ANIMAL MODELS OF NON CIRRHOTIC PORTAL HYPERTENSION (NCPH)

[1*]Moattar R Rizvi

ABSTRACT

Portal hypertension (PHT) is a common and serious clinical syndrome often associated with chronic liver diseases. Portal hypertension may be defined as portal pressure gradients of 12 mmHg or more in the veins of the portal system caused by obstruction in the liver from intrahepatic or extrahepatic portal venous compression or occlusion (often associated with chronic liver disease), causing enlargement of the spleen and collateral veins. In the western world, hepatic cirrhosis related to chronic hepatitis C and B and alcoholic cirrhosis are the conventional rationale for the development portal hypertension. Besides cirrhosis, a number of disorders collectively called as non-cirrhotic portal hypertension (NCPH) can also result in portal hypertension. Evaluation of non-cirrhotic portal hypertension is more difficult than cirrhotic portal hypertension, both from clinical and pathological perspectives.

ملخص: ارتفاع ضغط الدم البابي هو متلازمة سريرية شائعة وخطرة وغالبا ما ترتبط مع أمراض الكبد المزمنة. ويمكن تعريف ارتفاع ضغط الدم البابي عندما يصل الضغط في اوردة النظام البابي الي 12 ملم زئبق أو أكثر والناتج عن انسداد او الضغط علي الاوردة البابية داخل الكبد (والمصاحب عادة لأمراض الكبد المزمنة) ، مما يسبب في تضخم الطحال والأوردة الجانبية. في العالم الغربي، تشمع الكبد المتصل بالتهاب الكبد المزمن C و B وتليف الكبد الناتج عن الكحول من الاسباب التقليدية لارتفاع ضغط الدم البابي. بالإضافة الى تليف الكبد، هنالك اضطرابات اخري تسمى مجتمعة باسم ارتفاع ضغط الدم المدخل الغير مرتبط بتليف الكبد يؤدي أيضا إلى ارتفاع ضغط الدم البابي. تقييم ارتفاع ضغط الدم البابي الغير مرتبط بتليف الكبد هو أكثر صعوبة من تقييم ارتفاع ضغط الدم البابي الناتج من التليف الكبدي، من وجة النظر السريرية والمرضية

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*Correspondence: rajrizvi@yahoo.com
[1]Assistant Professor, Medical Physiology, College of Applied Medical Science Al-Majmaah University, Majma'ah, Kingdom of Saudi Arabia.

INTRODUCTION

Non-cirrhotic portal fibrosis (NCPF) and extra-hepatic portal vein obstruction (EHPVO) are very common in developing countries and almost always present only with characteristic features of portal hypertension. Non-cirrhotic portal fibrosis (NCPF), the equivalent of idiopathic portal hypertension in Japan and hepatoportal sclerosis in the United States of America, is a common cause of portal hypertension in India and accounts for 15-40% [8,21,37]. Its etiopathogenesis is still obscure, as patients present late when bleeding has already occurred from the varices. The disease is common in the lower or lower–middle socio-economic strata of society. Improved hygienic standards of living could explain the relative rarity of the disease in the West and declining incidence in Japan.

Table 1: General considerations in choosing animal models (modified from Mullen & McCullough)[4]

Reproducibility:	Rate of reproducing the model should be high with the Consistent time frame to attain desired state.
Specificity:	The model should bear only the characteristic anomalies without other complicating problems.
Costs:	Consider not only the direct costs, but also indirect costs such as animal housing (and, therefore, the time to achieve the desired state). An expensive but reliable model could be cheaper than a cheap but inconsistent model.
Safety:	Animal handling should involve no risk both to the person engaged in as well as the animal.
Size:	The size should be appropriate enough to have great deal of vascular study. The size also determines drug spending.
Ethics:	Different ethics committees can have different opinions about the acceptability of one model
Feasibility:	Whether the laboratory has the expertise, manpower facilities, etc, to generated or hand the model.

EXPERIMENTAL MODEL OF PORTAL HYPERTENSION

The inability to solve the enigma of portal hypertension shoots essentially from not being able to produce the disease experimentally in animals. Animal models, mimicking the human situation, have helped us in exploring the pathophysiology of portal hypertension as ethical considerations limit experimental procedures in humans. The use of animal models as an alternative source has allowed researchers to investigate the state of disease in ways which is inaccessible in a human patient. According to the Animal Care guidelines high-degree resemblance to human condition, high reproducibility and homogeneity and a low mortality, are the basic criteria for animal model of portal hypertension (Table1).

MODELS OF INTRAHEPATIC PORTAL HYPERTENSION

Several models of intrahepatic portal hypertension in animals have been developed.. The most common are models of cirrhosis from any causes, secondary biliary cirrhosis, idiopathic portal hypertension and schistosomiasis.

Table 2: Effect of arsenic exposure on serum albumin and aspartic lactic transaminase (ALT)

Experimental details	Albuin (g/dl)	ALT (IU/l)
Control	3.40±0.06	51±27
120 ppm for 3 months	3.32±0.50	101±56*
240 ppm for 3 months	3.02±0.30	136±82*
360 ppm for 3 months	3.24±0.90	56±49
360 ppm for 6 months	3.3±0.60	183±84*
500 ppm for 1.5 months	2.72±0.20*	56±12.2

*P <0.05; Values are expressed as mean ± SE

Table 4: Effect of oral arsenic feeding on liver histopathology of mice of different groups

Group (ppm)	Duration (months)	Fibrosis (%)	Inflammation (%)	Kupffer cell hyperplasia (%)	Normal (%)
Control	---	---	---	---	100
120	1.5	---	25	42	29
120	3.0	---	18	---	82
120	6.0	---	25	---	75
240	1.5	---	44	---	56
240	3.0	---	25	38	37
360	1.5	---	40	13	47
360	3.0	---	44	---	56
360	6.0	---	40	---	60
500	1.5	30	23	---	46

Whole liver compression – Dog model: Yamana et al[38] produced a unique model of whole liver compression in dogs where whole of the liver was wrapped and compressed with a tense ligature of polypropylene mesh or gauze. Both the intrahepatic resistance and portal venous pressure were raised without development of hepatorenal collaterals for nine weeks after the surgery. Since this whole liver compression method requires no complicated surgical maneuver, the experimental animals survived well.

Cirrhosis induced by carbon-tetrachloride (CCl_4): Currently, the carbon-tetrachloride (CCl_4) hepatotoxin-induced model of cirrhosis, mimicking human non-biliary cirrhosis, is considered the 'gold standard' for cirrhosis [22]. However, it is associated with major drawbacks such as low reproducibility resulting from a mortality rate averaging 30% during induction, poor homogeneity and a rather low resemblance to human cirrhosis, as typical features of human cirrhosis, such as nuclear atypia, mitoses and nodular parenchymal regeneration, resulting in a more typical distorted architecture, are not so prominent. Additionally, inhalation with CCl_4 seems to be the sole way of achieving a high yield of cirrhosis, which might pose potential health hazards for its investigator[6,7,22].

Secondary billiary cirrhosis induced by bile duct ligation: Chronic cholestasis produces secondary biliary cirrhosis in animals as it does in man. The chronic bile duct-ligated rat model (CBDL) is commonly used model of PHT [6]. This surgically induced model of cirrhosis can be used after 3–5 weeks and is therefore less costly than a hepatotoxin-induced liver damage model[10]. There are, however, several disadvantages: high mortality (> 40%), haemodynamic instability owing to the toxic effect of the cholestasis on renal function, and higher susceptibility for sepsis owing to the absence of bile in the digestive tract, exaggerated dilatation of the extrahepatic bile duct causing extrinsic compression on portal vascular structures, and the fact that CBDL rats do not show elevated portal venous inflow when studied under pentobarbital anaesthesia [6, 22].

Table:3 Collagen and 4-hydroxyproline (4-HP) levels in liver following different dosage of arsenic at 1.5, 3 and 6 months of treatment.

Duration (months)	Experi-mental group (ppm)	Hepatic collagen (µg/mg protein)	Hepatic 4-HP (µg protein)
1.5	Control	11.3±1.47	23.5±4.36
	120	24.3±0.71[a]	102.8±6.50[c]
	240	13.6±1.60	152.0±18.80[c]
	360	17.7±1.30[a]	70.5±1.30[b]
	500	87.8±8.90c	132.5±9.81[c]
3	Control	12.0±1.11	22.3±4.00
	120	26.4±2.90[a]	320.0±15.10[c]
	240	63.1±7.00[c]	193.0±11.80[c]
	360	71.8±5.80[c]	200.0±10.10[c]
6	Control	14.1±2.50	29.9±5.50
	120	595.4±130.0[a]	242.0±79.80[a]
	240	397.7±129.4[a]	164.8±9.80[a]
	360	159.3±3.90[a]	373.0±14.40[a]

P values: a <0.05; b<0.01; c<0.001; Values are expressed as mean ± SE

Table 5 Liver function Tests in Control and Arsenic-Exposed Mice at the End of Different Study Periods

Month	Group	T. Protein (g/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
3	Control (n = 5)	6.46±0.34	3.24±0.35	22.80±2.77	23.40±4.44	107.6±5.02
	Experimental (n = 6)	6.23±0.46	3.08±0.29	23.83±2.99	24.10±4.57	109.8±6.99
6	Control (n = 10)	6.58±0.58	3.42±0.45	24.30±3.80	23.50±2.27	108.6±8.03
	Experimental (n = 10)	6.50±0.73	3.17±0.48	26.30±4.49	26.00±5.29	111.7±5.83
9	Control (n = 5)	6.52±0.54	3.40±0.60	22.80±2.78	22.60±1.67	113.4±8.64
	Experimental (n = 5)	6.40±1.32	3.08±0.68	31.20±9.65	26.80±4.29	116.8±11.62
12	Control (n = 9)	6.36±0.91	3.06±0.45	25.00±4.27	23.50±2.78	121.5±6.8
	Experimental (n = 14)	6.66±0.86	3.03±0.61	34.60±6.93**	32.80±8.21*	128.4±12.02
15	Control (n = 8)	6.05±0.26	3.11±0.12	27.50±4.10	25.50+6.63	123.7±5.02
	Experimental (n = 6)	5.95±0.32	2.71±0.24*	40.80±5.60**	38.16±6.79*	164.6±10.94**

*p<0.01; **p<0.001

Cirrhosis induced by hepatotoxin thioacetamide (TAA): A potential alternative to these above-mentioned problems is the induction of cirrhosis in the rat with the hepatotoxin thioacetamide (TAA), which is suggested to have more in common with human cirrhosis[1]. To date the problem with TAA has been the heterogeneous production of cirrhosis (varying from 3 to 7 months [28]) when administered orally. To overcome these inconveniences, Li et al.[23] recently suggested an intoxication protocol over 12 weeks which adapts the dose of TAA in drinking water according to the weekly body weight, as originally described for intragastric administration of CCl_4 by Proctor[29]. W. Laleman in 2006 concluded that thioacetamide, adapt to weekly weight changes, leads to a homogenous, reproducible model of cirrhosis in the rat in 18 weeks, which is associated with all the typical characteristics of portal hypertension, including endothelial dysfunction[36].

Periportal fibrosis: Other cirrhosis or precirrhosis induced by alcohol have been described in the literature. It has been shown that portal hypertension develops in alcohol-fed baboons[24]. These baboons developed fatty liver and perivenular fibrosis. Cirrhosis and portal hypertension also developed in some monkeys with a

Table 6: Hepatic Glutathione and other Enzymes of the Antioxidant Defense system in Control and Arsenic-Exposed Mice at the End of Different Study Periods

Month	Group	G6PDH (nmole NADP reduced/min/ mg/protein	GR (umole of NADPH oxidation/min /mg protein)	GST (nmole produced/min/ mg protein)	Catalase (umole H202 reduced/min/mg protein	GSH-Px (umole NADPH oxidation/min /mg protein)
3	Control (n = 5)	10.32±0.230	24.38+0.89	118.02±1.12	6.78±0.22	8.28±0.38
	Experimental (n = 6)	11.04±0.56	27.39±2.66	130.05±11.04**	7.26±0.59	9.19±0.67^
6	Control (n = 10)	10.46±0.84	24.92±0.37	118.42±2.04	6.68±0.33	8.27±0.28
	Experimental (n = 10)	8.39±0.62#	25.43±1.31	121.85±7.30	6.34±1.05	7.49±0.94^
9	Control (n = 5)	10.05±0.41	24.99±0.83	117.41±1.87	6.78±0.27	8.29±0.20
	Experimental (n = 5)	7.02±0.29**	24.24±2.21	113.92±4.70	6.06±0.48*	7.01±0.61#
12	Control (n = 9)	9.80±0.25	25.04±0.44	117.73±2.24	6.74±0.29	8.03±0.24
	Experimental (n = 14)	6.39±0.64**	20.86±1.88**	102.68±6.90**	5.45±0.50**	5.95±1.01**
15	Control (n = 8)	9.94±0.26	24.93±0.79	122.28±4.22	6.47±0.26	8.02±0.55
	Experimental (n = 6)	5.02±0.87**	18.43±2.02**	88.72±18.0**	4.55±0.85**	5.20±1.12**

*p<0.05; ^p<0.02; #p<0.01; **p<0.001

diet for a period of 16 months lacking choline, low in protein (5%), and rich in cholesterol. In dogs it has been demonstrated that the repeated intraportal injection of a polyvinyl alcohol suspension over a 2-6 months period produces portal hypertension [2].

MODELS OF EXTRAHEPATIC PORTAL HYPERTENSION:

Portal Vein Stenosis Model: The most common animal model of prehepatic portal hypertension currently used is partial portal vein ligation. This model has been developed in the rats [4,5,35], mice [9, 14] and rabbits[3]. Neuhof in 1912 first consummate the production of portal hypertension in animals by partial constriction of the portal vein. This study was carried out in dog with the degree of constriction of portal vein up to 50%. After a period of recovery, the portal vein was further stenosed to 25% of its original diameter and finally after 6 days the portal vein was completely ligated. Neuhof clearly described anastomoses between the hepatic and diaphragmatic vessels, the gastric and esophageal veins, and the portal system and vena cava in the postmortem report at 34 days. Splenomegaly was also present but the remaining portal tract appeared to be normal. Later, Reynell stenosed the portal vein in rats with a 50% mortality rate[30]. Most of the surviving rats had portal hypertension. Between 1973-75 it was shown that the diameter of the stenosis and the body weight or age of the rat were critical in determining survival[25].

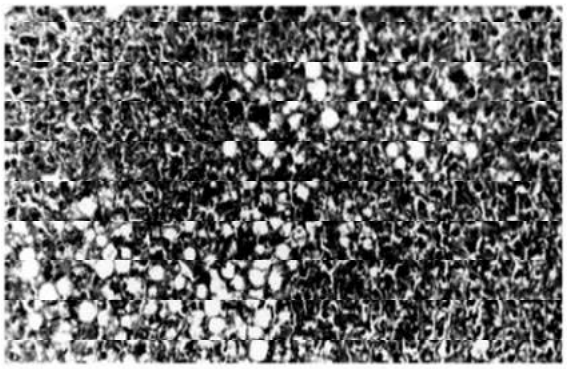


Fig 1: Liver histology of mice exposed to arsenic for 12 months. The histology shows fatty changes in the liver (hematoxylin-eosin, magnification X 100).

Portal vein occlusion using microspheres: A new experimental animal model for portal hypertension was developed by an intraportal injection of DEAE-cross-linked dextran microspheres (100±25 microns in diameter) in the female Japanese white rabbit characterized by elevation in portal pressure and portal systemic collateral (Komeichi H, 1991). Histology of the liver revealed portal obstruction by the injected microspheres in almost all portal triads, resulting in a foreign body granuloma. Since this model appears to show the two main conditions characteristic of portal hypertension, persistent elevation of portal pressure and both extra- and intrahepatic portal collaterals, mimicking those in humans, portal obstruction by injecting DEAE-cross-linked dextran microspheres into the portal vein of the rabbit could provide a versatile model for portal hypertension.

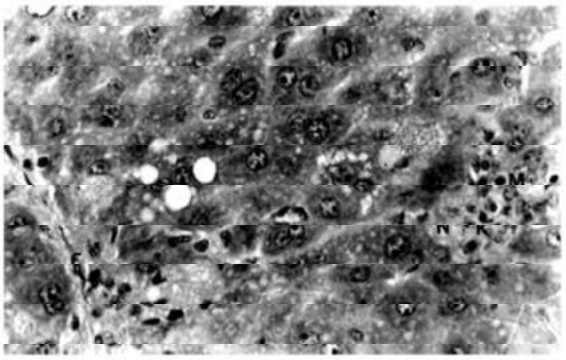


Fig 2. Liver histology of mice exposed to arsenic for 15 months. The histology shows hepatocellular degeneration and necrosis characterized by collections of mononuclear cells and Kupffer cells associated with injured hepatocytes. Streaky fibrosis is seen at one end of the liver lobule (hematoxylin-eosin, magnification X 400)

MODELS OF PRESINUSOIDAL PORTAL HYPERTENSION:

The exact mechanism for the development of idiopathic portal hypertension has not been clarified but the pathomorphological changes caused by this syndrome have been widely studied in animals. These lesions have been attributed to intra-abdominal infection.

MODELS OF IDIOPATHIC PORTAL HYPERTENSION (IPH):

Prolonged sensitization with egg albumin: Okabayashi and Suzuki produced idiopathic portal hypertensive models in rabbits characterized by splenomegaly and portal hypertension. They emphasized chronic antigenic stimulation as being the cause of splenomegaly with prolonged intravenous administration of egg albumin. Later, the experiment was conducted on dogs as the rabbit could not tolerate more than three intraportal injections. However, they could not provide the mechanism whereby the spleen reacts to chronic antigenic stimulation. The histological changes that occurred in these animals were characterized by early portal inflammation immediately followed by portal fibrosis, aberrant vasculature and disappearance of portal venules and were very similar to those in human IPH.

Prolonged sensitization with non-pathogenic E. coli: In rabbits, killed nonpathogenic E. coli were administered intraportally. The animals that received an intraportal mixture of killed E. coli and rabbit antiserum (aggregated E. coli) developed histological changes in the liver and portal hypertension[19]. Early inflammatory reactions in the portal area and parenchyma were followed by the development of portal fibrosis. Three intraportal challenges with aggregated E. coli were enough to produce marked portal fibrosis, splenomegaly, and portal hypertension. In dogs, repeated intraportal injections of a mixture of killed nonpathogenic E. coli and dog anti-E. coli serum induced portal fibrosis and intrahepatic presinusoidal portal hypertension[33].
However, these investigators have used repeated cannulation of the portal vein which may itself cause damage to the portal vein intima, portal pyemia and altered hemodynamic and histological picture in the animal. Another limitation of the model is the use of E. coli and anti-E. coli aggregate, which is not only unphysiological, but also the large aggregate can block the hepatic sinusoid causing a situation akin to portal vein thrombosis. Accordingly, alternative routes of introducing E. coli into the portal circulation have been proposed[17].

Schistosomiasis japonica models: Since schistosomiasis japonica and idiopathic portal hypertension share similar histological changes of the liver, Masayoshi K et al produced rabbit model by infecting with 200-300 Schistosoma cercariae percutaneously and subcutaneously. The angioarchitecture of chronic schistosomiasis japonica is characterized by narrowing, obstruction and obtuse angles of bifurcation of the peripheral portal veins and this disease is quite similar to IPH in both histological and angioarchitecture strongly suggest that portal change is the primary lesion of the hepatic disorder in IPH. However, splenomegaly invariably noted in IPH is not necessarily observed in chronic schistosomiasis japonica, suggesting that the portal system may be more extensive in IPH than in schistosomiasis japonica.

MODELS OF NON-CIRRHOTIC PORTAL HYPERTENSION (NCPH)

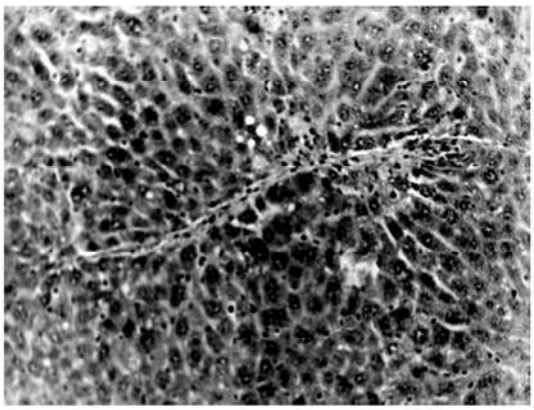


Fig 3: Liver histology of mice exposed to arsenic for 15 months. The histology shows streaky fibrosis in the liver (hematoxylin-eosin, original magnification x 100).

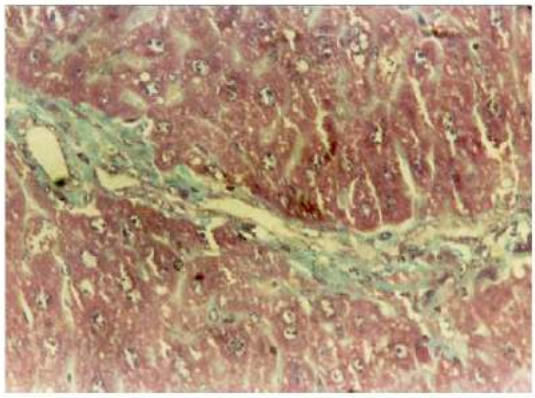


Fig 5: Liver histology of mice exposed to arsenic for 15 months. The histology shows mature collagen deposition spreading from the portal tracts through the liver lobules (Masson Trichrome x 400).

Chronic arsenic ingestion: Despite the establishment of an association of non-cirrhotic portal fibrosis with drinking of arsenic-contaminated water in several districts of West Bengal, India, elucidation of the mechanism of this disorder has remained conjectural. Chronic arsenic toxicity is a form of hepatic fibrosis that causes portal hypertension, but does not progress to cirrhosis. Hepatotoxic effects of arsenic in humans have been reported [14,26,32,34]. Injury to intrahepatic portal vein and even development of cirrhosis have been alleged to occur with prolonged usage of Fowler's solution containing sodium arsenite.

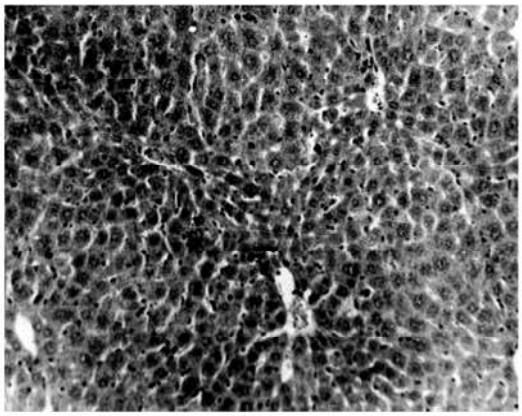


Fig 4 Normal liver histology of control mice at the end of 15 months of feeding As-free water (hematoxylin-eosin, magnification x 100).

Sarin SK et al in 1999 produced a reproducible and homogenous murine model of hepatic fibrogenesis and fibrosis without significant hepatocellular necrosis and inflammation through chronic arsenic feeding (Table 2). In addition they investigated the fibrogenic potential of chronic ingestion of different dosages of arsenic in mice. The study pointed that fibrogenesis could be induced within six weeks by arsenic. There was relatively more fibrosis with extended arsenic exposure associated with increased hepatic collagen deposition much more than hydroxyproline levels (Table 3-4). This fact was also supported by the histopathological studies which showed that the fibrosis and collagen deposition was more at 6 months. This result was specially obtained with arsenic dosage of 120 ppm. The fibrogenesis and fibrosis produced with this dosage at 6 months was near maximum that could be produced by feeding arsenic to mice. The mortality rate with 120 ppm dosage was near to the ground. Higher dosages of arsenic given for a short period of 6 weeks did enhance collagen synthesis and deposition; but there was no dose dependent increase with prolonged exposure.

The main conclusions of the study were: (i) prolonged oral arsenic ingestion in mice leads to significant hepatic fibrogenesis

and collagen synthesis with minimal hepato-cellular injury; (ii) arsenic ingestion alone is unlikely to cause non-cirrhotic portal fibrosis or cirrhosis of liver. This murine model of arsenic feeding could be used for the evaluation of new antifibrotic agents for the liver.

Guha Mazumdar et al described in a population from West Bengal that chronic arsenic toxicity leads to the development of hepatic fibrosis with portal hypertension in the absence of cirrhosis [12,13]. The group also demonstrated for the first time hepatic fibrosis due to long-term arsenic toxicity in a murine model of NCPF. In this study the mice received drinking water contaminated with arsenic (3.2 mg/L) or arsenic-free (0.01 mg/L, control) *ad libitum* till 3, 6, 9, 12, and 15 months. The result demonstrated that after 12 months of arsenic feeding, the liver weights increased significantly consistent with raised ALT and AST (Table 5). Arsenic feeding after 6 months showed significant decrease in hepatic glutathione and the enzymes glucose-6-phosphate dehydrogenase and glutathione peroxidase than the control group. Also, the plasma membrane Na^+/K^+ ATPase activity was reduced while lipid peroxidation increased significantly after 6 months of arsenic feeding. Hepatic catalase activity was significantly reduced at 9 months in the arsenic-fed group, while glutathione-S-transferase and glutathione reductase activities were also significantly reduced at 12 and 15 months (Table 6). Histopathology of the liver (Fig 1-5) remained normal for the initial 9 months, but showed fatty infiltration after 12 months of arsenic feeding. Fibrosis was only evident after 15 months. The main findings of the study were (i) long term arsenic toxicity produced hepatic fibrosis in murine model. (ii) Initial biochemical evidence of hepatic membrane damage, probably due to reduction of glutathione and antioxidant enzymes, may be seen by 6 months. (iii) Continued arsenic feeding resulted in fatty liver with serum aminotransferase and alanine aminotransferase elevated at 12 months and hepatic fibrosis at 15 months.

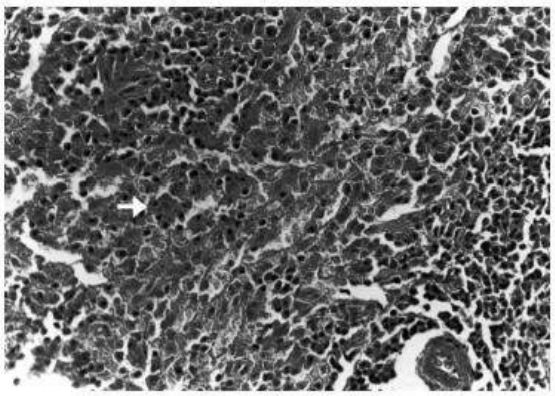


Fig 6: Photomicrograph showing a section from spleen with medullary congestion and (→) hemosidrin pigment (H&E x 160).

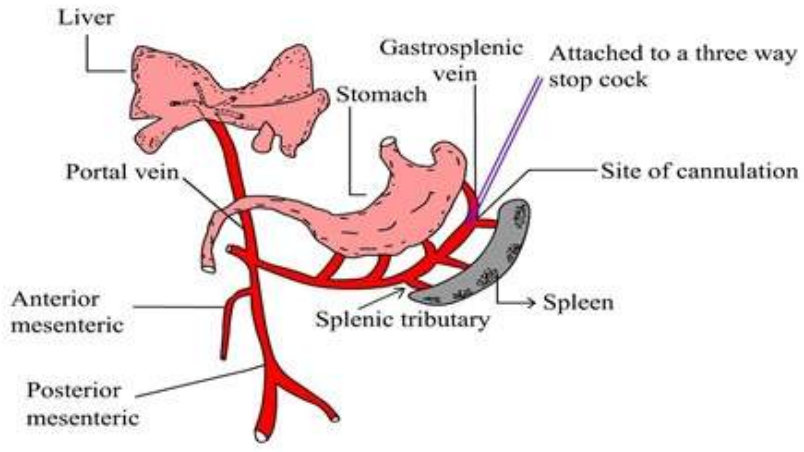


Fig 7: Indwelling cannula placed in gastrosplenic[27]

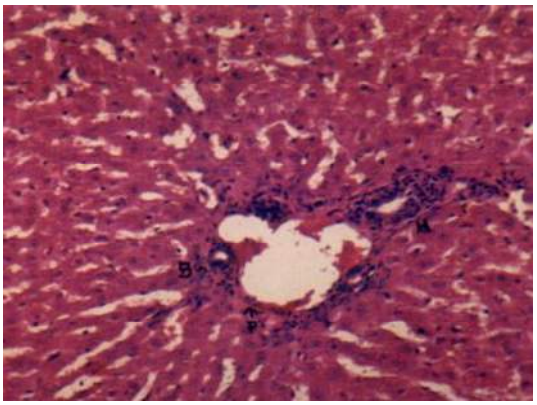


Fig 8: Photomicrograph of a section of liver showing normal hepatic parenchyma in NCPF[27]

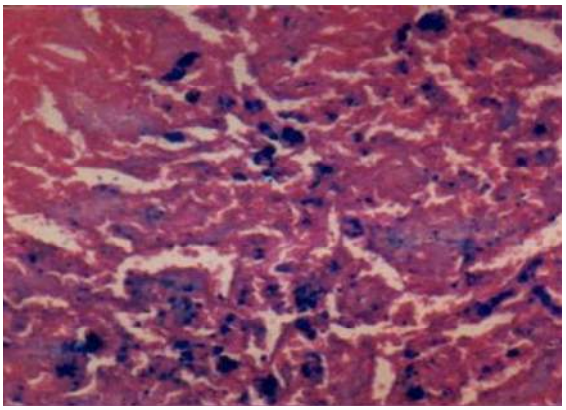


Fig 9: Photomicrograph showing a section from spleen with medullary congestion and hemosidrin pigment[27]

Repeated immunosensitization by rabbit splenic extract: A rabbit model of NCPF was developed by an intramuscular injection of splenic extract[15]. Six milligrams of the splenic protein thus prepared was mixed with Freund's complete adjuvant in a 1:1 ratio and injected intramuscularly into the experimental rabbits every 2weeks for 3 months. The control group of animals was sensitized by normal saline mixed with Freund's complete adjuvant in an equal ratio in the same manner. This animal model showed significant splenomegaly at one (0.63±0.19 vs 0.23±0.04 g; $p<0.05$), three (0.73±0.24 vs 0.38±0.10 g; $p<0.05$) and six (0.51±0.17 vs 0.23±0.04 g; $p<0.05$)

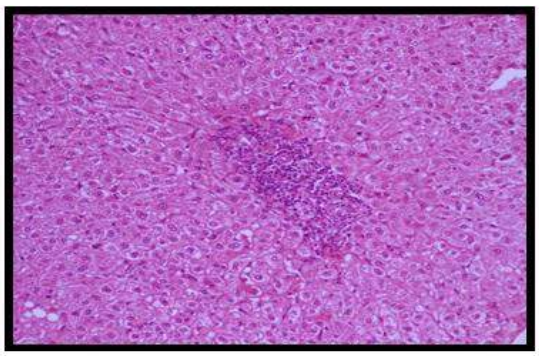


Fig 10: Small portal vein-obstructed with mild portal inflammation (+), H&E

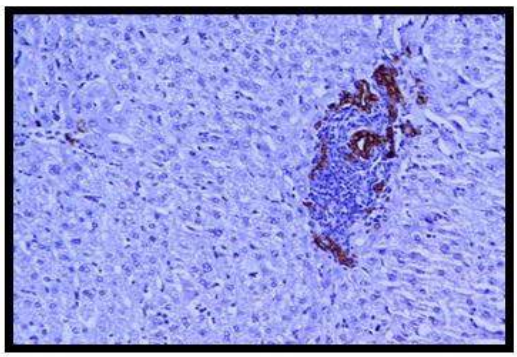


Fig 11: Small portal vein obstructed with mild portal inflammation (+), CK7, immunohistochemistry (IHC)

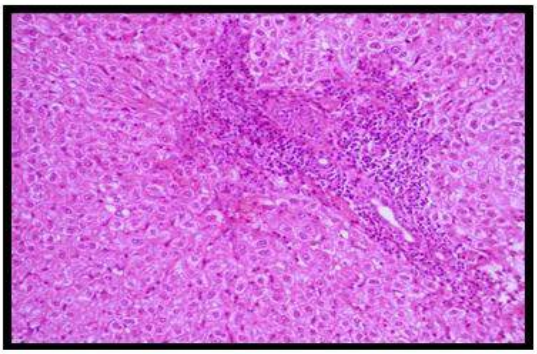


Fig 12: Medium portal vein with moderated portal inflammation (++), & compromised portal vein lumen,,H&E

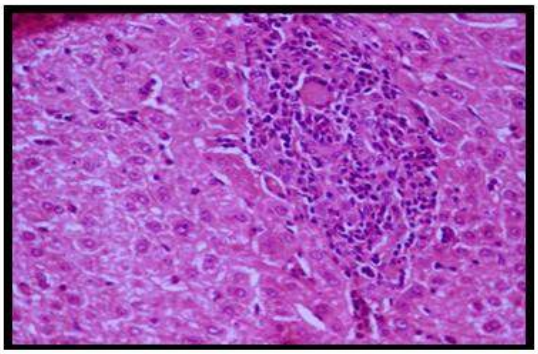


Fig 13: Small portal vein-obstructed with portal inflammation and giant cell, H&E

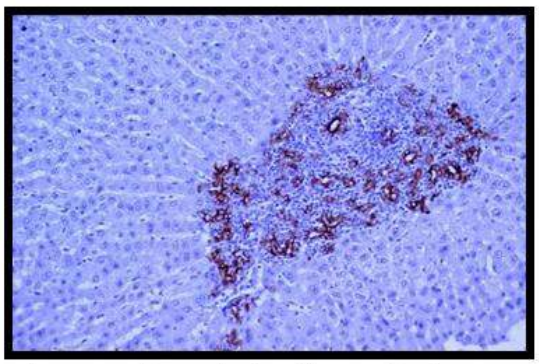


Fig 18: Small portal vein with completely obstructed portal vein showing Bile Duct proliferation

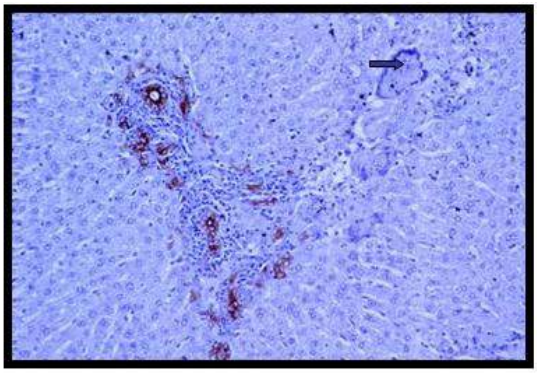


Fig 19: Portal tract with moderated portal inflammation (++) and giant cell (arrow)

with persistent rise in portal pressure at one (19.4±2.9 *vs* 10.4±2.2 mmHg; $p<0.05$), three (16.7±1.1 *vs* 7.2±3.6 mmHg; $p<0.05$), and six (20.3±5.4 *vs* 10.3±4.8 mmHg; $p<0.05$) months without hepatic parenchymal injury, quite akin to NCPF seen in humans. The histological examination of the liver specimen from NCPF rabbits showed mild portal and lobular inflammation, along with Kupffer cell hyperplasia. The major histological changes observed in the spleen from NCPF rabbits were fibrocongestive sp lenomegaly, that is, medullary congestion, thick-walled vessels and hemosidrin-laden macrophages (Fig 6). This study also proposes that repeated immunostimulation may have an important role in the pathogenesis of NCPF.

Repeated low dose endotoxinemia of portal circulation: Portal pyelephlebitis due to repeated abdominal infections and thrombosis in the portal circulation lead to obstruction of small and middle branches of portal vein and development of NCPF. Based on this hypothesis, Omanwar S et al[27] developed an animal model of NCPF by repeated low dose endotoxemia by injecting E. coli (heat killed) into the portal system of the animal through an indwelling cannula (placed in gastrosplenic vein) to understand the etiology and pathophysiology of this disease. Heat killed *E. coli* (LPS, 4 mg/kg b. wt) was injected through an indwelling cannula into the gastrosplenic vein (Fig 7) in pre-sensitized (1.5 mg E. coli protein/kg b. wt + Freund's complete adjuvant; 1:1 ratio; IM) rabbits. The control group of rabbits received normal saline in the same manner. The mean portal pressure in NCPF rabbits was significantly ($p<0.05$) higher compared to the control group at one (17.5 ± 3.4 Vs 10.4 ± 2.2 mmHg), three (17.8 ± 1.3 vs 7.2±3.6 mm Hg), and at 6 (19.8 ± 3.1 vs. 10.3 ± 4.8 mmHg) months. Similarly, the splenic weight in NCPF rabbits was significantly

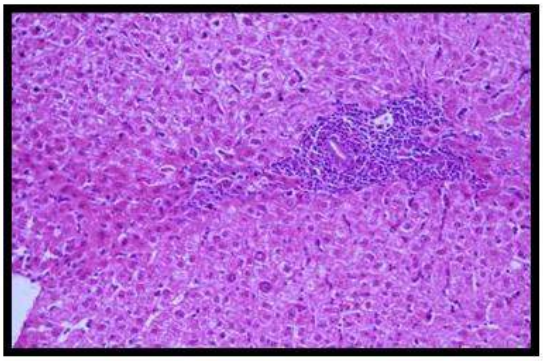


Fig 16: Medium portal vein with small lumen showing mild portal inflammation

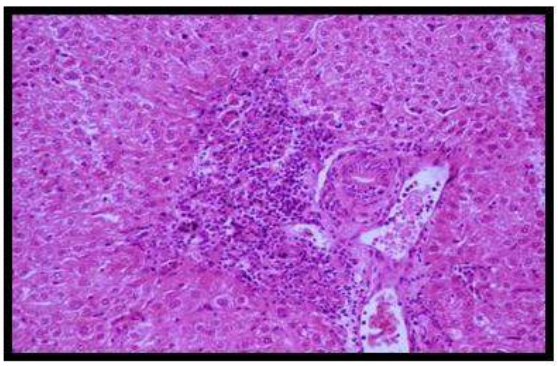


Fig 17: Medium portal vein with normal lumen showing mild portal inflammation

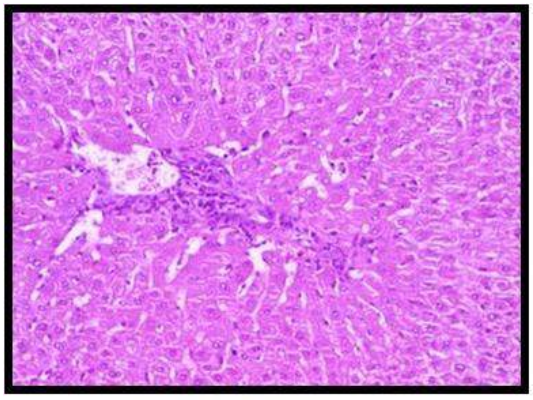


Fig 22: Dilated PV with dilated sinusoids (H&E)

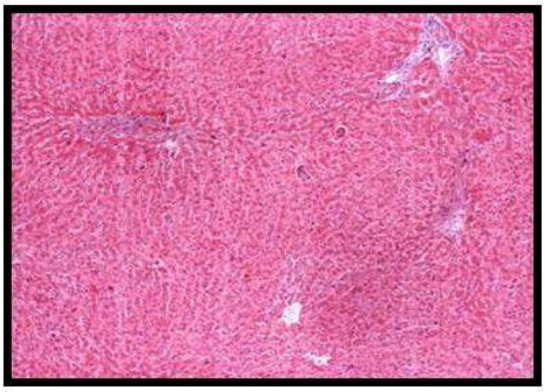


Fig 23: No significant fibrosis

($p<0.05$) greater than the control rabbits at one, three and six months. Absence of hepatic parenchymal injury and persistently elevated portal pressure makes this model ideal to investigate the vascular reactivity to various agents (Fig 8-9).

More recently, Sakhuja P et al[31] studied the liver histology in rabbit model of NCPF (induced by injecting LPS) especially with respect to the changes in portal veins. Five micron thick sections were stained with routine H & E and with Masson's Trichrome stain. Immunohistochemical staining with antibodies to Cytokeratin 7 using DAB as chromogen was also performed in all cases to identify bile ducts and portal tracts. Portal tracts (PT) were divided into small, medium and large based on size of accompanying bile duct. Portal veins (PV) in each PT were counted as normal if lumen was seen, or obstructed if PV was not identified or completely obscured by inflammatory cells. Number of obstructed Portal veins was then expressed as a percentage of total PTs. The model was characterized with splenomegaly and portal hypertension with normal liver function tests. Obstructed PVs were seen in 25-80% (mean = 54.12%) of small PTs in the experimental group in comparison to 15 – 26% (mean = 19.75%) in the control group. 5 of 8 rabbits in the experimental group showed greater than 50% obstructed PVs in the small PTs. Several PTs showed mononuclear inflammation with accompanying giant cells and granulomatous reaction causing obstruction to portal veins. Similar inflammation was observed focally in the lobular parenchyma. One animal showed active granulomatous inflammation in the medium sized portal veins. No significant fibrosis or cirrhosis was seen in any case. No significant inflammation or fibrosis was observed in the control group. The main conclusion of the study was that E.Coli induced portal hypertension is associated with obstructive

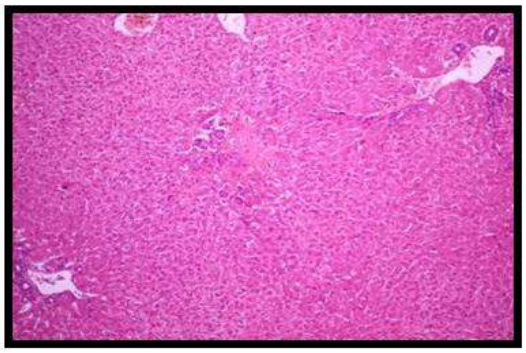


Fig 20: Dilated PV with dilated sinusoids and focal LI with giant cells (H&E)

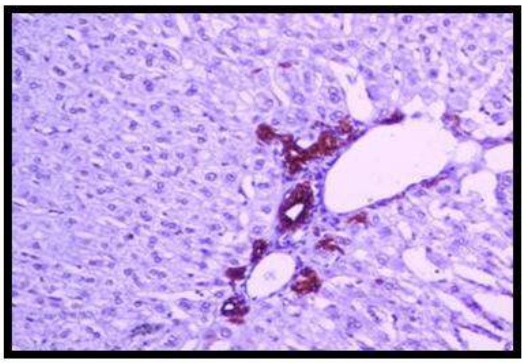


Fig 21: Dilated PV with dilated sinusoids (CK7, IHC)

venopathy in small portal veins, without accompanying fibrosis[31].

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