

Full Length Research Paper

Effects of aqueous extracts of *Mucuna sloanei* (Fabaceae) seed on haematological parameters of albino rats (*Rattus norvegicus*)

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Mucuna sloanei is a legume used as a soup thickener by communities in some parts of Africa countries. The effect of aqueous seed extract of *M. sloanei* on the haematological profile of normal albino rats was investigated for 28 days using standard methods. The results show no overall dose dependent significant difference ($p > 0.05$) in the serum levels of the white blood cell count, red blood cell count and its indices (haemoglobin content, pack cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) of the rats throughout the duration of the experiment when compared with the control. However, a dose independent significant reduction ($p < 0.05$) in the white blood cell level was observed in week 3, while the decrease observed in the remaining week was not significant. The effects of the interaction between the doses and duration of treatment were not statistically significant ($p > 0.05$) in all the haematological parameters tested. These findings suggest that consumption of the crude seed extracts of *M. sloanei* may not constitute any adverse effect on the haematological indices of the consumers. However, the significant reduction in white blood cell levels observed in week 3 could compromise the body's immunity and may predispose consumers to opportunistic and supra-infections in the long run.

Key word: *Mucuna sloanei* seed, aqueous extract, haematological parameters, albino rats.

INTRODUCTION

Several forest resources have been domesticated and today they are contributing in feeding the world's teeming population especially in the developing world. The growing demands in plant-based proteins for humans and livestock,

have elicited several researches on the possibilities of employing underutilized legumes as inexpensive and elegant source of protein than the conventional sources such as, soybean (*Glycine max*), groundnut (*Arachis*

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Abbreviations: RBC, Total red blood cells; Hb, haemoglobin; WBC, white blood cells; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; F-LSD, Fisher's least significant difference; SEM, standard error of mean.

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hypogea) and animal based proteins (Siddhuraju et al., 1995; Krause et al., 1996; Chel-Guerrero et al., 2002). *Mucuna sloanei*, an annual leguminous climber commonly called the “horse eye” or “hamburger” bean is one of such important legumes and is widely used among the various ethnic groups resident in Nigeria. It is variously called ‘ukpo’ by the Ibos; ‘karasuu’ by the Hausas; ‘yerepe’ by the Yorubas (Nwosu, 2011) and ‘ibabat’ by the Efiks of Nigeria (Obochi et al., 2007). *M. sloanei* seeds contain high protein, carbohydrate, crude fat and fiber contents (Akpata and Miachi, 2001) as well as a very rich amino acid profile (Ojiako et al., 2012). These constituents potentiate the seeds as a nutritious source of meal with high water binding capacity arising from the formation of hydrogen bonds between water and polar residues of the protein molecules (Obochi et al., 2007). The seeds are used as thickener in soups as well as a source of edible vegetable oil in many Igbo communities of Southeastern Nigeria (Afolabi et al., 1985; Ukachukwu et al., 2002). According to Ukachukwu and Obioha (1997), several rural populations of Nigeria, fall back on seeds of *M. sloanei* as soup thickeners during famine and scarcity of alternative soup thickeners such as melon.

M. sloanei seeds like all *Mucuna* species are very rich in many important bioactive substances such as L-3, 4-dihydroxyphenylalanine (L-DOPA) (Adebowale et al., 2005), which has been reported to be a potent precursor of the brain neurotransmitter, dopamine (Hornykiewicz, 2002; Kostrzewa et al., 2005; Nagatsua and Sawadab, 2009). Similarly, lectin from *M. sloanei* has been reported as an effective and suitable cell receptor signal inducer as a result of its agglutinating ability in various blood cells of humans, goat, cow and chicken (Obochi et al., 2007). The Efiks of South south Nigeria claim that consumption of seeds of *M. sloanei* lowers libido in men as well as possessing sedative properties (Obochi et al., 2007). Despite all these varied perceptions, there is paucity of information regarding the effects of this important food condiment on blood parameters in experimental animal *vis-à-vis* the possible risks associated with its consumption by humans. The present study was therefore initiated to ascertain the haematological problems associated with oral consumption of aqueous extracts of shade dried dehulled seeds of *M. sloanei*.

MATERIALS AND METHODS

Collection and preparation of *M. sloanei* crude seed extract

Dried and mature nuts of *M. sloanei* were purchased from local markets around Nsukka metropolis. The seeds were identified using the identification key of Anyanwu and Okoli (2004). They were dehulled, dried at room temperature and pulverized into fine powder using a milling machine (Honda: model 622, China). The method of extraction followed that of Akintayo et al. (2000). A total of 100 g of the powdered sample was introduced into 2000 ml flat bottom flask and 1500 ml of distilled water was added. The content was mixed thoroughly and left for about 24 h with an occasional shaking to

increase the extraction capacity. Thereafter, the soaked substance was filtered with a muslin clothe (number 60 mesh size) and concentrated to dryness. The solid extract was weighed and redissolved in normal saline according to the body weights of the animals for oral administration.

Procurement and management of experimental animals

Forty-eight (48) adult male albino rats weighing between 150 to 250 g were obtained from the Genetics and Animal breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats had no history of drug consumption (that is, they have not been used for any investigation). They were kept in stainless wire rat cages with dimensions (12 × 40 × 15 cm) equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats were fed with commercial growers chick mash (18% crude protein) made by Vital Feeds, Nigeria Limited and clean drinking water. They were allowed to acclimatize 14 days before the start of the experiment. All the animals were maintained under standard laboratory conditions for temperature, humidity and light throughout the experiment and were allowed unhindered access to food and water. The fecal droppings in the tray were removed daily. All the experiment was carried out under the approval of the Faculty Ethical Committee on Animal Research, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.

Toxicity test and experimental design

The LD₅₀ of the extract in mice was determined orally using Lorke (1983) methodology with slight modification. The forty eight (48) male albino rats were then assigned into four groups (A, B, C, and D) of 12 rats per group. Each group was further replicated three times comprising of 4 rats per replicate. The rats in group A (control) were fed with normal rat feed and 1 ml/kg body weight of normal saline *ad libitum*. The treatment groups, B, C and D were administered 100, 200 and 400 mg/kg body weight of the seed extract, respectively. All the doses were administered once daily orally for 28 days (four weeks) for all the groups using 1 ml syringe.

Collection of blood sample

About 5 ml of the blood samples was collected from each of the anaesthetized rats using the ocular puncture method described by Hoff (2000). This was done before the start of the experiment (Week 0) and at weekly intervals during treatment (weeks 1 to 4) for the various haematological profile tests.

Determination of haematological parameters

Total red blood cells (RBC) count, haemoglobin (Hb) content and white blood cells (WBC) count were determined according to the methods described by Sood (2006). The packed cell volume (PCV) was ascertained using the methodology of Coles (1986). A heparinized capillary tube was filled to approximately three fourth (¾) of its length with well-mixed anticoagulated blood. The coloured end of the capillary tube was sealed with plasticin. The capillary tube was then placed into a microhaematocrit centrifuge set at 7826 g for 5 min. The height of the packed cell as well as the total height in millimeter was measured using the haematocrit reader. The packed cell volume was then calculated using the formula:

$$\text{PCV (\%)} = \frac{\text{Height of red cell (mm)}}{\text{Total height (mm)}} \times 100$$

Table 1. Effects of the aqueous seed extract of *M. sloanei* on red cell indices of albino rats.

Parameter	Concentrations (mg/kg)	Duration (week)				
		0	1	2	3	4
MCV (μ^3)	Control	54.20±0.06 ^{a1}	54.30±0.10 ^{b1}	54.20±0.10 ^{a1}	54.33±0.07 ^{a1}	54.20±0.12 ^{a1}
	100	54.23±0.07 ^{a1}	54.03±0.03 ^{a1}	54.10±0.12 ^{a1}	54.17±0.15 ^{a1}	54.30±0.06 ^{a1}
	200	54.13±0.07 ^{a1}	54.07±0.07 ^{a1}	54.00±0.10 ^{a1}	54.10±0.15 ^{a1}	54.23±0.09 ^{a1}
	400	54.23±0.03 ^{a1}	54.33±0.03 ^{b1}	54.03±0.09 ^{a1}	54.17±0.03 ^{a1}	54.10±0.00 ^{a1}
MCH (Pg)	Control	18.10±0.00 ^{a1}	18.10±0.00 ^{ab1}	18.03±0.03 ^{a1}	18.10±0.00 ^{a1}	18.07±0.03 ^{ab1}
	100	18.07±0.03 ^{a1}	18.00±0.00 ^{c1}	18.03±0.03 ^{a1}	17.33±0.82 ^{a2}	18.10±0.00 ^{b1}
	200	18.07±0.03 ^{a1}	18.03±0.03 ^{ac1}	18.03±0.03 ^{a1}	18.03±0.03 ^{a1}	18.07±0.00 ^{ab1}
	400	18.10±0.00 ^{a1}	18.17±0.03 ^{b2}	18.03±0.03 ^{a1}	18.07±0.03 ^{a1}	18.00±0.00 ^{a1}
MCHC (g/dl)	Control	33.33±0.03 ^{a1}	33.33±0.03 ^{a1}	33.30±0.00 ^{a1}	33.33±0.03 ^{a1}	33.30±0.00 ^{a1}
	100	33.57±0.22 ^{a1}	33.37±0.03 ^{a2}	33.33±0.03 ^{a2}	33.33±0.03 ^{a2}	33.30±0.00 ^{a2}
	200	33.37±0.03 ^{a1}	33.37±0.3 ^{a1}	33.30±0.00 ^{a1}	33.37±0.03 ^{a1}	33.30±0.00 ^{a1}
	400	33.30±0.00 ^{a1}	33.37±0.03 ^{a1}	33.33±0.03 ^{a1}	33.30±0.00 ^{a1}	33.30±0.00 ^{a1}

Values with different alphabetic superscripts differ significantly ($p < 0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration.

Similarly the red blood cell indices namely, mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were determined according to the methods described by Baker et al. (2001). The reagents used for the analyses were all analytical grades.

Statistical analysis

Data accumulated was analyzed using the GENSTAT (VSN International, Hemel Hempstead, Herts, UK). Whereas, a one-way analysis of variance (ANOVA) was used to test the effect of treatment, a Two-way ANOVA was used to determine the interactive effects of treatment and duration. Fisher's least significant difference (F-LSD) was used in the separation of means of the different treatment groups. All results were expressed as mean \pm standard error of Mean (SEM), while values were considered significant at $p < 0.05$.

RESULTS

The oral LD₅₀ of the aqueous seed extract in the rats showed no mortality at the different doses of 1000, 3000 and 5000 mg/kg. However, such cage side characteristics as shivering, bulging eyes and dullness were observed in the rats administered the 5000 mg/kg dose. Table 1 shows the results of the weekly effects of the seed extracts of *M. sloanei* on the haematological parameters assayed, namely, red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV) level, haemoglobin (Hb) content and the red blood cell indices (MCV, MCH and MCHC) in the albino rats. There was no overall dose dependent significant difference ($p > 0.05$) observed in the levels of all the parameters in the weeks when compared with the control. However,

minimal variations were observed in the weekly levels of some of the parameters; whereas, a significant decrease ($p < 0.05$) was observed in the RBC levels of the rats administered 100 and 200 mg/kg in week 1, the WBC levels decreased significantly ($p < 0.05$) in all the dose levels in week 3. Similarly, there was a sharp increase in the mean WBC levels observed in week 2 of the animals given 200 and 400 mg/kg, followed by a somewhat marked sharp decreases in values from week 2 to 4, respectively (Table 1).

The PCV and Hb levels also decreased significantly ($p < 0.05$) in rats administered the dose levels of 100 mg/kg (weeks 1 and 3) and 200 mg/kg (week 1), respectively. In the same vein, minor variations were observed in the levels of the red cell indices. A significant decrease ($p < 0.05$) in the MCV level was observed in rats administered the doses of 100 and 200 mg/kg in week 1. Thereafter, a very slight increase in the mean values of MCV was observed in the rats administered 100 mg/kg from week 2 to 4. Similarly, the MCV levels of the rats that received 200 mg/kg slightly increased from week 3 to 4, respectively. The MCH level of those animals given 100 mg/kg body weight was also observed to show a significant decrease ($p < 0.05$) in week 1, followed by a further decline in week 3.

DISCUSSION

The possible toxic effect of aqueous extracts of *M. sloanei* seed on haematological profile of normal albino rats was investigated for a total of 28 days. Toxicity test using raw unprocessed seed extract of *M. sloanei*

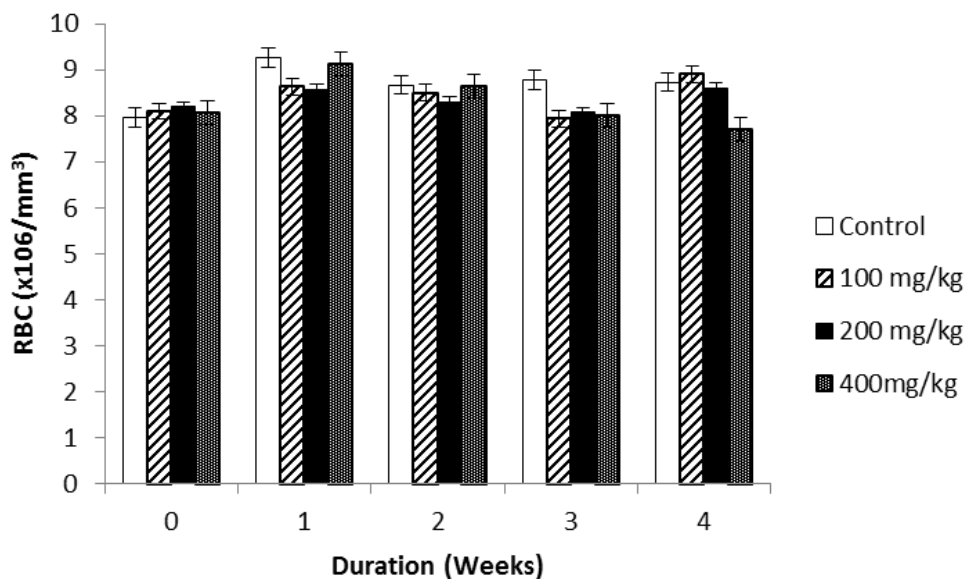


Figure 1. Effects of the aqueous seed extract of *M. sloanei* on red blood cell of albino rats.

showed that although at a dose level of 5,000 mg/kg, no death occurred, there was shivering, bulging of eyes as well as dullness in the rats. This observation contradicts the report of Egwurugwu et al. (2012) who recorded lethal effects of the *M. sloanei* seed extract at a dose level of 3,872.98 mg/kg in experimental rats. This discrepancy aptly calls for more studies to fully unravel the confusion surrounding the identity of the *Mucuna* species involved. Blood parameters are good indicators of physiological and nutritional status of animals, and changes in haematological parameters have the potential of being used to elucidate the impact of nutritional factor and additives supplied in diets of any living creature (Majid et al., 2010). Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal (Ashafa et al., 2009). It can also be used to explain blood relating functions of chemical compounds including plant extracts (Yakubu et al., 2007). The various haematological parameters investigated in this study are useful indices that can be employed to assess the toxic potentials of plant extracts/botanicals in living systems (Sunmonu and Oloyede, 2010). Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies (Olson et al., 2000). The packed cell volume (PCV), haemoglobin (Hb) and red blood cells (RBC) are associated with the total population of red blood cells (Ashafa et al., 2011).

However, except in week 1, there were no significant effects of the extracts at various dose levels (100, 200 and 400 mg/kg body weight) on the RBC and its indices

(Hb, PCV, MCV, MCH and MCHC) from the first to the last week of treatment when compared with the control (Table 1; Figures 1 to 4). This clearly indicates that there was no change in the rate of production of RBCs (erythropoiesis) as well as destruction of matured RBCs within the study period. It further showed that the extract did not have the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-Elsner et al., 2004). The little or non-significant effect on the RBC and Hb also implies that there was no change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the extract administration, since RBC and Hb are very important in transferring respiratory gases (De Gruchy, 1976). The significant reduction in the levels of RBC, PCV, HB and MCV (Table 1) when compared with the control at dose levels of 100 and 200 mg/kg in week 1 could be as a result of the animal's initial reaction to the assault by the extracts. The non-significant effects on these RBC indices further suggest that the average size of RBC (microcytes) as well as the haemoglobin weight per RBC were not affected. This implies that the aqueous seed extract does not seem to possess the potential of inducing anaemia following a prolonged period of administration. This is more so because MCV, MCH and MCHC levels have been found to be important indicators of anaemia diagnosis in most animals (Coles, 1986).

Similarly, the absence of observable significant effects of the extracts on the MCH, MCHC and MCV levels between the treatment groups within the duration of the present experiment may be an indication that neither the incorporation of haemoglobin into the red blood cells nor

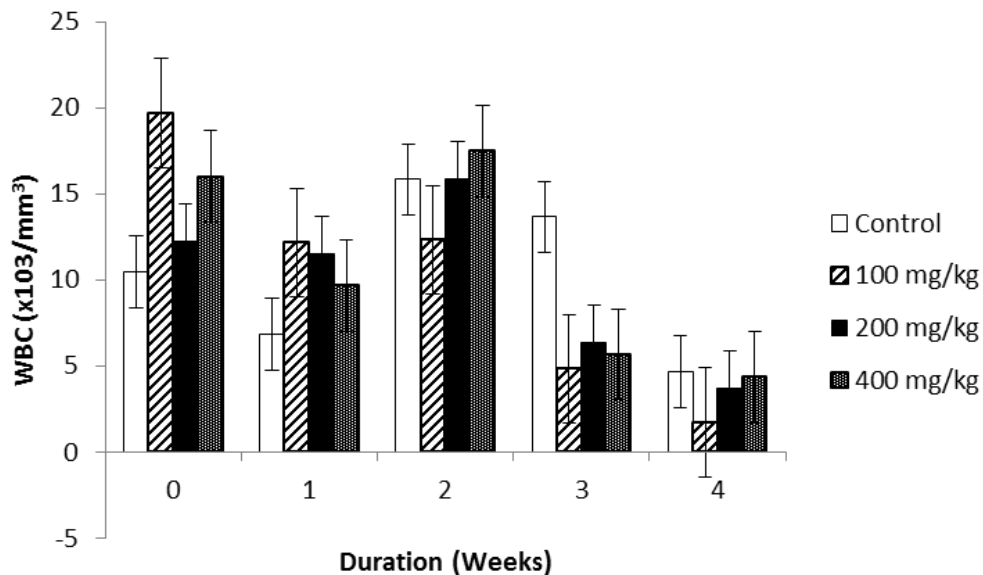


Figure 2. Effects of the aqueous seed extract of *M. sloanei* on white blood cell of albino rats.

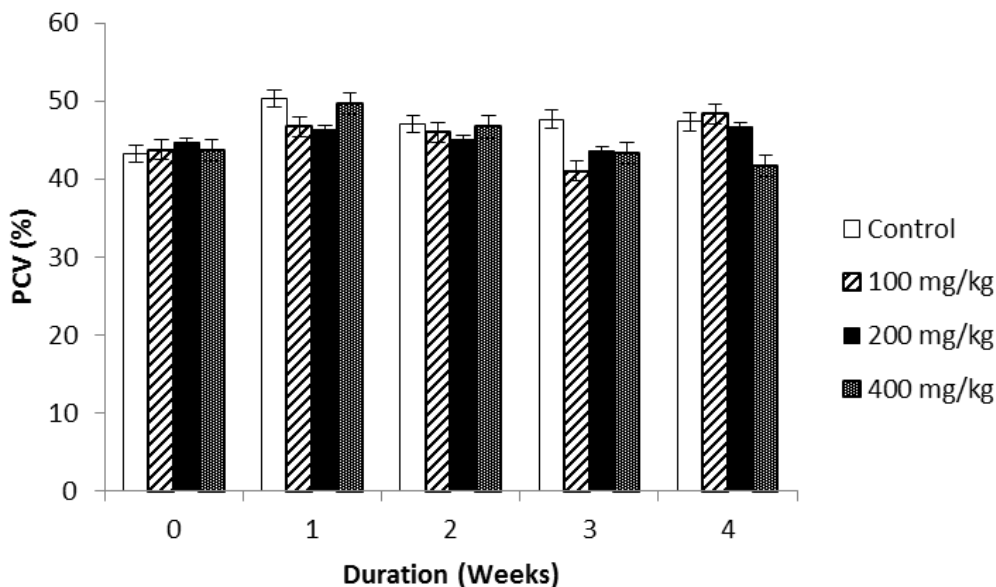


Figure 3. Effects of the aqueous seed extract of *M. sloanei* on pack cell volume (PCV) of albino rats.

the morphology and osmotic fragility of the red blood cells was altered (Adebayo et al., 2005). Nevertheless, the intermittently observed significant decreases in the values of these haematological parameters among treatment groups and the control, still falls within established rat haematological reference ranges (Johnson-Delaney, 1996).

The results obtained in the present research however, largely corroborate that obtained earlier for other *Mucuna* species in Nigeria. Odoh and Osadebe (2010) observed

that aqueous extracts of *Mucuna flagellipes* produced no significant changes in the values of haemoglobin (HB), red blood cell (RBC), white blood cell (WBC) and packed cell volume (PCV) of normal albino rats. Similarly, Adepolu and Odubena (2009) posited that extracts of *Mucuna pruriens* did not produce any significant difference in the packed cell volume (PCV) of albino rats. Contrarily, the data obtained in the present study is at variance with the findings of Esonu et al. (2001) who stated that feeding of raw *Mucuna* (Velvet bean) seed

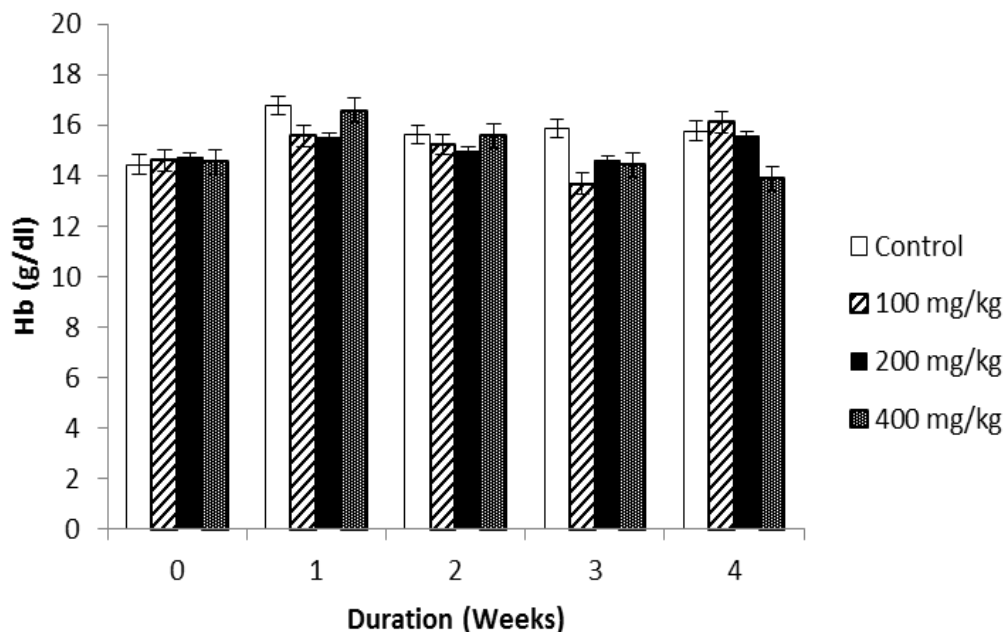


Figure 4. Effects of the aqueous seed extract of *M. sloanei* on haemoglobin level of albino rats.

meal resulted in deleterious effects on the performance and blood constituents of weaned pigs.

Nevertheless, the observed significant reduction in white blood cells in week 3 (Table 1) could compromise the body's immunity and may predispose consumers to opportunistic and supra-infections despite the acclaimed nutritional benefits of the *M. sloanei* seed. This is because the total WBC content contributes to the defense mechanism of the body (Lloyd and Mary, 1999). Any explanation for this observed discrepancy in the WBC level at present would at best be subjective. Hence, the need for more studies to actually unravel the active ingredient in *M. sloanei* implicated. However, it is instructive that the lack of significant differences between doses and duration of treatment portends that consumption of seeds of *M. sloanei* is beneficial even in the short term.

Conclusively, the study has demonstrated that the aqueous seed extract is safe for consumption since it does not cause any adverse effect on the haematological profile of rats. We therefore posit that, there is the need for further researches on the seed extracts of *M. sloanei* in order to fully understand the actual effects on the prolonged administration *vis-à-vis* its stability and suitability in clinical trials. Also, to fully explore the exact roles being played by the individual phytochemical constituent of *M. sloanei* seed in the general wellbeing of the body.

Conflict of interests

The author(s) did not declare any conflict of interest.

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