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Subchronic Toxicity of Ethanolic Extract Velvet Bean (*Mucuna pruriens*) from Indonesia

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ABSTRACT

The present study is aimed at investigating the subchronic toxicity of ethanol extract of *Mucuna pruriens* seeds from Indonesia. A total of 120 rats Wistar strain, both sexes, were used in this study. Both were divided into one control group, three treated groups, and two satellite groups. The extract in different doses was administered orally for 90 days for the treated groups, while 120 days for the satellite groups. The subchronic toxicity was evaluated using various parameters including death, motoric activity, body weight, haematology parameters, biochemical parameters, and organ weight along with the histopathological study. There was no mortality and no significant change in motoric activity, body weight, several haematological parameters, and glucose levels. Significant differences were found in levels of cholesterol, triglyceride, AST and ALT, BUN, and creatinine. Degeneration of some tissues was seen in the kidneys and the liver. The ethanol extract of *Mucuna pruriens* seeds from Indonesia has a reversible subchronic toxicity to the liver and kidneys.

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INTRODUCTION

Mucuna pruriens or velvet bean is native to tropical Africa and Asia and has been widely used in many countries as a medicinal plant (Deokar, Deore, & Kshirsagar, 2016; Natarajan, Narayanan,

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& Ravichandran, 2012). The seed has been used as a stimulant and nerve tonic to treat impotence and infertility, as well as in the treatment of Parkinson's disease (Kumar & Saha, 2013). The pods are reported as anthelmintic, and the roots are used in the treatment of neurological disorders and blood purification. Another report also mentioned this plant to have antioxidant activity (Agbafor & Nwachukwu, 2011) and can be used in the treatment of cholesterol (Enechi & Ozougwu, 2014), diabetes (Majekodunmi, Oyagbemi, Umukoro, & Odeku, 2011), cough (Sultana, Khan, & Azhar, 2013), rheumatism, gout, cancer, tumors, tuberculosis, asthma, cholera, diarrhea, dysentery, irritation, muscle pain (Deokar et al., 2016; Kumar & Saha, 2013; Natarajan et al., 2012) and to treat snake bites (Fung, Tan, Sim, & Aguiyi, 2012).

Various studies have reported the activity of *Mucuna pruriens* seeds as a promising antiparkinson due to the content of L-DOPA (Cassani et al., 2016; Kasture et al., 2009). L-DOPA is the precursor to the neurotransmitters dopamine and is able to cross the blood-brain barrier. Since Parkinson implies a decrease of dopamine, the presence of L-DOPA can help to increase the dopamine (Hefti & Melamed, 1980; Misra & Wagner, 2007). The highest content of L-DOPA was found in the seeds (Deokar et al., 2016). The seed of *Mucuna pruriens* from Indonesia has been reported to contain about 7.56 to 13.9% L-DOPA (Sardjono, Musthapa, Sholihin, & Ramdhani, 2012). It is also known that *Mucuna pruriens* contains alkaloids, saponins, flavonoids, steroids, triterpenoids, glycosides, reducing sugar, cardiac glycosides and tannins (Akindele & Busayo 2011; Eze, Mohammed, Musa, & Tanko, 2012; Hadimani et al., 2015; Kumar, Rajput, Dhatwalia, & Srivastav, 2009; Pandey & Pandey, 2016).

Toxicity studies have reported that the extract of *Mucuna pruriens* seeds at a dose of 2000 mg/kg body weight did not cause signs of toxicity, did not lead to weight loss and did not cause death in animals (Hadimani et al., 2015; Krishna, Manikyam, Sharma, & Sharma, 2016). The leaf extracts administered orally showed the LD_{50} value of more than 2000 mg/kg (Akindele & Busayo, 2011; Eze et al., 2012). However, the leaf extracts administered by intraperitoneal injection has LD_{50} value of 1509.46 mg/kg (Akindele & Busayo, 2011).

With regard to the use of the plant as an alternative medicine for Parkinson's treatment, toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. Previously the authors reported acute toxicity testing and revealed that the *Mucuna pruriens* seed ethanol extract from Indonesia administered orally is safe or non-toxic with $LD_{50} > 5000$ mg/kg (Sardjono, Musthapa, Sholihin, Qowiyah, & Rachmawati, 2017). In the interest of safety in the use of *Mucuna pruriens* as medicinal plants, and considering that humans more often consume the plant extract at lower dose but in longer time, thus it is necessary to assess the subchronic

toxicity that will provide information on health hazards that may result from repeated exposures to the extract. Unfortunately, there is no any report related to the subchronic toxicity of *Mucuna pruriens* seed extract from Indonesia. Therefore, this research aims to evaluate the subchronic toxicity of ethanol extract of *Mucuna pruriens* seed from Indonesia.

MATERIALS AND METHODS

Plant Material

Seeds of *Mucuna pruriens* were obtained from Bantul, Yogyakarta (07°44′04″S 110°12′34″E). The plant was authenticated in School of Life Sciences, Bandung Institute of Technology, Indonesia with 468/11.CO2.2/PL/2017 voucher specimen number.

Extraction of *M. pruriens* Seeds

The seeds were sun-dried and ground into powder. The powder was macerated with 70% ethanol for 3 × 24 hours at room temperature with daily solvent replacement. The liquid extract was evaporated at 40 °C under low pressure in a rotary vacuum evaporator. The dry extract was served as a suspension in 1% tragacanth, with several doses of extract.

Chemicals

Tragacanth, ethanol, distilled water, Turk solution, sodium citrate, formalin buffer solution and reagents for biochemistry test were obtained from local suppliers with technical and pro analyst quality.

Equipment

The equipment used were rotary evaporator, analytical balance, scale, mortar and stamper, needle oral for rat, syringes 3 cc, cage metabolism, platform for behavioural test, surgical equipment, Eppendorf tube, centrifuge Eppendorf, capillary tube for haematocrit, micropipette, hemocytometer, microscopes, counters, Sahli tube, UV-visible spectrophotometer, magnifying glass, microscope slides, glass cover, and commonly laboratory glassware.

Subchronic Toxicity Study

Animals. The rats of Wistar strain, 60 males and 60 females, aged six to eight weeks were obtained from the Bandung Institute of Technology. Variations of weight did not exceed 20% of the average weight.

Experimental Design. Both male and female rats were divided into six groups (six male groups and six female groups) with 10 rats in each group. The control group received tragacanth (1%) orally for 90 days. The three tested groups, received extract with dose of 50, 400, and 1000 mg/kg body weight respectively for 90 days. The satellite of control group received tragacanth (1%) for 90 days, then stopped and nourished until 120 days, and the satellite of high-dose group received extract with dose of 1000 mg/kg for 90 days, then stopped and nourished until 120 days. The extract was administered orally as much as 1 mL per 200 g of rat body weight (Jaijoy et al., 2011; Parasuraman, 2011; Yuet Ping, Darah, Chen, Sreeramanan, & Sasidharan,

2013). Behavioural and motoric activity was observed before and an hour after the administration of the extract on the first day until the 91st day for control and treated groups, and on the 121st day for satellite groups. Body weight was checked daily for 91 days for the control and tested groups and 121 days for the satellite groups. At the end of the study, the experimental rats were sacrificed. For haematological examination, blood samples collected from jugular vein were mixed with EDTA and for examination of clinical biochemistry, blood samples were centrifuged at 6000 rpm for 15 minutes. Haematological examination included examining the levels of hemoglobin, leukocytes, erythrocytes, platelets, haematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Examination of clinical biochemistry included the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, and triglycerides. Quantitative determination was done by using UV-visible spectrophotometer with enzymatic reagent.

Macroscopic Observation. Liver, spleen, kidneys, heart, lungs, pancreas, brain, stomach, testis (males), and ovaries (females) were washed and dried with paper absorbed. The organs were observed macroscopically and weighed. Organ weights in comparison to body weights were taken to obtain relative organ weights. The condition of the gastric mucosa was also observed macroscopically.

Histopathology Determination. Liver fixed in 10% formalin buffer was dehydrated in graded alcohol, embedded in paraffin, and cut into 4 μm thick sections. Haematoxylin-eosin was used to stain it (Yuet Ping et al., 2013).

Statistical Analysis

Values are expressed as mean±SD (n = 10). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD). All statistical analyses were performed using SPSS 22.0 software. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Subchronic Toxicity

Orally administered ethanol extract of *Mucuna pruriens* seeds for 90 days did not cause any mortality and did not cause a significant change in body weight (Figure 1 and Figure 2). However, there was 20% to 30% decrease in motoric activity and muscle strength in the treated groups of both sexes. A decrease in motoric activity and muscle strength is possible due to increase in body weight. The weight loss observed was possibly caused by stress during the experiment. Both male and female groups showed normal gastric mucosa condition and asserted that the ethanol extract of *Mucuna pruriens* is safe for the stomach. This finding is in line with previous report by Golbabapour et al. (2013).

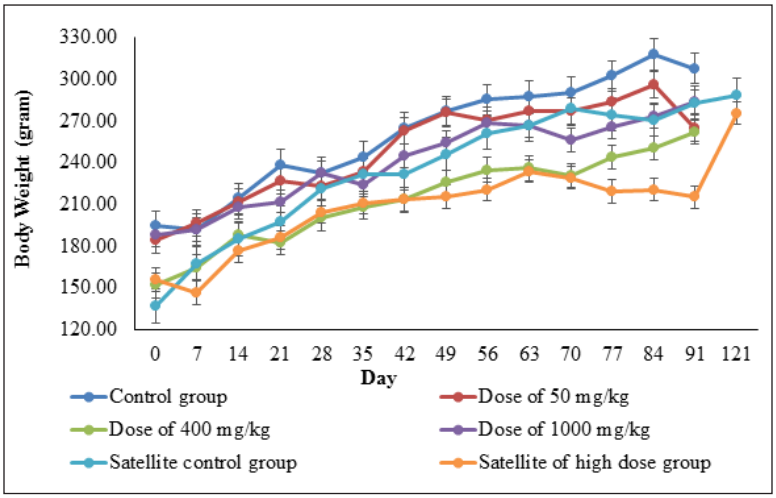

Figure 1. Profile of body weight gains in male rats during experiment

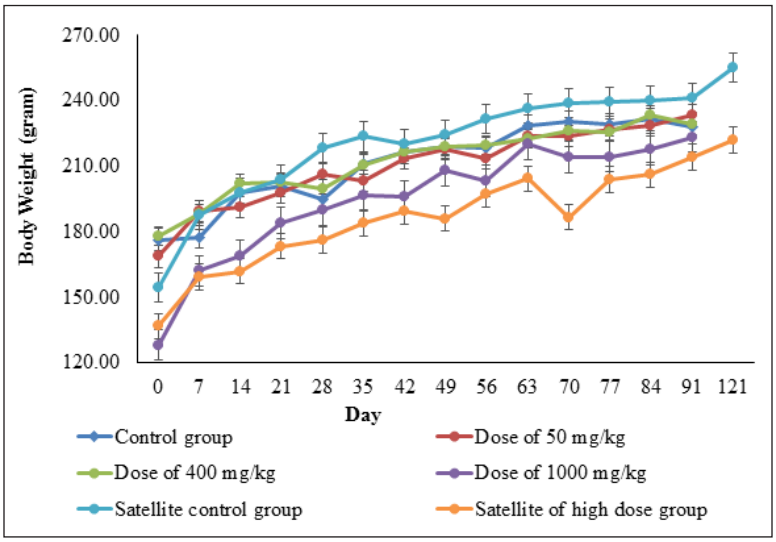

Figure 2. Profile of body weight gains in female rats during experiment

Haematology Parameters

Extract of *Mucuna pruriens* seeds did not affect most of the haematological parameters. Several parameters such as hemoglobin, erythrocytes, leukocytes, platelets, and haematocrit increased significantly, while the number of MCHC and MCH showed a decrease. Haematological examination results can be seen in Table 1. Increase in hemoglobin and erythrocyte is possibly caused by the minerals contained in *Mucuna pruriens* seeds such as Fe, Mg, Zn, Cu and ascorbic acid, which can help the formation of blood cell (Akindele & Busayo, 2011;

Table 1
Haematology parameters of male and female rats orally administered ethanol extract of Mucuna pruriens *seeds for 90 days*

Parameters	Control	Extract of Mucuna pruriens seeds (mg/kg)			Satellite groups	
		50	400	1000	Control	High-dose
Male						
Hemoglobin (gr/dL)	15,84±3.87	**18,44±2,31 (*P*=0,026[a])**	14,71±1,55 (*P*=0,330)	**12,12±2,64 (*P*=0,002[a])**	**12,09±1,00 (*P*=0,002[a])**	**16,38±1,75 (*P*=0,000[b])**
Leukocytes ($\times10^3\times mm^3$)	3,38±0.94	3,86±1,02 (*P*=0,471)	2,72±0,96 (*P*=0,322)	3,94±2,08 (*P*=0,401)	2,72±1,22 (*P*=0,322)	4,54±1,71 (*P*=0,368)
Erythrocytes ($\times10^6/mm^3$)	1,87±0.55	1,85±0,31 (*P*=0,965)	1,23±0,25 (*P*=0,167)	**4,12±2,97 (*P*=0,000[a])**	1,28±0,33 (*P*=0,203)	**2,3±0,53 (*P*=0,000[b])**
Platelets ($\times10^5/mm^3$)	2,52±0,69	2,00±0,20 (*P*=0,234)	2,90±0,42 (*P*=0,379)	**3,57±1,11 (*P*=0,016[a])**	**3,57±1,11 (*P*=0,016[a])**	2,93±0,83 (*P*=0,343)
Haematocrit (%)	42,85±5,37	**50,45±4,73 (*P*=0,035[a])**	43,68±4,48 (*P*=0,814)	**63,32±8,31 (*P*=0,000[a])**	44,61±10,52 (*P*=0,620)	**50,51±8,25 (*P*=0,000[ab])**
MCV (μ^3/sel)	2,52±0,95	2,78±0,42 (*P*=0,675)	3,69±0,86 (*P*=0,063)	2,43±1,43 (*P*=0,885)	3,72±1,32 (*P*=0,057)	2,30±0,60 (*P*=0,828)
MCHC (pg/sel)	37,43±9,93	36,60±3,44 (*P*=0,790)	33,89±4,14 (*P*=0,257)	**19,47±4,99 (*P*=0,000[a])**	**28,90±8,93 (*P*=0,007[a])**	**33,41±7,30 (*P*=0,000[b])**
MCH (g/dL)	92,54±36,31	101,04±11,99 (*P*=0,634)	122,99±21,26 (*P*=0,900)	**48,97±33,82 (*P*=0,016[a])**	100,89±29,71 (*P*=0,640)	74,82±19,18 (*P*=0,149)
Female						
Hemoglobin (gr/dL)	14,98±2,18	15,70±4,25 (*P*=0,53)	15,94±2,39 (*P*=0,408)	13,16±3,50 (*P*=0,118)	14,01±1,83 (*P*=0,403)	**10,56±1,49 (*P*=0,026[ab])**
Leukocytes ($\times10^3\times mm^3$)	3,52±1,48	2,54±0,60 (*P*=0,143)	**2,02±0,95 (*P*=0,026[a])**	**5,40±1,96 (*P*=0,006[a])**	3,90±2,04 (*P*=0,568)	4,18±1,86 (*P*=0,069)
Erythrocytes ($\times10^6/mm^3$)	1,43±0,41	1,17±0,38 (*P*=0,570)	1,33±0,56 (*P*=0,836)	1,96±1,33 (*P*=0,248)	1,32±0,45 (*P*=0,810)	1,45±0,49 (*P*=0,266)
Platelets ($\times10^5/mm^3$)	1,69±0,39	2,35±0,77 (*P*=0,125)	**2,69±0,70 (*P*=0,022[a])**	**3,10±2,58 (*P*=0,011[a])**	2,44±0,47 (*P*=0,084)	2,5±0,48 (*P*=0,169)
Haematocrit (%)	48,06±10,92	**40,85±6,74 (*P*=0,045[a])**	43,26±8,35 (*P*=0,179)	**61,81±4,03 (*P*=0,000[a])**	49,06±13,51 (*P*=0,778)	**45,44±1,91 (*P*=0,000[b])**
MCV (μ^3/sel)	3,66±1,39	3,99±1,95 (*P*=0,597)	3,82±2,05 (*P*=0,802)	3,92±1,38 (*P*=0,677)	4,26±2,09 (*P*=0,338)	3,46±1,10 (*P*=0,463)
MCHC (pg/sel)	32,45±8,17	38,46±9,26 (*P*=0,056)	37,83±7,76 (*P*=0,087)	**21,21±4,86 (*P*=0,000[a])**	29,88±6,83 (*P*=0,410)	**23,29±3,45 (*P*=0,004[a])**
MCH (g/dL)	111,04±35,95	145,87±55,88 (*P*=0,053)	144,58±80,29 (*P*=0,062)	82,47±31,24 (*P*=0,111)	119,20±45,07 (*P*=0,648)	80,27±28,93 (*P*=0,087)

Note: Values are expressed as mean±SD (n=10). $P < 0.05$ was considered significantly different using one-way ANOVA followed by least significant difference (LSD). [a]significant significantly different compared to control group. [b]significant significantly different compared to high-dose group

Table 2
Biochemistry parameters of male and female rats orally administered the ethanol extract of Mucuna pruriens *seeds for 90 days*

Parameters	Contol	Extract of Mucuna pruriens seeds (mg/kg)			Satellite groups	
		50	400	1000	Control	High-dose
Male						
Glucose (mg/dL)	120,80±15,59	133,9±18,42 (*P*=0,089)	135,70±20,11 (*P*=0,053)	110±24,64 (*P*=0,160)	116,90±16,26 (*P*=0,610)	111,8±18,20 (*P*=0,814)
Total Cholesterol (mg/dL)	58,23±33,45	100,90±38,41 (*P*=0,078)	**122,46±47,94 (*P*=0,008[a])**	**254,20±49,74 (*P*=0,000[a])**	**138,53±53,30 (*P*=0,001[a])**	**132,29±65,49 (*P*=0,000[ab])**
Triglyceride (mg/dL)	103,702±59,05	96,35±93,51 (*P*=0,775)	59,03±27,58 (*P*=0,085)	95,29±73,83 (*P*=0,744)	100,70±79,02 (*P*=0,907)	**46,19±23,13 (*P*=0,027[a])**
BUN (mg/dL)	25,02±3,61	**19,26±5,79 (*P*=0,007[a])**	27,71±2,71 (*P*=0,202)	**13,55±3,46 (*P*=0,000[a])**	25,73±4,48 (*P*=0,733)	**21,48±4,65 (*P*=0,000[b])**
Creatinine (mg/dL)	1,08±0,31	1,49±0,98 (*P*=0,148)	1,49±0,98 (*P*=0,148)	**2,00±0,89 (*P*=0,001[a])**	0,91±0,42 (*P*=0,845)	**1,02±0,39 (*P*=0,001[b])**
AST (U/L)	153,40±29,57	160,50±25,81 (*P*=0,564)	177,40±21,25 (*P*=0,053)	153,90±30,20 (*P*=0,968)	152,80±19,27 (*P*=0,961)	**180,60±34,26 (*P*=0,032[ab])**
ALT (U/L)	54,30±12,06	53,80±9,32 (*P*=0,925)	**74,30±10,21 (*P*=0,000[a])**	48,70±10,10 (*P*=0,291)	64,3±10,39 (*P*=0,061)	62,00±18,55 (*P*=0,148)
Female						
Glucose (mg/dL)	99,30 ±15,48	104,10±12,02 (*P*=0,530)	107,50±14,79 (*P*=0,285)	107,90±17,96 (*P*=0,262)	97,10±15,77 (*P*=0,774)	111,10±11,24 (*P*=0,676)
Total Cholesterol (mg/dL)	131,49±61,90	124,64±61,73 (*P*=0,776)	142,52±59,74 (*P*=0,646)	**276,40±52,72 (*P*=0,000[a])**	128,92±55,73 (*P*=0,915)	**80,35±53,42 (*P*=0,000[ab])**
Triglyceride (mg/dL)	119,52±80,20	96,93±66,56 (*P*=0,381)	**59,49±42,27 (*P*=0,021[a])**	**54,89±24,85 (*P*=0,013[a])**	83,05±24,78 (*P*=0,159)	**48,11±21,48 (*P*=0,006[a])**
BUN (mg/dL)	19,26±7,17	20,68±6,38 (*P*=0,499)	**25,27±3,83 (*P*=0,005[a])**	**23,61±2,60 (*P*=0,041[a])**	**23,96±4,61 (*P*=0,027[a])**	21,14±4,44 (*P*=0,241)
Creatinine (mg/dL)	1,18±0,46	1,95±0,45 (*P*=0,007)	1,30±0,50 (*P*=0,683)	**2,12±0,72 (*P*=0,001[a])**	1,15±0,55 (*P*=0,907)	**0,65±0,40 (*P*=0,000[b])**
AST (U/L)	143,1±28,42	112,60±25,92 (*P*=0,014)	117,80±18,71 (*P*=0,042)	107,40±15,32 (*P*=0,004)	**173,50± 47,04 (*P*=0,015[a])**	107,90±17,48 (*P*=0,005)
ALT (U/L)	40,8±8,65	**53,1±17,74 (*P*=0,022[a])**	48,4±9,67 (*P*=0,153)	47,2±14,88 (*P*=0,228)	49,80±6,48 (*P*=0,091)	44,2±6,02 (*P*=0,521)

Note: Values are expressed as mean±SD (n=10). P<0.05 was considered significantly different using one-way ANOVA followed by least significant difference (LSD). [a]significant significantly different compared to control group. [b]significant significantly different compared to high-dose group

Chukwudi, Simeon, & Chinyere, 2011; Ifemeje, 2016; Obioma, Emmanuel, Okechukwu, & Ifemeje, 2014; Tavares, Silva, Campos, Schuler, & Aquino, 2015). Increase in leukocyte is possibly triggered by a metabolic assault from alkaloid and/or phenolic contained in *Mucuna pruriens* (Chukwudi et al., 2011). However, an increase in leukocyte was still below normal levels (10,000/mm^3), which means it was not due to an infection.

Biochemical Parameters

The effect of ethanol extract of *Mucuna pruriens* seeds on biochemical parameters is presented in Table 2. There were no significant differences in glucose levels in male and female groups. The result in glucose levels supports the findings of the ability of *Mucuna pruriens* to maintain the levels of glucose (Bhaskar, Vidhya, & Ramya, 2008; Bhutkar & Bhise, 2013; Eze et al., 2012; Majekodunmi et al., 2011).

However, there were significant changes in total cholesterol and triglyceride levels. Many studies have reported that *Mucuna pruriens* has a powerful anti-cholesterol effect, which can be attributed to its high antioxidant activity. The hypocholesterolemic effects of *Mucuna puriens* may be due to several mechanisms, including inhibition of the cholesterol biosynthesis, prevention of low-density lipoprotein (LDL) oxidation by flavonoids, conversion of cholesterol to bile acids, and inhibition of cholesterol absorption from the intestine by the formation of complexes with compounds such as glycosides and saponins (Dhanasekaran, Tharakan, & Manyam, 2008; Enechi & Ozougwu, 2014). Besides helping to reduce LDL or bad cholesterol, triglyceride and total cholesterol levels, *Mucuna pruriens* also helps in increasing the level of high-density lipoprotein (HDL) or good cholesterol. Therefore, an increase in total cholesterol levels in this study is possibly due to the increase of HDL.

The level of BUN and creatinine in the treated groups showed significant changes too. These changes were reversible, indicated by the change of BUN and creatinine levels close to normal when the administration of the extract was stopped as shown in the satellite of high-dose groups. It indicated that the extract of *Mucuna pruriens* seeds did not cause any significant damage to the kidneys. This finding was also supported by the result of histopathologic assessment.

Further, the extract of *Mucuna pruriens* seeds induced significant changes of ALT. The level of ALT significantly increased, indicating hepatotoxic that causes liver damage (Giannini, Testa, & Savarino, 2005). However, an increase in ALT levels of the rats is relatively safe because the value is slightly higher than normal. This finding is in line with previous reports (Ezeja & Omeh, 2010; Obioma et al., 2014) that showed a hepatotoxicity of *Mucuna pruriens*. However, other reports showed hepatoprotective effects of *Mucuna pruriens* (Chukwudi et al., 2011; Obogwu, Akindele, & Adeyemi, 2014).

Macroscopic Observations (The Relative Organ Weight)

Based on the relative organ weight measurement, the female group showed significant differences both in kidneys and liver, while the male group only showed a significant difference in liver (Table 3). Compared to the results of its biochemical parameters, the significant difference in relative organ weight of the kidneys did not affect the function of the organ. This was also reinforced by the results of relative organ weight measurements of high-dose satellite group which did not show any significant difference with the normal group. It also indicated that toxicity effects of the extract are reversible. However, the significant differences in relative organ weight of liver in both male and female groups indicate toxicity of the extract to the liver.

Table 3
Relative organ weight for all groups after administration of extract of Mucuna pruriens *seeds for 90 days*

Organ	Contol	Extract of Mucuna pruriens seeds (mg/kg)			Satellite groups	
		50	400	1000	Control	High-dose
Male						
Liver	2,341 ± 0,262	2,430 ± 0,202 (P=0,489)	**2,998 ± 0,208 (P=0,000[a])**	**2,624 ± 0,267 (P=0,029[a])**	**2,815 ± 0,214 (P=0,000[a])**	**2,827 ± 0,144 (P=0,000[a])**
Heart	0,257 ± 0,027	0,319 ± 0,033 (P=0,272)	0,282 ± 0,014 (P=0,665)	0,299 ± 0,032 (P=0,458)	0,299 ± 0,032 (P=0,459)	0,311 ± 0,039 (P=0,837)
Lungs	0,637 ± 0,120	**0,835 ± 0,266 (P=0,012[a])**	0,587 ± 0,119 (P=0,522)	**0,842 ± 0,239 (P=0,009[a])**	0,657 ± 0,122 (P=0,797)	**0,618 ± 0,116 (P=0,004[b])**
Kidneys	0,566 ± 0,067	0,640 ± 0,059 (P=0,063)	0,612 ± 0,038 (P=0,250)	0,611 ± 0,068 (P=0,259)	0,614 ± 0,054 (P=0,226)	0,567 ± 0,052 (P=0,267)
Spleen	0,182 ± 0,046	0,161 ± 0,027 (P=0,407)	0,170 ± 0,061 (P=0,633)	0,181 ± 0,068 (P=0,965)	0,184 ± 0,073 (P=0,937)	0,228 ± 0,059 (P=0,066)
Stomach	0,447 ± 0,071	0,443 ± 0,052 (P=0,937)	0,443 ± 0,078 (P=0,930)	0,402 ± 0,141 (P=0,305)	0,469 ± 0,081 (P=0,605)	0,443 ± 0,051 (P=0,346)
Brain	0,624 ± 0,080	**0,730 ± 0,103 (P=0,034[a])**	0,711 ± 0,065 (P=0,081)	0,684 ± 0,104 (P=0,229)	0,669 ± 0,091 (P=0,366)	0,686 ± 0,064 (P=0,956)
Testes	0,478 ± 0,059	0,227 ± 0,084 (P=0,163)	0,535 ± 0,096 (P=0,320)	0,527 ± 0,035 (P=0,394)	0,532 ± 0,085 (P=0,345)	0,505 ± 0,061 (P=0,703)
Female						
Liver	2,571 ± 0,193	**3,292 ± 0,477 (P=0,000[a])**	**2,956 ± 0,173 (P=0,003[a])**	**3,139 ± 0,161 (P=0,000[a])**	**3,360 ± 0,489 (P=0,000[a])**	**3,154 ± 0,373 (P=0,000[a])**
Heart	0,322 ± 0,036	0,324 ± 0,056 (P=0,970)	0,313 ± 0,025 (P=0,869)	0,310 ± 0,034 (P=0,827)	0,463 ± 0,422 (P=0,014)	0,357 ± 0,373 (P=0,409)
Lungs	0,620 ± 0,177	0,653 ± 0,205 (P=0,666)	0,613 ± 0,131 (0,921)	0,628 ± 0,111 (P=0,919)	0,902 ± 0,228 (P=0,000)	0,680 ± 0,133 (P=0,503)
Kidneys	0,667 ± 0,095	**0,530 ± 0,177 (P=0,001[a])**	**0,565 ± 0,028 (P=0,011[a])**	**0,564 ± 0,097 (P=0,010[a])**	0,620 ± 0,142 (P=0,234)	0,624 ± 0,064 (P=0,130)

Table 3 *(continue)*

Organ	Contol	Extract of Mucuna pruriens seeds (mg/kg)			Satellite groups	
		50	400	1000	Control	High-dose
Female						
Spleen	0,375 ± 0,058	**0,232 ± 0,045 (P=0,000[a])**	**0,308 ± 0,040 (P=0,009[a])**	**0,305 ± 0,027 (P=0,006[a])**	**0,240 ± 0,102 (P=0,000[a])**	0,340 ± 0,062 (P=0,156)
Stomach	0,493 ± 0,072	0,580 ± 0,182 (P=0,050)	0,452 ± 0,154 (P=0,339)	0,465 ± 0,068 (P=0,511)	0,464 ± 0,053 (P=0,499)	0,508 ± 0,040 (P=0,320)
Brain	0,780 ± 0,149	**0,673 ± 0,240 (P=0,034[a])**	0,811 ± 0,055 (P=0,526)	0,806 ± 0,052 (P=0,601)	0,735 ± 0,115 (P=0,367)	0,847 ± 0,063 (P=0,408)
Ovaries	0,380 ± 0,117	0,458 ± 0,176 (P=0,172)	0,382 ± 0,183 (P=0,976)	0,431 ± 0,119 (P=0,369)	0,276 ± 0,069 (P=0,942)	**0,562 ± 0,247 (P=0,022[ab])**

Note: Values are expressed as mean±SD (n=10). P<0.05 was considered significantly different using one-way ANOVA followed by least significant difference (LSD). [a]significant significantly different compared to control group. [b]significant significantly different compared to high-dose group

Histopathologic Assessment

A mild glycogen depletion showed in the tissue of the kidneys of the treated groups. Figure 3 shows the tissue of the liver in the treated groups that had moderate glycogen depletion and enlargement of nuclei in some cells of the liver. Although this indicates toxicity effects of the extract, significant pathological changes in the kidneys and liver did not show the phase of necrosis (cell death), thus the kidneys and liver were still quite functional. The satellite of high-dose groups of both genders also showed recovery of the tissue, either in the kidneys and the liver asserted reversible toxic effects of the extract on the kidneys and liver.

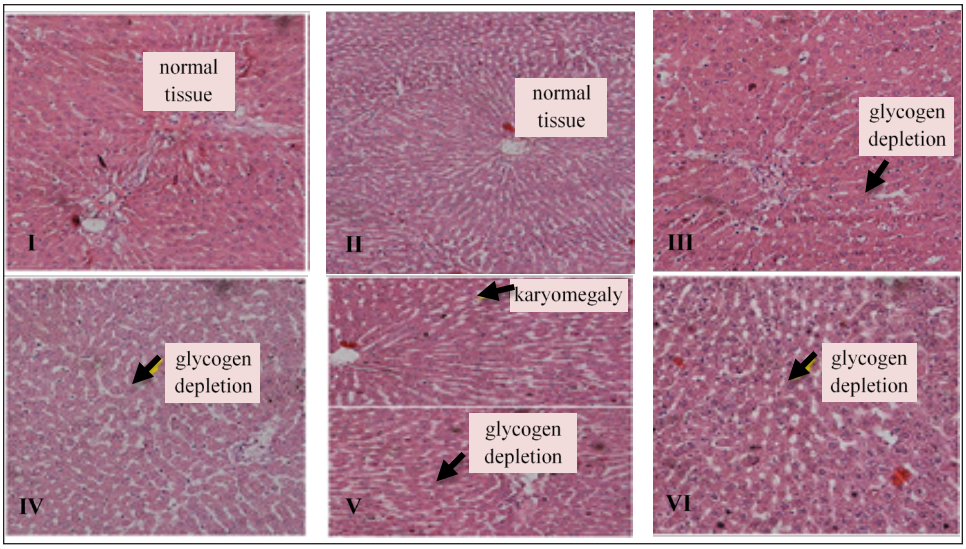

Figure 3. Histopathology of liver tissues

The source of liver are: (I) liver from the control group, (II) liver from satellite control group, (III) liver from a rat treated with 50 mg/kg extract of *Mucuna pruriens* seeds, (IV) liver from a rat treated with 400 mg/kg extract of *Mucuna pruriens* seeds, (V) liver from a rat treated with 1000 mg/kg extract of *Mucuna pruriens* seeds, and (VI) liver from the satellite of high-dose group.

CONCLUSION

Repeated oral consumption of ethanolic extract of *Mucuna pruriens* seed for 90 days did not cause death. The extract did not cause any change in food intake, water consumption, and body weight change. It also did not cause any significant change to the haematology and biochemical parameters. However, the extract caused glycogen depletion and enlargement of nuclei in some cells of the kidneys and liver but did not cause cell death. 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.

CONFLICT OF INTEREST STATEMENT

The authors declare that they did not face any conflict of interest while conducting this study.

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