

*Full Length Research Paper*

# Preliminary evaluation of the haematological effects of *Picralima nitida* saponin extracts on *Rattus novergicus*

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This study evaluated some aspects of haematological effects of *Picralima nitida* saponin extracts on *Rattus novergicus*. The effect of increasing dosage of plant extract on haematological parameter was investigated. Thirty six (36) adult male white (albino) rats weighing 200 to 250 g were used for the study. Soxhlet extract of crude saponin from *Picralima nitida* was extracted using routine methods. All haematological parameters assessed were determined before treatment of the animals and subsequently evaluated weekly for six weeks. Haematological parameters were determined with routine methods. The increasing dosages (300, 600 and 900 mg/kg) of *P. nitida* soxhlet extracts produced significant ( $P < 0.05$ ) changes in the haematological parameters of rats weekly for the 6 weeks of treatments when compared with those of the control rats. From the results of this experimental study, *P. nitida* saponin extract's ability to decrease WBC shows that it is not useful in boosting the immune system, fighting immunity dependent infections and leucopenia but its ability to increase PCV and RBC shows that it may be useful in the management of Anaemia. Further studies of *P. nitida* saponin extracts are therefore highly suggested for future investigations.

**Key words:** *Picralima nitida*, saponin, PCV, WBC, RBC.

## INTRODUCTION

Saponins are steroids or triterpenoid glycosides, common in a large number of plant products that are important in human and animals' nutrition. They are glycosides with distinctive foaming characteristics and are natural detergents found in certain plants (Ajali, 2004). Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature (Bachran et al., 2006). *Picralima nitida* (Apocynaceae) used in this study as a source of saponin is a deciduous tree of about 20 m in height with dense crown and widely distributed in the tropical rainforest of Africa (Keay et al., 1964). The fruit is broadly obovoid, smooth and glabrous measuring about 15 cm long and 10 cm in diameter. Each fruit contains flattened seeds embedded in pulp (Aguwa et al., 2001). Almost all parts of the plant are used in the treatment of all types of fever, as antitussive, for wound healings, as aphrodisiac (Arens et al., 1982), Trypanosomiasis treatment and as local

anaesthetic comparable to cocaine (Hamet, 1940). The aqueous extract of the *P. nitida* seed when analysed gave positive chemical reactions for glycosides, saponins, tannins, alkaloids, proteins and carbohydrates (Aguwa et al., 2001). In view of the varied applications of saponins to humans, it is important to evaluate some aspects of haematological effects of increasing dosage of crude seed extract of *P. nitida* on *Rattus novergicus* viz – a – vis PCV, RBC and WBC.

## MATERIALS AND METHODS

### Plant materials

The pods of *P. nitida* were bought from Orieamaiwe

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market in Umunoha, Mbaitoli L.G.A, Imo State, Nigeria. The plant was identified to species level (Gbile, 1980) at the Herbarium unit, Department of Botany, University of Nigeria Nsukka, where voucher specimens were kept.

### Animal model

Thirty six (36) adult male white (albino) rats weighing 200 to 250 g, bred in the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka were purchased and used for the study. They were fed *ad libitum* with water and 18% crude proteins (Guinea feed) commercial feed. They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage in the Postgraduate Research Laboratory of Department of Zoology, University of Nigeria Nsukka. All the animals were maintained under the standard laboratory condition for temperature,  $26 \pm 2^\circ\text{C}$ .

### Soxhlet extraction of saponin from *Picralima nitida*

The pods of *P. nitida* were broken and the seeds were obtained, sun-dried for two months and pulverized to coarse form. The pulverized seed were defatted in a soxhlet extractor set at  $30^\circ\text{C}$  using petroleum ether as the solvent (Nwobi et al., 2006). Maceration of the defatted seed in 80% methanol was done for 48 h and filtered afterwards. The methanol solvent was boiled off from the filtrate at  $70^\circ\text{C}$  using soxhlet extractor. The extract was packed into silica gel, poured into a chromatographic plate and allowed to dry before being extracted successively with chloroform, ethyl acetate and acetone to remove other non-saponin components. The saponin was extracted with water and boiled off at  $90^\circ\text{C}$ . The resinous product was precipitated in acetone for further removal of non-saponin component. The end product which foams when stirred in water as a simple test for saponin was kept in a refrigerator until administration.

### Experimental design

Randomized block design of four groups (A, B, C, D) replicated thrice. Each replicate had 3 rats. Group A received 300 mg/kg of plant extracts saponin while B and C received 600 and 900 mg/kg respectively on daily basis for six weeks through the intraperitoneal route. Group D served as the control and received 2.0 ml/kg of normal saline daily. All haematological parameters assessed were determined before the extract treatment of the animals and subsequently evaluated weekly for six weeks. Blood was collected from each of the rats from the orbital sinus using the infra orbital lateral canthus method with heparinised capillary tubes (Oyeyemi and Adeniji, 2009).

### Evaluation of haematological parameters

Routine haematological methods involving the use haematocytometer, microhaematocrit and centrifuge

were used to determine PCV, WBC, and RBC (Sood, 1999).

### Data analysis

The data collected were pooled and analysed for their central tendencies using descriptive statistic, values were given as mean  $\pm$  standard deviation of the observations. ANOVA and Fisher's Least Significant Difference (FLSD) were employed to test the significant differences ( $p < 0.05$ ) among treatment means. All analysis was done using SPSS for windows statistical software version 16. The resulting outputs were presented in tables and figures.

## RESULTS

### Effects on the packed cell volume (PCV)

Increasing dosages (300, 600 and 900 mg/kg) of *P. nitida* seed saponin extract produced a dose -independent, significant ( $P < 0.05$ ) increment in the packed cell volume levels of rats after six weeks of treatment when compared with that of the control rats (Table 1). *P. nitida* seed saponin extract at 300 mg/kg increased packed cell volume by 7.25% ( $36.67 \pm 1.00$  to  $39.33 \pm 1.80$ ) after six weeks of treatment, at 600 mg/kg it increased it by 4.2% ( $40.00 \pm 1.41$  to  $41.67 \pm 1.66$ ) whereas at 900 mg/kg it was increased by 7.26% ( $41.33 \pm 1.12$  to  $44.33 \pm 2.29$ ) (Table 1). These values were statistically different when their Fishers Least Significant Difference (1.382) was used to test the significance differences between the treatment means at  $P < 0.05$ . A closer look at the weeks of study revealed that there was no (0.00%) change on packed cell volume at the dose of 900 mg/kg between week 0 ( $41.33 \pm 1.12$ ) and week 1 ( $41.33 \pm 1.12$ ) and also no change (0.00%) was noticed in week 4 at the dose of 600 mg/kg. The most effective percentage increment in packed cell volume was observed at 300 mg/kg body weight.

### Effects on the white blood cell (WBC) count

Increasing dosages (300, 600 and 900 mg/kg) of *P. nitida* seed saponin extract produced an overall dose - dependent, significant ( $P < 0.05$ ) reduction in the white blood cell count of albino rats after six weeks of treatment when compared with that of the control rats in the control group (Table 2). It is observed that *P. nitida* at 300 mg/kg reduced the number of white blood cell by 1.16% ( $9097.1 \pm 37.34$  to  $8611.1 \pm 47.81$ ) at the end of the sixth week of treatment, at 600 mg/kg the reduction was at 3.03% ( $9177.8 \pm 97.18$  to  $8900.0 \pm 377.5$ ) whereas at 900 mg/kg it was reduced by 8.90% ( $9610.6 \pm 10.09$  to  $8755.6 \pm 278.9$ ) (Table 2). These values were statistically different when their Fishers Least Significant Difference (236.94) was used to test the significant difference between the treatment means at  $P < 0.05$ . The

**Table 1.** Effects of duration and dosage of *P. nitida* saponin seed extract on packed cell volume of *Rattus norvegicus*

Dosages (mg/kg)	Packed cell volume (%)						
	Duration in weeks						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Normal Saline	30.67±1.32	30.67±1.41 (0.00%)	30.44±1.33 (0.75%)	30.33±1.58 (1.11%)	30.22±1.20 (1.47%)	30.56±1.13 (0.36)	30.63±1.50 (0.13%)
300 mg	36.67±1.00	43.67±1.32 (-19.09%)	37.33±1.32 (-1.80%)	37.67±1.00 (-2.73%)	38.44±1.13 (-4.83%)	42.22±1.39 (-15.13%)	39.33±1.80 (-7.25%)
600	40.00±1.41	45.33±1.00 (-13.33%)	43.67±1.00 (-9.18%)	40.22±1.56 (-0.55%)	40.00±3.39 (0.00%)	46.89±1.76 (-17.23%)	41.67±1.66 (-4.18%)
900	41.33±1.12	41.33±1.12 (0.00%)	45.33±1.41 (-9.68%)	41.22±1.09 (0.27%)	45.78±1.09 (-10.77%)	47.44±1.01 (-14.78%)	44.33±2.29 (-7.26%)

Negative % change denotes an increase, positive % change a decrease. FLSD = 1.382, Figures in parenthesis = Percentage change within weeks of study.

**Table 2.** Effects of duration and dosage of *P. nitida* saponin seed extract on white blood cell count of *Rattus norvegicus*.

Dosages (mg/kg)	White blood cell count (mm <sup>-3</sup> )						
	Duration in weeks						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Normal Saline	9385.6±192.69	9400.0±200.0 (-0.16%)	9405.6±182.76 (-0.21%)	9405.6±172.2 (-0.21%)	9386.7±157.2 (-0.01%)	9396.7±153.8 (-0.12%)	9400.0±166.28 (-0.16%)
300	9097.1±37.34	8522.2±311.4 (6.32%)	8372.2±343.8 (7.97%)	8455.6±245.5 (7.05%)	8290.0±235.4 (8.87%)	9011.1±293.4 (0.95%)	8991.5±47.81 (1.16%)
600	9177.8±97.18	8211.1±145.29 (10.53%)	8445.6±282.7 (7.98)	8933.3±360.6 (2.66%)	8777.8±307.3 (4.36)	8733.3±264.6 (4.84%)	8900.0±377.5 (3.03%)
900	9610.6±10.09	8255.6±218.58 (14.10%)	8544.4±364.38 (11.09%)	8666.7±217.94 (9.82%)	8644.4±418.7 (10.05%)	8988.9±396.1 (6.47)	8755.6±278.9 (8.90%)

Negative % change denotes an increase, positive % change a decrease. FLSD = 236.94, Figures in parenthesis = Percentage change within weeks of study.

most effective percentage reduction in the number of white blood cell was observed at 900 mg/kg body weight.

### Effects on the red blood cell count

Increasing dosages (300, 600 and 900 mg/kg) of *P. nitida* seed saponin extract produced a dose-independent, significant ( $P < 0.05$ ) increment in the red blood cell count of rats after six weeks of treatment when compared with that of the control rats (Table 3). The number of red blood cell was reduced by (1.69%) in the first week of study but was increased by the same percentage (1.69%) in the third week. *P. nitida* seed saponin extract at 300 mg/kg increased red blood cell count by 2.88% ( $663.11 \pm 17.69$

to  $682.22 \pm 0.67$ ) after six weeks of treatment, at 600 mg/kg it increased the Red blood cell count by 2.26% ( $667.11 \pm 12.43$  to  $682.22 \pm 2.33$ ) whereas at 900 mg/kg it was increased by 3.23% ( $681.33 \pm 1.12$  to  $703.33 \pm 8.66$ ) (Table 3). These values were statistically different when their Fishers Least Significant Difference (5.524) was used to test the significant difference between the treatment means at  $P < 0.05$ . An in depth look at the dosage throughout the six weeks of study showed that the red blood cell count remained the same for dosages 300 ( $682.22 \pm 0.67$ ) and 600 mg/kg ( $682.22 \pm 2.33$ ) at the sixth week of treatment. The most effective percentage increment in number of red blood cell count was observed at 900 mg/kg body weight.

**Table 3.** Effects of duration and dosage of *P. nitida* saponin seed extract on red blood cell count of *Rattus norvegicus*.

Dosages (mg/kg)	Red blood cell count ( $\times 10^4/\text{mm}^3$ )						
	Duration in weeks						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Normal Saline	603.11 $\pm$ 4.26	606.11 $\pm$ 3.48 (-0.50%)	607.89 $\pm$ 3.10 (-0.79%)	608.33 $\pm$ 2.83 (-0.87%)	608.22 $\pm$ 2.39 (-0.85%)	607.11 $\pm$ 1.83 (-0.66%)	604.11 $\pm$ 1.96 (-0.17%)
300	663.11 $\pm$ 17.69	651.89 $\pm$ 1.45 (1.69%)	669.33 $\pm$ 11.00 (-0.94%)	674.33 $\pm$ 9.21 (-1.69%)	671.78 $\pm$ 1.86 (-1.31%)	669.0 $\pm$ 5.98 (-0.89%)	682.22 $\pm$ 0.67 (-2.88%)
600	667.11 $\pm$ 12.43	618.78 $\pm$ 3.56 (7.24%)	684.44 $\pm$ 5.27 (-2.60%)	662.0 $\pm$ 1.56 (0.77%)	682.00 $\pm$ 2.24 (2.23%)	607.22 $\pm$ 4.52 (8.98%)	682.22 $\pm$ 2.33 (-2.26%)
900	681.33 $\pm$ 1.12	693.00 $\pm$ 3.46 (-1.71%)	688.89 $\pm$ 6.27 (-1.11%)	692.33 $\pm$ 1.41 (-1.61%)	682.00 $\pm$ 2.00 (-0.10%)	684.11 $\pm$ 2.62 (-0.41%)	703.33 $\pm$ 8.66 (-3.23%)

Negative % change denotes an increase, positive % change a decrease. FLSD = 5.524, Figures in parenthesis = Percentage change within weeks of study.

## DISCUSSION

### Hematological effects

The administration of a chemical compound may bring about significant changes in the structure, function, metabolic transformations and concentration of biomolecules, enzymes and even metabolic pathways. These alterations which may be rapid or slow, may lead to different biochemical mechanisms producing observed pathological and clinical changes (Murray et al., 2000). Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood. The significant ( $P < 0.05$ ) increase by *P. nitida* crude saponin extracts of packed cell volume (PCV), Red blood cell count (RBC) at different dosages implies that the plant extracts have potential haematological effects. Its effects on RBC and PCV implies that there was a change in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissue, since RBC is very important in transferring respiratory gases (Degruchy, 1976). This suggested that the extracts have the potential to stimulate erythropoietin release in the kidney which is the humoral regulators of RBC production (Poleunakoric and Sikole, 1996; Sanchez-elsner et al., 2004). There has been no previous report on the hematological effects of *P. nitida* saponin extracts. WBC were significantly decreased an indication of a fall on the immune system by the extracts. The fact that *P. nitida* saponin extracts decreased WBC at different dosages suggested that saponin may be a bioactive agent that could cause decreased production of white blood cell. *P. nitida* crude saponin extracts may not be considered in the management of anemia leucopenia given its effects from this experimental study.

### Conclusions

From the results of this experimental study, *P. nitida*

saponin extract's ability to decrease WBC shows that it is not useful in boosting the immune system, fighting immunity dependent infections and leucopenia but its ability to increase PVC and RBC shows that it may be useful in the control of Anaemia.

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