ORIGINAL ARTICLE

The Ameliorative Effects of Aqueous Leaf Extract of Mucuna pruriens in lead acetate induced liver and bone marrow injury

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ABSTRACT

Introduction: Mucuna pruriens (velvet beans) is an unconventional legume commonly found in the tropical regions of Africa with several ethnomedicinal uses. The present study was designed to investigate the effects of aqueous extract of Mucuna pruriens leaves in rats intoxicated with lead acetate.

Method: Thirty rats were divided into six groups (five rats per group). Group I (control group) was given distilled water only, group II was intoxicated with lead acetate (100 mg/kg) only, group III was administered M. pruriens extract (600 mg/kg) only daily for a period of 28 days, while groups IV, V and VI animals were co -administered M. pruriens extractat at different concentrations of 300, 600 and 1200 mg/kg body weight daily for 28 days. After 28 days of experiment, the animals were euthanized. Blood samples were collected for some biochemical parameters and organs harvested for histology.

Results: The results showed that there were no significant differences between bilirubin, total protein, albumin and globulin in test and control animals, but significant increase ($P<0.05$) in ALT after 28 days of intoxication with lead acetate when compared with the control. However there was no significant difference in ALP activities. Administration of aqueous extract of M. pruriens leaves at a dose of 600 mg/kg body weight for 28 days showed a significant increase ($P<0.05$) in direct bilirubin, total protein, albumin, globulin, AST and ALT, but significantly reduced ALP activities.

Administration of the crude aqueous extract (300, 600 and 1200 mg/kg), did not show any ameliorating effect after 28 days when compared to rats intoxicated with lead acetate, but showed mild reduction in ALP activities with no significant changes in ALT and AST activities .

Conclusion: Mucuna pruriens extract possess ameliorative effect in rats intoxicated with lead acetate.

Keywords: Mucuna pruriens, lead acetate, histopathology

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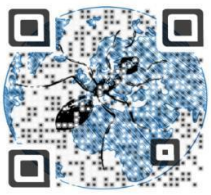

INTRODUCTION

Health problems caused by toxic environmental pollutants have become a global challenge due to increased urbanization and industrialization. Lead is considered as one of the major environmental pollutants and is amongst four metals that have the most detrimental effects on human wellbeing; the others being cadmium, mercury and arsenic[1]. Lead is a soft and malleable metal, which is considered a heavy metal. Lead has no known biological role in plants or animals and is extremely toxic to mammals and aquatic life. It can cause mental impairment in young children, causing neuropathy and hypertension in adults [2]. However there are several ways of preventing or treating lead poison, one of such ways is the use of metal chelating agents. This involves the use of compounds or substances that have the properties to mop up or reduce lead toxicity. Therefore, it is against this backdrop that this research seeks to evaluate the effect of medicinal plants to treat or ameliorate diseases caused by lead poisoning, and one of such plants is Mucuna pruriens (MP).

Mucunapruriens (velvet beans) is an unconventional legume commonly found in the tropical regions of Africa, India and West Indies[3]. It is an annual twining herb, found in bushes and hedges at damp places, ravines, scrubs, jungles throughout the plain of Nigeria. It is cultivated for its pods as vegetables and young leaves as fodder in the eastern part of Nigeria.

Mucuna pruriens is popularly known as agbara leaf and is used as a blood tonic traditionally[4]. Among the natives of eastern part, the use of Mucuna pruriens (agbara leaves) extract is a very common remedy for the treatment of anaemia.

M. pruriens possess valuable medicinal properties. The leaves and seeds are consumed for their nutritional value and are also used in folk medicine as a therapy for various diseases such as diabetes, arthritis, dysentery, infertility, obesity, anti-microbial, anti-inflammatory, anti-plasmodial, anti-epileptic and cardiovascular disorders from various clinical researches with animals [5, 6, 7, 8, 9, 10]. The pods are anti-helmintic. They have also been shown to demonstrate anti-parkinson properties probably due to the presence of levodopa (L-DOPA) (a precursor of neurotransmitter dopamine) and other phytochemicals such as 3 methoxy- 1,1 -dimethyl-6,7-dihydroxy- 1,2,3.4-tetrahydroquinoline and 3 -methoxy- 1,1 -dimethyl-7,8 -dihydroxy- 1,2,3.4-

tetrahydroquinoline [11].This study was therefore undertaken to evaluate the effects of aqueous leaf extract of Mucuna pruriens in rats intoxicated with lead acetate.

MATERIAL AND METHOD

Collection and identification of plant materials

Fresh leaves of Mucuna pruriens were collected from the back of Niger Delta Development Commission Hostel University of Benin, Benin City, Edo state. They were then identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology of the University of Benin.

Animals

Thirty male albino rats (Wistar strain) weighing 200±10 g were purchased from the animal house of the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were housed in galvanised rat cages and acclimatised for two weeks on guinea growers mash (Bendel Feed and Flour Mill, Ltd, Ewu, Nigeria) and allowed access to food and water ad libitum. The handling of the animals was in accordance with the principles of laboratory animal care [12].

Preparation of plant extracts

The leaves were washed, air-dried, macerated and then extracted with distilled water at room temperature for 48 hours with stirring at interval. The extracts obtained were concentrated to dryness at 40oC using a rotary evaporator under reduced pressure. The dried extracts were weighed and then stored at 4oC for subsequent analysis.

Experimental Design

The design consisted of thirty rats, divided into six groups of five rats each. The animals weighed between 200 ± 10g.

Group I (Control): Rats were given normal diet and distilled water.

Group II: Rats were administered Lead acetate (100mg/kg body weight) once daily for 28 days.

Group III: Rats were administered aqueous extract of Mucuna pruriens (MP) (600mg/kg body weight) once daily for 28 days.

Group IV: Rats were co -administered aqueous extract of Mucuna pruriens (300mg/kg body weight) and lead acetate (100 mg/kg) once daily for 28 days.

Group V: Rats were co -administered aqueous extract of Mucuna pruriens (600mg/kg body weight)

and lead acetate (100 mg/kg) once daily for 28 days.

Group VI: Rats were co -administered aqueous extract of Mucuna pruriens (1200mg/kg body weight) and lead acetate (100 mg/kg) once daily for 28 days.

To ensure accuracy of treatment, administration of the aqueous extract of Mucuna Pruriens was done using orogastric tube for 28 consecutive days (4 weeks).

Histopathology

The tissues (liver, kidney and bone) were fixed in 10% neutral buffered formalin for 3 -5 days. They were later dehydrated by passing through varying (increasing) concentrations of alcohol, 70% to 100%, cleared in xylene and then embedded in molten paraffin. Five micron (5µm) microtome sections were stained with hematoxylin and eosin dyes. The sections were examined under light microscope at high power magnification(x100 and x400) and photomicrographs taken[13].

Statistical Analysis

Data were expressed as (Mean ± SEM) of six replicates and were subjected to one way analysis of variance (ANOVA) using SPSS version 17.0 to compare standard error in mean and the individual comparisons were obtained by the Duncan Multiple Range Test (DMRT). A value of $P<0.05$ was considered significant [14].

RESULTS

Table 1 shows the effect of the aqueous extract of M.pruriens seeds on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities in rats intoxicated with lead acetate after 28 days of administration. Administration of lead acetate at a dose of 100 mg/kg body weight significantly increased ($P<0.05$) the activities of the ALT, but reduces AST activities after 28 days when compared with the control. However there was no significant difference in ALP activities. Administration of aqueous extract of M. pruriens leaves at a dose of 600 mg/kg body weight for 28 days showed a significant increase ($P<0.05$) in AST and ALT, but significantly reduced ALP activities (Table 1). Administration of the crude aqueous extract (300 and 600 mg/kg), showed ameliorating effects after 28 days when compared to rats intoxicated with lead acetate, through mild reduction in ALP activities with no significant changes in ALT and AST activities.

Table 1: Effect of aqueous extract of M.pruriens seeds on Serum Enzyme Markers in rats intoxicated with Lead Acetate

Treatment n=5	Dose (mg/kg Body wt.)	AST (U/l)	ALT (U/l)	ALP (U/l)
Group 1: Control		101.0 ± 3.00	8.33 ± 1.53	33.00 ± 2.65
Group II: Lead Acetate only	**100**	89.0 ± 10.00[a]	27.67 ± 0.58[a]	30.00 ± 4.58
Group III: Aq.Extract of MP seeds only	**600**	116.33 ± 3.51[b]	40.07 ± 3.06[b]	21.0 ± 2.65[b]
Group IV: Extract of MP +Lead Acetate	**300**	144.0 ± 5.00[b]	32.33 ± 1.55[b]	20.67 ± 1.53[b]
Group V: Extract of MP +Lead Acetate	**600**	128.67 ± 11.37[b]	31.0 ± 2.65[b]	16.76 ± 3.21[b]
Group VI: Extract of MP + Lead Acetate	**1200**	99.0 ± 2.65[a]	30.33 ± 3.51[a]	18.33 ± 1.53[b]

Values are Mean ± SD, n= 5 rats in each group, P<0.05, a as compared with the normal (control) group; b as compared with the lead acetate group only. AST - Aspartate amino transferase; ALT - Alanine aminotransferase; ALP - Alkaline phosphatase

Effect of Aqueous Extract of M.pruriens seeds on Serum Biochemical Molecule in rats intoxicated with Lead Acetate. There were no significant differences in direct bilirubin, total protein, albumin and globulin levels in rats intoxicated with lead acetate only compared with control, and no significant decrease in total bilirubin level. However, when administered aqueous extract of M.pruriens at dose of 600 mg/kg only, there was significant increase of the serum metabolites (Albumin, total bilirubin, direct bilirubin, total protein albumin and globulin).

Table 3.2: Effect of aqueous extract of M.pruriens seeds on Serum Biochemical Molecule in rats intoxicated with Lead Acetate

Treatment n=5	Dose (mg/kg Body wt.)	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Group 1: Control		0.50 ± 0.10	0.13 ± 0.58	0.77 ± 0.25	3.53 ± 0.06	3.23 ± 0.21
Group II: Lead Acetate only	**100**	0.33 ± 0.06	0.13 ± 0.58	0.77 ± 0.15	3.13 ± 0.12	3.23 ± 0.76
Group III: Aq.Extract of MP seeds only	**600**	0.43 ± 0.15	0.17 ± 0.58	7.60 ± 0.98[b]	3.13 ± 0.32	4.90 ± 0.10
Group IV: Extract of MP +Lead Acetate	**300**	0.37 ± 0.06	0.20 ± 0.10	6.97 ± 0.06[b]	3.20 ± 0.10	3.57 ± 0.12
Group V: Extract of MP +Lead Acetate	**600**	0.43 ± 0.06	0.20 ± 0.10	3.20 ± 0.10[b]	3.00 ± 0.90	3.63 ± 0.15
Group VI: Extract of MP + Lead Acetate	**1200**	0.40 ± 1.10	0.13 ± 0.58	3.57 ± 0.12[b]	3.00 ± 0.10	4.53 ± 0.47

Values are Mean ± SD, n= 5 rats in each group, P<0.05, a as compared with the normal (control) group; b as compared with the lead acetate group only. AST - Aspartate aminotransferase; ALT - Alanine aminotransferase; ALP - Alkaline phosphatase

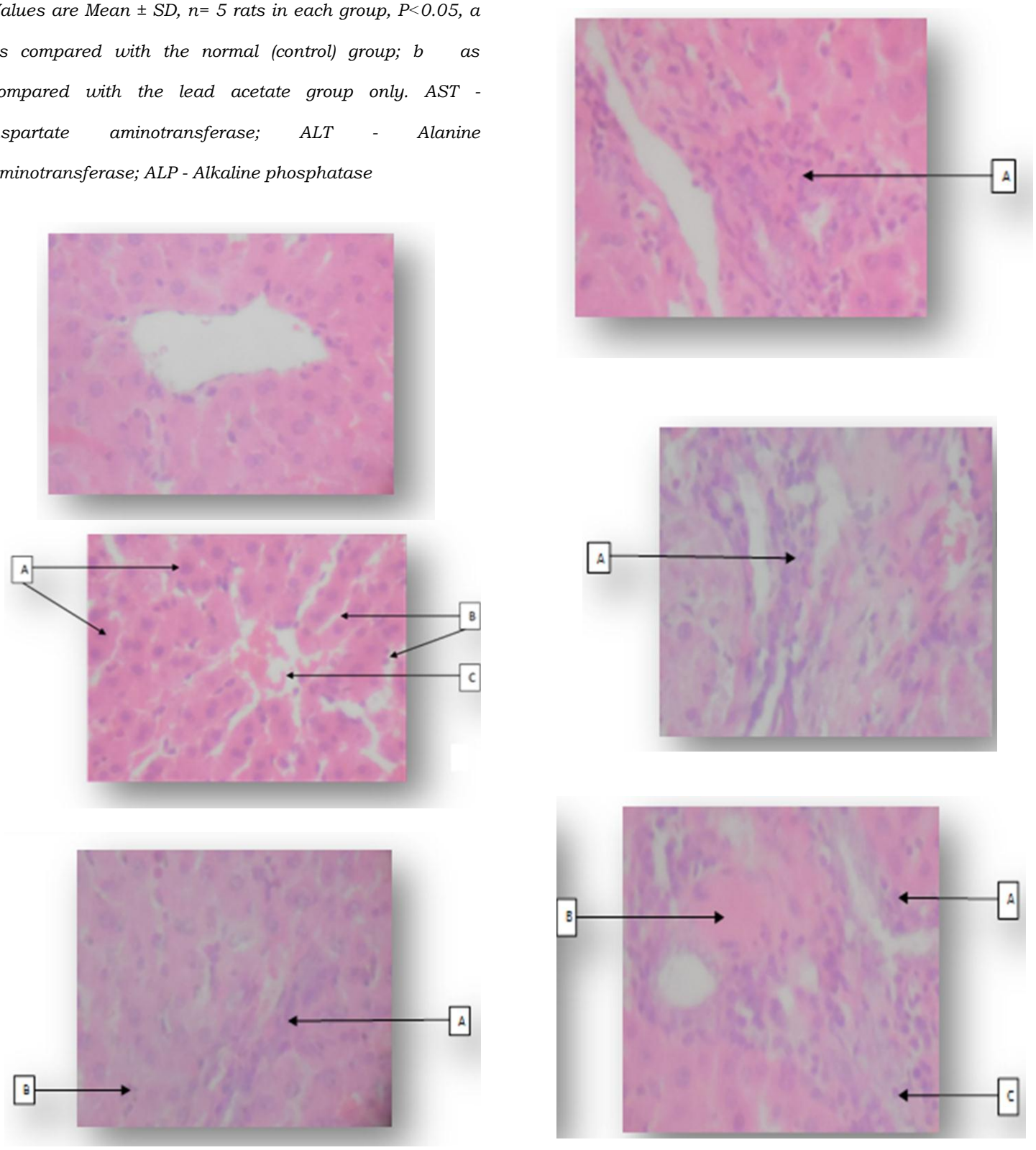

Fig. 1. Photomicrographs of liver sections from (a) Control, Rat liver composed of A, hepatocytes, B,

sinusoids and C, central vein, (b) Rats were given 600 mg/kg of MP extract only, with liver showing A, mild sinusoidal congestion, (c) Rats were given Lead acetate (100 mg/kg) only, with liver showing A, moderate periportal infiltrates of inflammatory cells, (d) Rats were given 300mg/kg of MP extract and Lead acetate (100 mg/kg), with liver showing A, mild periportal infiltrates of inflammatory cells and B, moderate kupffer cell activation, (e) Rats were given 600 mg/kg of MP extract and Lead acetate (100 mg/kg) showing A, mild periportal infiltrates of inflammatory cells, (f) Rats were given 1200 mg/kg of MP extract and Lead acetate (100 mg/kg)showing A, mild periportal infiltrates of inflammatory cells, B, mild vascular congestion and C, mild kupffer cell activation (H&E x 100).

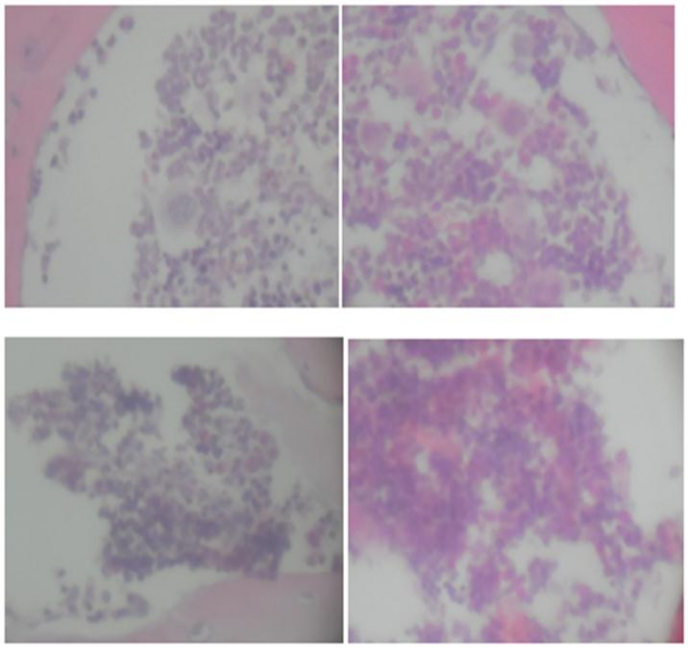

Fig. 2 Photomicrographs of bone sections from (a) Control, Rat bone marrow composed of A, bone trabecula, B, fat vacuoles, C, megakaryocytes and D, myeloid-erythroid cells, (b) Rats were given 600 mg/kg of MP extract only, with bone showing increased A, megakaryocytes, B, myeloid- erythroid cells and C, decreased fat vacuoles, (c) Rats were given Lead acetate (100 mg/kg) only, with bone marrow showing A, moderately decreased myeloid-erythroid cells, (d) Rats were given 300mg/kg of MP extract and Lead acetate (100 mg/kg), with bone marow showing A, mildly increased myeloid-erythroid cells (e) Rats were given 600 mg/kg of MP extract and Lead acetate (100 mg/kg), with rat bone marrow showing A, mildly increased myeloid-erythroid cells, (f) Rats were given 1200 mg/kg of MP extract and Lead acetate (100 mg/kg)with rat bone marrow showing A, moderately increased myeloid-erythroid cells (H&E x 100).

DISCUSSION

Lead (Pb) toxicity is a public health problem of global proportion because it is considered as one of the major environmental pollutants and one of the most deleterious toxins harmful to humans 1. Lead has adverse effects throughout the body. It can damage the kidneys, nervous system, and reproductive system and can cause high blood

pressure. Lead is especially harmful to fetuses and young children, whose brains are still undergoing development. Elevated blood levels of lead in children are a major preventable health problem. The higher the dose and the longer the duration of exposure, the greater the likelihood of damage. Elevated levels can lead to learning disabilities, behavioural problems, and mental retardation. Extremely high levels may lead to seizures and coma and can be fatal 15.However there are several ways of preventing lead poisoning, one of such ways is the use of metal chelating agents. This involves the use of compounds/substances that have the properties to mop up or reduce lead toxicity. It was based on this that this research sought to evaluate the effects of medicinal plants to treat/ameliorate injuries caused by lead poisoning.

Results from this study show the effects of aqueous extract of M. pruriens in serum biochemical hepatic markers. This result showed that rats intoxicated with lead acetate at a dose of 100 mg/kg after 28 days respectively led to the development of mild hepatic injury in rats. Serum activities of ALP was significantly increased ($P< 0.05$) in the rats treated with lead acetate only when compared with the rats in the control group. Elevation of ALP has been reported to be an index of hepatobiliary apparatus injury in rats 16, 17 while ALT and AST showed no significant differences. The administration of aqueous extract of M. pruriens leaf at a dose of 600 mg/kg only showed significant increase in AST and ALT activities, but a significant reduction in ALP activity. This result suggests that the extract has a mild effect at this dose, because high ALT and AST suggest hepatocellular injury. Administration of the extract at both 300 and 600 mg/kg was not able to totally attenuate the toxic effects of lead acetate. This is so because there were significant increases in AST and ALT activities when compared to rats administered lead acetate only (group 2). However there was a significant reduction in ALP levels when compared to group 2. The likely damage to the hepatobiliary apparatus caused by lead toxicity is rather prevented with the administration of the extract. Interestingly at the highest dose of 1200 mg/kg, there were marked reduction in AST, ALT and ALP activities when compared to rats administered with lead acetate only. This was in agreement with Muhammad et al 18, who stated that administration of M. pruriens leaf extract causes a dose dependent decrease ($P < 0.05$) in the level of the liver biomarkers (AST, ALT and ALP) when compared to the control.

There were no significant differences in all serum metabolites (total bilirubin, direct bilirubin, total protein albumin and globulin) intoxicated with lead

acetate only when compared to the control group. There were no significant differences (P>0.05) in total bilirubin, direct bilirubin, albumin and globulin in rats administered with aqueous extract of M.pruriens (600 mg/kg) only, when compared with the control(group I), but showed a significant increase in total protein levels. The raised total protein levels could be as a result of so many reasons, viz; the rats may have been dehydrated, infected with diseases or the plant extract may be toxic to the liver. Administration of the plant extracts at various doses of 300, 600 and 1200 show marked significant increases in total protein levels compared to rats intoxicated with lead acetate.

Furthermore, in the present study, histopathological evaluation of aqueous extract of various doses (300, 600 and 1200 mg/kg body weight) of M. pruriens leaves on the liver and bone marrow were examined. Photomicrographs of the liver in the control group, and group given Mucuna pruriens only (600 mg/kg) showed normal liver architecture with prominent hepatocytes, sinusoids and central vein, while rats exposed to lead acetate only (100 mg/kg) showed moderate infiltrates of mixed population of inflammatory cells, the inflammation was majorly seen around the portal region which is indicative of portal hepatitis in the liver. Similar investigations have also been reported by EL- Sokkary et al 19 and Sharma et al 20. They stated that the lymphocytic infiltration observed following rats intoxicated with lead acetate show evidence of cell irritability, inflammation and hypersensitivity to the lead. However co - administration of lead acetate and the plant extract in groups (IV, V and VI) showed marked reduction in the inflammatory cells around the portal region of the liver in the treated groups as compared to rats intoxicated in the lead acetate only group. This was in agreement with the work done by Lauk et al 21, which affirmed the possible anti-inflammatory effect of Mucuna pruriens. Furthermore, treatment groups administered with graded doses of Mucuna puriens and lead acetate respectively showed mild activation of kupffer cells in the hepatic sinusoids. This indicates activation of the local immune system at these doses.

Phillip and Gerson 22, opined that the bone cells for adults retains approximately 80-95 percent of lead in the bone, while in children approximately 70 percent is stored in bone, resulting in more soft tissue lead in children compared to adults .

Photomicrographic presentation of the histopathological slides of control and Mucunapruriens (600 mg/kg) only showed normal bone architecture involving the bone trabecula, fat

vacuoles, megakaryocyte and myeloid-erythroid cells, while rats intoxicated with lead acetate only, showed moderate reduction in the myeloid-erythroid series or colony of cells and absence of megakaryocytes, which is indicative of reduction in hematopoietic cell population. This correlated with the research carried out by Schuhmacher et al. 23, who stated that lead impairs the activity of pyrimidine 5' -nucleotidase, increasing the pyrimidine nucleotides in red blood cells and preventing the maturation of erythroid elements, which leads to decreased red blood cell counts and eventually reduction in hematopoietic cell population. Experimental groups given lead acetate and Mucuna pruriens (IV, V and VI) at low, moderate and high doses respectively showed a remarkable ameliorative effect in these groups as opposed to those exposed to lead, increasing the number of myeloid-erythroid colony of cells as well as the megakaryocyte number, in a dose dependent manner. The highest dose (1200 mg/kg) showed the most ameliorative effect, this result could be attributed to the higher values of iron, beta carotene, folate, thiamine, zinc, copper and riboflavin, indicating high haematological potentials, which was in agreement with the research done by Chikwendu et al 24, that Mucuna pruriens has the potential of preventing or treating reduction in haematopoietic cell population.

CONLUSION

The study shows the effect of lead on the bone marrow which affects the myeloid-erythroid cell colony in the bone marrow (hypoplasia) leading to reduction in hematopoietic cell population , in which Mucuna pruriens leaf extract demonstrated an ameliorate ability towards the effect of lead, attributed to its anti-anaemic property. In addition, it attenuated the inflammation induced by lead acetate in the portal region of the liver.

AUTHOR'S CONTRIBUTIONS

Eze,G.I, Innih, S.O, and Owolabi O.J carried out the laboratory work and histopathological analysis, while Oriakhi, K took part in the research work and prepared the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest whatsoever

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