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Research Article

A STUDY TO EVOLVE AN EFFECTIVE PARACETAMOL MODEL TO INDUCE HEPATOTOXCITY IN WISTAR ALBINO RATS

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ABSTRACT
Paracetamol has become a model toxin and a tool in biochemical and clinical toxicological research also, it is used as a model toxin for establishing the usefulness of *in vitro* models such as measuring the hepatoprotective activity of certain herbs indicated in ancient systems of medicine. In the present study the objective is to establish a paracetamol model with a pertinent look out for the dose, duration and route of administration. The present study was done on wistar rats, which were divided into six groups of six rats in each group. Group I and IV were the control groups which received sodium CMC per oral and intraperitoneal respectively. Group II and Group V were administered paracetamol at a dose of 1gm/kg bw per oral and intraperitoneal respectively for 7 days. Group III and Group VI were given paracetamol at a dose of 1gm/kg bw per oral and intraperitoneal respectively for 14 days. After 24 hrs of the last dose of PCM blood was collected from the retro-orbital plexus and analysed for biochemical parameters like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TBIL) and total protein (TPRO). Then the rats were sacrificed, livers were excised to measure the weights and volumes, followed by the histological processing and staining. Induction of liver toxicity with paracetamol resulted in the worsening of the biochemical parameters and histological picture. The results indicated that administration of PCM at a dose 1gm/kg bw for 14days per oral would be an effective model.

Key Words: *Paracetamol, Per Oral, Intraperitoneal, Biochemical Parameters and Histological Processing*

INTRODUCTION
Experimental induction of Liver Injury, which would be predictable, reproducible in animal models cannot be attained with ease. One of the intrinsic hepatotoxin causing reproducible dose dependent toxicity in the liver is paracetamol Linda (2011). A 3500, million 500 mg tablets of paracetamol were estimated to have been consumed in the year 2000 which was reported by (IMS Health, Sheen unpublished data). A very widely used over- the counter (OTC) antipyretic and analgesic drug with a chemical structure (4'-hydroxyacetanilide, *N*-acetyl-*p*-aminophenol, acetaminophen) is paracetamol. Paracetomal was discovered in Germany at the end of 19^{th} century Sheen (2002). If normal capacity of liver to detoxify acetaminophen is exceeded, which is done by normal mechanisms of glucuronidation and sulfation, oxidation into toxic metabolite N-acetyl-p-benzoquinone occurs by the cytochrome P450 system Linda (2011). The present study has been taken up to arrive at a successful toxicity induction procedure in wistar albino rats, as there is a wide range of dose and duration of administration of paracetamol in the available literature .

MATERIALS AND METHODS
Chemicals
Analytical grade chemicals were used in the experiment, 1% sodium carboxy methyl cellulose (Na.CMC) acquired from Sai chemicals and Paracetamol (Lambert). Biochemical parameters were estimated by using the diagnostic kits purchased from various manufacturers serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) from Excel Diagnositics Hyderabad, total serum bilirubin (TBIL) from Erba diagnostics and total serum protein

(TPRO) from Autospan diagnostics which were all performed on a semiautoanyliser of Erba company. Standard orogastric cannula was used for the oral administration of drug while intraperitoneal administration of drug was performed by the use of 24 gauze needle with a disposable syringe.

Test Animals

Wistar albino rats (180-210gm of weight) from Animal House of Mahaveer Agencies, Hyderabad, Andhra Pradesh are the study material. They were housed in stainless steel cages and kept in a room where a 12-hour light/dark cycle was maintained. They were allowed to have free access to water and standard pellet (National Institute of Nutrition) feed throughout the period of the experiment. All the weights were taken by using the Laboratory analytical balance, volume of the liver was measured by water displacement method using syringe accurate up to $1/40^{th}$ of milliliter.

Paracetamol Induced Liver Toxiciy

A very widely used over- the counter (OTC) antipyretic and analgesic drug with a chemical structure (4'-hydroxyacetanilide, *N*-acetyl*p*-aminophenol, acetaminophen) is paracetamol. Toxicity studies were carried following the OECD guidelines. Liver damage induced by administration of acetaminophen (Paracetamol) at a dose of 1g /kg body weight Manokaran (2008) but Shah *et al.,* (2010) gave 3gm/kg bw. Liver detoxifies paracetamol to a limit by the process of glucuronidation and sulfation, oxidation into toxic metabolite N-acetyl-p-benzoquinone occurs by the cytochrome P450 system this increases the values of the biochemical parameters as SGOT, SGPT, TBIL and TPRO (Kursad, 2007 and Sabir, 2008).

Toxcity Studies

Wistar albino rats were the study material. The rats were randomized and divided into six groups each group containing 6 animals .group I served as control for oral administration, in which 1ml of 1% sodium CMC was given per oral. Group II received paracetamol at a dose of 1gm/kg *bw p.o.* for 7 days while Group III received the same for 14 days. Group IV served as control for Intraperitoneal administration , in which 1ml of 1% sodium CMC was given ip for 7 days .Group V received paracetamol at a dose of 1gm/kg/bw i.p. for 7 days, while Group VI was administered the same for 14 days .

Assessment of Biochemical Parameters

Blood was collected from retro-orbital plexus, after 24 hrs of last administration and was allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minutes. The serum was analysed for SGOT/AST, SGPT/ALT, ALP, TB and TPR. Transaminase activity was measured by IFCC kinetic method, ALP by p-NPP kinetic Mono method, Bilirubin was estimated by Diazo method and Total protein was measured by Biuret method.

Histopathyology

Rats were sacrificed by cervical dislocation, liver was excised, weight and volume were determined and were immersed in more than 10 times volume of 10% formalin solution, after fixation for 1 week liver tissues were dehydrated in graded ethanol in ascending order from 50 to 100% followed by clearing in xylene solution then embedded with paraffin wax and blocks were made. Sections of 5-micron thickness are made using rotary microtome and mounted on to the slides .Staining was done by Haematoxylin - Eosin method and Periodic Acid Schiff's staining.

Statistical Analysis

All the results were expressed as Mean ± SEM. The statistical analysis was carried by one-way Analysis of Variance (ANOVA) followed by Dunnett's Multiple comparison tests using graph pad Prism software, $P < 0.05$ was considered as significant.

RESULTS

Significant change in the weights and volumes of the liver due to hepatotoxicity caused by paracetamol were observed as follows, the increase in the weight and volume was documented to follow an ascending sequence from control -per oral 7 days – i.p. paracetamol for 7days- per oral paracetamol for 14 days – highest values were seen in i.p. administration of paracetamol for 14 days .In the paracetamol treated rats there was a significant increase in biochemical markers (SGOT,SGPT, ALP and TBIL) when compared

Table 1: Showing various Biochemical parameters in paracetamol induced liver toxicity with a variation in route and duration of administration

Treatment design(n=6)	Liver weight in gms	Liver volume in ml	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	TP (IU/L)
1. Control (Sod CMC, p.o.)	6.983± 0.07419	6.050±0.01443	71.37± 0.5511	44.82±0.2555	169.7± 1.029	0.5233± 0.02275	9.293± 0.04248
2. Paracetamol 1gm/kg bw p.o. 7days	8.633± 0.1186***	6.179±0.04104	143.5±3.698***	98.48± 0.4394***	434.2± 2.165***	0.8567± 0.05402	6.233± 0.04773***
3. Paracetamol 1gm/kg bw p.o. 14 days	10.38± 0.1973***	9.250± 0.04282***	254.2±6.635***	219± 2.236***	619.3± 2.667***	6.317± 0.2738***	5.167± 0.1333***
4. Control (Sod CMC, i.p.)	7.017± 0.07032	6.092± 0.02713	71.08± 0.4362	44.60± 0.1317	169.8± 0.5548	0.5883± 0.05419	9.160± 0.04163
5. Paracetamol 1gm/kg bw i.p. 7days	8.290± 0.03751***	6.258± 0.04167	151.2±0.6009***	111.7± 1.961***	453.7± 2.985***	1.367± 0.04944**	6.070± 0.08062***
6. Paracetamol 1gm/kg bw i.p. 14days	13.55± 0.2306***	10.83± 0.08028***	278±3.795***	233.3±1.961***	633±3.454***	6.567± 0.2390***	5.017± 007491***

***P <0.01, ***P <0.001, as compared to the control group.*

to the values of the control group. As a consequence of the hepatic damage, production of the proteins decreased which was evident in the decreased values of the serum protein levels. These changes were depicted in the table 1. A mortality of 33.3% in group V and 50% in group VI occurred.

DISCUSSION

Though paracetamol is considered to be the safest non-steroidal anti-inflamattory drug available over the counter if used in recommended doses; it is also capable of producing hepatic damage on consuming single overdoses or chronic low dose (Prescott, 1971; Wilkinson, 1977 and Bonkovsky, 1994). Hence liver toxicity induction model was developed using this drug to study the hepatoprotective activity of other drugs. The normal levels of biochemical parameters **(**liver weight 6.983±0.074, volume 6.05±0.0143, SGOT 71.37±0.5511, SGPT44.82±0.25, ALP169.7±1.03, TBIL 0.52±0.055 and Tpro 9.29±0.043). Around 80% of ingested paracetamol at lower doses is detoxified as conjugates of sulfates and glucoronide without undergoing oxidation, while 5 % of it is oxidised into toxic metabolite N-acetyl-p-benzoquineimine (NAPQI) by the action of hepatic cytochrome P450. However the increase in the biochemical parameters is a consequence of the hepatic damage, due to the incapacitation of the cell membrane of hepatocytes to retain the enzymes, which leak into the blood stream resulting in the elevation of the serum values Madhukiran (2012). Further damage would result in the necrotic changes in the parenchyma of the liver. Except for the Serum total protein which showed decrease in serum values as hepatic damage resulted in the decreased production of protein rest of all the parameters have shown significant increase in the values in the following order that is group VI in which the rats were administered PCM intraperitoneal for 14 days showed maximum values similarly Adejova (2008) during the work of evaluating the hepatoprotective activity of *ascorbic acid* induced toxicity to the albino rat liver by the i.p injection of PCM for 14 days. Followed by group III Where PCM was given p.o for 14 days however Iqbal (2012) while assessing the hepatoprotective activity of *Feronia limonia* the author induced hepatotoxicity by administering PCM for 10 days. Next grade values in the present study were shown by group V in which the rats received PCM i.p for 7days while a marginal lower values were seen in group II where the rats were given PCM p.o for 7 days as depicted in table 1.

Histopathology

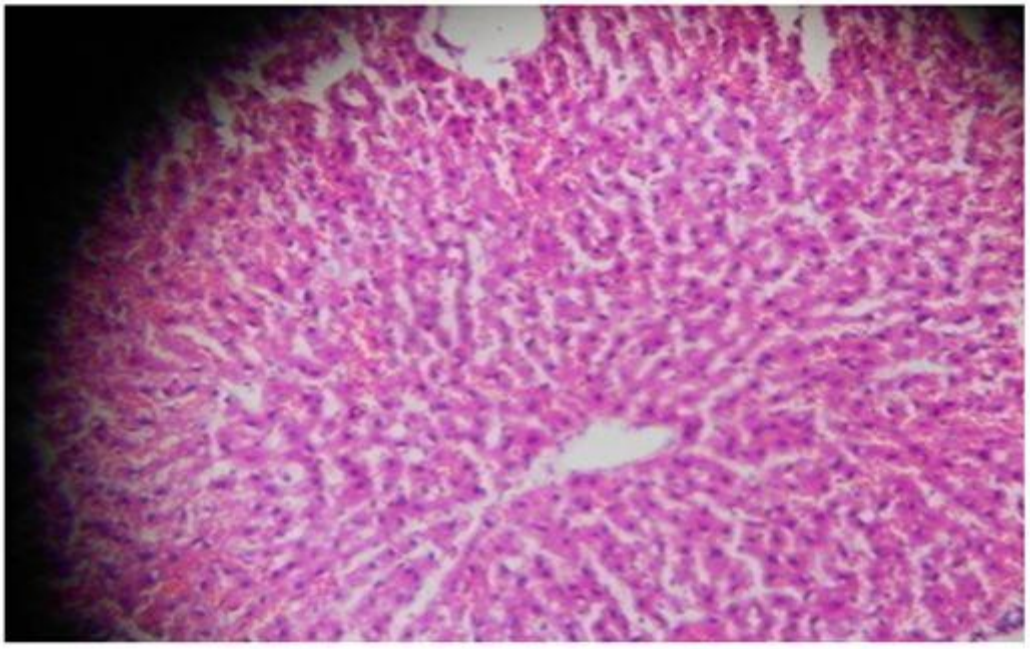

Figure 1: Showing the normal sinusoidal pattern of the liver of control group H and E Stain 40 magnifications

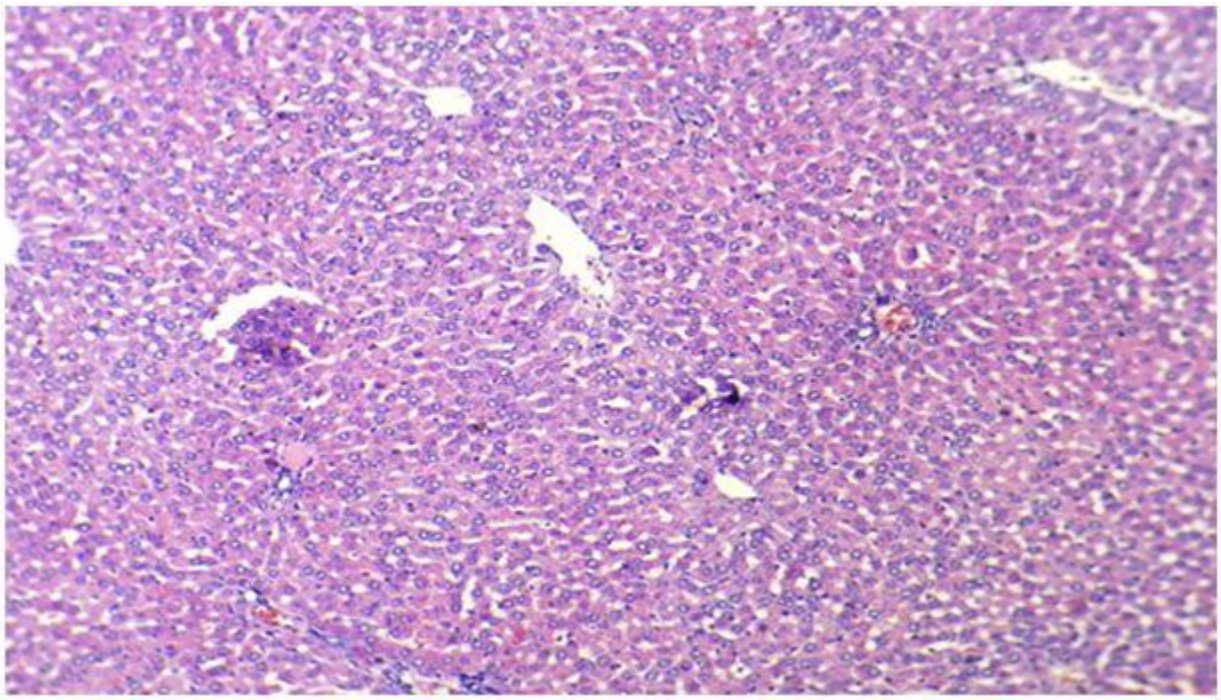

Figure 2: Showing the necrotic changes which are not wide spread group II PAS STAIN 40 magnifications

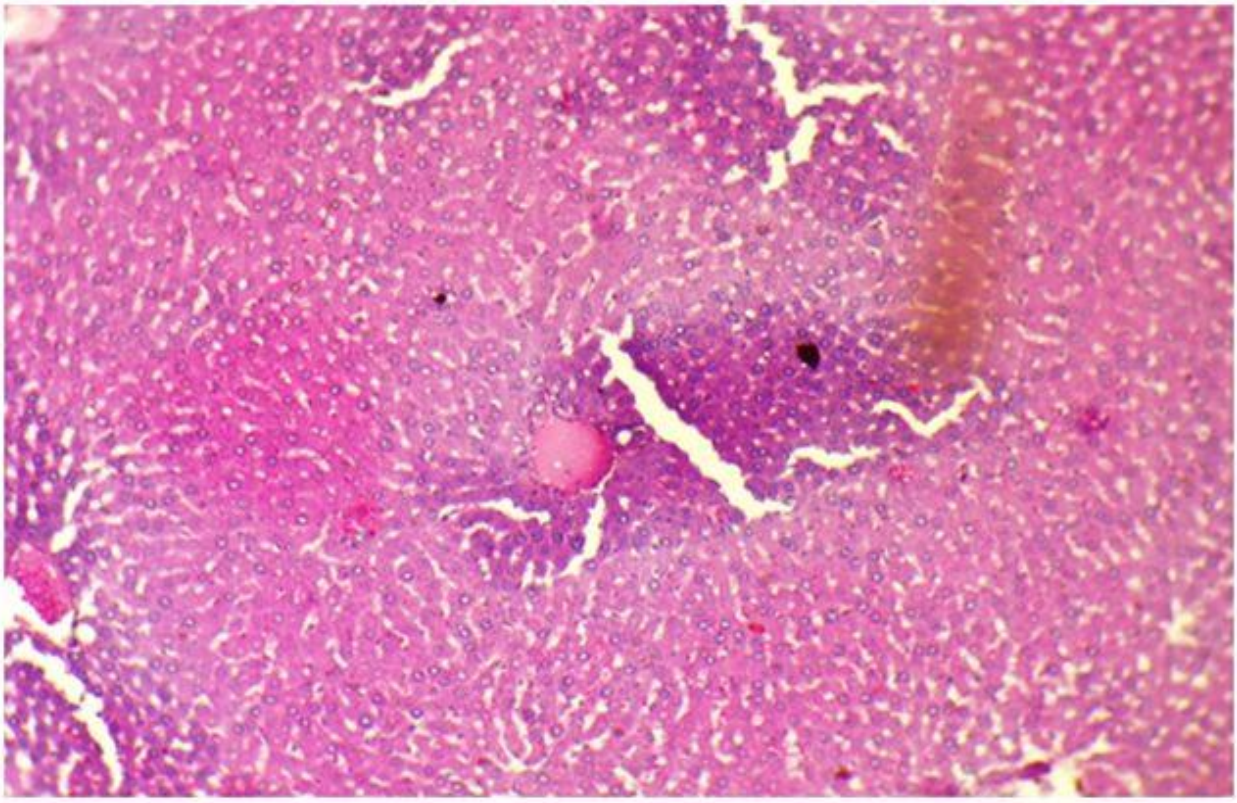

Figure 3: Showing wide spread necrosis of bridging type in groupd III 14 days pcm po H&E Stain 100 magnification

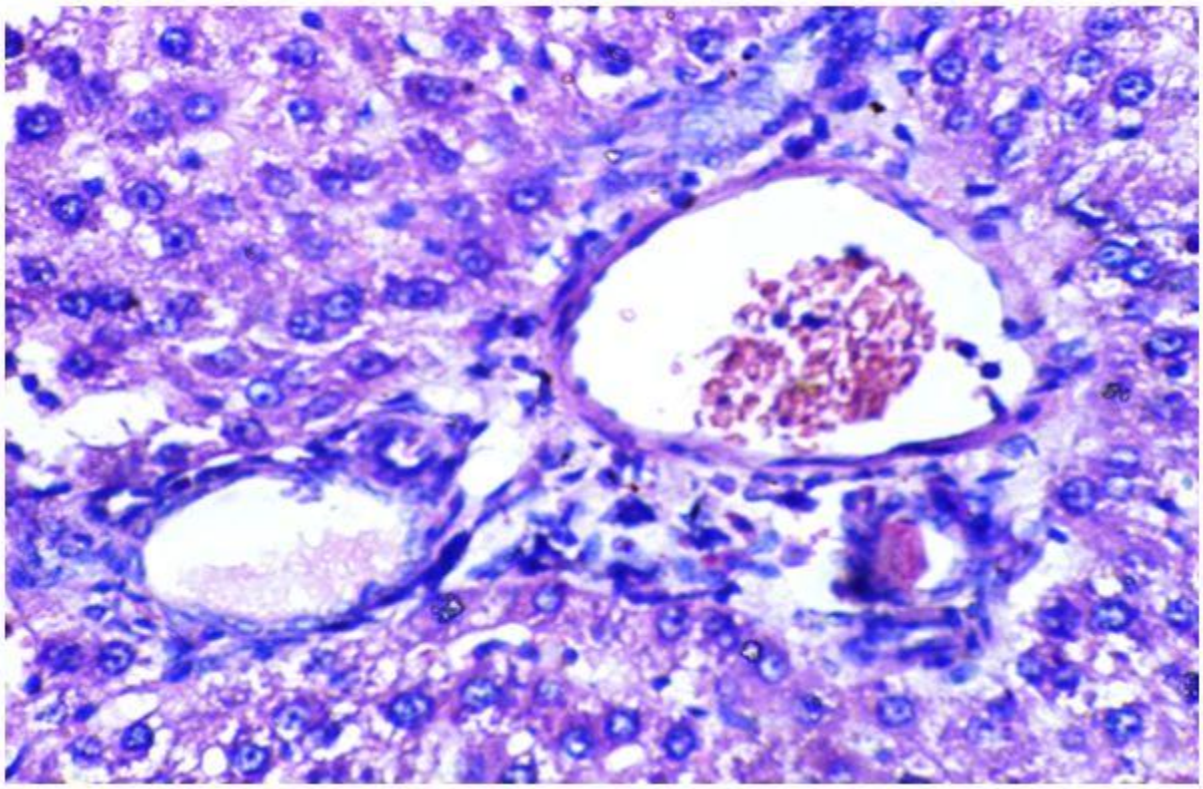

Figure 4: Showing the periportal necrosis which is not so extensive in the group V PCM /7days/i.p. PAS 400 magnification

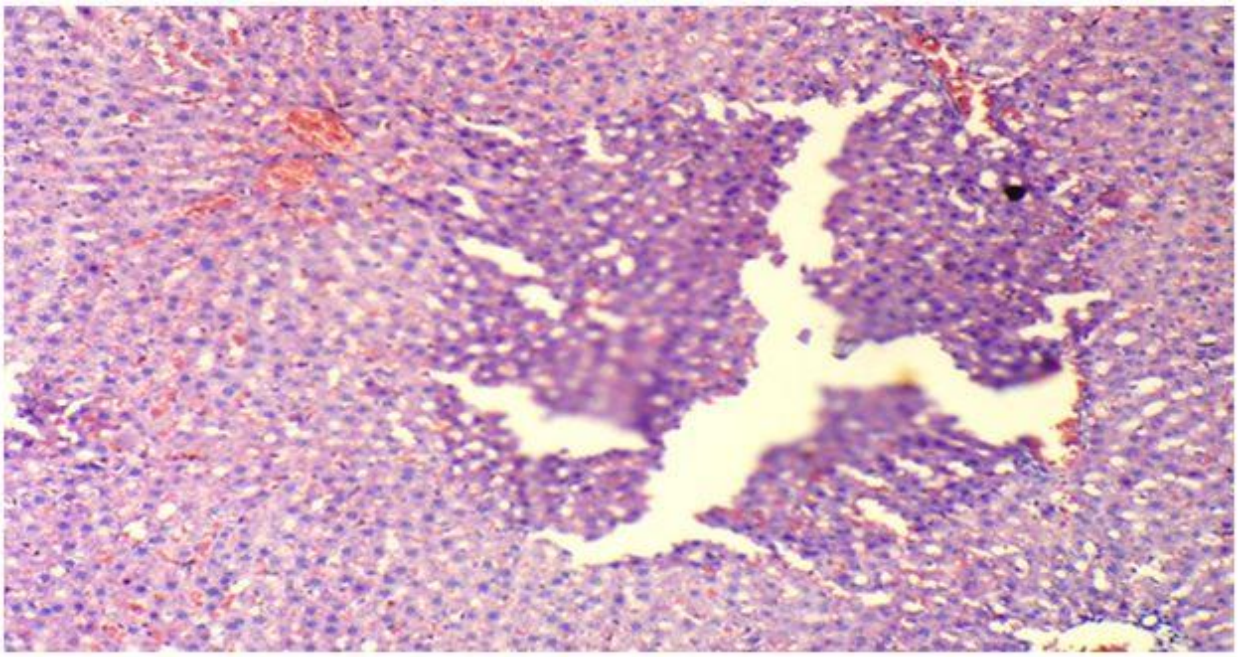

Figure 5: Showing the Extensive necrosis of the liver tissue in groupVI (pcm 14days ip) H and E staining at 100 magnifications

Histopathology findings were also in line with the serum parameters groups VI (Fig. 5) showed wide spread bridging type of necrosis with vacuoles while group III (fig 4) showed bridging type of necrosis with relatively lesser vacuole, group V (Fig. 3) showed periportal necrosis and in group II the section showed minimum necrosis evident in (Fig. 2) all these histological findings were compared with the control liver which showed normal sinusoidal pattern (Fig. 1).

Conclusion

As PCM model of induction of liver toxicity to evaluate hepatoptotective activity is being used extensively with a wide variation of dose, duration and route of administration, the present work is taken up as a part of PhD work of evaluating the hepatoprotective activity of Phyllathus niruri, to arrive at an appropriate PCM model of inducing toxicity to the rat liver. The results indicate that group III where PCM was administered at a dose of 1gm/kg/bw *p.o.* for 14 days would be appropriate, as measurable toxicity with zero mortality was observed.

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REFERENCES

Adejuwon AA and Joseph OO (2008). Protective Effect of Oral Ascorbic Acid (Vitamin C) Against Acetaminophen-Induced Hepatic Injury in Rats. *African Journal of Biomedical Research* **11** 183-190.

Arshed Iqbal Dar, Saxena RC and Bansal SK (2012). Assessment of hepatoprotective activity of fruit pulp of Feronia limonia(Linn.) against paracetamol induced hepatotoxicity in albino rats. *Scholars Research Library Journal of National Production Plant Resources* **2**(2) 226-233.

Bonkovsky HL, Kane RE, Jones DP, Galinsky RE and Banner R (1994). Acute hepatic and renal toxicity from low doses of acetaminophen in the absence of alcohol abuse or malnutrition: evidence for increased susceptibility to drug toxicity due to cardiopulmonary and renal insufficiency. *Hepatology* **19**(5) 1141.

Kursad Y *et al.*, (2007). Hepatoprotective effect of L-carnitine against acute acetaminophen toxicity in mice. *Experimental Toxicology Pathology* **59** 121-128.

Linda DF and Sanjay K (2011). Acetaminophen – Induced Fulminant Liver Failure Liver Pathology. *1st edition, Published by Demos Medical Publishing* 217-219.

Madhukiran P, Vijaya Raju A and Ganga Rao B (2012). Investigation of Hepatoprotective activity of Cyathea gigantean (Wall Hook) leaves against paracetamol-induced hepatotoxcity in rats. *Asian Pacific Journal of Tropical Biomedicine* 352-356.

Manokaran S, Jaswanth A, Sengottuvelu S, Nandhakumar J, Duraisamy R, Karthikeyan D and Mallegaswari R (2008). Hepatoprotective Activity of Aerva lanata Linn. Against Paracetamol Induced Hepatotoxicity in Rats. *Research Journal of Pharmaocology and Technology* **1** 398-400.

Prescott LF, Roscoe P, Wright N and Brown SS (1971). Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. *The Lancet* **1** (698): 519.

Sabir SM and Rocha JBT (2008). Water-extractable phytochemicals from Phyllanthus Niruri exhibit distinct invitro anti oxidant and in vivo hepatoprotective activity against paracetamol – induced liver damage in mice. *Food Chemistry* **111** 845-851.

Shah PA, Parmar MY, Thakkar VT and Gandhi TR (2009). Protective effect of Hordeum vulgare linn on acetaminophen-induced liver damage. *Journal of Young Pharmacists* **1**(4) 336-340.

Sheen CL *et al.*, (2002). *Paracetamol toxicity: epidemiology, prevention and costs to the health- care systerm. QJM: An International Journal of Medicine* **95** 609-619.
Wilkinson SP, Moodie H, Arroyo VA and William R (1977). Frequency of renal impairment in paracetamol overdose compared with other causes of acute liver damage. *Journal of Clinical Pathology* **30**(2) 144.