Effects of *Mucuna pruriens* Leaf Extract on the Liver of Wister Rats

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ABSTRACT

Introduction: *Mucuna pruriens* being one of the important medical plants in the world possesses valuable medicinal properties. **Objectives:** The study is aimed at determining the effects of *Mucuna pruriens* on the liver of wistar rats. **Methods:** a total number of twelve (12) healthy adult wistar rats of both sexes weighing 140g – 179g were used. The wistar rats were randomly assigned into four (4) groups (Group T_1, Group T_2, Group T_3 and Group T_4). Group T_1 was orally given water while group T_2, T_3 and T_4 were orally given 0.4mg, 1mg and 10mg of aqueous extract of Mucuna pruriens leaf respectively for 21 days. At the end of the experimental days, blood specimen was collected, the rats were sacrificed, and their liver was excised and immediately fixed in 10% formol saline. The liver tissues were processed and the slides were histologically examined. Liver function test (Total Bilirubin (TB), Direct Bilirubin (DB), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alkaline Amino Transferase (ALT), Total Bilirubin (TB) and Albumin) was carried out on the blood sample collected. The liver tissues were processed and the slides were histologically examined. **Results:** All the measured biochemical parameters; TB, DB, ALP, AST, ALT, TB and Albumin were found to be statistically significant among the treated groups (T_2, T_3, T_4) when compared with the control group (T_1). TP was positively correlated with weight measured within 1-7 days while other parameters were negatively correlated. TP was positively and significantly correlated to weight measured within 8-14days. Albumin, ALP, TB, DB were negatively correlated and insignificant while others were positively and insignificantly ($p>0.05$) correlated. Liver histology shows moderate glycogen depletion with some inflammatory cells. **Conclusion:** This study shows *Mucuna pruriens* to be a medicinal plant. However, notable increase in total protein and some inflammatory cells seen in the liver histology can indicate toxicity; but further studies on the on the toxicity effects and the lethal dose of *Mucuna pruriens* leaf extract are to be carried out, so as to guide its safe and efficient use.

Keywords: Aqueous, Bilirubin, Hypoalbuminemia, Liver, *Mucuna pruriens*.
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Author's contribution: this work was carried and approved in collaboration between all the authors and take responsibility for its integrity and accuracy. **ON** designed the study; **ON** sourced for funding; **ON** and **FSA** wrote the protocol; **ON, FSA, AAB and AFO** contributed in literature search; **ON, FSA, AAB** and **AFO** did the lab experiments; **ON** and **FSA** analysed the results; **FSA, AAB** and **AFO** did statistical analysis; **ON, FSA, AAB and AFO** contributed in discussion; **ON** and **FSA** supervised the study; **ON, AAB** and **AFO** drafted the manuscript; **ON** and **AAB** wrote the final manuscript; **ON, FSA, AAB and AFO** proof read the final version for publication.

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INTRODUCTION

Plant extracts have been used in various medicinal practices for the treatment of various diseases since antiquity[1]. A medicinal plant according to world health organization (WHO) is a plant which has one or more parts of its constituents, containing substance(s) that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[2]. *Mucuna pruriens* is thought to have originated from India. All parts of *Mucuna pruriens* possess valuable medicinal properties[3;4]. *Mucuna pruriens* popularly known as agbala leaf in igbo is used as a blood tonic for the treatment of anaemia[5;6]. Bilirubin is the yellow breakdown product of normal heme catabolism. It is the chief bile pigment found in the serum. The liver is the largest and most complex internal organ in the body. It plays an important role in the maintainer of internal environment through its multiple and diverse function. Liver is involved in several vital functions such as metabolism secretion and storage. Hepatitis or inflammatory disorder involves inflammation and change to the hepatocytes. Hepatitis is one of the most prevalent diseases in the world. Every year 18,000 people had been reported to die due to liver cirrhosis caused by viral hepatitis[7]. Liver has great capacity to detoxicate toxic substance and synthesizes useful principle. Therefore damage to liver inflicted by hepatotoxic agents is of grave consequence. Many medicinal plant/indigenous plant have been mentioned and well established as hepato-protective agents. *Mucuna pruriens* is a medical plant around the world [8;9]. It also showed that one of the uses is for erectile enhancing property. *Mucuna pruriens* being one of the important medical plants in the world may have effect on the liver. However limited studies are available on the effect of *Mucuna pruriens* extract on liver function of wistar rats. A clinical study confirmed the efficacy of the seeds of *Mucuna pruriens* in the management of Parkinson's disease by virtue of their L-Dopa content. The organs mostly affected by toxins from medicinal plants include the liver, heart and kidney [10;11]. Velvet leaves (*Mucuna pruriens*) are found in Asia including Malaysia, America and Africa. The leaves have been prescribed by traditional practitioners in Nigeria as an oral prophylactic for feeding animals (i.e. wistar rat)[12]. The prophylactic protective effect of *Mucuna pruriens* aqueous leaf extract (MPE) has been demonstrated in mice against Echis carinatus (aw-scaled viper) and wistar rat [13;14]. *Mucuna pruriens* is an invasive annual vine that poses a high risk to native environments. The species has been extensively cultivated as a popular soil-improver and forage plant, as well as a cover crop, as it smothers weed plants[15;16]. However, this species itself has the potential to invade and damage ecosystems, and is listed as "agricultural weed", "environmental weed", "garden thug", "naturalized", "sleeper weed", and "weed" in the Global Compendium of Weeds[17;18]. The species spreads by seeds. Considered native to Asia and an introduction to the Neotropics[19]. *M. pruriens* is known to be a serious weed in Mexico and Mozambique, a principal weed in Jamaica and Madagascar, and a common weed in Guatemala, Kenya, Micronesia, and Tanzania[20]. The non-irritating variety more commonly used in agriculture can apparently revert to the type species over time, a trait which heightens the risk of the species' introduction[21]. Hammerton,[22] reported it as a potential invasive species in the Bahamas, as it overgrows shrubs and small trees. *Mucuna pruriens* has been said to have valuable medicinal properties and being consumed in many part of the world especially in rural areas. The liver plays an important diverse functions in the maintenance of internal environment. The status of these organs can be evaluated by determining the activities of some

enzymes specific to these organs in serum. Elevated level of these enzymes in the serum is an indication of damage or health status of these organs. In the case of the liver, estimation of some enzymes in the serum like ALT, AST, and ALP can be seen as indicators of its functional status. Thus, this research was performed to assess the effects of *Mucuna pruriens* aqueous leaf extract on the liver of Wistar rats

MATERIALS AND METHODS

Study Location

This study was carried out at Nongo Kristu Sersha Tar (N.K.S.T.) 'Universal Reformed Christain Church' Len Gabrielse School of Medical Laboratory Science Mkar, Gboko Local Government Area Benue State

Experimental Design

The wistar rats were grouped into four (4) tagged as Group T1 (control), Group T2, Group T3, Group T4. Group 1 (control) T1R1, T1R2, T1R3; Group 2 (Test) T2R1, T2R2, T2R3; Group 3 (Test) T3R1, T3R2 and T3R3 and group 4 (Test) T4R1, T4R2 and T4R3. Mucuna Pruriens aqueous leaf extract was administered for 21 days. After which the liver was harvested and blood sample collected and analysed.

Harvesting of Mucuna Prurient Leaf

The leaves were washed, shade, dried and grinded into coaxed form and soaked with distilled water for 5 hours. The mixture was then filtered; the filtrate was evaporated to dryness at 40^0c in a water bath, the dried extract was kept in a clean, cool and dried container, and was used throughout the experiment. The extract was reconstituted in distilled water for a known weight of the dried filtered to obtain the desired concentration.

Administration of Extracts

Twelve (12) healthy wistar rats of both sexes were categorized into four groups, each consisting of three (3) rats and were orally administered as follows:
Group T1 (Control: T1R1, T1R2 and T1R3) were administered 20ml of distilled water for 21 days; Group T2 (T2R1, T2R2 and T3R3) were administered 20ml of 0.4mg of mucuna prurien aqueous leaves extract for 21 days. Group T3 (T3R1, T3R2 and T3R3) were administered 20ml of 1mg of mucuna prurien leaves extract for 21 days. Group T4 (T4R1, T4R2, and T4R3) were administered 20ml of 10mg of mucuna prurien aqueous extract for 21 days.

pH of Juice: Group one (Control), normal water pH 7.0**;** Group Two juice pH 7.6**;**Group three Juice pH 7.8**;** Group four Juice pH 8.0

Collection of Samples

Blood samples were collected from the marginal vein of the ear, from the central artery of the ear and the heart. 5 ml of blood was collected repeatedly from these sites[23]. The liver organs of the rats were also harvested, fixed in 10% formal saline and properly labelled.

Liver Function Tests

Liver function tests were carried out on serum separated from the blood samples collected for estimation of Albumin (ALB), Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate aminotransferase (AST), Total Bilirubin (TB), Direct Bilirubin (DB), and Total protein (TP) using standard test procedures (30, 31).

Histopathology Assessment

The harvested liver organs were processed, embedded, and then the tissue blocks were sectioned at 3µm thickness in the histopathology laboratory. The tissue sections were stained with three (3) different staining techniques which

include: Haematoxylin and Eosin (H and E), Perl's Prussian blue (PPB), and Periodic acid Schiff (PAS) technique.

RESULTS

Group 3 (1400.0±100.0) showed the highest measured weight for the wistar rat within 1-7 days, groups 4 within 8-14(1666.7±57.6) days and 15-21(1733.3±57.7) days.

Measured weight within 8-14days and 15-21 days were statistically significant (p=0.000) while 1-7days was statistically insignificant (p=0.627) (Table 1).

The results of serum biochemistry test (Table 2) shows albumin to be higher in group 1 (35.33±0.57) which is the control compare to the other groups. ALP was more raised in group 2 (244±0.57) and group 3 (239±2.00) respectively. ALT was found to be higher in group 3(239±2.00) compared to other groups. Group 3 (266±0.43) showed increased level of serum AST followed by group 2 (166±26.80) and group 3(134±11.43). TB was higher was higher in group 2(3.23±0.20) compared to other groups while DB was found to be higher in the control group (2.66±0.05). Highest level of serum TP was found in group 4(58.10±4.30) and group 3 (49.00±8.36) (Table 2). All the measured parameters were found to be statistically significant among the groups ALB (p= 0.003), ALP (P=0.001), ALT (P=0.001), AST (P=0.000), TB (P=0.013), DB (P=0.000), TP (P=0.015) (Table 2).

Table 3 shows that TP was positively correlated with weight measured within 1-7 days while other parameters were negatively correlated. There was no significance (p>0.05) between the measured parameters and the weight measured within 1-7 days. Albumin, ALP, ALT, TB, and DB were negatively correlated among which albumin and TB were statistically significant (p<0.05) when compared to weight measured within 8-14 days. TP was positively and significantly correlated to weight measured within 8-14days. Albumin, ALP, TBDB were negatively correlated and insignificant while others were positively and insignificantly (p>0.05) correlated.

Table 1: Multiple comparison (ANOVA) for the weight among the groups

Parameter	Group 1	Group 2	Group 3	Group 4	F Value	P value
weight 1-7days	1333.3±57.7	1333.3±57.4	1400.0±100.0	1366.7±57.7	0.611	0.627
weight 8-14days	1266.7±57.7	1433.3±57.4	1566.7±57.7	1666.7±57.6	27.000	.000
weight 15- 21days	1333.3±57.7	1433.3±57.6	1566.7±57.7	1733.3±57.7	27.000	.000

Table 2: Comparison of the biochemical parameters among the groups

Parameter	Group 1	Group 2	Group 3	Group 4	F	P value

	(Control)					
ALB	35.33±0.57	32.67±0.56	31.70±1.41	32.90±0.10	10.871	.003
ALP	237±1.00	244±0.57	239±2.00	236±2.51	14.933	.001
ALT	236±2.51	134±0.05	239±2.00	236±2.51	18.003	.001
AST	44±0.11	266±0.43	166±26.80	134±11.43	119.328	.000
TB	3.10±0.10	3.23±0.20	2.90±0.10	2.66±0.20	6.865	.013
DB	2.66±0.05	2.26±0.06	1.93±0.06	2.63±0.05	107.583	.000
TP	43.66±0.57	43.03±0.15	49.00±8.36	58.10±4.30	6.556	.015

[ALB - Albumin; ALP - Alkaline phosphatase; ALT- Alanine transaminase; AST - Aspartate aminotransferase; TB - Total Bilirubin; DB - Direct Bilirubin; TP - Total protein]

Table 3: Correlation between weight and the measured biochemical parameters

		ALB	ALP	ALT	AST	TB	DB	TP
weight 1-7days	Pearson Correlation	-.161	-.073	-.015	-.048	-.219	-.183	.356
	Sig. (2-tailed)	.617	.822	.963	.882	.493	.570	.257
weight 8-14days	Pearson Correlation	-.664*	-.207	.369	.305	-.620*	-.254	.704*
	Sig. (2-tailed)	.019	.518	.237	.335	.031	.427	.011
weight 15- 21days	Pearson Correlation	-.479	-.325	.227	.143	-.809**	-.026	.798**
	Sig. (2-tailed)	.115	.302	.477	.657	.001	.936	.002

*. Correlation is significant at the 0.05 level

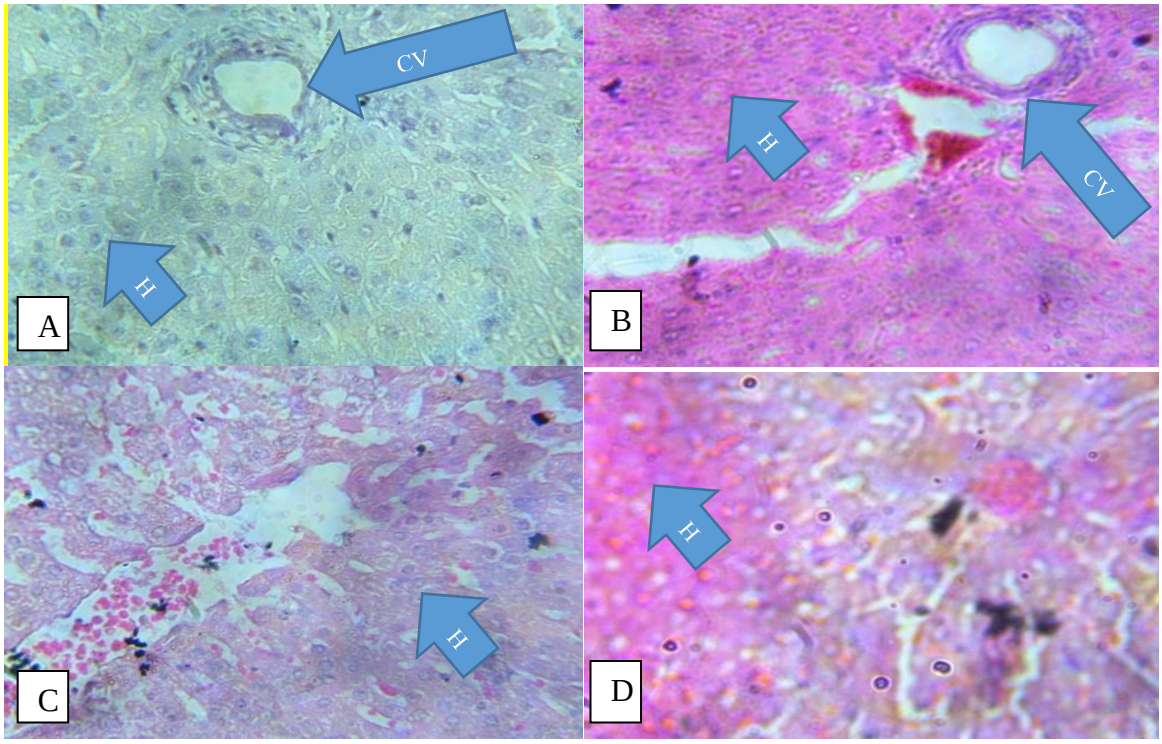

Figure 1 (**Liver controls Replicates TI GROUP)**: Normal liver histology with portal triad and hepatocytes (H) radiating from the central vein (CV). A-(PPB), C-(PAS), B and D - (H&E) X400

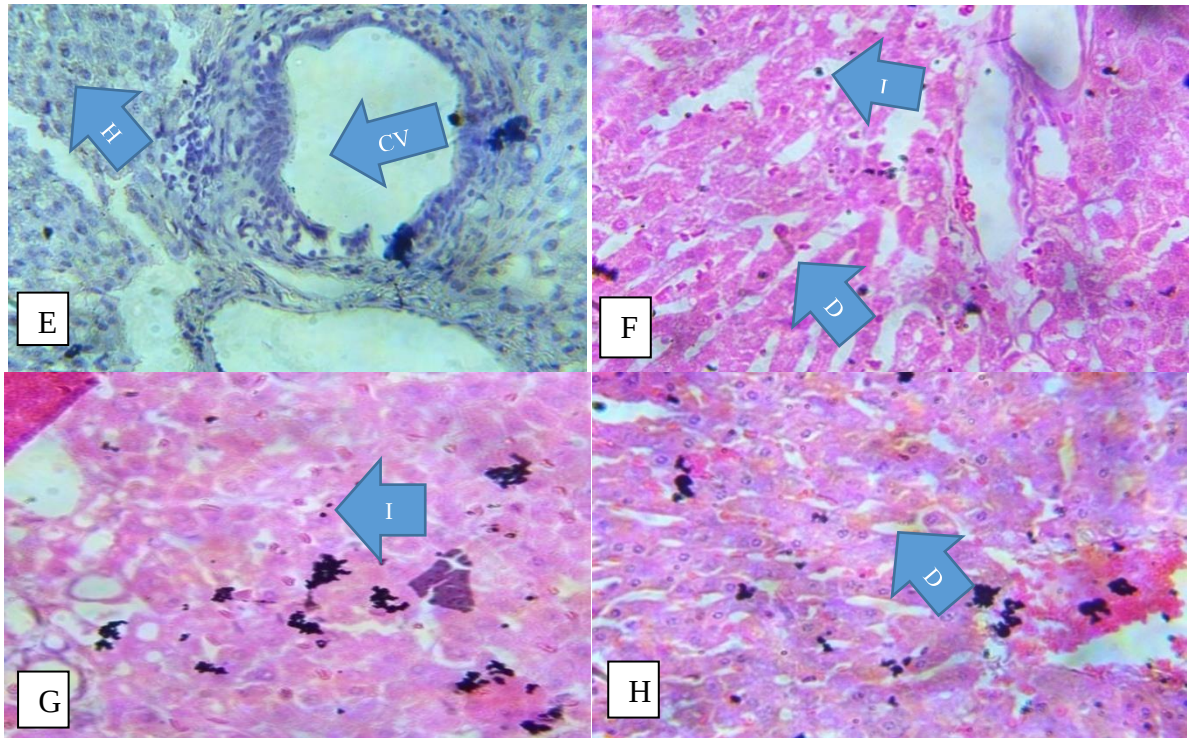

Figure 2 (**Liver Test group 2 replicates T2)**: Liver histology showing moderate glycogen depletion (D) with some inflammatory cells (I). E-(PPB), G-(PAS), F and H - (H&E) X400

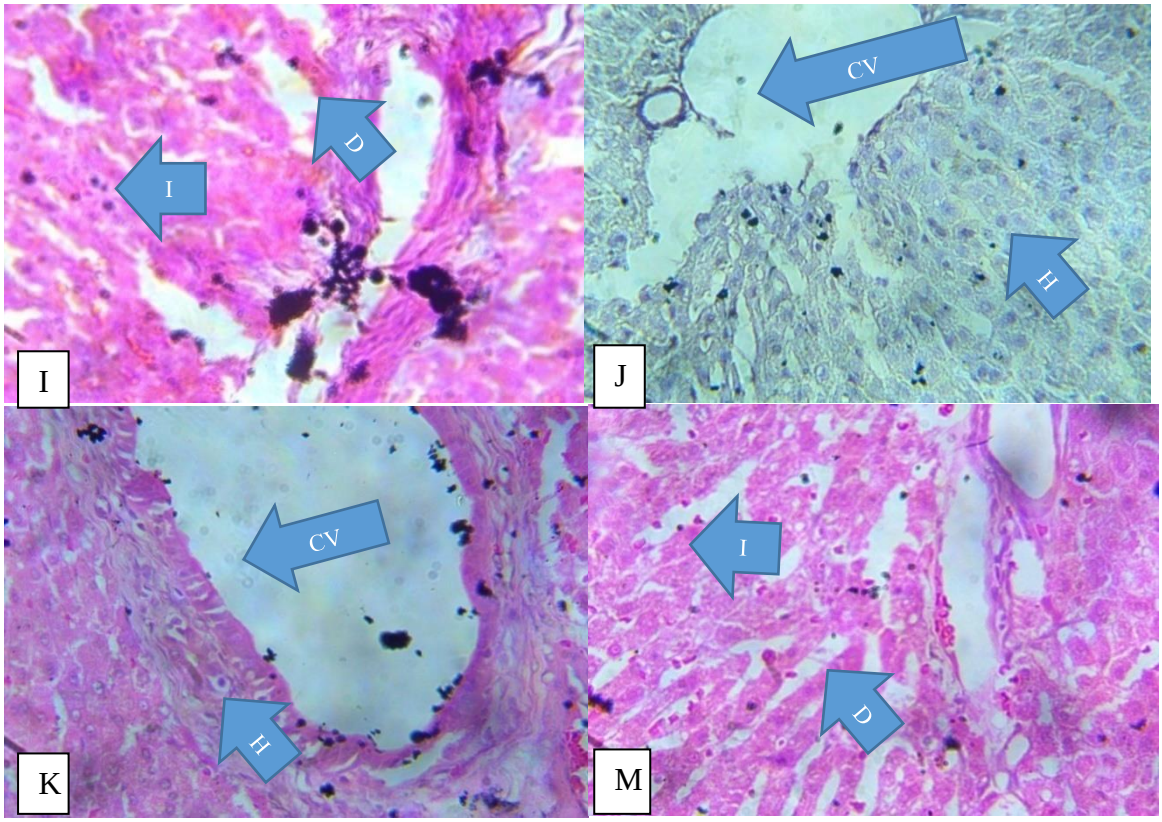

Figure 3 (**Liver Test group T3 Replicate)**: Liver histology showing moderate glycogen depletion (D) with some inflammatory cells (I). J-(PPB), K-(PAS), I and M - (H&E) X400

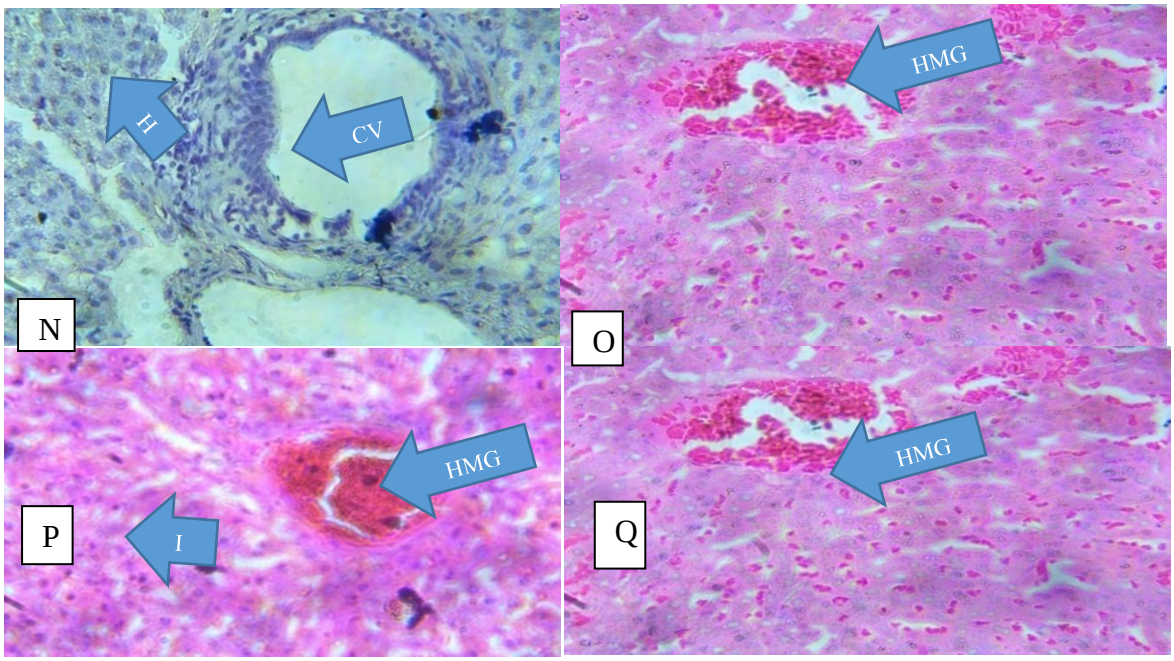

Figure 4 (**Liver Test group T4 Replicates)**: Liver histology showing moderate glycogen depletion with haemorrhagic (HMG) features and some inflammatory cells (I). N-(PPB), P-(PAS), O and Q - (H&E) X400

DISCUSSIONS

This study shows that the measured weight of the rats administered with *Mucuna pruriens* extract within 8-14days and 15-21 days were statistically significant (p=0.000) while those of 1-7days was statistically insignificant (p=0.627) which can mean that *Mucuna pruriens* extract can likely improve weight loss (Table 1). The renal threshold of both total and conjugated bilirubin is low and the elevation of serum bilirubin (total and conjugated) is indicative of hepatocellular damage. Total bilirubin was reduced in group administered with 1mg and 10mg of Mucuna extract. Reduction in bilirubin may indicate increase in liver activities in the metabolism of bilirubin. Though, it was slightly raised in group administered with 0.4mg *mucuna* extract. Direct bilirubin was significantly reduced (P<0.05) in within the groups. Albumin was reduced in all the groups when compared with control, continuous administration of this may lead to hypoalbuminemia which has a lot of clinical implications. AST was markedly raised in the subjects which could be as a result of damage to the parenchyma cells of the liver. ALT and ALP were slightly raised in some of the groups. This research is consistent with Ezeja and Omeh[24] who reported increase in liver enzymes and serum bilirubin with increase in the percent level of *Mucuna pruriens* inclusion in the feed prepared for albino rats. This study reported an increased level of serum total protein. This shows that *mucuna pruriens* leaf extract is of clinical importance in the synthesis of serum protein. ALT was markedly reduced in group administered with 0.4mg of Mucuna extract when compared with the control. This is in support with a research that said the reason for this decreased observation in enzyme level has been attributed to the fact *that Mucuna pruriens* is a known antioxidant[25]. Reduction in serum ALT in this group may suggest that the extract of *Mucuna pruriens* seeds contains significant antitumor and antioxidant activities in mice[26]. This is in line with the work carried out by Chukwudi[27] who ascribed the decrease in the liver enzymes activities to the antioxidant property of the *Mucuna pruriens*. Increased level of total protein was observed in this study. *Mucuna pruriens* can be said to have increased the synthetic ability of the liver and which is of medical importance. The histopathology photomicrographs of the groups tested with *Mucuna pruriens* extract (Figure 1-4) shows a mild glycogen depletion with some inflammatory cells present and there was a noticeable haemorrhage which can indicate toxicity effect of the extract; but the level of glycogen depletion observed in the liver was with no visible fatty changes or cytoplasmic vacuolization which could have clearly indicate severe toxic effect of the extract on the liver if present [29] There were also slight visible fibrotic features observed, which is indicative that the liver is function might be impaired on long run. This is accordance with the study by Ratnaningsih[28]on Sub-chronic toxicity of ethanolic extract of velvet bean which revealed that the liver histology tested with *Mucuna prurien* had moderate glycogen depletion and noticed enlargement of nuclei in some cell. Ratnaningsih *et al.* [28] also discovered that although the feature indicates toxicity effects of the extract but significant pathological changes in the liver did not show the phase of necrosis (cell death), thus the liver were still quite functional. Ratnaningsih *et al.*[28] also further stated in their study that the toxic effect is reversible characterised by recovery of the kidney and liver tissues in the satellite of high-dose groups after the extract was no longer administered.

CONCLUSION

Base on this study and review of relevant literatures; *Mucuna pruriens* aqueous leaf extract can improve weight loss, can be of

clinical importance in the synthesis of serum protein, can help liver activities especially in the metabolism of bilirubin, and can also be used as an antioxidant. The oral consumption of 10mg of *Mucuna pruriens* aqueous leave extract for 21 days does not cause death; however, continuous administration of the extract may lead to hypoalbuminemia and consumption of higher-dosage may be toxic to the liver. It is therefore recommended that further studies should be done focusing on the toxicity effect and the lethal dose of the extract on the body, so as to guide its safe and efficient usage.

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