

## Haematoprotective Effect of *Salacia nitida* Root Bark Extract on Ethanol-Induced Toxicity in Albino Rats.

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### Abstract

**Background:** Alcoholic decoctions of *Salacia nitida* root bark is orally taken by people with low socio-economic status in Southern Nigeria for treatments of malaria and typhoid fever. This study was carried out to evaluate the protective effect of *S. nitida* root bark extract on blood cell counts in rats challenged with ethanol.

**Materials and Methods:** A total of 48 rats, divided into 6 groups of 6 rats each were used for the study. Rats in group I were orally given 3 ml of physiological saline for 36 days, and group II rats given 1 ml each of 40 % ethanol daily for 18 days, followed by water for further 18 days. Groups III, IV, V and VI rats were orally given 3 ml of 25 mg/kg body weight/day of silymarin, 500 mg/kg body weight/day, 700 mg/kg body weight/day, and 900 mg/kg body weight/day of extract of *S. nitida* root bark accordingly for 18 days, followed by ethanol for further 18 days. Standard methods were used to evaluate the haematological parameters.

**Results:** Results showed that there were no significant changed ( $P > 0.05$ ) in levels of haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), platelet counts, absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and red cell indices in the experimental rats pretreated with extract, and significantly decreased ( $P < 0.05$ ) in the mean concentrations of these indices in the induction control rats compared to rats in placebo.

**Conclusion:** Therefore, extract from root bark of *S. nitida* prevented derangements in blood cell counts in the experimental rats due to excessive intake of ethanol.

**Keywords:** Blood cell counts, ethanolic extract, haematoprotective, root bark, *Salacia nitida*, toxicity.

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### I. Introduction

Moderate consumption of alcohol is said to be beneficial to the body by dissolution of blood clots and reduction of plaque deposits in arteries<sup>1,2</sup>, but alcoholics likely develop some medical problems that may affect erythropoiesis and its functions. Its abuse constituted about 3.5% of global disease burden<sup>3</sup>, and is among the leading cause of death globally<sup>4</sup>. Ethanol is absorbed from the intestines into the blood stream and circulated to other parts of the body including the liver<sup>5</sup>. Alcohol, when taken excessively has several devastating effects on the body cells, tissues and organs, causing major health problems and diseases. In the liver, alcohol is oxidized by alcohol dehydrogenase (ADH) to acetaldehyde that is subsequently oxidized to citrate by aldehyde dehydrogenase (ALDH). Intakes of too much alcohol affect the hematopoietic system directly by affecting the bone marrow, blood cell precursors, blood cells and thrombocytes<sup>6</sup>. It also results to poor nutritional states of the patients, which will result in deficiencies of some essential nutrients that will in turn, impair formation of blood cells including their functions, which may result to anaemia. Anaemia can bring about abnormal heart beats, fatigue, and shortness of breath, low immune responses, and impairment of thrombocytopoiesis, leading to bleeding, hemorrhagic stroke, and thrombosis due to poor formations of clotting factors<sup>6</sup>, and even reduction in mental ability. Excessive consumption of alcohol affect mean cell volume, MCV<sup>7</sup>, development of red cells with large vacuoles, and increase the levels of haemolysis of red blood cell (RBC). Formation of blood sludge, where red blood cells clumps together to block arterioles and capillaries and causing cells death by depriving them of oxygen and nutrients, is another effect of alcohol intake<sup>8,9</sup>. Excessive intake of alcohol may cause mal-absorption of folate thus leading to folic acid deficiency<sup>6</sup>. This could bring about improper division of blood cell precursors that could lead to formation of immature and non-functional blood cells (megaloblasts), a condition

that could result to pancytopenia (deficiency in RBCs, WBCs and platelets), megaloblastic anaemia, infections and haemorrhages.

*Salacia nitida* Linn Benth (Celastraceae) is a woody scandent shrub found in southern Nigeria, which is claimed to cure malaria, thus making it to play a vital role in the health services, especially among the low socio-economic class in Southern Nigeria. Alcoholic decoctions of *S. nitida* root bark are orally taken for the treatments of malaria and typhoid fever in folk medicine. The antimalarial activity and potential of *S. nitida* root bark to influence the state of anaemia in malaria infected mice has been proven<sup>10, 11</sup>. It has also been shown to contain phytochemicals with pharmacological activities including antioxidant and anti-inflammatory activities<sup>12</sup>. In view of its numerous uses therefore, the present study was designed to evaluate the haematoprotective effect of ethanolic extract from *S. nitida* root bark on alcohol-induced toxicity in albino rats, since no information on this work is found in any literature, thus the need for this study.

## II. Materials and Methods

**Chemicals and Reagents:** Chemicals and reagents used were all of analytical grades purchased from Austin Laboratory and store, Alakahia, Port Harcourt, Rivers state, Nigeria.

**Collection of plant materials and preparation of extract:** *Salacia nitida* was collected from Baapeele farm in Nyogor-Beeri, Khana local government area of Rivers state, Nigeria. It was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, (Voucher number UPHV-1033). Plants were pulled from the ground and roots removed, carried to the laboratory, washed and air-dried under shade for 2-3 hours. Barks were removed from the root and reduced into bits onto a clean leather material with machete and air-dried under shade for 7-8 days. The dried root barks were then pulverized with a hand grinding machine (Corona-16D). Extraction was done with Soxhlet extractor using 500 g of powdered root bark material and 2 litres of ethanol (80%) on a heating mantle regulated at 80°C. Extracts were concentrated to dryness using a rotatory evaporator (Heidolph 4000, Schwabach, Germany). The dry extract was kept in a refrigerator at 4°C until required for use.

**Experimental animals:** Sixty eight (68) albino rats weighing between 170 – 300 g of mixed sex were procured from the Department of Biochemistry animal house, University of Port Harcourt, Rivers State, Nigeria. The rats were maintained in plastic cages, under standard housing conditions of humidity (56– 68%), temperature (27 ± 2 °C), and 12:12 hours light/darkness cycle, with free access to animal feed (grower's mash) and clean water *ad libitum* for 10 days acclimatization period. Animals were maintained according to the United States National Institute of Health "Principle of Laboratory animals care"<sup>13</sup> and the University of Port Harcourt guidelines on use, care and maintenance of laboratory animals during the study. Physical appearances and feeding behaviors of animals were used to confirm the health status of the experimental rats.

**Acute toxicity test:** The acute toxicity (LD<sub>50</sub>) of the ethanolic extract from *S. nitida* root barks was studied according to Lorke<sup>14</sup> using twenty (20) rats. The rats were grouped into five (5) groups of four (4) rats (2 males and 2 females) each. Animals were fasted overnight prior to treatments and 2 ml each of 10, 500, 1000, 3000, and 5000 mg/kg of extract were orally administered to them accordingly. They were monitored for signs of toxicity including mortality for 72 hours. The LD<sub>50</sub> was calculated by taking the square root of the least dose that can cause mortality minus the highest dose that did not cause any mortality.

**Experimental Protocol:** Forty eight (48) albino rats were used for the study. They were grouped into 6 with 6 rats each and treated with intragastric metal gavage *per os* (*p. o*) as follows:

Group I: Normal control group (Placebo) - given 3 ml of physiological saline for 18 days + clean water *ad libitum* for another 18 days.

Group II: Ethanol induction control group (EICG) - given 1 ml of 40% ethanol daily for 18 days + clean water *ad libitum* for another 18 days.

Group III: Drug pre-treated group (DPG) - given 3 ml of 25 mg/kg body weight/day of silymarin for 18 days + 1 ml of 40% ethanol daily for another 18 days

Group IV: Extract pre-treated group-1 (EPG-1) – given 3 ml of 500 mg/kg body weight/day of ethanolic extract of *S. nitida* root bark for 18 days + 1 ml of 40% ethanol daily for another 18 days.

Group V: Extract pre-treated group-2 (EPG-2) - given 3 ml of 700 mg/kg body weight/day of ethanolic extract of *S. nitida* root bark for 18 days + 1 ml of 40% ethanol daily for another 18 days.

Group VI: Extract pre-treated group-3 (EPG-3) - given 3 ml of 900 mg/kg body weight/day of ethanolic extract of *S. nitida* root bark for 18 days + 1 ml of 40% ethanol daily for another 18 days.

Then twenty four (24) hours after treatments, animals were anaesthetized with formalin, sacrificed by cervical dislocation and blood pooled by venipuncture with sterile disposable needle and syringe into sample bottles treated with EDTA for analyses of hematological parameters.

**Assessment of hematological parameters:** The cyanomethaemoglobin method was used to determine hemoglobin (Hb), and packed cell volume (PCV) determined by haematocrit method<sup>15</sup>. Bulk dilution method was employed in the determination of red blood cell (RBC) count<sup>16</sup>, and total white blood cell (WBC) and

platelet count were determined as described by Cheesbrough<sup>15</sup>, while total white blood cell differentials (neutrophils and lymphocytes) was evaluated using the Romanowsky method as described by Cheesbrough<sup>15</sup> and converted to their absolute values (absolute neutrophil count, ANC and absolute lymphocyte count, ALC) according to Tefferi<sup>17</sup>. Also, red cell indices (mean cell volume, MCV, mean cell hemoglobin, MCH, and mean cell hemoglobin concentration, MCHC) were determined according to Cheesbrough<sup>15</sup>.

**Data analysis:** Results were expressed as mean values ± standard error of means (SEM). The data obtained were statistical analyzed with one-way analysis of variance (ANOVA) with the SPSS version 22 statistical package. Scheffe's post hoc test was used for multiple comparisons and results were considered significant at 95% confidence level ( $P < 0.05$ ).

### III. Results

Results for the study showed that excessive ethanol intake significantly decreased ( $P < 0.05$ ) the levels of Hb by -38.95%, PCV by -47.85%, RBC by -67.41%, WBC by -57.20%, platelet counts by -39.64%, neutrophils by -53.37%, and lymphocytes by -69.57% in the induction control rats in group II that were challenged with ethanol compared to the placebo (figure 1). There were no significant differences ( $P > 0.05$ ) observed in mean concentrations of Hb, PCV, RBC, WBC, platelet counts, neutrophil and lymphocyte counts in the experimental rats in groups III through VI pretreated with different doses of ethanolic extract from root bark of *S. nitida* and silymarin compared to those in normal rats in the placebo. From table 2, it is seen that there was significant increased ( $P < 0.05$ ) in levels of MCV by 37.52%, MCH by 45.58% and MCHC by 14.57% in rats in group II (induction control). Also, it was seen that pretreatments of alcohol toxicity in experimental rats in groups III through VI showed no significant differences ( $P > 0.05$ ) in MCV, MCH, and MCHC compared to red cell indices of healthy rats in the placebo. But there were significant differences seen between haematological parameters in the induction control rats in group II and experimental rats in extract pretreated groups III, IV, V and VI. It was also noticed that results obtained from the various experimental groups were dose-dependent.

**Table 1.** Effect of *S. nitida* root bark extract on haematological indices in albino rats challenged with high doses of ethanol, n = 6.

Group	Parameters						
	Hb (g/dl)	PCV (%)	RBC ( $\times 10^{12}/l$ )	WBC ( $\times 10^9/l$ )	Platelet counts ( $\times 10^9/l$ )	ANC ( $\times 10^9/l$ )	ALC ( $\times 10^9/l$ )
I (Placebo)	12.58 ± 3.55	41.57 ± 2.46	9.88 ± 5.11	8.75 ± 3.23	334.23 ± 5.21	4.10 ± 2.66	5.88 ± 6.37
II (40 % ethanol)	7.68 ± 4.36*	21.68 ± 3.21*	3.22 ± 3.07*	3.57 ± 2.15*	201.75 ± 6.10*	0.78 ± 3.79*	0.73 ± 3.78*
III (25 mg/kg)	10.70 ± 1.55**	35.09 ± 1.95**	8.21 ± 1.74**	7.01 ± 1.51**	309.75 ± 3.81**	3.01 ± 0.81**	4.88 ± 5.65**
IV (500 mg/kg)	10.31 ± 4.67**	29.80 ± 0.27**	6.95 ± 2.61**	6.80 ± 1.79**	299.15 ± 3.24**	2.67 ± 1.42**	4.97 ± 2.01**
V (700 mg/kg)	10.62 ± 1.79**	31.02 ± 3.77**	7.15 ± 3.07**	7.05 ± 3.90**	305.76 ± 4.77**	2.90 ± 2.40**	5.09 ± 2.20**
VI (900 mg/kg)	11.00 ± .36**	35.17 ± 3.10**	8.09 ± 2.90**	8.09 ± 2.90**	318.54 ± 4.28**	3.04 ± 1.80**	5.27 ± 3.47**

Results = mean ± SEM; \*, \*\* = significant values ( $P < 0.05$ ) compared to placebo and induction control; Hb = haemoglobin, PCV = packed cell volume, RBC = red blood cell, WBC = white blood cell, ANC = absolute neutrophil count, ALC = absolute lymphocyte count.

**Table 2.** Effect of *S. nitida* root bark extract on red cell indices in albino rats challenged with high doses of ethanol, n = 6.

Group	Red Cell Indices		
	MCV (fl)	MCH (pg)	MCHC (g/dl)
I (Placebo)	42.07 ± 1.12	12.73 ± 3.02	30.26 ± 3.32
II (40 % ethanol)	67.33 ± 3.17*	23.39 ± 1.49*	35.42 ± 2.11*
III (25 mg/kg)	42.74 ± 1.29**	13.03 ± 4.11**	27.68 ± 2.58**
IV (500 mg/kg)	42.88 ± 2.98**	14.83 ± 1.74**	28.00 ± 1.86**
V (700 mg/kg)	43.39 ± 1.22**	14.85 ± 3.31**	28.40 ± 1.31**
VI (900 mg/kg)	43.47 ± 3.61**	13.60 ± 1.81**	28.76 ± 2.14**

Results = mean ± SEM; \*, \*\* = significant values ( $P < 0.05$ ) compared to placebo and induction control; MCV = Mean cell volume, MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration.

#### IV. Discussion

The results for this study revealed that excessive consumption of ethanol and other alcoholic beverages have some deleterious effects on the body. Changes in haematological parameters are useful indices for routine diagnostic evaluation of the health status of an individual<sup>18</sup>. It is seen from table 1 that treatment of rats in group II with ethanol brought about significant decrease in the number of blood cells. This might be due to the ability of alcohol to suppress haematopoiesis, and folic acid deficiency due to poor nutritional habits because alcohol is an appetite suppressant<sup>19</sup>. Reduction in the level of Hb seen in this work might result from ethanol interference with proper absorption and incorporation of iron into Hb molecules of RBCs, thereby causing iron deficiency that could cause anaemia<sup>6</sup>. Too much drinking of alcohol can also lead to sideroblastic anaemia, macrocytosis, and haematocytosis<sup>6</sup>, which might be another reasons for the decreased in RBC levels seen in this work. Changes in RBC counts could be linked to low Hb levels<sup>15</sup>. The reduced level of PCV recorded in this work shows that the experimental rats in group II were anaemic<sup>15</sup>. The observed reduction in the number of WBC, platelets, and white cell differentials in the group II rats in this study is an indication that alcohol interferes with the synthesis and functions of WBCs. Too much alcohol intake also retard neutrophil and lymphocyte activities, cause neutropenia and lymphocytopenia which play vital roles in immune responses, and causes low thrombocytopenia and thrombocytopenia as well<sup>6, 20</sup>. The study also shows that high intakes of alcohol raised the levels of red cell indices in the induction control rats of group II. An increase in MCV is linked to chronic liver disease, or vitamin B<sub>12</sub> or folate deficiency<sup>6, 21</sup>. Increased in MCV and MCH are indications of macrocytic anaemia, while increased in MCHC is indication of spherocytosis<sup>15</sup>.

The haematoprotective effect of *S. nitida* root bark extract seen in this work might be due to phytochemical compounds with bioactive activities present in the extract. The root bark of *S. nitida* has been reported to contained catechin, epicatechin, rutin, kaempferol, lunamarine, ribalinidine, spartein, sapogenin, tannins, phenols, phytates and anthocyanins as phytochemicals with antioxidant activity<sup>12, 22</sup>. The antioxidative activity of the extract containing the above mentioned bioactive compounds might quenched the oxidation of iron (II) in haemoglobin of RBCs<sup>23</sup>, by scavenging any Fe<sup>3+</sup> formed, and which brought about the proper incorporation of iron into haemoglobin molecules. Also, the formation of reactive oxygen species (ROS) in the cell membranes of RBCs due to alcohol and its cytotoxic metabolites<sup>24, 25</sup> are attenuated as a result of pretreatments with the extract. Therefore, the present of phytochemicals with bioactive properties in the root bark extract might be responsible for the haematoprotective actions of the root bark extract.

#### V. Conclusion

This study revealed that pretreatments of ethanol-induced toxicity with different doses of *S. nitida* root bark extract protected the albino rats challenged with ethanol from developing anaemia, thus confirming that *S. nitida* root bark extract has the potential to influence the state of anaemia, thus supporting the use of this plant part in folk medicine.

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