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HEPATOPROTECTIVE ACTIVITY OF AN AYURVEDIC HERBOMINERAL COMPOUND YAKRITSHULA VINASHINI VATIKA

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ABSTRACT

Yakritshula Vinashini Vatika (YSV) is a herbo-mineral compound used in the management of Liver disorders. The formulation is mentioned in the latest classics, Bhaishajya ratnavali. It is a combination of Navasadara and Saindhava lavana and 6 herbal ingredients with Putikambu swarasa (Chirabilva) levigation. Though herbo-minerals are highly acclaimed for their therapeutic efficacy. In the present study an attempt has been made to evaluate the hepatoprotective activity of Yakritashula vinashini vatika. The hepatoprotective potential was evaluated in CCl4 induced hepatotoxicity (in rat model). The test drug was given in two different doses to the experimental rats for duration of 28 days. Group I (Control) received gum acacia per oraly for 28 days and the toxicant CCl4 was used to induce hepatotoxicity at a dose of 1 ml/kg body weight as 1:1 mixture with olive oil (Group II). YSV in the dose of 200 and 400 mg/kg body weight/day orally (Group III & IV). Silymarin 100mg/kg (standard drug-Group V) and Prophylactic (Group VI) YSV 200 mg/kg.b.w. Biochemical parameters like SGOT, SGPT, Lipid profile, Total Protein and Billirubin were evaluated. The Hepatoprotective effect of YSV was evaluated by the assessment of biochemical parameters such as SGOT, SGPT ALP, Total Billirubin, Total Protein & histopathological studies of Liver.

Keywords: Carbon tetrachloride, Hepatoprotective activity, Silymarin.

INTRODUCTION

Liver diseases are considered as fatal & life threatening. It creates a serious challenge to public health. According to the latest W.H.O. data published in April, 2011 death due to liver disease in India has reached 2.3% of total deaths. India stands 27th in the world. There was no safe hepatoprotective drug available for the treatment of liver disorders [(1)]. Yakritashula Vinashini vatika[(1)] is one of such herbo-mineral compound, which is mentioned in Bhaishajya ratnavali in Pleehyakrita rogadhikara. Its therapeutic indications are Yakrit, Gulma, Pleehodara vyadhi with the anupana of karvellaka swarasa at a dose of badarsthi pramana i.e. near about 750 mg in weight. The existing drugs can cure most of the diseases. Still there is a never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic activity than the existing drugs. The present study intended to evaluate Yakritashula vinashini vatika for its hepatoprotective activity.

OBJECTIVES

To evaluate Hepatoprotective activity of Yakritashula vinashini vatika by animal

experimentation by inducing hepatotoxicity by CCl_4.

MATERIAL AND METHODS

Materials

Collection and identification of raw materials

The ingredients of YSV were procured from the pharmacy attached to NIA, Jaipur, & identified or authenticated by expert of the P.G. Department of Dravya Guna, NIA, Jaipur. The drug **(**Yakritahsula vinashini vatika) was prepared in P.G. Department of Rasashastra & Bhaishajya Kalpana, as per the procedure.

Table No. 1: Showing Ingredients of *Yakritashula vinashini vatika*:

S.No	Name	Origin	Latin Name	Part used	Proportion
1.	Navasadara	Mineral	Ammonium chloride	-	1 Kasha
2.	Saindhava lavana	Mineral	Rock salt halite / Chloride of sodium	-	2 Kasha
3.	Chitraka	Herbal	*Plumbago zeylanica*	Rt.bark	10 Kasha
4.	Kokilaksha	Herbal	*Asteracantha longifolia*	Seed	10 Kasha
5.	Rohitaka	Herbal	*Tacoma undulate*	Bark	10 Kasha
6.	Yavani	Herbal	*Tachyspermum ammi*	Fruits	10 Kasha
7.	Putikambu (Swarasa)	Liquid for levigation	*Holoptelia integrifolia*	Leaves.	Q.S.

Processing of formulation (Test drug):

The collected material was shade dried and powdered using mixer grinder. Care was taken to avoid fungal contamination while drying and handling by wearing gloves. The powder mixed was triturated for 4 hours by mixing Putikambu (Chirabilwa) and dried. The process was repeated for 7 times, when the mass become homogenous the pills were prepared by hand molding. Then the Material was weighed and stored in an air tight container.

Experimental study:

- **Drugs : ** YSV 200, & 400 mg/kg body weight
- **Silymarin : ** (Silybon-140-Micro lab Limited) from R.N. Medical store**.**
- **Chemicals and Instruments: (**Chemicals-From Metro trading Corporation, Pharma plaza, Jaipur.**)**

S.No.	Chemicals	Instruments
1.	Carbon tetrachloride	Weighing scale
2.	Chloroform	Syringe & Needle
3.	Diethyl ether	Gloves & Mask
4.	Formaldehyde	Feeding needle
5.	Olive oil	Picric acid for marking

Selection of Animals and Animal care:

Wistar strain albino rats of either sex, weighing 120-150 gm housed in polypropylene cages kept in the animal house of NIMS Institute of Pharmacy, Nims, University, Jaipur. They were maintained under standard husbandry conditions (temperature 23±2 ◦C, relative humidity 55±10% and 12-h light: 12-h dark cycle) during experiments. Animals were allowed to take standard laboratory feed and water. They were given a week

times to get acclimatized to the laboratory conditions. Initial body weight of each animal was recorded.

The protocol is approved by animal ethics committee of the institution constituted (AECC) for the purpose as per CPCSEA guidelines. IAEC Clearance no.-NU/NIP/ IAEC/12/001

Marking: Rats were marked with picric acid on their tail for easy identification. They were recorded as 1, 2, 3, 4, 5.

Route of administration: Oral

Calculation of dose:

The dose was calculated by extrapolating the human dose to animal based on the body surface area ratio by referring to the table of Paget and Barnes (1964).Thus the dose conversion formula in animals is human dose multiplied by 0.018 (conversion factor for rats) and the resultant product will be further multiplied by 5 to obtain the dose per kg. Normal human dose of *YSV* according to text is badaraasthi. So in SI unit, it was near about 750 mg.

Dose conversion [(2)]

Human Dose x 0.018(conversion factor for rats) x 5

750x 0.018x5=67.5gm/kg

It can be stated as 6.75 mg/100 gm body wt of rats.

Preparation of drugs and solutions:

- **Drug:** The suspension was prepared in 5 % gum acacia. *YSV* 200 & 400mg/kg b. w. was added to 5 ml of Gum acacia and mixed it well in mortar and pestle to form a uniform suspension.
- **CCl_4:** It was used at a dose of 1ml/kg body weight by i. p. route. Solution of CCl_4 was prepared by making 1:1 dilution with olive oil.
- **Silymarin:** It was used at a dose of 100 mg/kg body weight. Stock solution of 200 mg/ml was prepared to carry out the experiment.

➢ **Study Protocol:** Pharmacological activity of YSV had been segmented as following

➢ **Experimental design:**

Induction of hepatopathy[4]:

Liver damage was induced in rats by suspension of CCl_4 at the dose of 1 ml/kg b. w. for first 7 days (i. p.) dilution with olive oil in ratio of 1**:**1 (v / v) , to the all the groups except group I.

Hepatoprotective activity:

Albino Wistar rats (100–150 gm) of either sex were used for study. Animals were divided into six groups, each group containing five animals. Study was carried out for 28 days. **Group I** served as normal control and received Gum acacia (5%) p.o. for 28 days. **Group II** served as induction control, **Group III** to **VI** received *YSV* 200 and 400 mg/kg body weight and **Group V** silymarin 100 mg/kg body weight respectively for 28 days by oral route. CCl_4 1 ml/kg, i. p., 1:1 dilution with olive oil was administered for 7 day to the animals of all the groups except group I.

Table No 2 : Experimental design

Groups	Treatment
Vehicle Control	Gum acacia (5 ml/kg b. w. p. o.) for 28 days.
Negative control	CCl_4 (1 ml/kg b. w. i. p.) was given for 7 days.
Test Drug 1 (YSV 200) (200mg/kg b/w)	Trial drug (200 mg/kg b. w. p. o.) for 28 days + CCl_4 (1 ml/kg b. w. i. p.) for 7 days.
Test Drug 2 (YSV 400)	Trial drug (400 mg/kg b. w. p. o.) for 28 days + CCl_4 (1 ml/kg b. w. i.

(400mg/kg b/w)	p.) for 7 days.
Standard	Silymarin (100 mg /kg b. w. p. o.)[7] for 28 days + CCl_4 (1 ml/kg b. w. i.p.) for 7 days.

Note: Dose of drug was carried out according to OECD Guidelines for the testing of Chemicals (ATC method (TG 423)(5).

Collection of Blood, Serum & Tissue Sample:

The animals were kept starved on the 29th day. On the next day, after recording their body weight and blood was collected by retro orbital puncture. The blood was allowed to clot, & then centrifuged at 3000 rpm for 20 min. Sera sample were collected for biochemical parameters like SGOT, SGPT, SALP and Lipid profiles.

The animals were sacrificed by using Diethyl ether and the abdomen was cut open to remove the liver.

Investigation of Biochemical parameters:

1. Serum Glutamic Oxaloacetic Transaminase (SGOT)[5] and
2. Serum Glutamic Pyruvic Transaminase (SGPT)[5]
3. Alkaline phosphatase (ALKP)[6]
4. Total bilirubin (TBL)[7]
5. Unconjugated bilirubin[7] = Total - Conjugated bilirubin.

Aspartate aminotransferase (AST), alanine aminotransterase (ALT).

All the determinations were carried out using standard kits using UV comparison test and & Graph Pad Instat statistical program. P- value <0.05 was regarded as statistically significant.

RESULT

A)Body weight & weight of liver:

No animal died during CCl_4 administration period. The administration of CCl_4 caused a significant decrease (13.29%) in the body weight of rats as compared with the control rats and also the animals co-treated with *YSV* (200, 400 mg/kg b.w.) for four weeks lost weight 5.38%, 0.60 % respectively during the experimental period. Whereas animal treated with standard showed increase (5.31%) in body weight, which is non-significant.

b) Assessment of hepatoprotective activity

YSV400- (400mg/kg bw) showed hepatoprotective activity in carbon tetrachloride induced hepatic damage in rat. Table No. 3 showed a significant

spectrophotometer.

Investigation of Lipid Profile :[8]

Cholesterol, Triglycerides, HDL, LDL, VLDL etc.

Histopathological Study:

A portion of the liver was cut into two to three pieces approximately of 6mm size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5µm thickness were cut and stained with haematoxylin–eosin. The stained sections were made into permanent slides and examined under high resolution microscope with photographic facility and photomicrographs were taken.

Then the sections were observed (at Reliable Diagnostic Centre, Malviya Nagar, Jaipur) under microscope with various magnifications to note down the changes in the microscopic features of the tissues studied.

STATISTICAL ANALYSIS

The data were expressed as mean ± SEM. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple

increase in the level of liver enzymes like SGOT, SGPT, ALP and bilirubin in CCl_4 intoxicated animals when compared with that of the control group of rats. CCl_4 induced animals treated with YSV200, were slowly recovered from hepatic injury and were evidenced by lower level of liver enzymes. These results were also observed in silymarin treated group. There was no change in control group.

Table No.3. Showing Effect of Yakritashula vinashini vatika (200, 400 mg/kg b.w.) on the activity of Liver marker enzymes in the serum of rats.

S.N.	Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)
1.	Vehicle Control	59.000 ±10.213	72.200± 22.940	166.60 ± 13.014	1.148 ± 0.2873	7.748 ± 0.9610
2.	Negative control	342.20 ±53.538^{+++}	256.40 ± 42.141 $^{+++}$	327.00 ± 15.789 $^{+++}$	3.854 ± 0.1040^{+++}	4.840 ± 0.5335^{+}
3.	YSV200	272.40 ±42.261 ^{NS}	199.40 ±18.885 ^{NS}	248.40 ± 15.917**	2.882 ± 0.03040**	6.140 ± 0.3763^{NS}
4.	YSV400	160.60 ±23.541 *	149.20 ±16.608 *	188.60 ± 9.796***	2.100 ± 0.1279***	6.460 ± 0.6728 ^{NS}
5.	Standard	153.06 ±20.260 **	141.20 ±16.506 *	159.80 ± 9.346***	1.530 ± 0.1148***	8.772 ± 0.6290**

- Values are mean ± SEM. (N= 5 animals in each group).
- NS=not significant (P>0.05), * P<0.05, ** P<0.01, *** P<0.001 significantly different from the group treated with CCl_4 and + P<0.05, +++ P<0.001 significantly different from the control.

Table No 4. Showing percent of mean (% mean) in various Groups:

Parameters	Groups			
	Negative control	YSV200	YSV400	Standard
SGOT	-480	20.51	53.06	55.27
SGPT	-255.12	28.58	41.80	44.92
ALP	-96.27	24.03	42.32	51.13
T.B.	-235	25.22	45.51	60.30
T.P.	37.53	-26.85	-33.47	-81.23
Chol.	-381.19	34.0	38.4	45.8
TG	-119.21	31.66	36.49	28.80
HDL	21.03	-29.53	-82.08	-65.13
LDL	-80.29	30.52	33.41	34.75
VLDL	-93.07	26.05	25.73	45.99

1. Carbon tetrachloride group significantly increased the serum level of SGOT (480%), SGPT (255.12%), ALP (96.27%), Total Billirubin (235%), Cholesterol (381.19%), Triglycerides (119.21%),LDL (80.29%) and VLDL (93.07%) as shown in Table No.7.36.
2. At the dose of 200 mg/kg b.w. *YSV*, increase in the value of the parameters was 20.51%, 28.58%, 24.03%, 25.22%, 26.85%, 34%, 31.66%, 29.53%, 30.52%, 26.05% respectively compared with Toxic control group.
3. *YSV* 400 showed percentage of recovery as -53.06%, 41.8%, 42.32%, 45.51%, 33.47%, 38.4%, 36.49%, 82.08%, 33.41%, 27.73%, respectively compared with Toxic control group.
4. Standard group showed percentage of recovery as 55.27%, 44.92%, 51.13%, 60.30%, 81.23%, 45.8%, 28.8%, 65.13%, 34.75%, 45.99%% respectively compared with Toxic control group.

DISCUSSION

In animal experimentation no casualties have been observed and the weight was recorded before and after treatment. The weight of the 2 group's standard has showed increase in weight. The aim of the study was to examine if treatment of drugs from *YSV* was capable of eliciting hepatoprotective in CCl_4-intoxicated rat model of hepatotoxicity. $CC1_4$ intoxication was found leading to many cellular and tissue abnormalities. As a result of these abnormalities, alterations were observed in several biochemical constituents in experimental animals. In this study, $CC1_4$ exposure has been found to result in alteration in the activities of conventional hepatic marker enzymes i. e. AST (SGOT), ALT (SGPT), ALP, & T. Billirubin, T. Proteins.

In biochemical parameters, negative control group showed very highly significant result for SGOT, SGPT, ALP, Total Billirubin & significant for Total Protein. YSV200 test drug1, group showed highly significant for ALP parameters, significant for, Total Billirubin and non-significant for SGOT, SGPT and Total Protein. YSV400 test drug2 group showed very highly significant result for ALP, Total Billirubin, significant for SGOT, SGPT, and non-significant for Total Protein. Standard group showed very highly significant result for ALP, Total Billirubin, highly significant for Total Protein, SGOT and significant for SGPT.

Additionally, it was observed that histopathological changes indicating liver damage after $CC1_4$ administration. It has been reported by previous findings that $CC1_4$ causes necrosis, fatty infiltration, and degeneration of Hepatocytes in liver. It has also been reported that $CC1_4$ causes apoptosis in liver. Therefore Histopathological findings in the liver due to $CC1_4$ administration are in agreement with previous studies.

Table No.5 showing level of significance in various parameters.

Groups	Non-Significant	Significant	Highly Significant	Very Highly Significant
Negative control	HDL	Total Protein	LDL	SGOT, SGPT, ALP, Total Bilirubin, Cholesterol, Triglycerides, VLDL
YSV200	SGOT, SGPT, Total Protein, HDL, LDL, VLDL	Cholesterol, Toatal Bilirubin	Triglycerides, ALP	----
YSV400	Total Protein, VLDL	SGOT, SGPT, LDL, HDL	Cholesterol,	ALP, Total Bilirubin, Triglycerides
Standard	HDL	LDL, SGPT	Triglycerides, Total Protein, SGOT	Cholesterol, VLDL, ALP, Total Bilirubin
Prophylactic	Total Protein, HDL	SGOT	SGPT	ALP, Total Bilirubin, Cholesterol, Triglycerides, LDL, VLDL

Histopathological findings:

Results of histopathological studies provided corroborative evidence for biochemical analysis. Histology of liver section of normal control animal (Group 1) exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus & nucleolus (Figure 1 of plate no.11).Whereas that of CCl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes & congestion of sinusoid and apoptosis (Figure no.2, 3, 4). *YSV* at a dose of 200 mg/kg b.w. showed moderate to weak activity in protecting the liver cells from CCl_4-injury (Figure no-6).*YSV* had shown very potential Hepatoprotective activity at a dose of 400 mg/kg b.w.

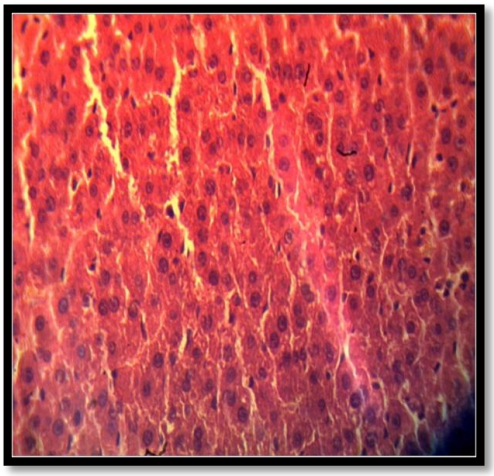

Figure. 1. Liver section of normal control showing normal architecture and radiating Hepatocytes (H & E * 40)

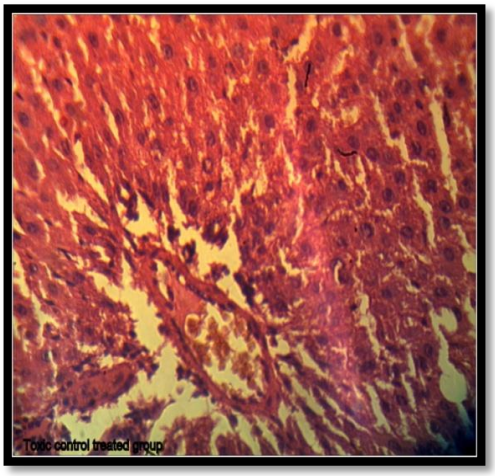

Figure.2. Liver section from rat Toxic control (CCL_4) shows fatty infiltration and degeneration of cells, necrosis. (H & E * 40)

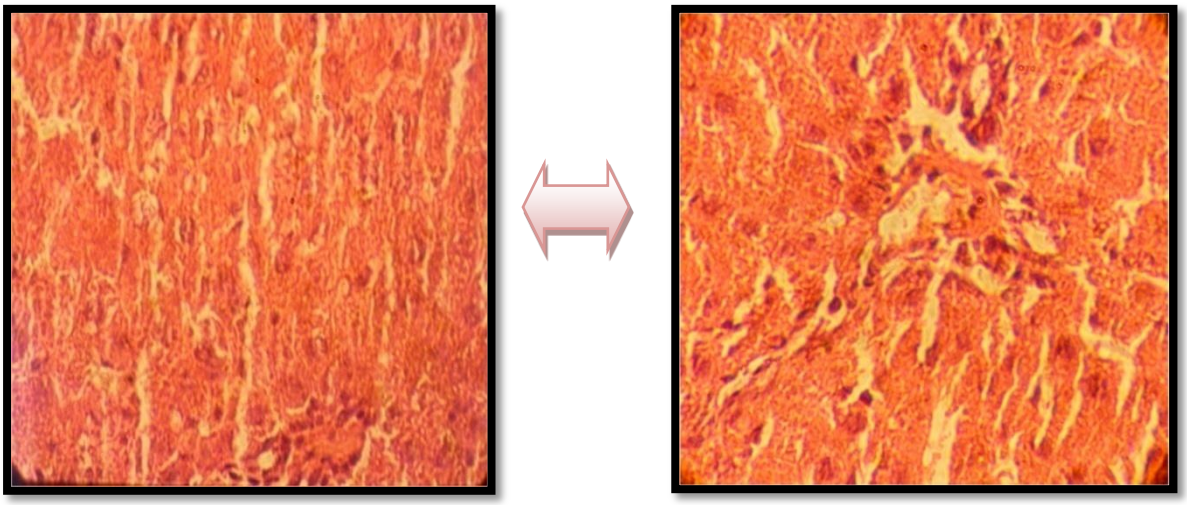

Figure.3-4. Liver section from Toxic control shows fatty infiltration and steatosis of cells (H & E * 100)

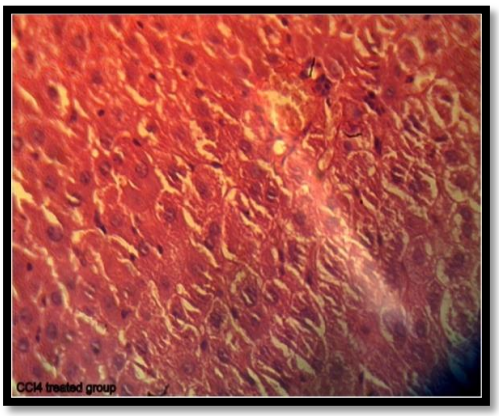

Figure. 5. Liver section from rat Toxic control shows apoptosis, fatty infiltration of cells (H & E * 40)

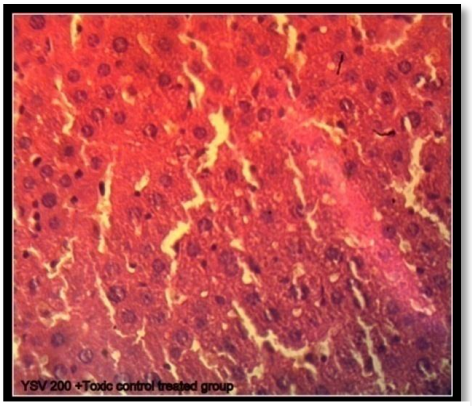

Figure6. Liver section from rat treated with *YSV* 200 (H & E * 40)

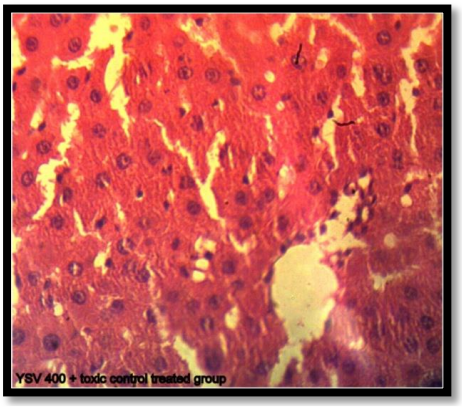

Figure.7 Liver section from rat *YSV* 400(H & E * 40)

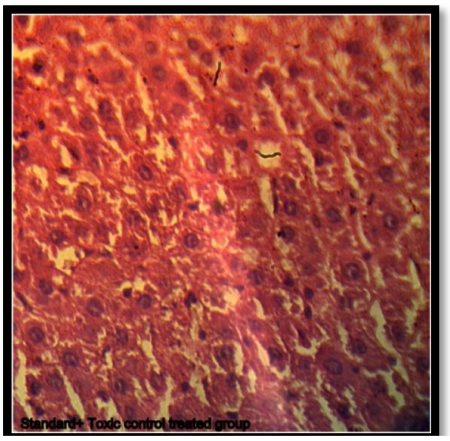

Figure. 8 Liver section from Standard (H & E * 40)

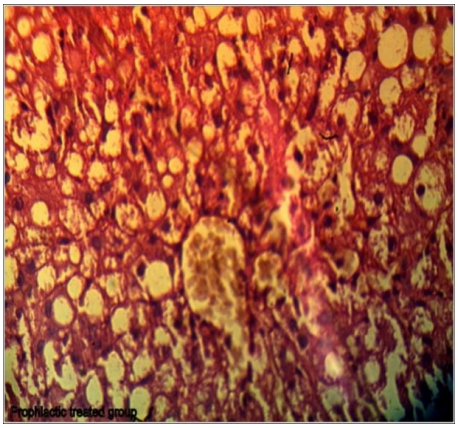

Figure. 9 Liver section from rat treated with YSV 200 mg/kg b.w. (H & E * 40)

CONCLUSION

The biochemical & histological results demonstrated Hepatoprotective activity, as indicated by the restoration of altered values of different parameters in hepatotoxic rats to near-normalcy at the dose of 400 mg / kg body weight of Yakritashula vinashini vatika. Histopathological observations of the liver tissues of various experimental groups further corroborated the biochemical findings observed in this study.

REFERENCES

1.AFI from Bhaishajya Ratnavali of Yakrit-pleeha rogadhikara 62-63.

2.Paget, G.E. and barnes, J.M. Toxicity tests. In evaluation of drug activities: Pharmacometrics (eds Lawrence, D. R. and Bacharach, A.L.), Academic press, London,1964pp.140-61.

3. OECD guidelines for the testing of chemicals (Acute oral toxicity –up and down procedure). Adopted 23rd march 2006. [cited 2008 Jun 20]; Available from: www.oedc.org.

4. 51. Sanmugapriya E, Venkataraman S. Studies on hepatoprotective and antioxidant actions of Strychnos potatorum Linn. Seeds on-CC14-induced acute hepatic injury in experimental rats. J Ethnopharmacol 2006 Apr 21;105(1-2):154-60.

5. Kalpana Patil,Alka mall. Hepatoprotective activity of Mentha arvensis Linn. Leaves against CC14 induced liver damage in rats. Asian Journal of Tropical Disease(2012) S223-S226.

6. Reitman's. Frankel S.A. Calometric method for the determination of SGOT and SGPT. Amer.J.Cline.Path.1957;28:56-63.
7. The method of King and Armstrong Determination of serum and bile phosphatas eactivity.Can.Med.Asso.J.1934;31:56-63.
8. TBL and conjugated Liver function In: Fundamental of clinical chemistry, (Ed) Tietz N.W.3rd edition. W.B. saunders company, Philadelphia 1987;729-761.
9. Fring CS and Dunn R. A colorimetric method of determination of total lipids based on sulfophosphate vanillin reaction Am J Clin Path 1970, 4, 53 89..

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