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Original Research Article

Haematological and Biochemical Examination of Pulcherrimin A isolated from *Caesalpinia pulcherrima* stem bark

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ABSTRACT

Caesalpinia pulcherrima has been utilised in the treatment of gastritis inflammation, diarrhoea dysentery, flatulence, ulcers, hepatitis, uterine dysfunction, rheumatism, haemorrhages and many other infections. The study evaluated the haematological and biochemical effects of pulcherrimin A isolated from *C. pulcherrima* stem bark. The compound, pulcherrimin A was administered to Wistar rats at doses of 2, 4 and 8 mg/kg body weight (bw) respectively for 28 days. The total blood count, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), total protein and total bilirubin were evaluated. The results showed a significant difference in white blood cell count at 2 mg/kg for female rats. Red blood cells and haemoglobin for both sexes appeared normal relative to control. The changes observed for platelets in all treated groups for both sexes were not significantly different from the control ($p>0.05$). The liver enzymes for the female rats appeared normal at all treatment doses, however, the glutamyltranspeptidase in male rats significantly increased at 2 mg/kg. The triglyceride and very low-density lipoproteins significantly decreased ($p<0.05$) for the female rats at 4 mg/kg. Findings from this study showed that pulcherrimin A have potential beneficial effects to reduce triglycerides and could facilitate the prevention of heart and liver related diseases.

***Keywords*:** *Caesalpinia pulcherrima*, Pulcherrimin A, Haematology, Biochemical parameter, Liver, Wister rat.

Introduction

Medicinal plant is widely considered by the human civilization of all race and culture as the primary source of medicine. Over the years, these plants have proven to be an inexhaustible storehouse of not just foodsource, but also as a therapy for diseases.[1]Reports have established that secondary metabolites like the phenolics, alkaloids, flavonoids, terpenoids and saponins which are produced by plants, are the active compounds that dictates their healing potency.[2-4]Despite the extensive use of medicinal plants, the safety and effectiveness of their constituents are yet to be fully explored, hence, a more thorough survey is required for evaluation and standardization of herbal formulations and their components.[5]
Some studies[6,7] have reported that medicinal plants contain toxins that can cause high alterations in total white blood cell count (WBC), reduction in haemocrit, haemoglobin and red blood cells (RBC), or increase in liver enzymes and total proteins as well as diseases conditions of the kidney and liver.

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However, in some cases, they do not have detrimental effects on haematological and biochemical parameters.[8,9] In addition, haematological and biochemical parameters are also influenced by other factors that may include gender, food, drugs, age, pathogens and toxic compounds.A full blood count on animal cells, can reveal the general health status about an individual. An abnormally low or high blood counts may suggest the presence of several forms of toxicity or disease conditions.[7,10,11]
C. pulcherrima, commonly known as 'Peacock flower', is broadly used in traditional medicine for treating different diseases including inflammatory, malarial, and microbial diseases.[12-14] The presence of diverse bioactive compounds of the flavonoids, terpenoids, alkaloids, tannins, glycosides and saponins have been shown in the phytochemical investigation of the stem bark.[14]
Due to wide application and continued intake of *C. pulcherrima* stem bark extracts, there is also the need to carry out studies with the aim to investigate and evaluate its potential toxic effects. This may be achieved by subjecting the extracts and pure isolates of *C. pulcherrima* to research in order to validate its potency.
This study is an extension of a preliminary study done on the stem bark of *C. pulcherrima*, which led to the isolation and characterisation of pulcherrimin A.[4]The study focused on haematological and biochemical evaluation of pulcherrimin A isolated from *C. pulcherrima* stem bark. To the best of our knowledge, this is the first report on the haematological and biochemical evaluation of pulcherrimin A isolated from *C. pulcherrima* stem bark.

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Materials and Methods

Experimental animals

Forty (40) young healthy Wistar rats (both sexes), of 9-12-week-old weighing about 150-250 g were used. The animals were purchased from the Animal House unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were maintained under standard environmental conditions (23–25°C, 12 h/12 h light/dark cycle) and had free access to standard pellet diet and water *ad libitum* according to the National Institute of Health (NIH) Guide for the care and use of laboratory Animals[15]and the Ethical approval number (LS19107) was issued by the Faculty of Life Sciences, University of Benin, Nigeria. Animals were acclimatized to laboratory environment for seven (7) days before the study commenced.

Sub-acute toxicity assay

The sub-acute toxicity study was carried out using the method described by Cornel *et al*.[16] with slight modification. Forty (40) healthy Wistar rats were divided into 4 groups of 10 rats each. The animals in Group A were administered distilled water only and considered as control. Animals in Group B, C and D were administered pulcherrimin A orally at the dose of 2, 4 and 8 mg kg(bw/day) respectively for twenty-eight (28) consecutive days. After the 28-day treatment, the animals were excised under inhaled chloroform anaesthetic. Blood samples were collected through cardiac puncture into EDTA and nonheparinized containers for haematological and biochemical analysis, respectively.

Haematological analysis

Blood samples collected were analysed[7]using automated haematology analyser (Mythic 18 by Orphee, Switzerland). Parameters checked include white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hgb), haematocrit (HCT), pack cell volume (PCV), platelet count (PLT), mean corpuscular haemoglobin concentration, (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), lymphocytes (LY).

Biochemical analysis

Serum blood samples were analysed[7]for aspartate transaminase (ALT), alanine transaminase (AST), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), total protein (TP), total bilirubin (TB) using (GesanChem 200, USA) automated machine.

Statistical analysis

Values for results were expressed as mean ± SEM. Analysis of variance (One-way ANOVA) was done to compare the differences of means from multiple groups followed by Dennett's post tests using SPSS version 20.0. A mean difference was considered significant at $p< 0.05$.

Results and Discussion

Aside the known pharmacological actions of *C. pulcherrima*, a comprehensive information about the toxicological effect of its constituents is yet to be studied. Consequently, the study was carried out to ascertain subacute toxicity of pulcherrimin A in experimental animals. Evaluation of blood parameters can be used to reveal the level of harmful effect posed by foreign compounds, and this can be ascertained from data obtained in animal studies.[3,17-20]

Haematological Study

White blood cells (WBC) are immune cells. A low WBC could indicate infection, chronic inflammation, bone disorder, drugs or nutrient deficiencies. While an increase in WBC can indicate fight against infection, acute stress, reaction to drug that increases WBC production or a decrease of the bone marrow, causing abnormally high production of WBC. In the female rats, there was a significant ($p<0.05$) and insignificant increase in WBC and LY at 2 mg/kg respectively. A slight increase was observed at 8 mg/kg, and normal for 4 mg/kg relative to control, suggesting that pulcherrimin A may not be dose dependent. (Table 1).The primary function of red blood cells (RBC) is transport of oxygen to the body cells and liver carbondioxide to the lungs. The oxygen and carbondioxide carrier composed in the RBC is the haemoglobin (Hgb). A low RBC count indicates anaemia, while kidney disease or performance enhancement drugs may cause a high RBC count. The values for RBC and Hgb for all treated groups were observed to be normal compared to control. There was no significant variation observed (MCH, MCHC, HCT and MCV) for all treated groups relative to control groups (Table 1 and 2).

Table 1: Effect of Pulcherrimin A on Haematological parameter(s) in Female rats

Parameters	Control	2 mg/kg/day	4 mg/kg/day	8 mg/kg/day
WBC x 10^3/µL	7.70 ± 2.30	14.45 ± 0.55*	7.80 ± 2.10	9.85 ± 1.95
RBC x 10^6/ µL	6.10 ± 0.22	7.04 ± 0.47	5.64 ± 0.53	6.10 ± 0.72
Hgb. g/d	16.35 ± 0.25	17.90 ± 1.30	15.00 ± 0.80	15.75 ± 0.25
PLT x 10^3/ µL	785.5 ± 55.50	1157 ± 139.5	747.5 ± 47.50	1111 ± 345.5
HCT%	44.30 ± 0.20	48.00 ± 4.20	47.80 ± 1.40	43.45 ± 1.25
MCV. fL	72.75 ± 2.95	68.05 ± 1.45	74.80 ± 3.60	74.70 ± 3.90
MCH. pg	26.85 ± 1.35	25.35 ± 0.15	27.25 ± 0.45	27.30 ± 1.50
MCHC. g/dL	37.90 ± 0.60	37.25 ± 0.55	35.85 ± 0.35	36.20 ± 0.50
LY x 10^3/ µL	5.00 ± 0.30	8.15 ± 0.65	5.15 ± 0.35	6.95 ± 0.15

Values are mean ± SEM (n=5). One-way ANOVA with post-hoc Dunnett's test was applied. * represents significance of $p < 0.05$ compared with control. WBC, white blood cells; PLT, platelets; Hbg, haemoglobin; HCT, haematocrit; LY, lymphocytes; MCV, mean corpuscular volume; RBC, red blood cells; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin.

Platelets (PLT) are blood cells responsible for blood clothing. A low PLT count might increase the risk of uncontrolled or prolong bleeding, while a high PLT count may lead to abnormal blood clothing. What may cause PLT imbalance includes; anaemia, heavy alcohol, viral infections, and drugs. In the female rats, a slight increase was observed in PLT for treated groups at 2 and 8 mg/kg, while 4 mg/kg was normal compared to control. This suggests that pulcherrimin A does not cause high imbalance for PLT at moderate dose. Unlike in the female rat where WBC was significantly increased at 2 mg /kg, the male rat showed a different pattern; at 2 mg/kg, there was an insignificant decrease in WBC and LY in comparison to control. The RBC for all treated groups was normal relative to control. The values for Hgb and PLT were observed to be normal with control and insignificant variations for groups at 2 and 8 mg/kg respectively. There was no significant variation observed (MCH, MCHC, HCT and MCV) in all treated groups relative to control (Table 1 and 2).

The serum biochemical analysis was carried out to evaluate possible alterations in the liver enzymes and function in order to find possible pathological changes caused by pulcherrimin A compared to control rats.

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Table 2: Effect of Pulcherrimin A on Haematological parameter(s) in Male rats

Parameters	Control	2 mg/kg/day	4 mg/kg/day	8 mg/kg/day
WBC x 10^3/ µL	12.09 ± 2.60	7.00 ± 1.00	13.65 ± 0.15	6.40 ± 0.40
RBC x 10^6/ µL	6.84 ± 0.76	6.21 ± 0.19	7.52 ± 0.16	6.40 ± 0.08
Hgb. g/dL	18.25 ± 1.25	17.00 ± 0.00	18.40 ± 0.50	16.90 ± 0.10
PLT x 10^3/ µL	787.5 ± 97.50	615.0 ± 39.00	783.5 ± 50.50	655.0 ± 69.00
HCT%	48.95 ± 3.35	45.90 ± 0.80	47.80 ± 1.40	45.80 ± 0.40
MCV.pg	71.90 ± 3.10	74.00 ± 1.00	63.55 ± 0.55	71.60 ± 0.30
MCH. g/dL	26.85 ± 1.05	27.35 ± 0.85	24.45 ± 0.15	25.85 ± 0.35
MCHC g/dL	37.20 ± 0.00	37.00 ± 0.60	38.45 ± 0.05	36.85 ± 0.15
LY x 10^3/ µL	8.20 ± 0.50	6.15 ± 1.05	7.70 ± 0.50	5.85 ± 0.35

Values are mean ± SEM (n=5).One-way ANOVA with post-hoc Dunnett's test was applied. * represents significance of $p < 0.05$ compared with control. WBC, white blood cells; PLT, platelets; Hbg, haemoglobin; HCT, haematocrit; LY, lymphocytes; MCV, mean corpuscular volume; RBC, red blood cells; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin.

Liver Enzymes and Lipids

AST and ALT are enzymes found in the liver, heart, muscles, kidney, brain and red blood cell. Normally AST and ALT levels are low, however, when there is liver damaged the level of AST and ALT increase in the blood. A high AST or ALT is a sign of liver damage or damage to another organ like the heart or kidney. ALP and GGT are also important enzymes. ALP is found mostly in the liver and bones; it helps to break down proteins, liver function and bone development. While the GGT helps the liver to metabolize drugs and toxins; it is found majorly in the liver and present in the gall bladder, spleen, pancreas and kidneys. Elevated levels for ALP and GGT are caused for liver disease, bone disorder or bile ducts. All liver enzymes for both female and male rats in all treated groups were observed to be normal in comparison to control, except for the significant increase observed for GGT and TP at 2 mg/kg in the male rats (Table 3 and 4).The observed significant rise ($p<0.05$) in GGT can be attributed to the study compound present during its metabolism in the liver[21] while the significant increase ($p<0.05$) in TP may be as a result of the protein content present in the feed diet,[21] a slight increase was also observed in TP among all treated groups in both sexes.

Table 3: Effect of Pulcherrimin A on Liver Function of the Female rats

Parameters	Control	2 mg/kg	4 mg/kg	8 mg/kg
ALT (U/L)	26.97 ± 0.21	23.83 ± 0.16	23.72 ± 0.06	26.81 ± 0.17
AST (U/L)	45.33 ± 0.29	43.74 ± 0.07	43.89 ± 0.14	45.94 ± 0.10
ALP (U/L)	13.77 ± 0.45	14.73 ± 0.22	13.62 ± 0.37	14.96 ± 0.84
GGT (U/L)	0.54 ± 0.01	0.60 ± 0.02	0.54 ± 0.07	0.57 ± 0.01
TB (U/L	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.07 ± 0.00
TP (U/L)	12.96 ± 0.20	14.97 ± 0.37	13.26 ± 0.49	13.16 ± 0.29

Values are mean ± SEM (n=5).One-way ANOVA with post-hoc Dunnett's test was applied. * represents significance of $p < 0.05$ compared with control. AST, Aspartate aminotransferase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma glutamyltranspeptidase; TB, Total bilirubin; TP, Total protein.

Table 4: Effect of PulcherriminA on Liver Function of the Male rats

Parameters	Control	2 mg/kg	4 mg/kg	8 mg/kg
ALT (U/L)	23.40 ± 0.13	24.78 ± 0.31	24.16 ± 0.31	23.09 ± 0.30
AST (U/L)	44.06 ± 0.14	44.36 ± 0.32	45.40 ± 1.74	43.66 ± 1.18
ALP (U/L)	13.67 ± 0.32	14.34 ± 0.07	14.61 ± 0.58	13.92 ± 0.12
GGT (U/L)	0.66 ± 0.02	0.77 ± 0.01*	0.75 ± 0.00	0.65 ± 0.01
TB (U/L)	0.06 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00
TP (U/L)	12.16 ± 0.17	14.38 ± 0.26*	12.16 ± 0.47	12.89 ± 0.19

Values are mean ± SEM (n=5). One-way ANOVA with post-hoc Dunnett's test was applied. * represents significance of $p < 0.05$ compared with control. AST, Aspartate aminotransferase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma glutamyltranspeptidase; TB, Total bilirubin; TP, Total protein.

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Cholesterol is essential for cell building and vital for synthesis of hormones and vitamin D. Excess calories, alcohol or sugar are turned into triglycerides and are stored in body for energy when needed. However, studies have consistently linked high cholesterol (low HDL and high LDL/VLDL) and triglycerides levels with cardiovascular diseases, atherosclerosis, diabetes, high blood pressure, heart disease, heart attack and stroke.[22-24]In the lipid profile for female rats (Table 5), there was a significant decrease($p<0.05$) in the level of triglycerides and VLDL at 4 mg/kg when compared to control. An insignificant decrease in Cholesterol was observed for treated groups at 2 and 4 mg/kg respectively. The High density lipoprotein (HDL) was observed to slightly increase across all treated groups in comparison to control. In the male rats (Table 6) there was no significant change in all the treated groups compared to control. However, in the female rats, there was a significant decrease ($p<0.05$) in TG and VLDL levels and an insignificant decrease and increase in Cholesterol and HDL levels respectively at 4 mg/kg. This is an indication that pulcherrimin A could be recommended for use at moderate dose.

Table 5: Effect of Pulcherrimin A on Lipid Profile of the Female rats

Parameters	Control	2 mg/kg	4 mg/kg	8 mg/kg
Triglyceride (mg/dL)	245.4 ± 3.47	216.0 ± 1.73	199.2 ± 0.53*	289.3 ± 1.39
Cholesterol (mg/dL)	87.50 ± 0.87	64.37 ± 0.55	61.97 ± 0.56	88.48 ± 0.66
HDL (mg/dL)	10.96 ± 0.02	16.53 ± 0.06	15.47 ± 0.72	11.34 ± 0.52
LDL (mg/dL)	27.46 ± 0.19	14.64 ± 0.84	16.68 ± 0.06	29.28 ± 0.14
VLDL (mg/dL)	49.36 ± 0.75	43.20 ± 0.35	39.85 ± 0.11*	47.87 ± 0.28

Values are mean ± SEM (n=5); * represents significance of $p < 0.05$, HDL, High Density lipoprotein; LDL, Low Density lipoprotein; VLDL, Very Low-Density lipoprotein.

Table 6: Effect of Pulcherrimin A on Lipid Profile of the Male rats

Parameters	Control	2 mg/kg	4 mg/kg	8 mg/kg
Triglyceride (mg/dL)	217.1 ± 3.17	216.5 ± 1.44	213.2 ± 1.01	204.4 ± 4.03
Cholesterol (mg/dL)	71.17 ± 2.21	64.17 ± 2.75	71.17 ± 0.14	70.84 ± 1.97
HDL (mg/dL)	14.21 ± 0.46	15.53 ± 0.52	15.37 ± 0.37	15.47 ± 0.31
LDL (mg/dL)	19.14 ± 0.01	14.34 ± 0.67	13.17 ± 0.71	19.49 ± 0.19
VLDL (mg/dL)	42.82 ± 0.22	49.30 ± 0.29	42.62 ± 0.20	40.88 ± 0.81

Values are mean ± SEM (n=5); * represents significance of $p < 0.05$, HDL, High Density lipoprotein; LDL, Low Density lipoprotein; VLDL, Very Low-Density lipoprotein.

Conclusion

The study revealed that pulcherrimin A has little or no effect on the blood parameters tested and could prevent risk of heart related disease at moderate dose (4 mg/kg/day) compared to lower and higher doses (2 mg/kg/day and 8 mg/kg/day). Hence, for optimal effect, the formulation is relatively safe at dose of 4 mg/kg/day for a 28-day period whereas the 2 mg/kg/day and 8 mg/kg/day should be used with caution.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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