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Histological Assessment and Antimicrobial Investigation of Pure Compound; 3,5,6,7-Tetrahydroxy-19-Vouacanoic Acid;(3*β*, 5*α*, 6*β*, 7*β*)-form,6,7-Dibenzoyl (Pulcherrimin A) Isolated from *Caesalpinia pulcherrima* Stem Bark

Osahon K. Ogbeide[1]*, Isaac U. Akhigbe[1], Charles A. Unuigbe[1], Osayemwenre Erharuyi[2], Vincent Imieje[2], Gabriel O. Benjamin[3], Kingsley Ikeke[4], Bosede Ayeni[4], Emmanuel Irabor[1], Joseph B. Owolabi[1,5], Abiodun Falodun[2]

[1]*Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*
[3]*Department of Pharmacology, Faculty of Physical Sciences, University of Benin.Benin City, Nigeria*
[4]*Department of Science Laboratory Technology, Edo State Polytechnic, Usen, Nigeria*
[5]*Department of Chemistry, School of Sciences, the Federal University of Technology, Akure, Nigeria*

ABSRTACT

This study was aimed to investigate the histological effect and antimicrobial activity of a compound [3,5,6,7-tetrahydroxy-19-vouacanoic acid;(3β, 5α, 6β, 7β)-form,6,7-dibenzoyl] previously isolated from *Caesalpinia pulcherrima* stem bark prior to this study. Different doses (2, 4 and 8 mg/kg) of the compound was administered to Wistar rats (both sexes) for a 28-day period. After the 28-day administration, vital organs (liver, heart, kidney, lung, uterus and testes) were harvested from the animals to ascertain their organ weight variations and histology. Microbial activity of the compound was investigated (using zone of inhibition test and minimum inhibitory concentration) for the microorganisms (*Bacillus subtilis, Klebsiella pneumoniae*, *P. aeruginosa*, *Staphylococcus aureus*, *Candida. albican* and *Trychophyton rubrum*). The observed weight changes in organs for all treated groups in relative to control was not significant at $p < 0.05$ and no observed toxic abnormalities for all organs in treated groups upon their gross examination in relative to control. Highest zone inhibition was observed at 100 mg/kg for all tested microorganisms (*B. subtilis*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *C. albican* and *T. rubrum*). with their corresponding MICs 11, 13, 16, 33, 30 and 31 mg/ml respectively. This study suggests that the compound could be a potential candidate in view of developing new antimicrobial agent and potential novel drug as a vasodilator.

Keywords: *Caesalpinia pulcherrima*, Pulcherrimin A, Wister rats, Histology, Microorganisms.

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Introduction

Man has always sought for ways to improve his health quality, eradicate, and prevent disease conditions that always attack him, and for many years, natural products have been recognised as one of the major sources of medicine across the world for management and prevention of diseases.[1-4] Also, over the years, the use of plant derived drugs has proven to be a good choice of drug for all kinds of diseases as it has better cultural adaptability with the human body and pose lesser side effects,[5] as compared to conventional drugs that have shown incidences of relapse, side effects and drug-body interaction,[5,6] Newman and Cragg[3] reported that prior to 2007, about 50% of all standard drugs registered over a 25-year timeline were sourced from natural products and their synthetic derivatives.

*Corresponding author. E mail: kennedy.ogbeide@uniben.edu
Tel: +2348055949282

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Histology, originally derived from the Greek root words; histos (tissues) and logos (study)[7] is a science that deals with the study of body organs and tissues at the microscopic level. It is a useful tool in clinical medicine and research for providing key information about biological tissue function (normalities and abnormalities) and certain disease diagnostics in toxicological studies. Over the years, it has been used to reveal exciting findings on animal growth, physiology, tissue diseases as well as investigation to different disease states.[7-9]

The rise of resistance of human pathogenic microorganisms to available antibiotics has augmented over the years, due to the haphazard usage of antimicrobial drugs.[10] This impends the intended prevention and management-cure for the widespread infections caused by these microbes, and in addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hyper sensitivity, immunosuppression and allergic reactions.[10-12]

Caesalpina pulcherrima commonly known as 'Peacock flower or Bride of Barbados' belongs to the family Fabaceae, is traditionally used in treating various diseases which include; dysentery, malaria, injury infections, ulcer, diarrhoea and hepatitis.[13,14] Phytochemical investigations on different parts of the plants have led to the isolation and characterisation of active secondary metabolites (pulcherrimins A-F, pulcherrins A-R and many other terpenoids) with medicinal properties such as anti-inflammatory, antimicrobial, antioxidant, analgesic, anticancer, and antimalarial activities.[15-22] This work is an extension of the preliminary study done on the stem bark of *C. pulcherrima*, which led to the isolation and characterisation of the compound.[23] The compound; 3,5,6,7-tetrahydroxy-19-vouacanoic acid; (3β, 5α, 6β, 7β)-form,6,7-dibenzoyl ($C_{34}H_{36}O_9$, 589.2449g) with the

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common name pulcherrimin A, was isolated as a white amorphous power with seventeen degree of unsaturation. The structure elucidation was successful by comparison of spectral data with that of literature.[24] The compound demonstrated a good antimalarial potential in a chemosuppressive assay. In the last study on haematological and biochemical examinations, pulcherrimin A showed no toxic abnormalities for the tested blood parameters, in addition to this, it displayed a beneficial effect in reducing triglycerides and facilitating the increase in high density lipoprotein (HDL).[25] Hence, this study focused on antimicrobial activity and histological assessment of pulcherrimin A isolated from *C. pulcherrima* stem bark. To the best of our knowledge, this is the first report on this study for pulcherrimin A isolated from *C. pulcherrima* stem bark.

Methods

Experimental animals

Forty (40) healthy Wistar rats (both sexes), of 9-12-week old weighing about 120–160g were used in this study. The animals were purchased from the Animal House unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The study was approved by the Ethical Review Board of Faculty of Life Sciences of University of Benin (LS19107). 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.[25,26]

Experimental design

Toxicity assay

The toxicity study was carried out using the method described by Cornel *et al*.[27] with slight modification. The 40 Wistar rats were divided into 4 groups of 10 in each. The animals in Group 1 were administered distilled water only and considered as control. Animals in Group 2, 3 and 4 were administered intraperitoneally 2, 4 and 8 mg/kg (bw/day) of pulcherrimin A respectively for 28 consecutive days.[26] At the end of the 28-day treatment, the animals were sacrificed under inhaled chloroform anaesthesia. The organs were excised, weighed and histologically examined.

Ethics approval

All procedures of the experiment were approved by the Ethical Review Board of Faculty of Life Sciences of University of Benin (LS19107).

Histological assessment

Organs of the heart, liver, kidney, lungs, ovary and testis were obtained from the animals and fixed in 10% normal-saline. Afterwards, these organs were dehydrated in graded alcohol, inserted in paraffin, and cut into 4-5 µm thick sections. Haematoxylin-eosin was used to stain the sections for photomicroscopic assessment using a Model N-400ME photomicroscope.[28] Slides were examined using *the 40X, and 100X objectives.*

Test microorganisms

The microorganisms (*K. pneumoniae, S. aureus, P. aeruginosa, B. subtilis, C. albican* and *T. rubrum*) used were obtained from stock cultures of clinical isolates sourced from the Department of Pharmaceutical Microbiology laboratory, Faculty of Pharmacy, University of Benin, Nigeria. All the test microorganisms were authenticated and sub-cultured from stock into sterile nutrient broth/ Sabouraud Dextrose broth and incubated overnight at 37ºC for bacteria and at room temperature (25±2ºC) for 72 hours for fungi. After incubation, overnight broth culture was adjusted to 0.5McFarland standard to give an inoculum size of approximately 10^8 cfu/mL and a further one in hundred serial dilutions (1:100) using normal saline solution to yield approximately 10^6 cfu/mL.

Antimicrobial sensitivity tests: zone of inhibition

Antimicrobial sensitivity test was carried out using agar-well diffusion method (Vinothkumar *et al*.[29] Mueller Hinton agar (BIOTECH, TM 339, India) and Sabouraud dextrose agar (BIOTECH, TM 387, India). were the media used as growth media and were prepared according to the manufacturer's instructions. A stock concentration of sample (pulcherrimin A) was constituted by weighing 1 g of the and dissolving it in 10 ml of 10% DMSO to obtain a stock concentration of 100 mg/mL. Other concentrations were made by appropriate dilutions and variation of specific volumes obtained from the stock concentration. Wells of 7 mm in diameter were made into seeded Mueller Hinton /Sabouraud agar plates using a flamed (sterile) cork borer. Prior to seeding, the turbidity of the overnight broth culture of each isolate were adjusted to match 0.5 McFarland turbidity standard and diluted (1:100) to give approximately $1x10^6$cfu/mL microbial suspension. Sterile swab sticks were then dipped into the standardized microbial suspension and gently streaked on the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. This was achieved by rotating the plate 90 degrees followed by 45 degrees with continuous streaking, and finally by streaking round the diameter of the agar. The 7 mm wells were filled with different volumes of the stock concentration and lower dilutions of pulcherrimin A corresponding to 100, 80, 60, 40 and 20 mg/mL concentrations. The same quantity of 10% DMSO served as negative controls while the standard drugs (1µg/mL of Ciprofloxacin for bacteria plates and 10µg/mL of Ketoconazole for fungal plates) served as positive controls. The plates were left to stand for 1 hour on workbench to allow diffusion of extract before incubating bacterial plates overnight at 37ºC and the fungal plates at room temperature (25±2ºC) for 72 hours. The diameter of clear zone was observed, measured in mm (millimeters) and recorded. The experiments including controls were done in triplicates and the mean zones of inhibitions calculated.

Determination of minimum inhibitory concentration (MIC)

The broth dilution method[30-32] was used to determine the MIC of the sample and standard antimicrobial drugs on susceptible organisms. From the sample stock concentration of 100 mg/mL, lower concentrations were prepared by separate incorporation into the Mueller Hinton and Sabouraud Dextrose broth at different volumes to obtain a range of concentrations of between 10 - 40 mg/mL for the test sample. Similar procedure was followed to obtain a concentration range of 0.001 - 2µg of the standard antibiotics (positive controls) and a prepared broth with 10% DMSO without the test sample nor standard antibiotics was used as (negative control). Then 100 µL (microliters) volume of two-fold dilution of 0.5MacFarland turbidity standard of microbial suspensions (10^6cfu/mL) obtained from overnight broth were separately used to inoculate the various sample concentrations or dilutions in test-tubes and all tubes were appropriately incubated. After incubation, tubes were observed for turbidity/growth and plated separately on agar plates and appropriately incubated after which the lowest concentration at which there was no observable bacterial/fungal growth was recorded as the MIC.

Statistical analysis

The results are expressed as mean ± SEM (standard error of mean). Values were subjected to one-way analysis of variance followed by Dunnett's test for comparison of group data. Statistical significance was considered as $p \leq 0.05$

Results and Discussion

The weight of organs (heart, lung, liver, kidney, uterus and testes) were obtained from both treated groups and control after the 28 days of oral administration of pulcherrimin A. As shown in Tables 1 and 2, the weight of the lung increased slightly in all treated groups for the female rats as well as a slight decrease in the liver at 2 mg/kg. In the male rats, a slight decrease was observed for the lung at 2 and 4 mg/kg respectively as well as a slight increase in the heart and kidney at 4 mg/kg. All the increases and decrease in the organ weight were minor changes and the difference may have been due to the variation in the size of internal organ or body weight of the animals.[33,34] Histological examinations were performed to further confirm whether or not the organs or tissue have been damaged.

There were no macroscopic or microscopic changes in the internal organs for both sexes of rats in all treated groups relative to control. A

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similar study conducted by Pramanik and Chatterje (2009),[35] revealed that variations in weight of organs observed upon administration of PITC-2 compound isolated from *Pluchea indica* showed no organ or tissue damage.

Plate 1 showed normal hepatocytes (liver cells) for both female and male in both control and treated group 2 mg/kg. However, fatty changes (which are mainly composed of triglycerides) were observed in the hepatocytes for treated groups 4 and 8 mg/kg (female and male). The centriole which aids in cells division was shown to be normal across all groups for both sexes of rat. Plate 2 showed normal prominent renal cells of the kidney and cells for both control and treated groups. Normal myocardial fibres and prominent coronary artery was for the heart (Plate 3) for both sexes in both sexes. Plate 4 revealed the lungs to be normal in all treated groups compared to normal. Plate 5 and 6 showed normal endometrial gland (uterine gland) in all groups including control, along with a reduced proliferative endometrium for the control and 2 mg/kg groups which was mild for groups 4 and 8 mg/kg in the female. While normal testicular cells were observed for all treated groups and control. The liver is an organ which detoxifies various metabolites, synthesizes proteins and produce biochemicals necessary for digestion.[36,37] The hepatocytes are liver cells that have direct access to the liver's blood supply through small capillaries called sinusoids (allows blood from between the hepatic artery and the portal vein).[37] Hepatocytes carry out many metabolic functions, including, the production of bile, protein synthesis and storage, transformation of carbohydrates and synthesis of cholesterol.[37] In Plate 1, fatty changes in the liver were observed for groups of 4 and 8 mg/kg respectively. This supports the changes in fats (triglycerides) observed by Ogbeide *et al.*[26] for the latter doses. No change was observed in the lower dose as it appeared to be normal with that of the control. The kidney performs the essential function of removing waste products from the blood and regulating the water fluid levels.[38] (NIH, 2022). The renal parenchyma is the functional part of the kidney that includes the renal cortex and renal corpuscle. The renal corpuscles aids in blood filtration (filters out water, ions, molecules, waste and toxins) of the nephron of the kidney. While the renal cortex which is the outer part of the kidney, provides space for easy passage for blood between the renal artery and vein as well as capillaries to the kidney.[38,39] Plate 2 showed normal prominent renal cells for both control and treated groups.

Table 1: Relative Organ Weights of Toxicological Evaluation in Female Rats

Parameters	Heart (g)	Lungs (g)	Liver (g)	Kidney (g)	Uterus (g)
Control	0.55 ± 0.07	1.25 ± 0.07	5.20 ± 0.00	0.45 ± 0.07	0.60 ± 0.28
2 mg/kg	0.60 ± 0.14	1.45 ± 0.07	4.60 ± 0.42	0.45 ± 0.07	0.40 ± 0.14
4 mg/kg	0.60 ± 0.00	1.50 ± 0.28	5.00 ± 0.42	0.55 ± 0.07	0.45 ± 0.07
8 mg/kg	0.55 ± 0.07	1.45 ± 0.01	5.35 ± 0.21	0.50- ± 0.14	0.50 ± 0.14

Values are presented as mean ± SEM. For statistical analysis one-way ANOVA with post-hoc Dunnett's test was applied at $p < 0.05$.

Table 2: Relative Organ Weights of Toxicological Evaluation in Male Rats

Parameters	Heart (g)	Lungs (g)	Liver (g)	Kidney (g)	Testes1 (g)
Control	0.70 ± 0.14	1.55 ± 0.07	6.45 ± 1.20	0.55 ± 0.07	1.30 ± 0.00
2 mg/kg	0.60 ± 0.21	1.25 ± 0.21	5.15 ± 0.64	0.50 ± 0.00	1.20 ± 0.00
4 mg/kg	0.90 ± 0.57	1.15 ± 0.35	6.00 ± 1.13	0.70 ± 0.14	1.25 ± 0.07
8 mg/kg	0.80 ± 0.14	1.50 ± 0.14	6.15 ± 0.49	0.55 ± 0.07	1.30 ± 0.00

Values are presented as mean ± SEM. For statistical analysis one-way ANOVA with post-hoc Dunnett's testwas applied at $p < 0.05$.

Female liver **Male liver**

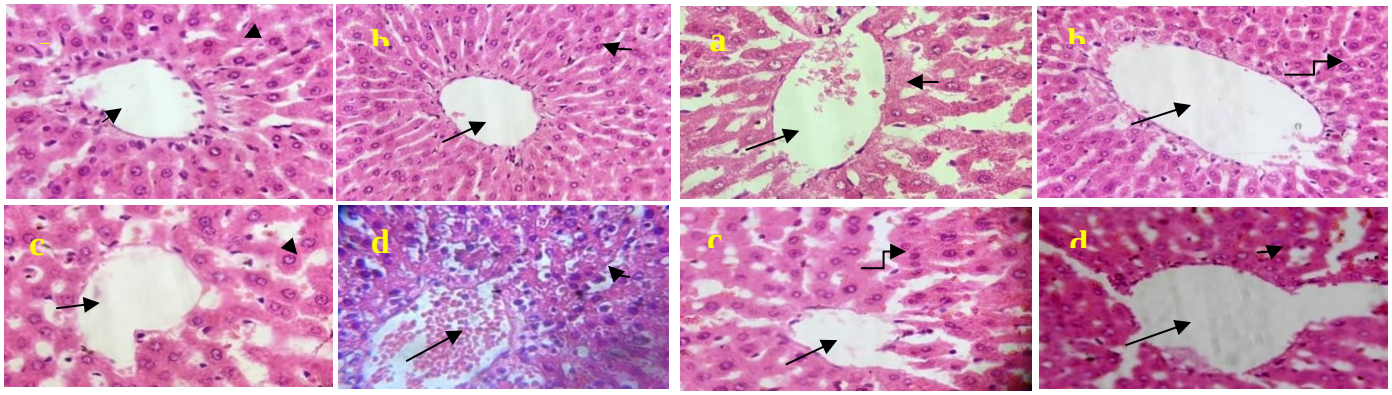

Plate1: Effect of pulcherrimin A on female and male hepatic cells

- **a. Control**: liver reveal prominent histology feature showing centriole (long arrow) well fenestrated sinusoids and hepatocytes and nucleus (short arrow).
- **b. 2 mg/kg**: liver reveal prominent histology feature showing centriole (long arrow) well fenestrated sinusoids and hepatocytes and nucleus (short arrow).
- **c. 4 mg/kg**: liver reveal centriole (long arrow) well fenestrated sinusoids and hepatocytes with fatty changes and vacuolated nucleus (short arrow).
- **d. 8 mg/kg**: liver reveal centriole (long arrow) well fenestrated sinusoids and hepatocytes with fatty changes and vacuolated nucleus (short arrow).

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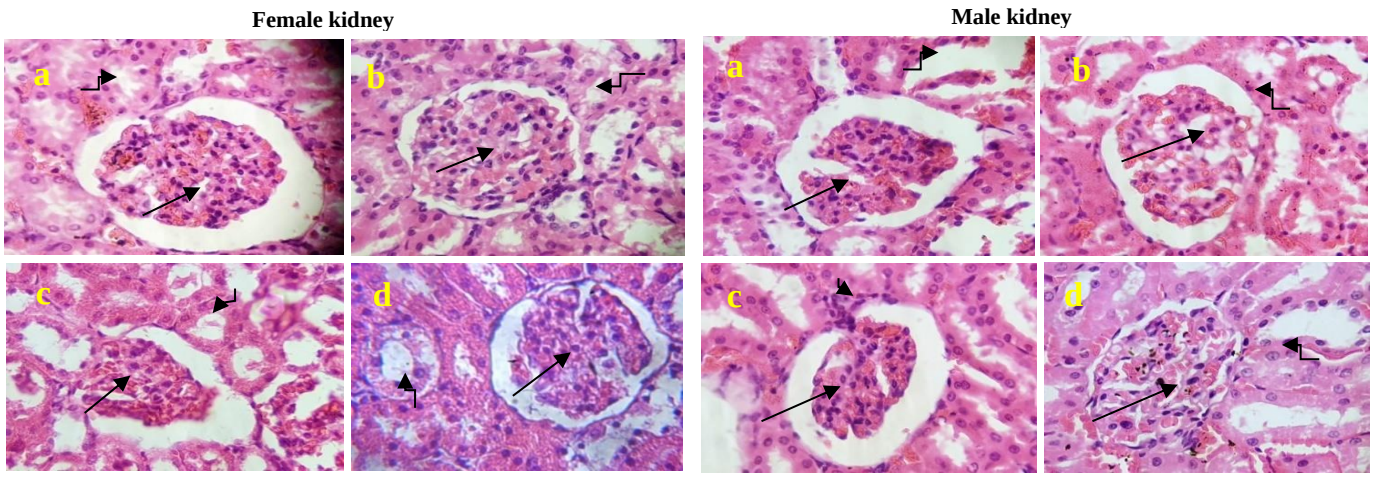

Plate 2: Effect of pulcherrimin A on female and male renal cells

- **a. Control**: kidney section showed normal histological features. The section indicated a detailed cortical parenchyma (short arrow) and the renal corpuscles appeared as dense rounded structures (long arrow).
- **b. 2 mg/kg**: kidney section showed normal histological features. The section indicated a detailed cortical parenchyma (short arrow) and the renal corpuscles appeared as dense rounded structures (long arrow).
- **c. 4 mg/kg**: kidney section showed detailed cortical parenchyma with not so prominent tubules (short arrow) and the renal corpuscles appear dense rounded and atrophied (long arrow).
- **d. 8 mg/kg**: kidney section showed detailed cortical parenchyma with not so prominent tubules (short arrow) and the renal corpuscles appear dense rounded and enlarged (long arrow).

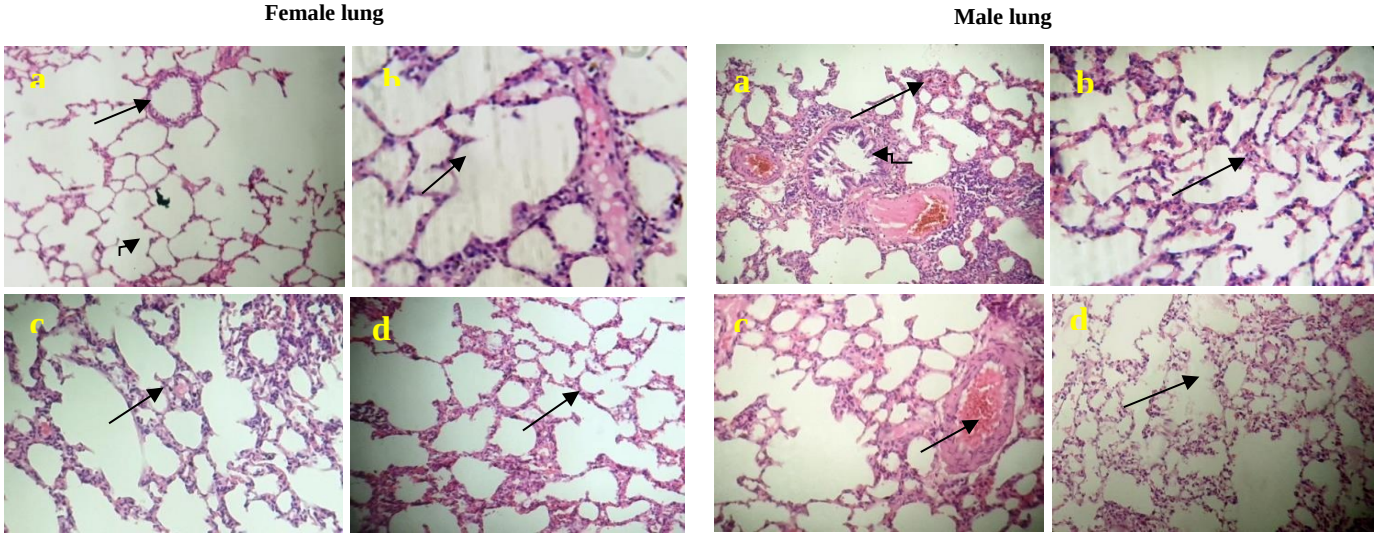

Plate 3: Effect of pulcherrimin A on female and male lungs

- **a. Control**: Male lung histology shows prominent alveolar sac (long arrow) and alveolar ring with well outlined bronchiole (short arrow) and artery. The alveolus appears distinct.
- **b. 2 mg/kg**; lung histology shows prominent alveolar sac (long arrow) and alveolar ring with well outlined. The alveolus appears distinct.
- **c. 4 mg/kg**: lung histology shows alveolar bronchiole (long arrow) and alveolar ring with well outlined. The alveolus appears slightly thickened.
- **d. 8 mg/kg**: lung histology shows prominent alveolar sac (long arrow) and alveolar ring with well outlined. The alveolus appears slightly thickened.

The heart helps in the pumping and circulation of blood through the entire body via blood vessels.[40] For every heartbeat, blood (carrying oxygen and nutrients) is sent to all the cells in body. The myocardial fibres are cardiac muscle tissues that keep the heart pumping through involuntary functions, while the coronary artery is a blood vessel that transports oxygenated blood to the heart muscles.[40] Vasodilation is the increase in width or widening of a blood vessel thus causing the relaxation of smooth muscles within the inner walls of the blood vessels (veins and arteries) thereby causing a free and increased flow of blood.[41,42] In a nutshell, the expansion of an artery causes instant decrease in heart rate and blood pressure hence, the use of chemical substances (arterial dilators) to treat angina, hypertension and heart failure.[42,43] Most compounds with vasodilator activity (vincamine and papaverine) are alkaloids, flavonoids or terpenoids.[44] In **Plate 3** the dilated coronary artery observed at 4 mg/kg may be caused by pulcherrimin A in comparison with control. This suggests pulcherrimin A could be a vasodilator and help in reducing the risk of heart related diseases.[42] On the other hand, other treated groups and control showed normal heart function. The lungs help in the process of gas exchange called respiration or breathing. The alveolar sacs are sacs of many alveoli, which are the cells that exchanges oxygen and carbondioxide in the lungs.[44] **Plate 4** revealed the lungs to be normal in all treated groups compared to normal. The slightly thickened alveolus may be from allergic reactions of pulcherrimin A or environmental particulates.[46] However, reports have revealed that the lung and heart interact together[47] to feed the body system with oxygenated blood. Any damage done to or experienced by one organ, of either the lung or the heart, would most likely affect the other.

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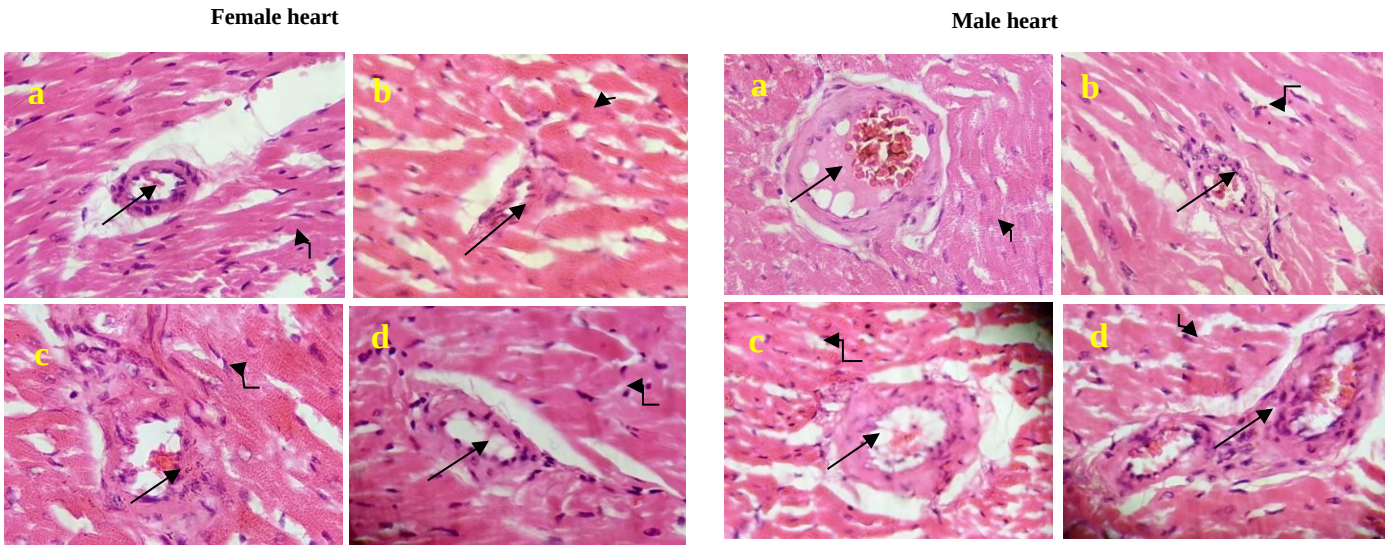

Plate 4: Effect of Pulcherrimin A on female and male cardiac cells

- **a. Control**: heart composed of bundles of myocardial fibres (short arrow), interstitial space and prominent coronary artery (long arrow)
- **b. 2 mg/kg**: heart composed of bundles of myocardial fibres (short arrow), interstitial space and prominent coronary artery (long arrow).
- **c. 4 mg/kg**: heart composed of bundles of myocardial fibres (short arrow), interstitial space and dilated coronary artery (long arrow).
- **d. 8 mg/kg**: heart composed of bundles of myocardial fibres (short arrow), interstitial space and prominent coronary artery (long arrow).

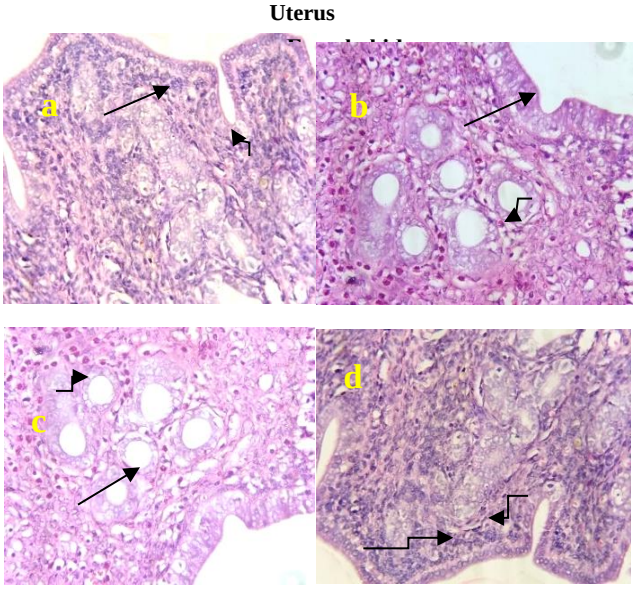

Plate 5: Effect of pulcherrimin A on female uterus

- **a. Control**: uterus reveals prominent endometrial glands which appear simple in circular forms with visible slightly reduced proliferative endometrium.
- **b. 2 mg/kg**: uterus reveals prominent endometrial glands which appear simple in circular forms with visible slightly reduced proliferative endometrium.
- **c. 4 mg/kg**: uterus reveals prominent endometrial glands (long arrow) which appear simple in circular forms with mild mononuclear proliferation in the endometrium (short arrow).
- **d. 8 mg/kg**: uterus reveals prominent endometrial glands (long arrow) which appear simple in circular forms with mild mononuclear proliferation in the endometrium (short arrow).

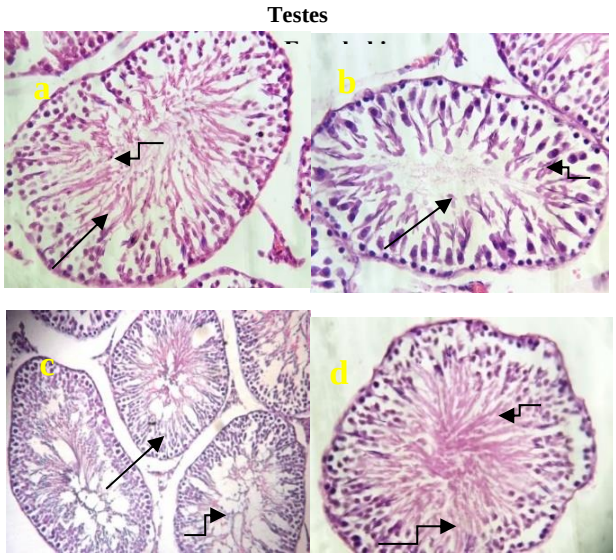

Plate 6: Effect of pulcherrimin A on male testicular cells

- **a. Control**: sections of the seminiferous tubules show moderately circular, transverse with stratified seminiferous epithelium showing cells of the spermatogenic series and spermatozoa within the lumen.
- **b. 2 mg/kg**: sections of the seminiferous tubules show moderately circular, transverse with stratified seminiferous tubules showing cells of the spermatogenic series and spermatozoa within the lumen (short arrow).
- **c. 4 mg/kg**: sections of the seminiferous tubules show moderately circular, transverse with stratified seminiferous tubulesshowing cells of the spermatogenic series and spermatozoa within the lumen (short arrow).
- **d. 8 mg/kg**: sections of the seminiferous tubules show moderately circular, transverse with stratified seminiferous tubules showing cells of the spermatogenic series and spermatozoa within the lumen (short arrow).

Base on the fact that no damage was observed in the heart across all groups, the thickened alveolus was not considered to be of a significant concern. An endometrium is the innermost lining layer of the uterus and function to prevents the adhesion between the opposed uterine walls.[48] Proliferative endometrium is the multiplication and spreading of cells in the uterus. This happens when the oestrogen levels are increases; this can be caused by hormonal contraceptives, and health related issues such as obesity, ovarian tumour or liver disease.[48,49] **Plate 5** showed normal endometrial gland (uterine gland) and testicular cells in female and male respectively.

Ibeh and Uraih (2003),[50] classified antimicrobial activity based on inhibition zone at ≤10 mm, (the organism is resistant), 11-15 mm (intermediate effect) and 16> mm (the organism is susceptible). In the antimicrobial assay, pulcherrimin A exhibited antimicrobial activity as a measure of its increasing doses against all tested microbes. The highest microbial inhibition was observed at the highest at all dose of 100 mg/mL.

Pulcherrimin A at 100 mg/ml, (Table 1) displayed antibacterial as well as antifungal activities against, *B. subtilis, S. aureus, K. pneumonia, P. aeruginosa, C. albicans* and *T. rubrum* respectively. Such a finding supports the traditional use of this plant (*C. pulcherrima*) in the treatment of infectious diseases and injuries.[13,14] It was observed to be active against all tested bacteria, particularly the common pathogens (*S. aureus, K. pneumonia, P. aeruginosa*) which are mostly wide spread in hospitals and home-communities. These organisms can cause infections in any parts of the body, ranging from the ear, lungs, liver, kidney and/or urinary tracts, this has greatly increased the mortality rate worldwide.[51] Among the microorganisms tested, *B. subtilis* was the most sensitive to Pulcherrimin A with an MIC of 11 mg/mL followed by *S. aureus*, and *Klebsiella pneumoniae* with 13 and 16 mg/mL respectively (Table 2).

At 20 mg/ml, no inhibition was observed for *P. aeruginosa*, *C. albicans* and *T. rubrum* (Table 1) which correspond to their observed higher MICs as compared to *P. aeruginosa, S. aureus* and *B. subtilis*.

Terpenoids have been reported to exhibit antibacterial activity among anti-tumour, anti-inflammatory, antiviral, antimalarial and antidiabetic.[52] So, the activity observed for the study compound could be attributed to the class of secondary metabolite it belongs.

In the search for treatments against resistant strains of disease pathogens, pulcherrimin A could be used as a drug or a lead compound in combination with other compounds that have demonstrated potency against these organisms. It could also be used in cream or lotion formulations for skin infections caused by tested organism (*C albican* and *T. rubrum*). However, other toxicity and clinical studies for pulcherrimin A are required to confirm its efficacy.

Table 1: Antimicrobial activity of pulcherrimin A at different concentrations

Organisms	Zones of Inhibition (mean ± S.E.M mm)							
	Concentrations (mg/mL)					CIP	KET	DMSO
	20	40	60	80	100	(1µg/ml)	(1µg/ml)	(10%)
S. aureus	3.6 ± 0.4	7.0 ± 0.0	11.5 ± 0.5	15.0 ± 1.0	18.3 ± 0.7	30.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
B. subtilis	5.5 ± 0.5	10.3 ± 0.7	14.0 ± 1.0	18.5 ± 1.5	24.5 ± 0.5	34.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
K. pneumoniae	1.5 ± 0.5	3.8 ± 0.7	7.0 ± 1.5	12.3 ± 1.7	16.6 ± 0.2	33.5 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
P. aeruginosa	0.0 ± 0.0	5.5 ± 0.5	7.0 ± 0.0	10.2 ± 0.4	13.0 ± 1.0	32.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
C. albicans	0.0 ± 0.0	4.0 ± 1.3	7.1 ± 0.0	11.8 ± 1.2	14.0 ± 1.0	0.0 ± 0.0	33.2 ± 0.8	0.0 ± 0.0
T. rubrum	0.0 ± 0.0	3.8 ± 0.0	7.0 ± 1.0	10.0 ± 0.0	13.1 ± 0.9	0.0 ± 0.0	31.5 ± 0.5	0.0 ± 0.0

S.E.M = Standard Error of Mean, 0.0 ± 0.0 =No activity, CIP = ciprofloxacin, KET = Ketoconazole

Table 2: Minimum inhibitory concentrations (MIC) of pulcherrimin A against the test organisms

Organisms	Test Compound	Ciprofloxacin	Ketoconazole
	MICs (mg/mL)	MICs (µg/mL)	
S. aureus	13	0.04	NA
B. subtilis	11	0.01	NA
K. pneumoniae	16	0.08	NA
P.aeruginosa	33	0.1	NA
C. albicans	30	NA	1.6
T. rubrum	31	NA	1.1

Key: NA (Not Applicable)

Conclusion

The results revealed no significant effect for all organs examined and the histological results showed no toxic damage for all organs across all treated groups in relative to control. At 4 mg/kg, pulcherrimin A was observed to dilate the coronary artery, an indication that it could be used in treatment or prevention of heart related diseases. Life threatening diseases such as meningitis, dermatitis, infections of wounds, eyes, ears, urinary tracts, gastrointestinal tracts, etc. that are caused by *S. aureus*, and *K. pneumoniae* were observed to be sensitive to pulcherrimin A.

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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