Cardisoma guanhumi as a Therapeutic Candidate: Assessing Hypo-lipidemic and Histological Effects in *Bordetella pertussis* Infected Swiss Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: *Bordetella pertussis* is the causative organism of an acute human respiratory tract disease known as *pertussis* – an endemic disease globally with reported cases in both developing and developed countries. This study evaluated the hypo-lipidaemic and histological effects of the crab specie *Cardisoma guanhumi* extracts on *bordetela pertussis* infected Swiss mice.
Methods: One hundred and twenty-two (122) animals were divided into five groups in the study. Groups 1 and 2 were the normal and negative controls respectively, while groups 3 and 4 received (300mg/kg and 600mg/kg) of the extract and group 5 received 4000mg/70kg (57.14 mg/kg) of erythromycin. Blood and kidney samples were collected on days 0, 6, 12 and 18for lipid profile analysis and kidney histological examination.

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Results: The result shows that Animals exposed to *B. pertussis* inoculum dose (5.0x10^{5}cfu/ml) without treatment caused a decrease in the plasma level of high density lipo-protein cholesterol (HDL) and an increase in the plasma levels of total cholesterol, triglycerides and low density lipo-protein cholesterol (LDL). However, treatment with *Cardisoma guanhumi* extract reversed the observed effect thereby producing a gradual increase in HDL levels and decrease in triglycerides, cholesterol and LDL levels with no adverse effect on the kidney histology.

Conclusion: The study reveals significant lipid profile alterations caused by *B. pertussis* infection in Swiss mice, highlights the potential lipid-lowering effects of *Cardisoma guanhumi* extract, and indicates the absence of apparent kidney damage from the infection.

1. INTRODUCTION

For centuries, *Bordetella pertussis* has posed a significant global health challenge, affecting individuals of all ages and leading to severe outcomes, especially in infants, children, and adults [1]. Despite extensive vaccination efforts, pertussis continues to be a public health concern, particularly in developing regions of Africa, Asia, and South America, where it remains a major cause of infant mortality [2].

"The pathogenicity of *B. pertussis* lies in its ability to produce toxins that paralyze cilia and induce inflammation in the respiratory tract, obstructing the clearance of pulmonary secretions" [3]. Additionally, pertussis antigens play a role in evading host defenses by promoting lymphocytosis while impairing chemotaxis [4]. "The global burden of pertussis is substantial, with an estimated 24.1 million cases and 160,700 deaths in children under five years of age worldwide" [5]. Nigeria bears a significant portion of this burden, accounting for 4.5% of global pertussis cases, highlighting the urgent need for effective control strategies [6]. Surprisingly, pertussis infections have been on the rise among adolescents and adults over the last two decades [7-9]. Outbreaks have been reported in various regions, including California, where individuals of white ethnicity are disproportionately affected [7].

Moreover, the emergence of *B. pertussis* strains resistant to commonly used antibiotics like erythromycin, azithromycin, and clarithromycin poses additional challenges in combating the disease [10]. Macrolides, particularly erythromycin, have long been the first-line antibiotics for pertussis treatment and post-exposure prophylaxis, but cases of resistance have been documented since 1994 [5].

Given the current scenario, there is a growing interest in exploring unconventional medicines that offer effectiveness against *B. pertussis* while being easily accessible and affordable. The medicinal crab, *Cardisoma guanhumi*, emerges as a potential source for such remedies due to its ethno-medicinal importance. Crabs, in general, have been recognized for their medicinal value, with studies demonstrating the cholesterol and triglyceride-reducing effects of chitin-enriched crab shells and the cough-suppressing properties of Chitosan Crab extract of *Uca tangeri* [11,12]. "In Latin America, *Cardisoma guanhumi* has been traditionally used to treat asthma, bronchitis, wounds, and boils, while *Goniopsis cruentata* has been employed to address epilepsy and venereal diseases" [13].

B. pertussis continues to pose a global health threat, particularly affecting vulnerable infants, and the emergence of antibiotic-resistant strains further complicates disease management. The exploration of alternative treatments from natural sources like *Cardisoma guanhumi* holds promise in providing solutions in the fight against pertussis. Such investigations into novel therapeutic options not only address the challenge of antibiotic resistance but also emphasize the potential of traditional remedies from natural resources to combat this persistent infectious disease. The findings may open up new avenues for developing effective and sustainable treatments for pertussis, especially in regions where access to conventional healthcare is limited.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

Cardisoma guanhumi were collected at the Buguma Creek because of its abundance in the area. "The Buguma Creek is a tributary of the Bonny Estuary which is located southeast of the Niger Delta between longitude 6º 51'E and 49.8'Eand latitude 4º 43'N and 47.8'N in Asari-

Toru Local Government Area of Rivers State. The creek system consists of the main creek channel with other associated interconnecting creeks which are interconnected and surround Buguma and Ido communities. The creek serves as a source of tidal water for Nigerian Institute for Oceanography and Marine Research/Buguma Brackish Water Experimental Fish Farm, which was constructed between 1963 and 1966 under the auspice of FAO. The New Calabar River brings the salty ocean water to the fish ponds with the diurnal tidal flows" [14]. A more detailed description of the study area and water quality have been given by Ogbeibu and Oribhabor [15]. The mangrove vegetation of the area comprises of species like: *Rhizophora racemosa, Avicennia germinans, Laguncularia racemosa and Conocarpus erectus.*

2.2 Sample Collection and Identification

Cardisoma guanhumi was caught using a trap. The samples collected were transferred into perforated plastic containers to allow for air during transportation and was transported to the Pharmacognosy Research Laboratory, Department of Pharmacognosy, University of Port Harcourt. The samples were identified using Food and Agriculture Organization species identification sheets for fresh water and marine crab species. The identified sampled species are presented in Fig. 1.

2.3 Isolation of Test Organism

The test organism *Bordetella pertussis* (ATCC®9340™) was gotten from the American Type Culture Collection (ATCC), USA. The culture media used for isolation according to ATCC is medium 35: Bordet Gengou/Broth medium from a human clinical specimen at a growth temperature of $37^{o}C$ in an aerobic atmosphere. The product was received freeze-dried at -20°C to -50°C and stored at -80°C. The bacterium (*Bordetela pertussis*) was reconstituted using Regan-Lowe agar (Charcoal blood Agar) in the Department of Microbiology Laboratory, University of Port Harcourt.

2.4 Method of Extraction

According to Shahidi and Synowiecki [16], 60 each of the freshly collected crabs (*C. guanhumi*) were sacrificed and the shell separated from the meat, then washed with tap water to remove all impurities. The crab shells and meat were then transferred to the oven (Memmert UN55plus Laboratory Oven) and dried at 70 ^{o}C until they were completely dry. Using a laboratory mortar and pestle, the dried crab shells and meat were ground together and sieved (ASTM 35 Mesh) into the size of 500μm. The guidelines for care and use of animals were followed in the research study.

Fig. 1. *Cardisoma guanhumi*

2.5 Carotenoids Extraction

Forty grams of the sieved crab shell was measured using WANT precision electric weighing balance (HZQ-A30 model) into a beaker and 200ml of cod liver oil was added and stirred with magnetic stirrer for 20minutes until it was completely mixed. The beaker was then transferred into a water bath at a temperature of $60^{o}C$ and allowed for 30 minutes to allow for proper separation of the carotenoids. The mixture was then filtered with a white handkerchief to drain off the oil and the residue transferred into a beaker for further extraction.

2.6 Deproteinization

"The residue from the carotenoids extract was treated with 2% potassium hydroxide (KOH) at a ratio of 1:20 w/v and was stirred continuously for 2 hours at a temperature of $90^{o}C$ to remove protein from the crab. The sample was filtered and the residues were continuously washed with distilled water until the pH became neutral i.e., pH=7. This was done to ensure that all the salt had been removed after removing the protein. The deprotienized crab was transferred into an oven and dried at $60^{o}C$ until it was completely dry" [16].

2.7 Demineralization

"2.5% w/v of hydrochloric acid was used at room temperature (23°C) for 6 hours to remove the

mineral content of the deproteinized crab materials at a ratio of 1:20 w/v. The samples were filtered and washed with tap water until the pH was neutral. The demineralized crabmaterial were then transferred to the oven and dried at a temperature of 60 ^{o}C until completely dried" [16].

2.8 Decolouration and Dewatering

The demineralized crab material was treated with 300ml acetone for 10 minutes and dried for 2hrs at an ambient temperature and the residues were removed to achieve decolourization. The decolourized sample was washed in running water, filtered and dried at 60 ^{o}C until it was completely dried to obtain crab chitin [16].

2.9 Deacetylation of Chitin

Deacetylation of chitin was carried out using the method of Yen et al. [17]. The obtained chitin was treated with 40% w/v aqueous sodium hydroxide in the ratio of chitin to the solution 1:15 w/v at 105 ^{o}C in a water bath (Water Bath-hilmedics H-420 model) for 2 hours. Thereafter, the chitin was filtered with filter pump and washed with deionized water until pH was neutral to obtain chitosan. The obtained chitosan was then dried in the oven at 60°C for 2hrs. The dried chitosan was preserved in a well labeled bottle and kept for the experiment.

2.10 Preparation of Culture Media

Nutrient broth and nutrient agar were prepared by adding 28g of nutrient agar to one liter of distilled water and the mixture heated at 60°C until it was completely dissolved while 13g of nutrient broth was added to one liter of distilled water and properly mixed with a stirrer, this was used to culture the bacterial population when checking for the microbial flora of the animals. They were prepared and distributed into test tubes, bottles and universal bottles respectively, and sterilized by autoclaving at 121°C for 15 minutes.

2.11 Antibiotic and Extract Concentration Preparation

The extract solution for the study was prepared by dissolving 0.5g of the extract in 1ml of di-methyl-sulfoxide (DMSO) solvent to have a stock concentration of 500mg/ml from where dilute solutions were made from distilled water, while erythromycin (manufactured by Wellona Pharma) solution was prepared by dissolving 500mg of the tablet in 21.87ml of distilled water based on the fact that 70kg (70000g) adult takes 4000mg of erythromycin daily for severe case of whooping cough. Hence the above concentration was prepared for an average weight animal of 25g, i.e., 25g×4000mg/70000g=1.429mg. This means 25g received 1.429mg/ml or 2.858mg/0.5ml (1.429×2) or 5.716mg/0.25ml. 5.716mg/0.25ml.

2.12 Preparation of Bacterial Suspension

The turbidity of each of the bacterial suspension to determine the microbial population was prepared to match 0.5 McFarland standards. The McFarland standard was prepared by dissolving 0.5g of BaCl$_2$ in 50ml of distilled water to obtain a 1% (w/v) solution of Barium chloride and mixed with one percent sulphuric acid as follows: 0.5 ml of the 1% BaCl$_2$ solution was mixed with 99.5 ml of 1% H$_2$SO$_4$ solution. The turbidity of the 0.5 McFarland Standards was measured with the aid of a spectrophotometer (UV5Bio model) at a wavelength of 625 nm to read an optical density between 0.08-0.10 which represents a bacterial cell density of approximately 1.5 x 10^8 CFU/ml or a range of (1.0 x10^8-2.0 x 10^8) CFU/ml. This solution was transferred to a screw-capped bottle sealed with paraffin to prevent evaporation due to exposure to air. Bacterial suspensions were prepared and tested against the McFarland standards until they reached the absorbance of the McFarland standard to determine the microbial load.

2.13 Anti-bacterial Preparation to Match the McFarland Standards

The method used for this preparation was the one used by Taye et al. [18]. *B. pertussis* was collected from the colony of an agar plate and transferred into 5ml broth of Mueller Hilton medium and kept in an incubator at 37 ^{o}C for a minimum of 12 hours. This was also incubated at the same time with plain Mueller Hilton broth medium to confirm if the medium was already contaminated. The optical density of plain and bacteria containing media were determined in cuvettes using same wavelength as the McFraland standards with the aid of a spectrophotometer. To obtain a bacterial cell density of 1.5x10^{8}CFU or a range of 1.0x10^8-2.0x10^8 CFU/ml, the absorbance of the bacterial suspension was adjusted with the plain suspension to equilibrate with the McFarland standards. This was used for the inoculation of the animals and nutrient agar in the different bioassays [19].

2.14 Anti-microbial Sensitivity Test

The agar well method of the agar diffusion technique was used in this study to determine the antibacterial activity of the crab extracts as described by Adegoke and Adebayo-Tayo [20]. The nutrient agar (Diffco) that was used was prepared by adding 28g of nutrient agar to one liter of distilled water and the mixture heated until it was completely dissolved and the extracts were then tested against *Bordetella pertussis* as follows: Three 6 mm wells were made into each agar plate using a sterile metal Cork borer. Then, 100μl of the standard drug erythromycin was placed in one well, the extract in another well and dimethyl sulfoxide (DMSO) was placed in the third well on each plate. The experiment was carried out in triplicate for each extract of the three crab species tested. All plates were incubated at 37°C for 48 hours and the zones of inhibition were measured in millimeters with the aid of a meter rule. The diameter of the zones of inhibition in the triplicate plates were then measured by calculating the difference between the core borer (6 mm) and the diameters of inhibition as described by Hewitt and Vincent [21]. The activity indices were calculated as the division of zone of inhibition of the extract by that of the standard drugs following the method of Singh et al. [22].

2.15 Determination of Minimum Inhibitory Concentration MIC

The MIC or minimum inhibitory concentration test determines antimicrobial activity of a material against specific bacteria. Agar well dilution method as described by Aida and Rosa [23] was used to determine the minimum inhibitory concentration (MIC). Extract dilutions with different concentrations (200mg/ml, 100mg/ml, 70mg/ml, 50mg/ml, 30mg/l etc.) were measured and analyzed. The lowest concentration (highest dilution) of test agent preventing appearance of turbidity (growth) is considered to be the minimal inhibitory concentration (MIC). At this dilution the test agent is bacteriostatic and was assessed by measurement of the zones of inhibition formed around the wells.

2.16 Determination of Minimum Bactericidal Concentration (MBC)

The minimal bactericidal concentration (MBC) or the minimum lethal concentration (MLC) of an antibacterial is defined as the maximum dilution of the product that will kill a test organism. To determine the minimum bactericidal concentration, the cultures from the plates showing no vivid growth were streaked onto newly prepared nutrient agar plates with the test organism (*Bordetella pertussis*) and both plates were allowed for 24hours in an incubator at a temperature of 37°c. The extract concentration that did not show a clear growth was seen as the minimum bactericidal concentration.

2.17 Pilot Study

Before the actual study, a pilot study was carried out to determine the route of inoculation that will produce infectious condition and the length of time for observable signs of infection in mice. Four animals each were given the intraperitoneal and intranasal routes of inoculation with 0.5ml of the inoculum dose of $5.0x10^{5}$ CFU/ml of the bacteria (*B. pertussis*). A confirmatory test was carried out by isolation of the organism from the blood sample. This served as a guide for the main study.

2.18 Experimental Design

A total of one hundred and twenty-two (122) animals (Swiss mice) were divided into five groups for the curative treatment study. Group 1 (normal) had 10 animals, group 2 (negative control) had 28animals; groups 3-5 consisted of 28 Swiss mouse each. Group 1 served as the normal control without treatment but was fed with the normal animal feed and water *ad libitum*. Group 2 (negative control group) consisted of *B. pertussis* inoculated mice without treatment. Group 3 consisted of *B. pertussis* infected mice treated with low dose (300mg/kg) of *Cardisoma guanhumi* extract; group 4 also consisted of *B. pertussis* infected mice treated with high dose (600mg/kg) of *Cardisoma guanhumi* extract, while group 6 consisted of *B. pertussis* infected mice treated with 4000mg/70kg of erythromycin. Samples of blood were taken from seven animals at intervals of six days and at the end of the eighteen days. The lipid profile status was analyzed, while the kidney tissues were collected for histopathological studies.

2.19 Blood Collection and Dissection

At the end of the experiment, blood was collected from each mouse by cardiac puncture method. The blood was immediately transferred

into appropriately labeled sample bottles containing anticoagulant for lipid analysis.

2.20 Histopathology Studies

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the kidneys which were then transferred into 10% chloroform, and it was later trimmed down to a size of 2mm to 4mm thickness. This was done to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker [24], including, fixation, dehydration, clearing, impregnation, embedding, sectioning and staining with hematoxylin and eosin (H&E), and finally mounting.

3. RESULTS

3.1 Effect of Extracts of *Cardisoma guanhumi* extract (CGE) on Lipid Profile and Kidney Histo-architecture on Post-Inoculation Treatment of *B. pertussis* infected Mice

Table 1. Effect of CGE Post – Inoculation Treatment on Cholesterol (mmol/l) in *B. pertussis* infected mice

Group	Day 0	Day 6	Day 12	Day 18
NC	1.32±0.000	1.32±0.000	1.32±0.000	1.32±0.000
NEC	1.97±0.061	2.01±0.076	2.11±0.006	2.13±0.006
SD	1.97±0.061	1.74±0.021	1.52±0.010	1.32±0.000
LDCGE	1.97±0.061	1.93±0.025^{ac}	1.86±0.025^{abc}	1.78±0.021^{abc}
HDCGE	1.97±0.061	1.92±0.030^{abc}	1.69±0.006^{abc}	1.50±0.015^{abc}

a= Significant (p<0.05) between test groups and normal control
b= Significant (p<0.05) between test groups and negative control
c= Significant (p<0.05) between test groups and standard drug
NC= Normal control (Animal fed with normal feed and water)
NEC= Negative control (Animal infected with Bordetella pertussis without treatment)
SD= 400mg/70kg Erythromycin
LDCGE= 300mg/kg Cardisoma guanhumi
HDCGE= 600mg/kg Cardisoma guanhumi

Table 2. Effect of CGE Post – Inoculation Treatment on Triglycerides (mmol/l) in *B. pertussis* infected mice

Group	Day 0	Day 6	Day 12	Day 18
NC	0.80±0.000	0.80±0.000	0.80±0.000	0.80±0.000
NEC	1.93±0.058	1.97±0.058	2.03±0.058	2.10±0.000
SD	1.93±0.058	1.57±0.058	1.13±0.058	0.80±0.100
LDCGE	1.93±0.058	1.80±0.000^{abc}	1.63±0.058^{abc}	1.50±0.200^{abc}
HDCGE	1.93±0.058	1.67±0.058^{abc}	1.33±0.115^{abc}	1.10±0.100^{abc}

a= Significant (p<0.05) between test groups and normal control
b= Significant (p<0.05) between test groups and negative control
c= Significant (p<0.05) between test groups and standard drug
NC= Normal control (Animal fed with normal feed and water)
NEC= Negative control (Animal infected with Bordetella pertussis without treatment)
SD= 400mg/70kg Erythromycin
LDCGE= 300mg/kg Cardisoma guanhumi
HDCGE= 600mg/kg Cardisoma guanhumi

Table 3. Effect of CGESHE Post – Inoculation Treatment on HDL (mmol/l) in *B. pertussis* infected mice

Group	Day 0	Day 6	Day 12	Day 18
NC	1.02±0.000	1.02±0.000	1.02±0.000	1.02±0.000
NEC	0.91±0.012	0.54±0.020	0.49±0.031	0.31±0.036
SD	0.91±0.012	0.95±0.006	0.97±0.012	1.01±0.017
LDCGE	0.91±0.012	0.91±0.006^{abc}	0.92±0.006^{abc}	0.94±0.006^{abc}
HDCGE	0.91±0.012	0.92±0.006^{abc}	0.96±0.012^{ab}	1.00±0.012^{b}

a= Significant (p<0.05) between test groups and normal control
b= Significant (p<0.05) between test groups and negative control
c= Significant (p<0.05) between test groups and standard drug
NC= Normal control (Animal fed with normal feed and water)
NEC= Negative control (Animal infected with Bordetella pertussis without treatment)
SD= 400mg/70kg Erythromycin
LDCGE= 300mg/kg Cardisoma guanhumi
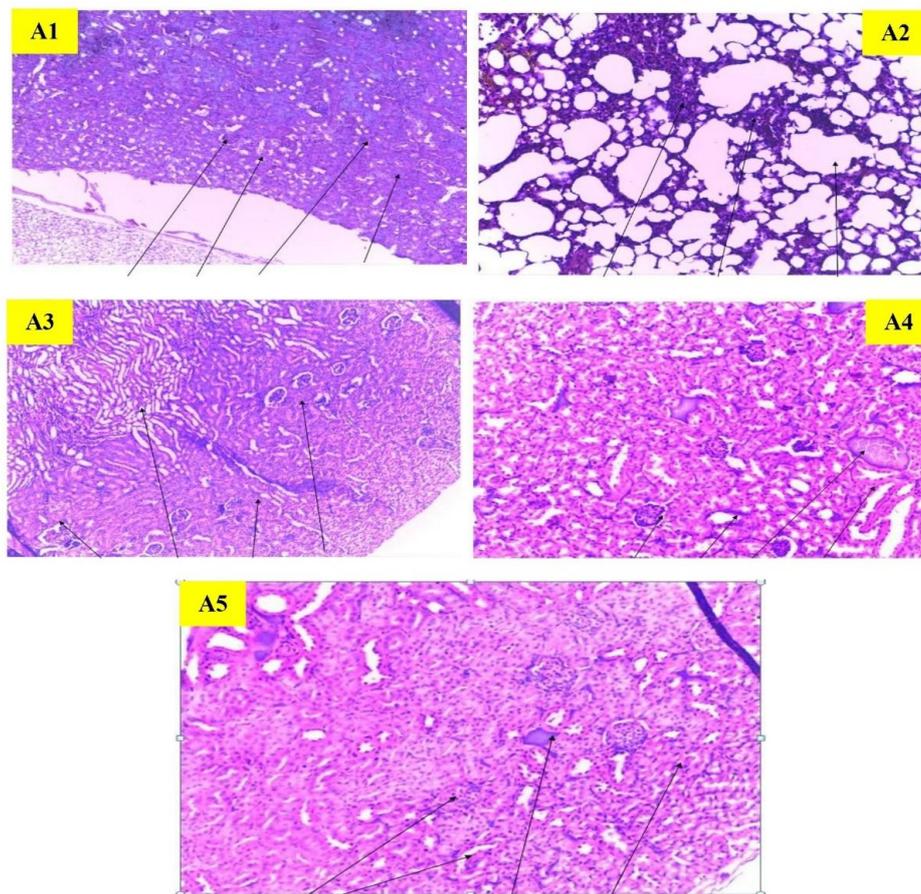*HDCGE= 600mg/kg Cardisoma guanhumi*

Fig. 2. Photomicrograph of kidney tissue on day 6
(A1) Photomicrograph of kidney tissue of normal mice showing glomeruli, renal tubules blood vessels and interstitium with no obvious histological alteration. (A2) Photomicrograph of B. pertussis infected kidney without treatment showing no obvious Histological alteration. (A3) Photomicrograph of B. pertussis infected kidney treated with 400mg/70kg Erythromycin showing no obvious Histological alteration. (A4) Photomicrograph of B. pertussis infected kidney treated with 300mg/kg Cardisoma guanhumi showing no obvious histological alteration with clear glomeruli, renal tubules and blood vessels. (A5) Photomicrograph of B. pertussis infected kidney treated with 600mg/kg Cardisoma guanhumi showing no obvious Histological alteration with vivid glomeruli, renal tubules and interstitium

Table 4. Effect of CGEPost – Inoculation Treatment on LDL (mmol/l) in B. pertussis infected mice

Group	Day 0	Day 6	Day 12	Day 18
NC	0.43±0.000	0.43±0.000	0.43±0.000	0.43±0.000
NEC	0.88±0.015	0.91±0.020	0.95±0.010	0.96±0.026
SD	0.88±0.015	0.82±0.015	0.52±0.030	0.40±0.021
LDCGE	0.88±0.015	0.87±0.006[abc]	0.81±0.015[abc]	0.78±0.021[abc]
HDCGE	0.88±0.015	0.86±0.012[abc]	0.57±0.035[ab]	0.40±0.010[b]

a= Significant (p<0.05) between test groups and normal control
b= Significant (p<0.05) between test groups and negative control
c= Significant (p<0.05) between test groups and standard drug
NC= Normal control (Animal fed with normal feed and water)
NEC= Negative control (Animal infected with Bordetella pertussis without treatment)
SD= 400mg/70kg Erythromycin
LDCGE= 300mg/kg Cardisoma guanhumi
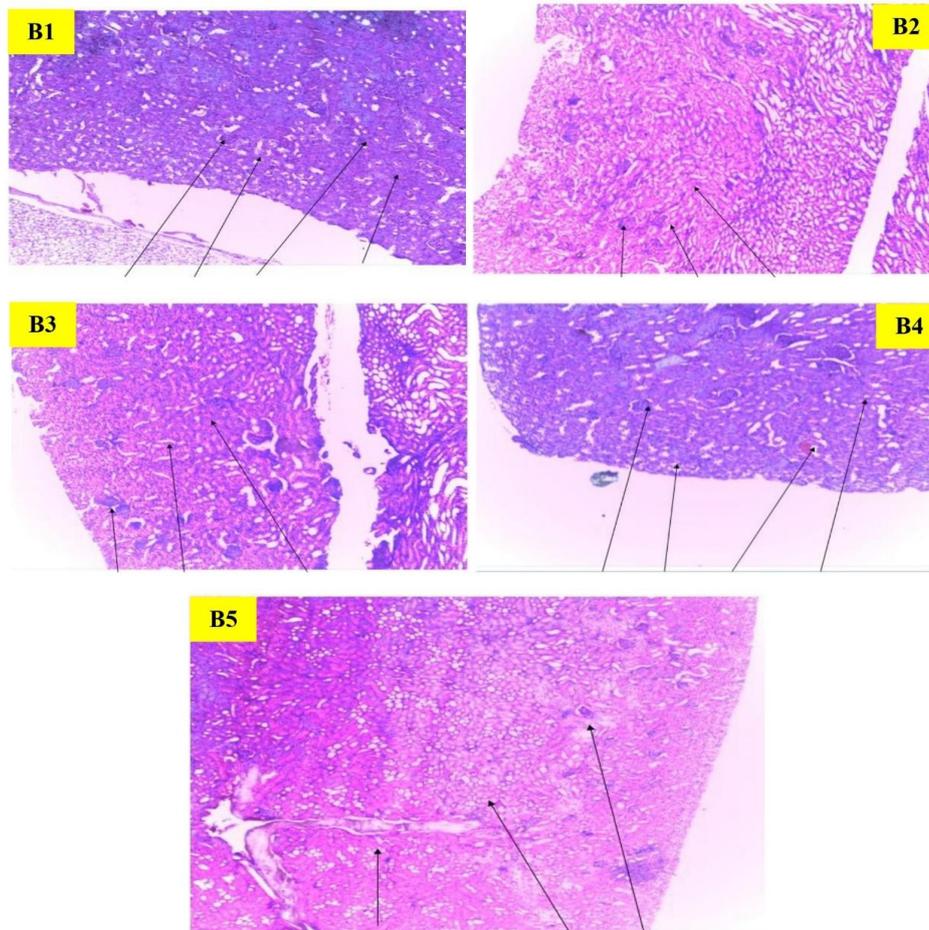*HDCGE= 600mg/kg Cardisoma guanhumi*

Fig. 3. Photomicrograph of kidney tissue on day 12
(B1) Photomicrograph of kidney tissue of normal mice showing glomeruli, renal tubules blood vessels and interstitium with no obvious histological alteration. (B2) Photomicrograph of B. pertussis infected kidney without treatment showing no obvious histological alteration. (B3) Photomicrograph of B. pertussis infected kidney treated with 400mg/70kg Erythromycin showing no obvious histological alteration showing clear glomeruli and renal tubules. (B4) Photomicrograph of B. pertussis infected kidney treated with 300mg/kg Cardisoma guanhumi showing no obvious histological alteration with obvious glomeruli, renal tubules and blood vessels. (B5) Photomicrograph of B. pertussis infected kidney treated with 600mg/kg Cardisoma guanhumi showing no obvious histological alteration showing blood vessels, renal tubules and glomeruli
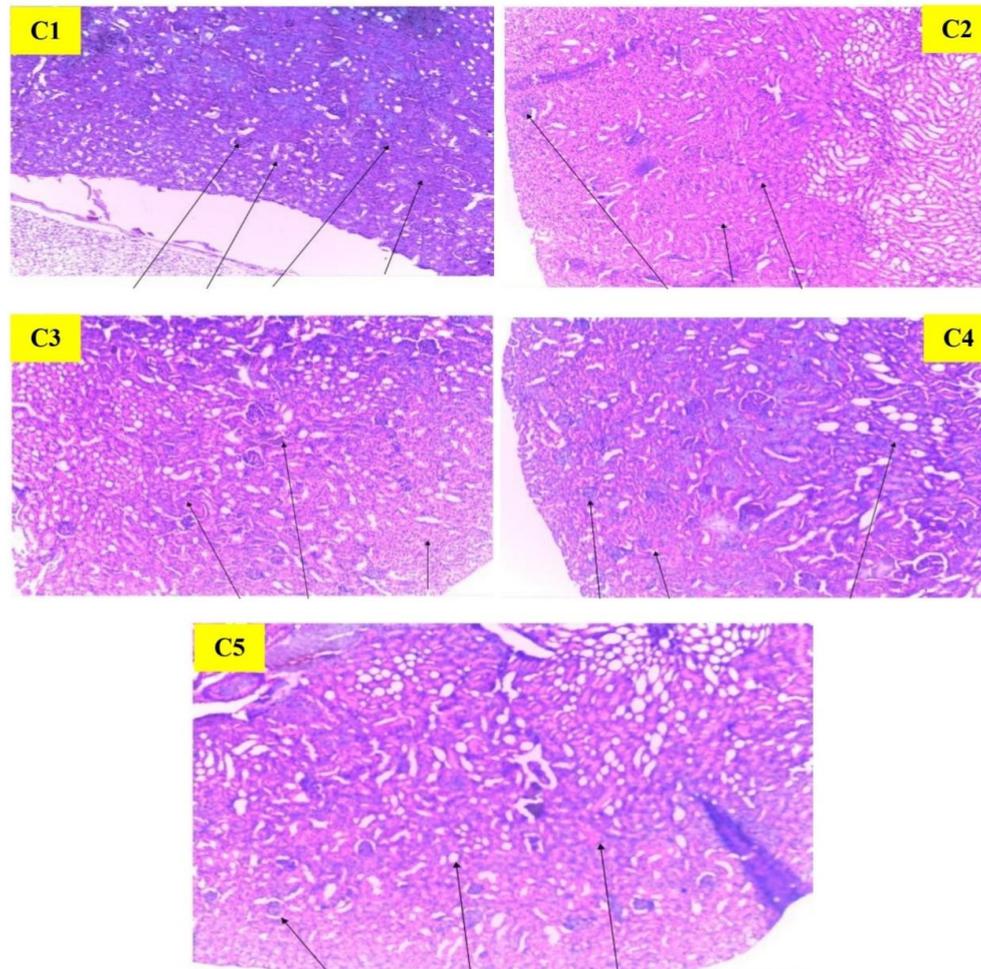
Fig. 4. Photomicrograph of kidney tissue on day 18

(C1) Photomicrograph of kidney tissue of normal mice showing glomeruli, renal tubules blood vessels and interstitium with no obvious histological alteration. (C2) Photomicrograph of B. pertussis infected kidney without treatment showing no obvious histological alteration. (C3) Photomicrograph of B. pertussis infected kidney treated with 400mg/70kg Erythromycin showing no obvious histological alteration with obvious glomeruli, renal tubules and blood vessels. (C4) Photomicrograph of B. pertussis infected kidney treated with 300mg/kg Cardisoma guanhumi showing no obvious histological alteration with glomeruli and renal tubules showing clearly. (C5) Photomicrograph of B. pertussis infected kidney treated with 600mg/kg Cardisoma guanhumi showing no obvious histological alteration with clear glomeruli and renal tubules

The result shows that the inoculation of an infective dose of *B. pertussis* produced a significant (P<0.05) decrease in the plasma level of high density lipoprotein-cholesterol (HDL) and an increase in the concentration of total cholesterol, triglycendes and low density lipoprotein-cholesterol (LDL). However, treatment with CGE and erythronmycin caused a reversal in the trend thereby leading to a gradual increase in HDL levels and decrease in triglycerides, cholesterol and LDL levels. The results reveal a significant difference (p<0.05) between the treatment groups, negative control and the normal control in total cholesterol, triglycerides,

HDL and LDL on the 6th, 12th and 18th day. However, there was no significant difference (P>0.05) on day 18 in HDL and LDL when compared with the normal control and standard drug. The results are presented in Tables 1, 2, 3 and 4. The results for the evaluation of the kidney histology are presented in Figs. 2 to 4. The histological structure of the control animals showed normal structure of the kidney tissue with glomeruli properly placed in the cortex of the tissue, clear blood vessels and renal tubules. There was no obvious histological alteration that was observed. In a similar manner, those infected with *B. pertussis* also showed no

pathological changes, but rather the glomeruli are properly placed in the cortex of the tissue throughout the period of the study. The same observations were obtained for the treatment groups for the period of the study. This result revealed that *B. pertussis* has no adverse effect on the kidney architecture of Swiss mice.

4. DISCUSSION

"Increased triacylglycerol levels as well as increase in VLDL and reduced HDL cholesterol levels are characteristic changes that have been reported to occur during infection" [25,26]. According to Tiirola, et al. [27] and Nonogaki, et al. [28], "elevation of plasma triacylglycerol level could be the result of either increased VLDL production or decreased VLDL clearance". Nonogaki, et al. [28] and Hardardottir, et al. [29] have remarked that "experiments in rats and mice showed that even at low doses, bacteria lipopolysaccharide (LPS) can rapidly stimulate VLDL production by increasing adipose tissue lipolysis, increasing hepatic de novo fatty acid synthesis, and decreasing hepatic fatty acid oxidation, which he said, these changes provide fatty acid substrate for esterification into triacylglycerols and assembly into VLDL particles in the liver". Yet in another account, Xu& Nilsson [30] remarked that injection of bacteria or LPS into rats had been reported to significantly inhibit the clearance of LDL from the circulation while Wright et al. [31] and Sakaguchi & Sakaguchi [32] associated "this effect to be responsible for the marked increase VLDL-triacylglycerol". Finally, Solomon et al. [33], concluded that "this mechanism may also have been responsible for the increased plasma and erythrocyte free fatty acids observed in the experimental rats infected with Salmonella as the increase in free fatty acids was associated with increased LDL-VLDL and plasma triacylglycerols". To corroborate these findings, Isirima, et al. [34] reported "an increase in triacylglycerol levels and reduced HDL cholesterol levels in Wistar rats infected with *Salmonella typhi* bacteria". This present study corroborates these findings because it was observed that inoculation of an infectious dose of *B. pertussis* produced a significant ($p<0.05$) decrease in the plasma level of high density lipoprotein-cholesterol (HDL) and an increase in the concentration of total cholesterol, triglycerides and low density lipoprotein-cholesterol (LDL) but treatment with CGE and erythronmycin reversed this trend thus leading to a gradual increase in HDL levels and decrease in

triglycerides, cholesterol and LDL levels. These findings also agree with the report of Solomon et al. [33] who reported that *B. pertussis* infection causes dyslipidemia with an increase in serum levels of triglycerides, cholesterol (hyper-triglyceridemia and cholesterogenesis) and Omeh et al. [35] who also reported a significant ($p<0.05$) increase in LDL-cholesterol level and a reduction in HDL-cholesterol that is statistically significant. The reversal effects of CGE, could be due to an increase in LDL-cholesterol clearance, enhanced fatty acid oxidation and a decreased adipose tissue lipolysis suggesting a hypo-lipidaemic and cardio-protective effect of the extracts against degenerative disease [36]. This study revealed that infection with *B. pertussis* does not adversely affect the kidneys and the doses of the extract and erythromycin do not also affect the kidneys.

5. CONCLUSION

The study highlighted the significant alterations in lipid profiles induced by *B. pertussis* infection in Swiss mice and demonstrates the potential beneficial effects of *Cardisoma guanhumi* extract in reversing these changes. The findings suggest that *Cardisoma guanhumi* may have lipid-lowering properties and could be explored further for its potential therapeutic use in managing lipid-related cardiovascular risks associated with *B. pertussis* infection. Furthermore, the observation that *B. pertussis* infection did not result in apparent kidney damage in Swiss mice is reassuring; however, it warrants further investigation to ascertain the long-term effects on renal health. This study contributes to our understanding of the pathophysiological effects of *B. pertussis* infection and opens new avenues for the development of novel interventions to mitigate its adverse effects on lipid metabolism and cardiovascular health.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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