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Research Article

A STUDY TO EVALUATE PROPHYLACTIC HEPATOPROTECTIVE EFFECT OF PHYLLANTHUS NIRURI AGAINST THE PARACETAMOL INDUCED LIVER TOXICITY IN ALBINO RATS

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ABSTRACT
The objective behind the study ***was t***o evaluate the efficacy of *Phyllanthus niruri* (*P. niruri*) aqueous extract as a prophylactic hepatoprotective agent against paracetamol (PCM) induced liver toxicity in albino rats. Five groups of six animals in each group of rats with a weight of 180- 210 gms were the experimental material. Group I - Served as normal control, administered sodium CMC for all the eight days. Group II rats were the negative control, treated only with PCM at a dose of 2.5 gm/kg bw on 8th day. Group III animals were administered silymarin at a dose of 100mg /kg bw for 8days and PCM at a dose of 2.5 gm/kg bw on 8th day, while group IV is the treated group which was given P. NIRURI aqueous extract at a dose of 200mg/kg bw followed by PCM of 2.5gm/kg bw on 8th day. Group V rats were administered with P. NIRURI at a dose 400mg/kg bw for 8days and PCM at a dose of 2.5gm/kg bw on 8th day. Biochemical examinations included total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), and alkaline phosphatse (ALP). Histopathological evaluation of liver was done using Hematoxylin and eosin staining. The results of five groups were statistically significant. Examination of liver weight and volume showed changes. Histopathological picture is in line with the biochemical parameter changes. *Phyllanthus niruri* aqueous extract at a dose of 400mg/kg bw showed higher prophylactic hepatoprotective effect in PCM induced hepatotoxicity than P. NIRURI at 200mg/kg bw and silymarin at a dose of 100mg/kg bw.

Keywords: *Paracetamol, Phyllanthus neruri, Per Oral, Biochemical Parameters and Histological Processing*

INTRODUCTION
Liver is in continuous cellular turnover, due to occurrence of careful removal of senescent and damaged cells with simultaneous repopulation. As liver cells are exposed to portal blood which is unfiltered, they are prone to insults from gut derived, diet derived in addition to blood borne insults. In drug induced liver diseases, viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, cholestasis and vascular liver diseases, the prominent feature is apoptosis (Malhi, 2008). While in massive ischemia, oxidative stress and xenobiotics, causing liver injury necrosis occurs (Jeaschke, 2003). Hepatic adaptation is a process which compensates the deteriorating effects on liver by inducing protective mechanisms in either antioxidant pathway or anti apoptotic pathways. Traditional systems of medicine like Ayurveda have served the medical needs of people of India, people of developed countries have also used native herbs since ages and even now in 21st century these are popular. According to the WHO estimates, around 80% of the population of the developing countries relies on traditional medicine with a progressing global market value of US $ 62 billion. International agencies like WHO, ICS and APCTT have emphasized the need of evaluating these herbs using the present day scientific techniques Neraliya (2004). It is estimated that liver diseases are among the top ten killer diseases in India, causing lakhs of deaths every year, and considerable number of patients suffering from chronic liver problems, needing recurrent hospitalization and prolonged medical attention. *Phyllanthus niruri* (*P. niruri*) of euphorbiaceae family occurs as winter weed (kharif) draws researcher's interest presently for its hepatoprotective effect and studies confirming its positive role in hepatitis drug induced liver diseases. *P. niruri* is also mentioned in Ayurveda in the treatment of jaundice (kamala).The present study is to evaluate the efficacy of *P. niruri* aqueous extract as a pretreatment prophylactic drug in paracetamol induced hepatotoxcity in albino rats.

Aim of the study: To study the prophylactic effect of "*P. niruri"* on the hepatic damage induced by paracetamol on albino wistar rats and to ascertain the same with various parameters.

MATERIALS AND METHODS

Animals

Wistar albino rats (180-210gm of weight) from Animal House of Mahaveer Agencies, Hyderabad, and 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 feed throughout the period of the experiment.

Chemicals

All the chemicals used were of analytical grade, 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 semi auto-analyser of Erba Company. Standard oro-gastric cannula was used for the oral administration of drug.

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 1/40th of millilitre.

Toxicity Studies

Acute toxicity study was carried as per OECD guidelines. A maximum of 4000 mg/kg (p.o) of *P. niruri* did not cause any mortality on observation for first 24hr till the next 14 days. A maximum Safe dose hence was fixed as 400mg/kg which happens to be 1/10th of maximum safe dose and a descending 2 fold interval dose of 200mg and 400mg/kg respectively by the weight of Albino rat.

Induction of Liver Damage

Liver damage was induced by an oral administration of Paracetomal (acetaminophen) of 2.5gm/kg body weight (Mitchell, 1973). Liver detoxifies paracetamol to a limit by the process of glucuronidation and sulphation, oxidation into toxic metabolite N-acetyl-p-benzoquinone occurs by the cytochrome P450 system. This increases the values of the biochemical parameters such as SGOT, SGPT, TBIL and TPRO (Kursad, 2007; Sabir, 2008).

Aqueous Extract Phyllanthus Niruri

Acquired from GR HERBAL EXTRACTIONS with the following specifications: Phyllanthus Niruri D.E, Common Name: Bhui-Amla

Parts Used: Whole Herb, Ratio: 10:1. It is a Brown coloured powder with bitter taste with a solubility of 94.56% in water, a PH of 5.53 and a bitter percentage of 4.68% on assay. Microbiological Test is in compliance with the specifications.

Experimental Design

Wistar albino rats were the study material. The rats were randomized and divided into six groups each group containing 6 animals.

Group 1: Normal control group received 1% sodium carboxy methyl cellulose (Na.CMC) 5ml/kg bw p.o.

Group 2: Liver damage induced, by giving paracetamol (paracetamol control) 2.5gm/kg p.o single dose on 8^{th} day.

Group 3: received silymarin at a dose of 50mg /kg bw p.o. for 7 days followed by a single dose of PCM at a dose of 2.5gm/kg/p.o. on 8^{th} day after 30minutes of silymarin administration

Group 4: Prophylactic group received *P. niruri* at doses of 200mg for 7days followed by a single dose of paracetamol 2.5gm/kg bw on 8^{th} day after 30 minutes of *P. niruri* administration.

Group 5: Prophylactic group received *P. niruri* at doses of 400mg for 7days followed by a single dose of paracetamol 2.5gm/kg bw on 8^{th} day after 30 minutes of *P. niruri* administration.

Analysis of Biochemical Parameters

After 24 h of the last treatment, blood was collected from all the rats from retro-orbital plexus. Blood was allowed to clot for 1h at room temperature and serum was separated by centrifugation at 3000 rpm for 15 min. The serum was collected and analyzed for various biochemical parameters like SGOT/AST, SGPT/ALT, ALP, TB and total protein (TP).

Serum transaminase activity was measured using "Rietman and Frankel method." ALP and the serum bilirubin were determined by using "method of Scand." The TP was measured by using "method of Lowry *et al.*,

Analysis of Histopathological Changes

A 50% of rats from all the groups were left for natural regeneration. Another 50% of rats in each group were euthanized 48hrs after last dosing; the livers were isolated, cleared off blood and were immersed in 10% neutral formalin solution and allowed to fix for 1 week. The liver bits of around 5 mm thickness were dehydrated with a sequence of ethanol solutions, and embedded in paraffin. Sections of 4 microns were made using rotary microtome and staining was done with "H&E stain" accordingly and the observations in slides and the blood investigations were recorded.

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 AND DISCUSSION

Results

Significant variations were observed in weight, volume and biochemical parameters of the liver due to hepatotoxicity and hepatoprotective activity of PCM and *P. niruri*, silymarin respectively. A significant increase ($P<0.001$ when compared to normal control group) in the weight, volume, serum SGOT, SGPT, ALP, bilirubin were observed in only paracetamol treated animals (group II), while serum protein levels were decreased ($P<0.001$). In animals administered with *P. niruri* 200mg/kg, 400mg/kg, and silymarin 100mg/kg, a decrease in SGOT, SGPT, ALP, bilirubin and an increase in the total protein that followed an ascending sequence, was observed. As a consequence of the hepatic damage, production of the proteins decreased, which was evident in the decreased values of the serum protein levels in only PCM treated group, unlike as *P. niruri* and silymarin treated groups. These changes were depicted in the table 1.

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

Treatment group	Liv wt (g)	Liv vol (ml)	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	TP (IU/L)
Control	6.93±0.12	6.21± 0.09	70.65±1.10	46.47±0.84	171.3±1.303	0.55±0.01	9.15±0.17
Paracetamol	7.9±0.09***	6.91±0.07***	255.7±3.40***	134.8±1.70***	440.8±3.07***	0.99±0.05***	5.59±0.09***
Silymarin (100 mg/kg)	7.37±0.04***	6.41±0.06***	125.3±1.17***	92.67±0.61***	234.5±1.80***	0.56±0.01***	8.37±0.03***
P. niruri (200 mg/kg)	7.58±0.10***	7.18±0.04**	131.3±0.49***	94.50±0.50***	251.8±2.99***	0.66±0.009***	7.90±0.03***
P. niruri (400mg/kg)	7.24±0.03***	6.23±0.03***	114.8±1.40***	88.17±0.70***	234.0±1.46***	0.56±0.01***	8.34±0.02***

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

Histopathology: Hepatic histology of normal control group I (Figure 1) is showing normal layout of sinusoids, hepatic cells arranged in plates around the central vein , and with no congestion of blood vessels . Periportal necrosis spreading across zone 3 between two adjacent hepatic lobules is evident in

group II (only pcm administered) liver tissue (Figure 2). Decreasing extent of necrosis, congestion of blood vessels, vacuolated hepatic degeneration and fatty changes are seen in group III (sylimarin 100+Pcm) (Figure 3), group IV(PN200+Pcm) (Figure 4) and group V (PN400+Pcm) (Figure 5) respectively.

Discussion

Though paracetamol is considered to be the safest non-steroidal anti-inflammatory 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; Bonkovsky, 1994). Hence single dose paracetamol model is used to evaluate the prophylactic hepatoprotective effect of *P. niruri*. The normal levels of biochemical parameters are liver weight 6.983±0.074, volume 6.05±0.0143, SGOT 71.37±0.551, SGPT 44.82±0.25, ALP169.7±1.03, TBIL 0.52±0.055 and TPRO 9.29±0.043.

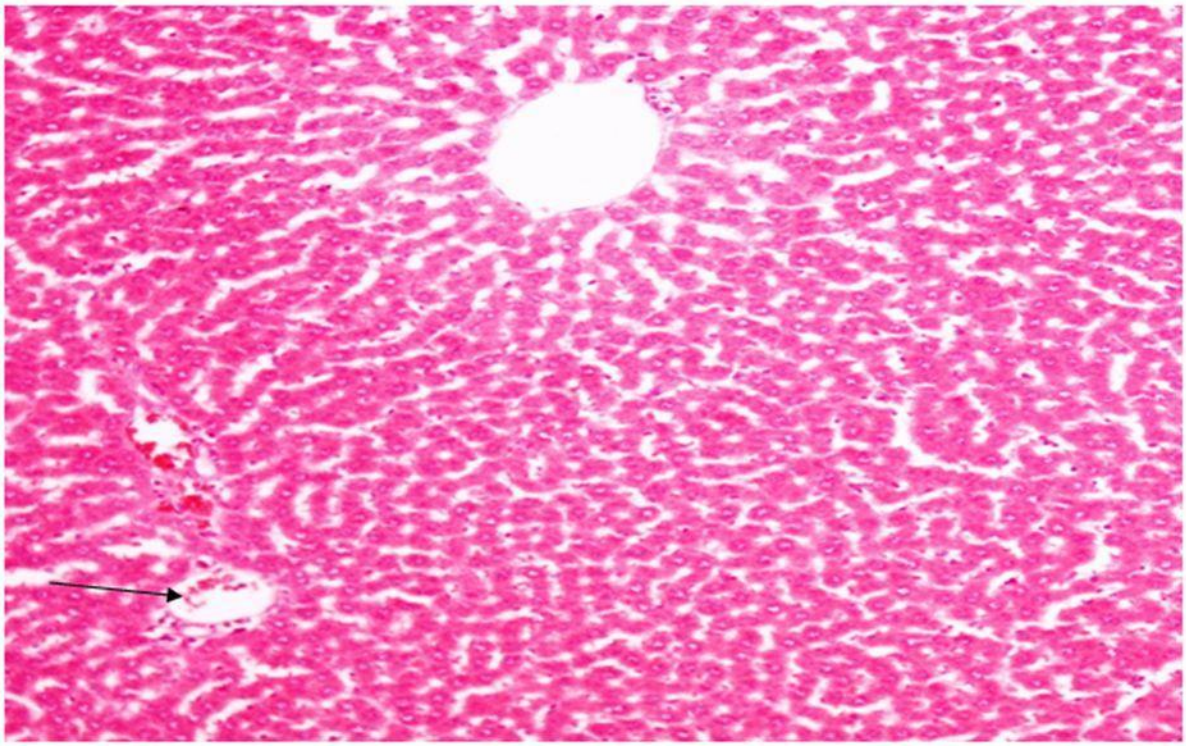

Figure 1: Showing the normal sinusoidal pattern of the liver of control group I H& E Stain X200

Acetaminophen (APAP; *N*-acetyl-*p*-aminophenol) is a very frequently used antipyretic and analgesic considered to be a safe drug at therapeutic dose. However, over dose not only causes the increase in the biochemical parameters due to hepatic damage, a resultant of incapacitation of hepatocyte cell membrane to retain the enzymes, which appear in the blood stream resulting in the elevation of the serum values (Madhukiran, 2012) but also produces a centrilobular hepatic necrosis. APAP induced toxicity occurs by initial hepatic metabolism of APAP by cytochrome P450 to the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Dahlin *et al.,* 1984). Further damage would result in the necrotic changes in the parenchyma of the liver. Except for the Serum total protein, which showed a decrease in serum values as hepatic damage resulted in the decreased production of protein, rest of all the parameters have shown significant increase.

Histology of the control group showed normal architecture. Tissues of animals treated with only paracetamol (group II) showed fatty changes and necrosis prominent in the periportal region, while group IV and V showed relatively less necrosis and inflammatory changes when compared to group III indicating that Phyllanthus niruri has a significant prophylactic hepatoprotective effect.The biochemical

markers and histopathology results are mutually supporting . The following Scientists have confirmed the hepatoprotective role of phyllanthus niruri (Mary, 2007) used nimusilide model (Micah, 2012) observed hepatoprotective activity of pn in rabbits and manjrekar (2008) verified its protective role in carbon tetra chloride induced liver damage along with other organs.

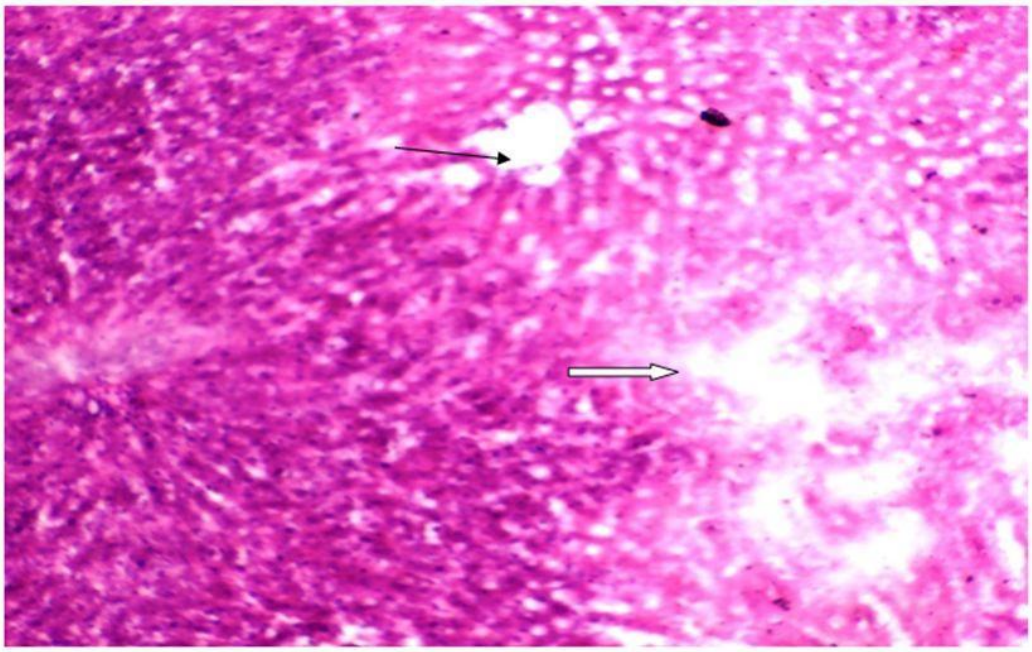

Figure 2: Showing the Extensive necrosis (white arrow) of the liver tissue group II(Only pcm at 2.5gm /bw on 8th day) H& E Stain X200

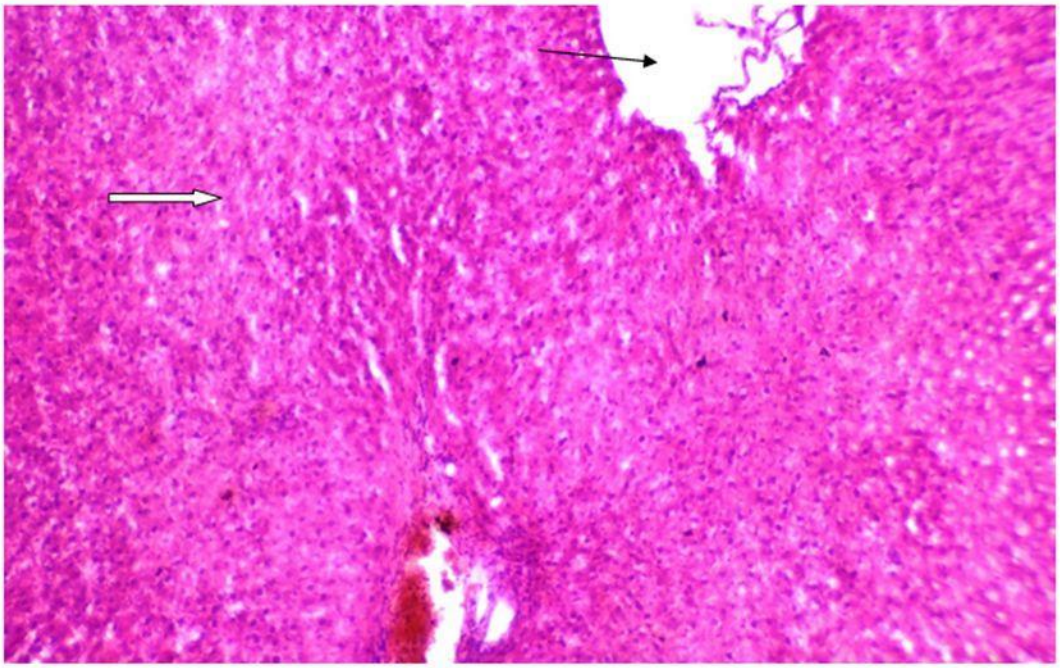

Figure3: Showing diffuse necrosis (white arrow) and steotosis congestion and dilation of blood vessels (black arrow) of the liver tissue group III(Siliymarin 100MG+ PCM at 2.5gm/bw on 8th day) H& E Stain X200

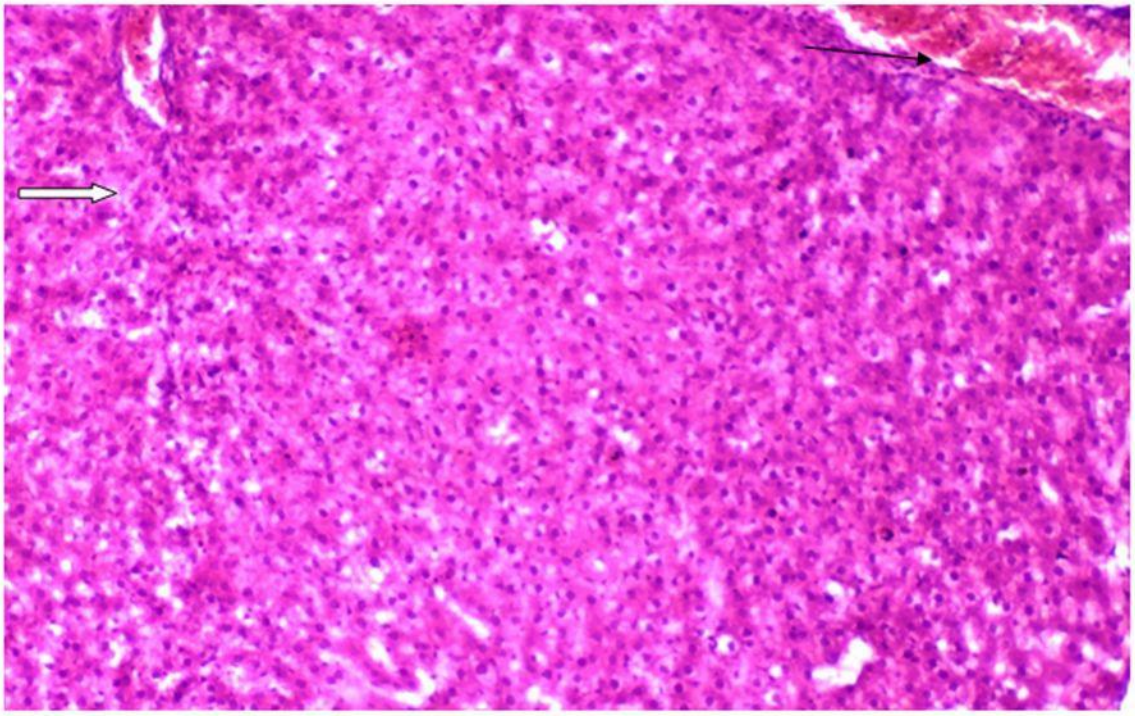

Figure 4: Showing diffuse necrosis (white arrow) and steotosis congestion blood vessels (black arrow) of the liver tissue group III(Phyllanthus niruri 200MG+ PCM at 2.5gm/bw on 8th day) H& E Stain X200

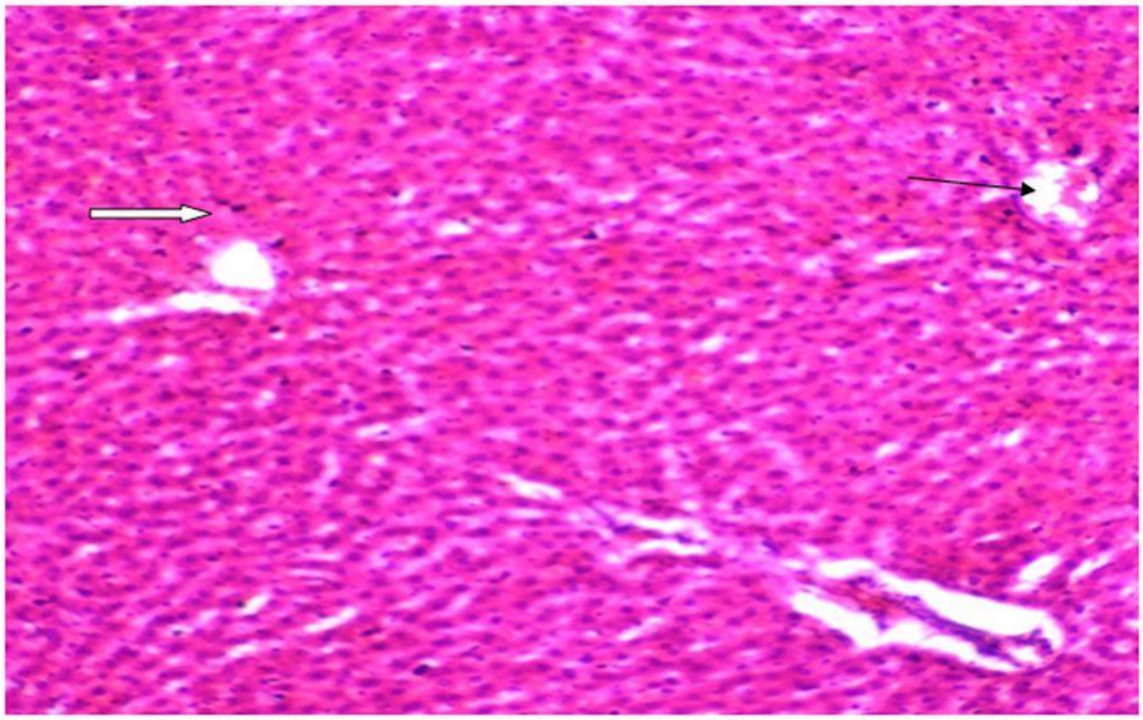

Figure5: Showing minimal necrosis (white arrow)and less congestion congestion and dilation of blood vessels (black arrow) of the liver tissue group III(Phyllanthus niruri 400+ PCM at 2.5gm/bw on 8th day) H& E Stain X200

Conclusion

The present study illustrated the hepatoprotective effect of phyllanthus niruri which is very widely available plant, this can be used as an adjuvant in any health supplement preparation to enhance the functional capabilities of the liver in general . Furthering the study, mode of action of Phyllanthus niruri has to be established by using immunohistochemistry technique. Studies to evaluate its role as an antidote in pcm poisoning and also its protective efficacy when administered concomitantly along with paracetamol in its prolonged therauptic use are to be carried out.

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