

**CHRONIC TOXICITY OF RAW LYON'S BEAN (*Mucuna
cochinchinensis*) IN BROILERS**

**[TOXICIDAD CRÓNICA DEL FRIJOL LYON (*Mucuna
cochinchinensis*) POLLOS DE ENGORDA]**

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SUMMARY

One hundred and fifty 3-week old broilers were subjected to long period of feeding on diets containing raw Lyon's bean (*Mucuna cochinchinensis*) with the aim to studying the toxicity of the bean on blood profile, carcass characteristics and pathological changes. Five isocaloric and isonitrogenous diets were formulated to contain 0, 2, 4, 8 and 16g/kg (that is, g *Mucuna* per kg feed) and fed to the birds for 12 weeks. Inclusion of *Mucuna* beans in broiler diets caused significant ($P<0.01$) reduction of RBC, PCV and Hb. On carcass characteristics, only the liver was affected by the dietary treatments. The liver weight was significantly ($P<0.05$) depressed at first slaughter (3rd week). Also, liver sections of birds fed *Mucuna* diets at all levels of inclusion consistently showed pathological lesions at all stages of slaughter.

Key words: *Mucuna cochinchinensis*, chronic toxicity, broilers, haemoglobin, packed cell volume, red blood cell.

RESUMEN

Ciento cincuenta pollos de engorda de 3 semanas de edad fueron empleados en una prueba de alimentación de largo plazo con dietas conteniendo *Mucuna cochinchinensis* cruda con el fin de estudiar la toxicidad de este frijol en el perfil sanguíneo, características de la canal y cambios patológicos. Se formularon cinco dietas isocalóricas e isonitrogenadas que contenían 0, 2, 4, 8 y 16 g/kg (g *Mucuna* por kg alimento) y se alimentaron las aves por 12 semanas. La inclusión de *Mucuna* en la dieta de los pollos de engorda causó una reducción ($P<0.01$) de Células Rojas Sanguíneas, Volumen de paquete celular y Hemoglobina. En la canal, solo el hígado fue afectado, siendo este menor ($P<0.05$) al primer sacrificio (3^a semana). Cortes histológicos del hígado de aves consumiendo *Mucuna* mostraron lesiones patológicas en todo el periodo experimental.

Palabras clave: *Mucuna cochinchinensis*, toxicidad crónica, pollos de engorda, hemoglobina, volumen de paquete celular, células rojas sanguíneas.

INTRODUCTION

Many leguminous crops in Nigeria belong to the neglected or under-utilized legumes. These include jack bean, sword bean, yam bean, *Mucuna* beans, pigeon pea, kidney bean, lima bean, bambara nut, cowpea, among many others (Ologhobo, 1992; Anumnu, 1990; Udedibie and Nwaiwu, 1987; Oyenuga, 1968; Hutchinson and Dalziel, 1954). The common feature about all these little known legumes is their high content of various naturally occurring constituents, which affect their nutritional quality. Olomu (1995) has described these natural constituents as endogenous toxic substances because they are part of the normal feedstuff. These anti-nutritional factors include haemagglutinins, protease inhibitors (like trypsin inhibitor), cyanogens, tannins, dopamine, L-Dopa, antivitamins, lipoxygenase, nicotin, phytates, goitrogens, and serotonin (Oke *et al.*, 1996; Ologhobo,

1992; Balogun and Fetuga, 1989; Udedibie and Nwaiwu, 1987; Duke, 1981; Hashim and Idrus, 1977; Piper and Tracy, 1910; Bort, 1909).

The presence of these antinutritional factors in a feedstuff confers toxicity action to such feedstuff. *Mucuna cochinchinensis*, a member of the genus *Mucuna*, has been reported to contain a good number of these factors (Carew *et al.*, 2000; Del Carmen *et al.*, 2000; Flores *et al.*, 2000; Ukachukwu and Obioha, 2000; Ukachukwu *et al.*, 1999; Ukachukwu and Obioha, 1997). Ukachukwu *et al.* (1999) reported that raw *Mucuna* extracts were not acutely toxic to broiler birds. However, they reported that the birds that received the extracts showed some other signs of toxicity, which included dizziness and diarrheic droppings. They opined that the non-acute toxicity of the raw seed extracts does not preclude the fact that raw *Mucuna* could be chronically toxic when eaten for

a long period of time. This work therefore, aims at investigating toxicity of raw *Mucuna* bean on broilers when they are exposed to such a bean for a long time.

MATERIALS AND METHODS

Two hundred (200) unsexed Anak strain day-old broiler chicks were raised in a deep litter house on a common broiler starter diet (Pfizer Feed^{Registered Trade Mark}) for 21 days. After the 21 days 150 of them were selected and randomly allocated to five dietary treatments using 30 birds per treatment in a completely randomized design (CRD) experiment. The five diets respectively contained raw *M. cochinchinensis* seeds at five levels of 0%, 0.2%, 0.4%, 0.8% and 1.6%. These translated to 0g/kg, 2g/kg, 4g/kg, 8g/kg and 16g/kg (that is, g *Mucuna* per kg feed). The feeding trial lasted for 12 weeks (4th – 15th week of age). The diets were formulated to be isocaloric and isonitrogenous as follows: (1) Weeks 4 - 6 (22%CP and 2.9Mcal. ME/Kg) (Table 1), and (2) Weeks 7 – 15 (20%CP and 2.9Mcal ME/Kg) (Table 2). Throughout the entire experiment feed and water were offered *ad libitum* on daily basis.

Three birds per dietary treatment were slaughtered at intervals of 21 days. So, there were four slaughters at

3rd, 6th, 9th and 12th week of experimental feeding, which corresponded to 6th, 9th, 12th and 15th week of age. The blood of the sacrificed birds was used for haematological studies including Packed Cell Volume (PCV), Red blood cells (RBC) count and Haemoglobin (Hb) determination. Some of the organs of the sacrificed birds were used for carcass characteristics and histopathological studies. The organs were weighed and examined grossly and histopathologically for any lesions arising from possible toxicity due to the dietary treatments. The organs examined were liver, spleen, kidney, heart and pancreas. They were preserved in formalin. The organs were fixed in 10% formal saline for a maximum of 24h, processed and infiltrated with paraffin wax. Sections (5 microns thick) were cut, stained with haematoxylin and eosin (H and E) and examined with the light microscope for pathological lesions.

Data collected on carcass characteristics and hematological conditions were subjected to analysis of variance (ANOVA) in CRD, and the Duncan's new multiple range test (DNMRT) was applied to partition means, where necessary (Duncan, 1955; Snedecor and Cochran, 1980; Gomez and Gomez, 1985), as packaged in the MSTATC (1993) computer software.

Table 1: Composition of the treatment diets containing different levels of raw *Mucuna cochinchinensis* fed at starter phase

Ingredients	Treatment diets				
	Control	0.20%	0.40%	0.80%	1.60%
<i>M. cochinchinensis</i> , %	-	0.20	0.40	0.80	1.60
Maize, %	51.90	51.82	51.75	51.56	51.17
Wheat offal, %	8.00	8.00	8.00	8.00	8.00
Soybean meal, %	33.00	32.88	32.75	32.54	32.13
Fish meal, %	2.00	2.00	2.00	2.00	2.00
Bone meal, %	3.00	3.00	3.00	3.00	3.00
Oyster shell, %	1.50	1.50	1.50	1.50	1.50
Salt, %	0.35	0.35	0.35	0.35	0.35
Vit./Min. Premix*, %	0.25	0.25	0.25	0.25	0.25
Calculated:					
Crude protein, %	22.00	22.00	22.00	22.00	22.00
ME (Mcal/Kg)	2.90	2.90	2.90	2.90	2.90

1Kg of premix contains: Vitamin A (5,000,000 I.U.), Vitamin D3 (1,000,000 I.U.), Vitamin E (16,000mg), Vitamin K3 (800mg), Vitamin B1 (1,200mg), Vitamin B2 (22,000mg), Niacin (22,000mg), Calcium pantothenate (4,600mg), Vitamin B6 (2,000mg), Vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (48,000mg), Iron (40,000mg), Zinc (32,000mg), Copper (3,400mg), Iodine (600mg), Cobalt (120mg), Selenium (48mg), Anti-oxidant (48,000mg).

Table 2: Composition of the treatment-diets containing different levels of raw *Mucuna cochinchinensis* fed from 7th – 15th week of age

Ingredients	Treatment diets				
	Control	0.20%	0.40%	0.80%	1.60%
<i>M. cochinchinensis</i> , %	-	0.20	0.40	0.80	1.60
Maize, %	55.65	55.57	55.50	55.31	54.92
Wheat offal, %	10.00	10.00	10.00	10.00	10.00
Soybean meal, %	27.25	27.13	27.00	26.79	26.38
Fish meal, %	2.00	2.00	2.00	2.00	2.00
Bone meal, %	3.00	3.00	3.00	3.00	3.00
Oyster shell, %	1.50	1.50	1.50	1.50	1.50
Salt, %	0.35	0.35	0.35	0.35	0.35
Vit./Min. Premix* , %	0.25	0.25	0.25	0.25	0.25
Calculated:					
Crude protein, %	20.00	20.00	20.00	20.00	20.00
ME (Mcal/Kg)	2.90	2.90	2.90	2.90	2.90

1 Kg of premix contains: Vitamin A (5,000,000 I.U.), Vitamin D3 (1,000,000 I.U.), Vitamin E (16,000mg), Vitamin K3 (800mg), Vitamin B1 (1,200mg), Vitamin B2 (22,000mg), Niacin (22,000mg), Calcium pantothenate (4,600mg), Vitamin B6 (2,000mg), Vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (48,000mg), Iron (40,000mg), Zinc (32,000mg), Copper (3,400mg), Iodine (600mg), Cobalt (120mg), Selenium (48mg), Anti-oxidant (48,000mg).

RESULTS AND DISCUSSION

Carcass characteristics (Tables 3a-d)

Dressed weight percentages of birds were not significantly affected ($P>0.05$) by the inclusion levels at any of the slaughter stages. Also there were no significant ($P>0.05$) differences among the levels of inclusion at all the slaughter stages in the percent weights of most of the organs investigated. It was only in the liver that significant ($P<0.05$) differences were observed among treatment levels at first slaughter. The 16,000ppm diet depressed percentage weight of liver when compared with the 0ppm diet. The other inclusion levels did not produce any significant depression. Also, there were no significant depressions at subsequent slaughters.

The non-significant ($P>0.05$) dressed weight percentages of birds suggest that the inclusion of *M. cochinchinensis* beans did not affect relative meat yield of birds at any of the slaughter stages. The non-significant ($P>0.05$) differences in the organs investigated could also mean that none of the organs was a direct target organ of the toxic factors contained in the bean. It could also suggest that the inclusion levels of the beans in the diets of the broilers (even the 16,000ppm) did not produce the various toxic factors at high enough levels to cause morphological changes of the organs. The significant ($P<0.05$) differences observed in the liver leads to a suspicion that the liver might be a target organ of the toxic factors contained in the beans. This is expected since the liver is the primary organ responsible for metabolism of any toxic

elements that have been absorbed into blood circulation. If complete detoxification of the toxic factor(s) takes place in this organ, they could get to other organs in less toxic or virulent form. Thus, they would place so less a demand on the functionality of these organs that their morphological integrity would not be interfered with. One of the toxic factors found to be contained in the *M. cochinchinensis* bean is haemagglutinin (Ukachukwu and Obioha, 1997) and liver is a target organ of this toxic component (Ikegwuonu and Bassir, 1976; Stead *et al.*, 1966; Salgakar and Sohonie, 1965). The non-significant ($P>0.05$) depression of liver at slaughters 2 - 4 suggests that some inactivation of haemagglutinin by pepsin may have occurred (Liener and Rose, 1953), but that all the haemagglutinins could not be inactivated (Goddard and Mendel, 1929).

Histopathological changes

Pathologic changes were observed only in the liver sections of broilers the selected from diets containing *Mucuna* beans. The lesions were similar at all levels and at every slaughter. A typical lesion is therefore, shown in plate 1. This reveals that diets containing raw *Mucuna* beans caused wild area of periportal necrosis with some mononuclear cell infiltration, while the centrilobular areas showed vacuolation and degeneration of hepatocytes. These observations contrasted with liver sections of broilers on the control (no mucuna) diet, which were devoid of histopathological changes (Plate 2). The severity of the lesions in the portal areas in all groups that fed on

diets containing *Mucuna* is related to its anatomical role as the focus of blood supply to the lobule through the hepatic portal vein from the gastro-intestinal, and hepatic artery from the aorta. Consequently, ingested toxic materials getting into the liver have contact first with hepatocytes in these areas. In line with the important function of the liver as an organ of detoxification, hepatocytes in the periportal areas suffer most from toxic insults. In this situation, toxic

substances present in the *Mucuna* beans (especially haemagglutinins) must have been responsible for the histopathological changes found in the liver sections. The known deleterious effects of haemagglutinins include zonal necrosis of liver (Salgakar and Sohonie, 1965) and complete damage of the liver (Ikegwuonu and Bassir, 1976; Stead *et al.*, 1966).

Table 3. Effect of prolonged feeding of *Mucuna cochinchinensis* on the carcass characteristics of broiler chicks

Organ [#]	TREATMENT					SEM
	0%	0.20%	0.40%	0.80%	1.60%	
a) First slaughter after 3 weeks of experimental feeding						
Dressed, %	49.43(57.70)	51.10 (59.21)	49.98(58.61)	49.03(56.98)	50.66(59.78)	1.88 ^{ns}
Kidney, %	6.19 (1.14)	5.67 (0.98)	5.74 (0.99)	5.85 (1.03)	5.68 (0.98)	0.18 ^{ns}
Spleen, %	2.30 (0.16)	2.38 (0.17)	2.19 (0.15)	2.66 (0.22)	2.23 (0.15)	0.19 ^{ns}
Liver, %	11.01 (3.64 ^a)	10.52 (3.30 ^{ab})	9.74 (3.28 ^{ab})	10.40 (2.83 ^{ab})	9.16 (2.52 ^b)	0.35 [*]
Pancreas, %	3.59 (0.39)	3.48 (3.37)	3.40 (0.35)	3.74 (0.43)	3.45 (0.37)	0.26 ^{ns}
Heart, %	4.44 (0.60)	4.53 (0.63)	4.74 (0.68)	4.69 (0.67)	4.10 (0.51)	0.21 ^{ns}
Lungs, %	5.27 (0.88)	4.97 (0.75)	4.42 (0.60)	4.63 (0.65)	4.76 (0.69)	0.31 ^{ns}
b) Second slaughter after 6 weeks of experimental feeding						
Dressed, %	55.62(68.10)	54.60(66.46)	54.48(66.25)	54.31(65.99)	55.02(67.06)	0.90 ^{ns}
Kidney, %	5.20 (0.82)	5.11(0.79)	5.54 (0.95)	4.74 (0.69)	5.28 (0.85)	0.27 ^{ns}
Spleen, %	2.42 (0.18)	1.92 (0.11)	2.18 (0.15)	2.73 (0.23)	2.58 (0.20)	0.25 ^{ns}
Liver, %	8.45 (2.74)	8.38 (2.27)	9.46 (2.25)	8.65 (2.16)	8.65 (2.14)	0.44 ^{ns}
Pancreas, %	2.94 (0.26)	2.05 (0.28)	2.95 (0.27)	3.07 (0.29)	3.03 (0.28)	0.10 ^{ns}
Heart, %	4.10 (0.51)	3.99 (0.49)	4.12 (0.52)	3.87 (0.46)	3.83 (0.45)	0.22 ^{ns}
Lungs, %	4.57 (0.64)	4.58 (0.64)	4.29 (0.56)	4.16 (0.53)	4.64 (0.66)	0.16 ^{ns}
c) Third slaughter after 9 weeks of experimental feeding						
Dressed, %	56.71(69.31)	56.67(69.81)	55.82(68.43)	54.41(66.11)	56.41(69.35)	0.55 ^{ns}
Kidney, %	4.90 (0.73)	5.07 (0.79)	5.13 (0.80)	4.84 (0.71)	4.69 (0.67)	0.18 ^{ns}
Spleen, %	2.19 (0.15)	2.14 (0.14)	2.26 (0.16)	2.43 (0.18)	1.81 (0.10)	0.15 ^{ns}
Liver, %	8.40 (2.34)	8.56 (2.24)	8.51 (2.20)	8.85 (2.13)	8.26 (2.07)	0.34 ^{ns}
Pancreas, %	2.77 (0.24)	2.72 (0.23)	2.98 (0.27)	2.81 (0.24)	2.90 (0.26)	0.21 ^{ns}
Heart, %	3.90 (0.46)	3.80 (0.44)	3.87 (0.46)	3.92 (0.47)	3.92 (0.47)	0.18 ^{ns}
Lungs, %	4.63 (0.66)	4.21 (0.54)	4.81 (0.70)	4.31 (0.57)	4.26 (0.55)	0.47 ^{ns}
d) Fourth slaughter after 12 weeks of experimental feeding						
Dressed, %	54.88(66.85)	55.49(67.85)	54.67(66.52)	55.25(67.52)	56.99(70.29)	1.29 ^{ns}
Kidney, %	5.09 (1.10)	4.73 (0.70)	4.71 (0.70)	4.59 (0.60)	4.79 (0.70)	0.24 ^{ns}
Spleen, %	2.40 (0.18)	3.39 (0.41)	2.72 (0.23)	2.07 (0.13)	2.12 (0.14)	0.48 ^{ns}
Liver, %	8.52 (2.18)	8.39 (2.12)	8.10 (2.00)	7.33 (1.73)	7.54 (1.63)	0.37 ^{ns}
Pancreas, %	2.96 (0.27)	2.87 (0.25)	2.94 (0.27)	2.45 (0.18)	2.43 (0.18)	0.19 ^{ns}
Heart, %	3.62 (0.40)	3.90 (0.46)	3.78 (0.44)	3.96 (0.48)	4.01 (0.49)	0.20 ^{ns}
Lungs, %	4.13 (0.52)	4.23 (0.55)	4.90 (0.76)	4.64 (0.67)	3.94 (0.48)	0.22 ^{ns}

* Means on the same row followed by the same superscript are significantly the same at * (P<0.05) or ** (P<0.01)

All expressed as percentage of liveweight of chicks

Note: Figures in parentheses are the untransformed or original figures while the ones outside the parentheses are the transformed figures using the Arc Sine Transformation method.

Hematological conditions (Table 4a-d)

At first slaughter only the 16g/kg diet caused significant (P<0.01) reduction of red blood cells (RBC). At subsequent slaughters inclusions of *M. cochinchinensis* beans even at 2g/kg caused significant (P<0.01) reductions of both RBC and packed cell

volume (PCV) of blood. They also caused reduction in the quantity of hemoglobin (Hb) though the reduction was significant (P<0.01) only at the second slaughter.

The hematological picture suggests that haemagglutinin could be implicated. The raw *M.*

cochinchinensis has earlier been found to have hemagglutinating activity of up to 4267 HU/g (Ukachukwu and Obioha, 1997). Ologhobo *et al.* (1993) reported localization of haemagglutinin in a base soluble protein fraction of lima bean. They fed

the base soluble protein fraction to broilers and observed consistent reduction of RBC and Hb in the birds. They concluded that this suggests the direct involvement of haemagglutinins.

Table 4: Chronic toxicity effect of raw *Mucuna cochinchinensis* on the haematological conditions of broilers

Parameter	TREATMENT					SEM
	0%	0.20%	0.40%	0.80%	1.60%	
a) First slaughter after 3 weeks of experimental feeding						
PCV, %	35.66 (34)	36.42 (35)	34.64 (32)	34.84 (33)	35.44 (34)	1.47 ^{ns}
Hb, g/100ml	11.33	11.77	10.77	10.90	11.17	0.81 ^{ns}
RBC (x10 ³)	243 ^a	227 ^a	227 ^a	224 ^a	120 ^b	7.20 ^{**}
b) Second slaughter after 6 weeks of experimental feeding						
PCV, %	40.78 (42.70 ^a)	34.85 (32.70 ^b)	34.65 (32.3 ^b)	34.24 (31.70 ^b)	33.21 (30.00 ^b)	0.74 ^{ns}
Hb, g/100ml	14.2 ^a	10.87 ^b	10.40 ^b	10.20 ^b	9.97 ^b	0.39 ^{**}
RBC (x10 ³)	296 ^a	227 ^b	219 ^b	217 ^b	152 ^c	7.67 ^{**}
c) Third slaughter after 9 weeks of experimental feeding						
PCV, %	39.43 (40 ^a)	36.25 (35 ^{ab})	35.26 (33 ^b)	35.06 (33 ^b)	35.45 (32 ^b)	0.72 ^{**}
Hb, g/100ml	12.30	11.67	11.10	11.07	10.63	0.54 ^{ns}
RBC (x10 ³)	298 ^a	258 ^b	239 ^{bc}	230 ^c	222 ^c	5.75 ^{**}
d) Fourth slaughter after 12 weeks of experimental feeding						
PCV, %	39.43 (40 ^a)	36.67 (36 ^b)	35.46 (34 ^{bc})	35.06 (33 ^{bc})	33.41 (30 ^c)	0.52 ^{**}
Hb, g/100ml	12.60	11.83	11.40	11.20	10.30	0.42 ^{ns}
RBC (x10 ³)	296 ^a	227 ^b	219 ^b	217 ^b	152 ^c	7.67 ^{**}

* Means on the same row followed by the same superscript are significantly the same at * (P<0.05) or ** (P<0.01)

Note: Figures in parentheses are the untransformed or original figures while the ones outside the parentheses are the transformed figures using the Arc Sine Transformation method.

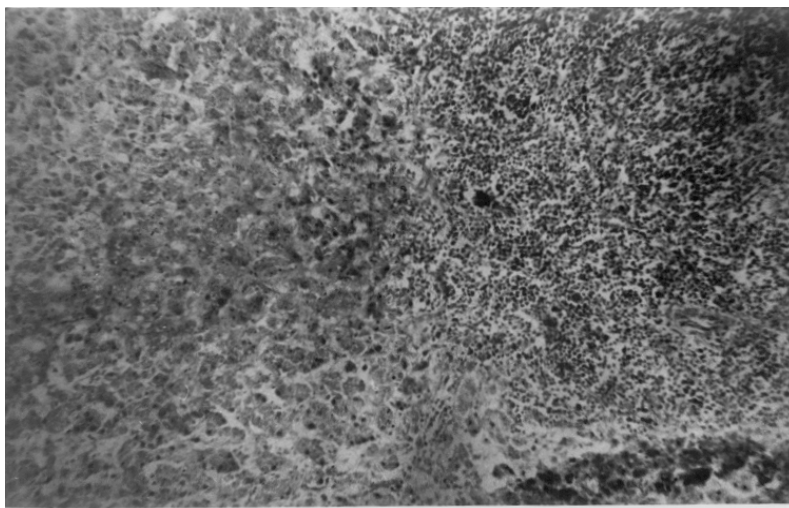


Plate 1: Liver section of broiler fed diet containing raw *Mucuna* (RMD). Note wild area of periportal hepatocytes necrosis (right) and centrilobular zone of hepatocytes degeneration (left). H and E stain, x320.

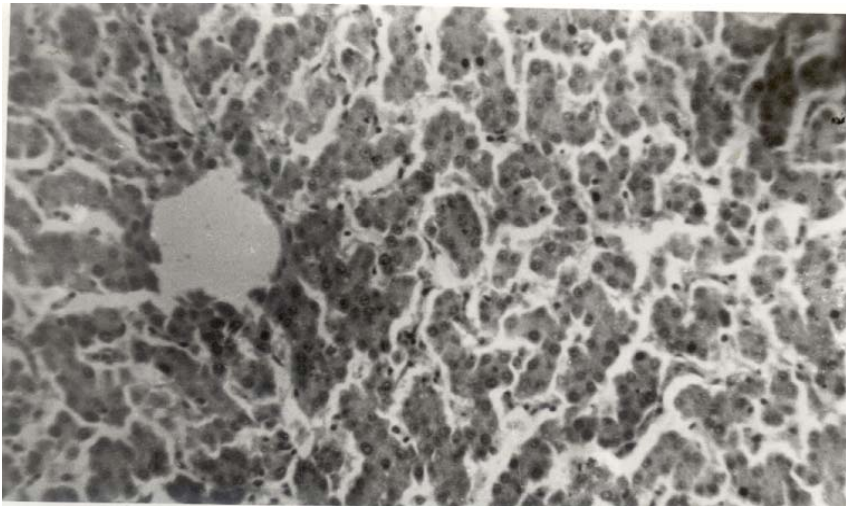


Plate 2: Liver section of broiler fed diet containing no *Mucuna* (NMD). Note normal lobular architecture, hepatocytes and sinusoids. H and E stain, x320.

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