosa (moraceae) commonly known as peepal tree is distributed throughout India. It is popular indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy and used treditionaly as, analgesic[4], anti-inflammatory[4,5], wound healing[5], antioxidant[5,6], anticonvulsant[7], asthma[8], antidiabetic, and ulcer[9], liver and kidney diseases[10]. Preliminary phytochemical screening of *F. religiosa* barks, showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. The barks of *F. religiosa* also showed the presence of bergapten, bergaptol, lanosterol, β-sitosterol, stigmasterol, lupen-3-one, β-sitosterol-d-glucoside (phytosterolin), vitamin k1, β-, leucocyanidin-3-0-β-D-glucopyrancoside, lupeol, lupeol acetate, α-amyrin acetate.[11]

Literature revealed that plant containing lupeol (triterpenoids)[12,13], flavonoids[14], phenolic compounds were responsible for hepatoprotective activity and the *F. religiosa* also having these constituent so it was hypothesized that it may also be able to protect the liver. Keeping this view the present study scientifically validated the traditional use of *Ficus religiosa* for liver disorders.

2. MATERIAL AND METHODS

2.1 Plant material: Stem bark of *Ficus religiosa* is obtained from fields of Bhopal, Madhya Pradesh, India. The plant was authenticated by Professor Dr. Ziaul hussen, Assistant professor, department of botany, safia College of science, Bhopal. Voucher specimen no –199/Bot/safia/10. The stem bark of *Ficus religiosa* were dried under shade, powdered with a mechanical grinder and passed through sieve No. 40 and extracted with soxhlet apparatus using petroleum ether for about 48 hour. After defatting, the marc was dried in hot air oven at 50^0C packed in soxhlet apparatus and further successively extracted with ethyl acetate, methanol. The aqueous extract was prepared by cold maceration (72 hour). The solvent were removed from the extracts under reduced pressure by using rotary vacuum evaporator. The % yields were found to be 1.28% of the petroleum ether extract, 2.12% of ethyl acetate extract,15.36% of methanol extract and 8.20% for *Ficus religiosa* was recorded.

2.2 Drugs and Chemicals: Standard Kit of Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT) and Alkaline phosphatase (ALP) were obtained from Span Diagnostics Ltd. Paracetamol was obtained as gift sample from Torrent Research center, Ahmedabad, Silymarin was obtained as gift sample from Cadila Pharma Ltd; India. All other reagents used for the experiments were of high analytical grade.

2.3 Phytochemical screening: In order to detect the various constituents present in the different extracts of *Ficus religiosa*, such as glycosides, steroids, carbohydrates, proteins, amino acids, triterpenoids, alkaloids, flavonoids, phenolic compounds and tannins.In order to detect the various constituents present in the different extracts of Ficus religiosa

these were subjected to preliminary phytochemical screening.[15]

2.4 Animal: Normal healthy male albino wistar rats weighing from 150-200g were used for the study and housed individually under standard condition of temperature (25 ± 1^0 C), 12 hr light/dark cycle and feed with standard pellet diet and water ad libitium. The study was permitted by the Institutional animal ethical committee of VNS Institute of pharmacy, Bhopal (M.P.).
The animal care was taken as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India. The ethical committee number is 778/03/C/CPCSEA.

2.5 Acute Toxicity Study: A safe oral dose of *Ficus religiosa* extract was determined through the acute oral toxic test in rats as described by the Organization of Economic Co-Operation and Development (OECD) as per 420 guidelines (OECD Guidelines for the Testing of Chemicals, 2010). The *Ficus religiosa* extracts, at different doses up to 2000 mg/kg, was prepared and then administered (p.o.). Animals were observed for behavioral changes, any toxicity and mortality up to 48 h. One-tenth of this dose were selected as the therapeutic dose for the evaluation[9, 16].

2.6 Evaluation of Hepatoprotective activity: Determination of hepatoprotective activity (curative aspect) in Paracetamol induced hepatotoxicity[17].
Wistar albino rats weighing between 150-200g, divided into 7 groups, and each group containing 6 animals.
Group 1- Normal control (vehicle treated, p.o for 10 days)
Group 2- Toxicant (Paracetamol 2g/kg daily p.o for 03 days and from 4th-10th day vehicle only)
Group 3- Paracetamol 2g/kg daily p.o for 03 days, Standard Silymarin (100 mg/ kg) p.o from 4th-10th day.
Group 4- Paracetamol 2g/kg daily p.o for 03 days, Pet ether extract (200 mg/kg) p.o from 4th-10th day.
Group 5 - Paracetamol 2g/kg daily p.o for 03 days, Ethyl acetate extract (200 mg/kg) p.o from 4th-10th day.
Groups 6 - Paracetamol 2g/kg daily p.o for 03 days, Methanol extract (200 mg/kg) p.o from 4th-10th day.
Group 7 - Paracetamol 2g/kg daily, p.o for.03 days, Aqueous extract (200 mg/kg) p.o from 4th-10th day.
The animals were sacrificed by cervical dislocation after 24 hours after the last dose administration. The blood samples were collected by cardiac puncture in heparinized microfuge tubes. The blood samples thus collected were immediately centrifuged at 2200rpm for 15 minutes. When serum clearly separated out, the serum was analyzed for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin were estimated using commercially available kits by the method proposed by Reitman and Frankel [18,19].

2.7 Histopathology study: Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 5µ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and

eosin. The sections were examined microscopically for the evaluation of histopathological changes[20].

2.8 Statistical analysis: The data are expressed as mean ±SEM. Statistical differences between means were determined by one- way ANOVA followed by Dunnett's't' test. Values of $P<0.05$ were considered as significant.

3. RESULTS

All the Extracts thus obtained were concentrated under vacuum, dried, weighed and stored in desiccators. The yields were found to be 3.2g (1.28%w/w of crude drug) of petroleum ether extract with semisolid mass of Blackish green color, 5.3g (2.12 %w/w of crude drug) of ethyl acetate extract with Blackish green color semisolid mass, 38.4g (15.36 %w/w of crude drug) of methanol extract with Reddish orange color semisolid mass, 20.5g (8.2%w/w of crude drug) of water extract with dark green color semisolid mass for *Ficus religiosa* was recorded.

In qualitative analysis, various chemical tests were performed for the identification of common phytoconstituents in *Ficus religiosa* extracts. Preliminary phytochemical screening was performed for each extract. (**Table 1**)

The *Ficus religiosa* extracts did not cause any mortality up to 2000 mg/kg and were considered as safe.

The effect of methanol and aqueous extracts of *Ficus religiosa* on normal liver function, it was found to be non toxic at the selected dose (200 mg/kg) since the parameters SGPT, SGOT, ALP and total bilirubin were within like that of control. Paracetamol intoxication in normal rats elevated the levels of SGPT, SGOT, ALP, and total bilirubin significantly, indicating acute centrilobular necrosis. The rat treated with alcoholic extract and aqueous extract showed a significant reduction in all the biochemical parameter elevated by paracetamol. Ethyl acetate and pet ether extract showed moderate reduction of all three biochemical parameters. This reduction in biochemical parameter exhibited by alcoholic and aqueous extracts is similar when compared with that of silymarin. Reduction of all biochemical parameters against the hepatotoxin is given in **Table 2**, **Figure 1, 2, 3 & 4.**

The histopathological profile of the rat treated with alcohol and aqueous extracts showed no visible changes confirming the safety of the extract at selected dose. Histopathological examination of liver section of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal space. In the liver section of the rats intoxicated with paracetamol, there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. The liver section of the rats treated with pet. ether and ethyl acetate extracts and intoxicated with paracetamol showed moderate hepatoprotective activity while rats treated with silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity. (**Figure 5**)

4. DISCUSSION

Most of the hepatotoxic chemicals damage liver cells either by lipid peroxidation or by other oxidative stress mechanisms induced cellular damage.

Activation of some enzymes in the Cytochrome P-450 system such as CYP-2E1 also leads to oxidative stress and injury to hepatocytes, bile duct cells

causing accumulation of bile acid inside liver and which in turn promotes further liver damage.

Acetaminophen (APAP) is a safe, effective and widely used antipyretic–analgesic drug however;

An overdose can induce severe hepatotoxicity in experimental animals and humans. Acetaminophen dose is metabolized in the liver via glucuronidation and sulfate conjugation. The remaining 10 - 15% undergoes oxidative metabolism via cytochrome P450 isoenzymes (CYP) 2E1 and 1A2, which produces the hepatotoxic metabolite, N-acetyl-p-benzoquinonimine (NAPQI). Excessive administration of APAP can cause over production of ROS during formation of N-acetyl-pbenzoquinoneimin(NAPQI) by cytochrome P450. This mechanism has been suggested to participate in the development of oxidative stress and injury in APAP-induced hepatotoxicity[21].

Chronic administration of drug (paracetamol) to rats increased the levels of marker enzymes like

ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells[22]. The silymarine and the methanolic extract of *Ficus religiosa* significantly decreased the paracetamol induced elevated levels of the enzymes in the treatment group, indicating the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin after treatment with *Ficus religiosa* indicated the effectiveness of the extract in the normal functional status of the liver.

Histopathological analyses were good in agreement with the biochemical changes. Many research findings. Therefore, there is a possibility that the extract of *Ficus religiosa* may possess hepatoprotective activity.

5. CONCLUSION

The methanolic extract has shown the ability to maintain the normal functional status of the liver.

From the above preliminary study, we conclude that the methanolic extract of *Ficus religiosa*, is proved to be one of the herbal remedies for liver ailment.

REFERENCES

1. Action Plan for Liver Disease Research, National Digestive Diseases Information Clearing house 2 Information Way Bethesda, MD 2009. 20892-3570
2. Adewusi EA, Afolayan AJ, A review of natural products with hepatoprotective Activity, Journal of Medicinal Plants Research 2010. 4(13): 1318-1334.
3. Parmar SR, Vashrambhai PH, Kalia K, Hepatoprotective Activity of Some Plants Extract Against Paracetamol Induced Hepatotoxicity In Rats, Journal of Herbal Medicine and Toxicology. 2010; 4 (2): 101-106.
4. Verma N, Chaudhary S, Garg KV, Tyagi S, Anti-Inflammatory and Analgesic Activity of Methanolic Extract of Stem Bark of *Ficus religiosa*, International Journal of Pharma Professional's Research. 2010 October; 1(2):145.
5. Charde MR, Dhongade JH, Charde SM and Kasture AV, Evaluation of Antioxidant, Wound Healing And Anti- Inflmmatory Activity of Ethanolic Extract of Leaves of *Ficus religiosa* International Journal Pharmaceutical science and Research 2010;1(5).
6. Sirisha N, Sreenivasulu M, Sangeeta K, Chetty MC, Antioxidant Properties

of Ficus Species –A Review, International Journal of Pharmtech Research. 2010 Oct-Dec; 2(4): 2174-2182.

7. Patil MS, Patil CR, Patil SW, Jadhav RB, Anticonvulsant activity of aqueous root extract of *Ficus religiosa*, J ethanopharmacol. 2010 Sep 15. 66(3):277-82.
8. Ahuja D, Bijjem KR, Kalia AN, Bronchospasm Potentiating Effect of Methanolic Extract of *Ficus religiosa* Fruits In Guinea Pigs, J Ethanopharmacol. 2011,133:324.
9. Mohammed SAK, Syed AH, Abdul MMJ, Zainul AZ and Mohib K, Anti-Ulcer Activity of *Ficus religiosa* Stem Bark Ethanolic Extract In Rats, Journal of Medicinal Plants Research. ISSN 1996-0875, 2011 Febuary 4; 5(3):354-359.
10. Makhija IK, Sharma IP, Khamar D, Phytochemistry and Pharmacological properties of F*icus religiosa*: An overview Annals of Biological Research. 2010; 1 (4): 171-180.
11. Joseph B and Justin SR, Phytopharmacological and Phytochemical Properties of Three Ficus Species - An Overview, International Journal of Pharma and Bio Sciences, 2010 Oct-Dec; 1(4).
12. Prasad S, Kalra N, Shukla Y, Hepatoprotective Effects of Lupeol and Mango Pulp Extract of Carcinogen Induced Alteration In Swiss Albino Mice, Molecular Nutrition & Food Research. 2007; 51(3):352-9.
13. Margareth BCG, Miranda JS, Biological Activity of Lupeol, International journal of biomedical and pharmaceutical sciences. 2009, 46-66.
14. Nema AK, abhinav agrawal,varsha kashaw, Hepatoprotective Activity Of *Leptadenia reticulate* Stems Against Carbon Tetrachloride Induced Hepatotoxicity In Rats. Ijp 2011 June;43(3).
15. Khandelwal KR, Practical Pharmacognosy Techniques and Experiments, Nirali Prakashn. Pune, 2005.
16. OECD/OCDE 420 guideline for testing of chemicals Adopted 17th, Acute Oral Toxicity – Fixed Dose Procedure. 2001 Dec.
17. Pal RK and Manoj J, Hepatoprotective Activity of Alcoholic and Aqueous Extracts of Fruits of *Luffa Cylindrica* Linn In Rats, Annals of Biological Research, 2011; 2 (1):132-141.
18. Takate SB, Pokharkar RD, Chopade VV, Gite1 VN, Hepato-Protective Activity of The Ethyl Acetate Extract of *Launaea Intybacea* (*Jacq) Beauv* In Paracetamol Induced Hepato-Toxicity In Albino Rats, International Journal of Pharmaceutical Sciences Review and Research, 2010; 1(2): 72.
19. Biu AA, Yusuf SD and Rabo JS, Aqueous Extract of Neem (*Azadirachta Indica*) as a Treatment For Poultry Coccidiosis In Borno State, Nigeria, African Scientist. 2006 September 30; 7(3).
20. Kanchana N and Sadiq AM, Hepatoprotective Effect of *Plumbago Zeylanica* on Paracetamol Induced Liver Toxicity In Rats, International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(1).
21. Larson AM, Polson J, Fontana RJ, The Acute Liver Failure Study Group. Acetaminophen-Induced Acute Liver Failure: Results of A United States Multicenter, Prospective Study. Hepatology 2005; 42:1364 - 72.
22. Mitra SK, Venkataranganna MV, Sundaram R and Gopumadhavan S, Protective Effect of HD-03, A Herbal

Formulation, Against Various Hepatotoxic Agents in Rats. J. Ethnopharmacol 1998; 63: 181-186.

Table – 1: Chemical tests of *F. religiosa* bark extract

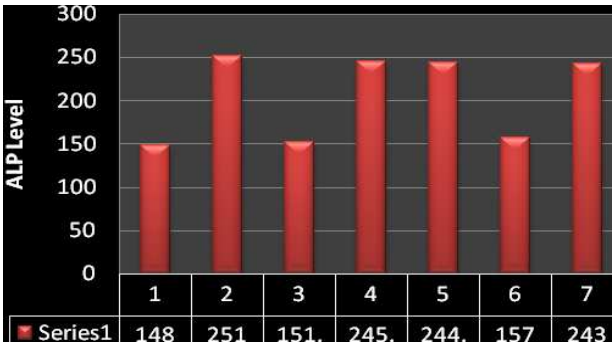
S.no	Chemical test	Pet ether	Ethyl acetate	methanol	Water
1	**Alkaloid**				
A	Mayer test	-	+	-	-
B	Dragendroff's test	-	+	-	-
2	**Flavonoids**				
A	Ammonia test	-	-	+	-
B	Shinoda test	-	-	+	-
3	**Phenolic compounds**				
A	Vanillin test	-	-	+	++
B	Acetic Acid test	-	-	++	+
C	Gelatin Test	-	-	+	-
4	**Tannin Test**				
A	Lead acetate	-	-	++	+
B	Ferric chloride	-	-	+	-
5	**Glycosides test**				
A	Borntrager test	-	-	-	+
B	Tollen test	-	-	+	-
C	Killer killani test	-	-	-	+
D	Saponine glycosides	-	-	+	+
E	Coumarin glycosides	-	-	+	-
6	**Steroids test**				
A	Libermann test	-	-	++	-
B	Salkowski test	-	-	++	-
7	**Carbohydrates test**				
A	Molish test	+	-	+	+
B	Fehling test	+	-	+	+
C	Benedict,s test	+	+	+	+
8	**Protein and amino acid**				
A	Biuret test	+	-	+	+
B	Ninhydrin test	+	-	+	+
9	**Triterpenoid**				
A	Salkowski test	-	-	+	-
B	Sulfur powder test	-	-	+	+

Table No.2 Effect of PEEFR, EAEFR, MEFR and AQEFR on biochemical parameters in Paracetamol induced hepatotoxic rats

Parameter	Normal	PCM (2g/kg)	Std.(silymarin) (100mg/kg)	PEEFR (200mg/kg)	EAEFR (200mg/kg)	MEFR (200mg/kg)	EQEFR (200mg/kg)
ALP	148±1.06	251.83±3.52	151.5±0.76**	245.33±2.46ns	244.17±1.33*	157±1.32**	243±0.82*
SGOT	47.5±1.33	108.33±2.23	51.5±1.65**	100.17±2.57*	99.17±2.59*	53.83±1.35**	55±1.88**
SGPT	57.67±1.89	113.17±2.29	62.5±2.42**	109.17±1.64ns	105.5±1.33*	66.17±2.30**	77.67±0.08**
Total Bil.	1.05± 0.08	5.43±0.13	1.27±0.08**	5.1±0.14 ns	4.92±0.14*	1.42±0.05**	4.9±0.14*

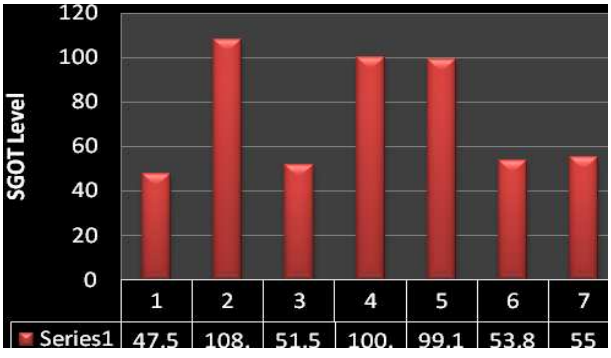
n = 6, Significant at P < 0.05, 0.01** and 0.001***, ns = not significant*
PCM – Paracetamol, PEEFR- Pet.ether extract of F.religiosa, EAEFR- Ethyl acetate extract of F. religiosa, MEFR- Methanolic extract of F. religiosa and EQEFR- Aqueous extract of F. religiosa

Figure -1: Effect of Pet. Ether, Ethyl Acetate, Methanol and Water Extract of *Ficus relogiosa* on ALP Level in PCM induced hepatotoxic rats



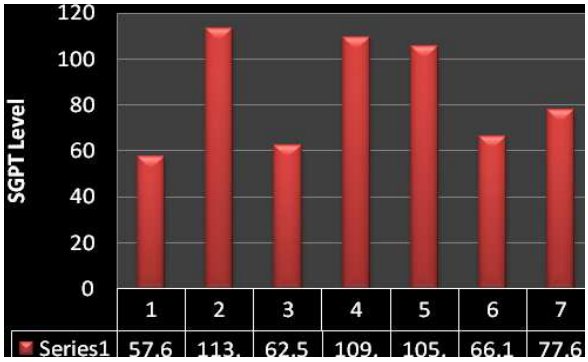
1= Normal Group
2= Paracetamol Group
3= Std. (Silymarin) Group
4=Pet Ether Group
5= Ethyl Acetate Group
6= Methanol Group
7=Water Group

Figure -2: Effect of Pet. Ether, Ethyl Acetate, Methanol and Water Extract of *Ficus relogiosa* on SGOT Level in PCM induced hepatotoxic rats



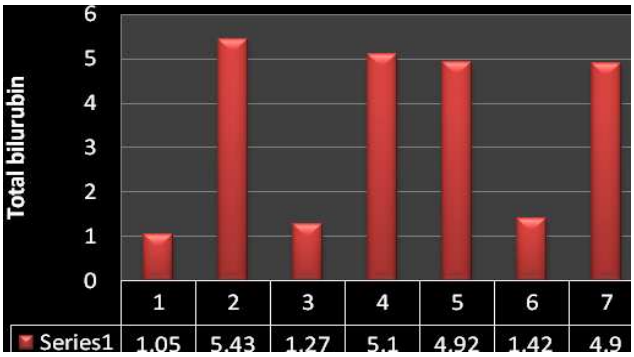
1= Normal Group
2= Paracetamol Group
3= Std. (Silymarin) Group
4=Pet Ether Group
5= Ethyl Acetate Group
6= Methanol Group
7=Water Group

Figure -3: Effect of Pet. Ether, Ethyl Acetate, Methanol and Water Extract of *Ficus relogiosa* on SGPT Level in PCM induced hepatotoxic rats



1= Normal Group
2= Paracetamol Group
3= Std. (Silymarin) Group
4=Pet Ether Group
5= Ethyl Acetate Group
6= Methanol Group
7=Water Group

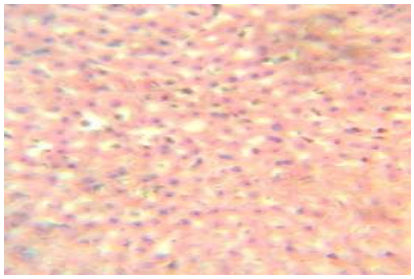
Figure -4: Effect of Pet. Ether, Ethyl Acetate, Methanol and Water Extract of *Ficus relogiosa* on Total Bilurubin Level in PCM induced hepatotoxic rats



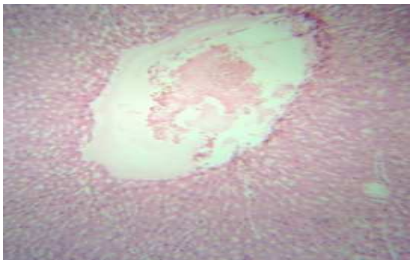
1= Normal Group
2= Paracetamol Group
3= Std. (Silymarin) Group
4=Pet Ether Group
5= Ethyl Acetate Group
6= Methanol Group
7=Water Group

Figure 5: Histopathology of Liver

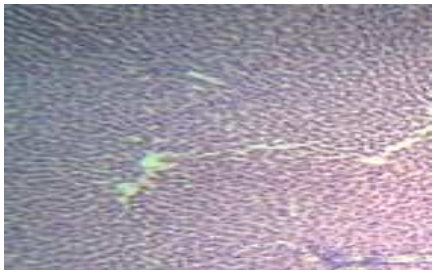
Histopathology of Liver shows normal hepatic tissue **(a)** normal control group, (b) PCM induced damage in hepatic tissue, **(c)** Effect of Silymarin on PCM induced hepatic damage, **(d)** Effect of PEEFR (High) dose on PCM induced hepatic damage, **(e)** Effect of EAEFR (High) dose on PCM induced hepatic damage, **(f)** Effect of MEFR (high) dose on PCM induced hepatic damage**, (g)** Effect of AQEFR (high) dose on PCM induced hepatic damage.



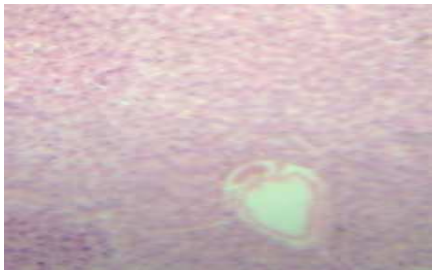
(a)



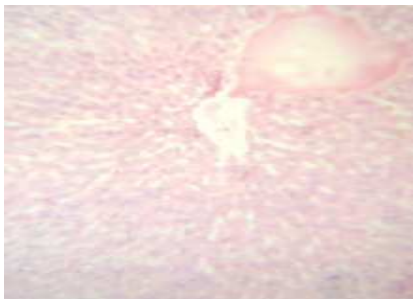
(b)



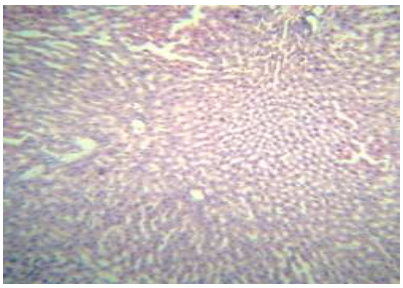
(c)



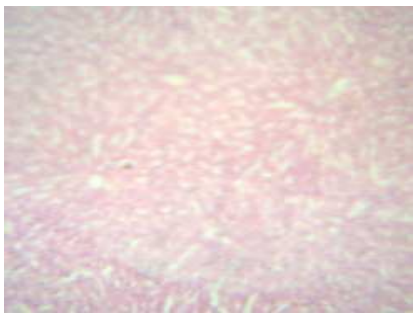
(d)



(e)



(f)



(g)