OPEN ACCESS
Scholars International Journal of Traditional and Complementary Medicine
Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) | ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Hypo-Lipidaemic and Histological Effects of *Goniopsis pelli in Bordetella pertussis* Infected Swiss Mice

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DOI: 10.36348/sijtcm.2023.v06i06.003 | **Received:** 18.06.2023 | **Accepted:** 23.07.2023 | **Published:** 29.07.2023

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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 anti-bacterial potentials of the crab specie (*Goniopsis pelli*) extract on *bordetela pertussis* in Swiss mice. ***Methods*:** One hundred and twenty-two (122) animals (Swiss mice) were divided into five groups for the curative treatment study. Groups 1 and 2 were the normal and negative controls respectively, while groups 3 and 5 received (300mg/kg and 600mg/kg) of the extract and group 5 received 4000mg/70kg of erythromycin. Blood and kidney samples were collected on 0, 6th, 12th and 18thday for lipid profile analysis and kidney histological examination. ***Result*:** 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 lipoprotein cholesterol (HDL) and an increase in the concentration of total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL). However, treatment with *Goniopsis pelli* extracts 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*:** *Goniopsis pelli* has the potentials of ameliorating lipid abnormalities and plays a therapeutic role in improving lipid profiles associated with the bacterial infection.

Keywords: *Goniopsis pelli, Bordetella pertussis*, lipid profile, Bacteria infection, Histology, Swiss mice.

INTRODUCTION

Bordetella pertussis, the bacterium responsible for pertussis, has been a significant global pathogen for over 1,600 years. It affects individuals of all ages, from infants to adults (Dorji *et al.*, 2017). Despite high vaccination coverage, pertussis remains a public health concern worldwide and is a leading cause of infant mortality (Xing *et al.*, 2014). When the bacteria colonize the respiratory epithelial cells, they produce toxins that paralyze the cilia and cause inflammation in the respiratory tract. This leads to impaired clearance of pulmonary secretions (Sheridan *et al.*, 2012). Pertussis antigens appear to enhance lymphocytosis while impairing chemotaxis, enabling the bacteria to invade the host's defenses (Center for Disease Control [CDC], 2011).

Pertussis is endemic globally, with reported cases in both developed and developing countries (Dobson, 2010). In 1999, there were an estimated 48.5 million cases of pertussis and approximately 295,000 deaths reported worldwide (Eby *et al.*, 2013). In 2010 alone, there were 16 million cases and 195,000 deaths, with a significant number of deaths occurring in Africa (Mooi *et al.*, 2012; Cherry and Seaton, 2012). In 2013, approximately 136,000 cases were reported globally (Rodgers *et al.*, 2013). Infants under the age of 1 year are particularly vulnerable, accounting for 19% of reported cases in 2003 and 25 deaths in 2010 (Tanaka *et al.*, 2003; Winter *et al.*, 2014). In the United States, pertussis has become endemic and is now considered the most common vaccine-preventable disease (Eshofonie *et al.*, 2015; Cherry, 2015).

Recently, there has been a rise in antibiotic-resistant strains of *B. pertussis*, posing a serious global concern, especially in developing countries with poor socio-economic conditions that facilitate the spread of the disease. Studies have shown increased resistance of *B. pertussis* to erythromycin, a commonly used macrolide antibiotic (Li *et al.*, 2015; Luo *et al.*, 2014; Guillot *et al.*, 2012). Macrolide-resistant *B. pertussis* has been reported in Iran, China, the United States,

Taiwan, and Africa (Hill *et al.*, 2016; Ekong *et al.*, 2015; Marchand-Austin *et al.*, 2014; Dinu *et al.*, 2014). In addition, there are isolates of *B. pertussis* in Japan that are resistant to quinolones, which are alternative drugs to macrolides (Ohtsuka *et al.*, 2009). Therefore, alternative therapeutic agents of natural origin need to be explored. One such potential source is the medicinal crab, specifically the species *Goniopsis pelli*, which has known antibacterial properties (Sriphuthorn, 2000). Crabs have been found to possess medicinal value, such as reducing cholesterol levels and suppressing coughs (Dobson, 2010; Isirima and Uahomo, 2023). In Latin America, crabs like *Cardisoma guanhumi* and *Goniopsis cruentata* have been used to treat asthma, bronchitis, wounds, and venereal diseases (Alves and Alves, 2011). Due to the growing trend of antibiotic resistance, unconventional medicine that is effective against *B. pertussis* and accessible and affordable is needed, making medicinal crabs an area worth exploring.

MATERIALS AND METHODS

Description of the Study Area

The sample (*Goniopsis pelli*) were collected at Sivibilagbara along the Dor Nwezor channel of Bodo Creek due to its abundance in the area. Sivibilagbara is a protected mangrove swamp and on four isolated, open, unvegetated intertidal flats along the Dor Nwezor channel of Bodo Creek with latitude 4º35'26.3"-4^{o}36'29.7"Nand longitude 7º15'30.2"-7^{o}16'50.9"E. Bodo Creek is a network of brackish water creeks flanking Bodo city on the upper reaches of the Andoni-Bonny River estuary system in Rivers State, Nigeria. The configuration and hydrology has been described by Onwugbuta-Enyi *et al.*, (2008).

Sample Collection and Identification

Goniopsis pelli were collected at low tide in the mangrove shores by hand picking. 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 Figure 1 below.

Figure 1: *Goniopsis pilli*

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 -80oC. The bacterium (*Bordetela pertussis*) was reconstituted using Regan-Lowe agar (Charcoal blood Agar) in the Department of Microbiology Laboratory, University of Port Harcourt.

Method of Extraction

According to Shahidi and Synowiecki (1991), 60 each of the freshly collected crabs (*G. pelli*) 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 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 into the size of 500µm.

Carotenoids Extraction

Forty grams of the sieved crab shell was measured using WANT precision electric weighing balance 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 30mins 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.

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 2hours 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 (Shahidi and Synowiecki, 1991).

Demineralization

2.5% w/v of hydrochloric acid was used at room temperature (23^{o}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 (Shahidi and Synowiecki, 1991).

Decolouration and Dewatering

The demineralized crab material was treated with 300ml acetone for 10mins 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 (Shahidi and Synowiecki, 1991).

Deacetylation of Chitin

Deacetylation of chitin was carried out using the method of Yen *et al.*, (2009). 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 for 2hrs. 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^{o}C for 2hours. The dried chitosan was preserved in a well labeled bottle and kept for the experiment.

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^{0}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.

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 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.

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_2SO_4 solution. The turbidity of the 0.5 McFarland Standards was measured with the aid of a spectrophotometer 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 bottlesealed with paraffinto 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.

Anti-bacterial preparation to match the Mcfarland standards

The method used for this preparation was the one used by Taye *et al.*, (2011). *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 12hrs. 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 (UV5Bio model). To obtain a bacterial cell density of 1.5x10^8CFU 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 (Agyare *et al.*, 2013).

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 (2009). 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^{o}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 (1989). 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*., (2002).

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 (2001) 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 bacterio static and was assessed by measurement of the zones of inhibition formed around the wells.

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^{o}C. The extract concentration that did not show a clear growth was seen as the minimum bactericidal concentration.

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.

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 *Goniopsis pelli* extract; group 4 also consisted of *B. pertussis* infected mice treated with high dose (600mg/kg) of *Goniopsis pelli* 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.

Challenging Apparently Healthy Animals with *Bordetella Pertussis* Infective Dose

One hundred and twelve animals were intraperetonially challenged with the infective dose of *Bordetella pertussis* which was calculated to be 5.0 x 10^{5}cfu/ml. After infection had set in, seven animals of the negative control were sacrificed using an anesthesia (diethyl ether) in a desiccators and the other animals from the other treatment groups were given two times daily of the various doses of the extract as well twice daily of erythromycin. Healthy mice infected with *B. pertussis* but not treated served as control.

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.

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 (1945) and Isirima and Uahomo (2023), including, fixation, dehydration, clearing, impregnation,

embedding, sectioning and staining with hematoxylin and eosin (H&E), and finally mounting.

Method of Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (IBM SPSS). Data were expressed as Mean ± Standard error of mean. Inferential statistics was performed using one-analysis of Variance (ANOVA) followed by the Dunnett method to determine significant differences among the groups. Statistical significance was considered at $p<0.05$.

RESULTS

Effect of extracts of *Goniopsis pelli* extract (GPE) on Lipid Profile and Kidney Histo-architecture on Post-Inoculation Treatment of *B. Pertussis* infected mice

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 GPE 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 differences ($p<0.05$) 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 plates 1-13. The histological structure of the control animals showednormal 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.

Table 1: Effect of GPE 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	
LDGPE	1.97±0.061	1.91±0.021^{abc}	1.91±0.010^{abc}	1.90±0.010^{abc}	
HDGPE	1.97±0.061	1.90±0.015^{abc}	1.78±0.035^{abc}	1.73±0.012^{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
LDGPE= 300mg/kg *Goniopsis pelli*
HDGPE= 600mg/kg *Goniopsis pelli*

Table 2: Effect of GPE 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
LDGPE	1.93±0.058	1.90±0.000^{abc}	1.87±0.058^{abc}	1.77±0.058^{abc}
HDGPE	1.93±0.058	1.80±0.000^{abc}	1.73±0.058^{abc}	1.60±0.000^{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
LDGPE= 300mg/kg *Goniopsis pelli*
HDGPE= 600mg/kg *Goniopsis pelli*

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

	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
LDGPE	0.91±0.012	0.91±0.000^{abc}	0.92±0.010^{abc}	0.94±0.006^{abc}
HDGPE	0.91±0.012	0.91±0.006^{abc}	0.93±0.006^{abc}	0.96±0.006^{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
LDGPE= 300mg/kg *Goniopsis pelli*
HDGPE= 600mg/kg *Goniopsis pelli*

Table 4: Effect of CGE, GPE and SHE Post – 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
LDGPE	0.88±0.015	0.87±0.006^{abc}	0.81±0.015^{abc}	0.77±0.045^{abc}
HDGPE	0.88±0.015	0.86±0.006^{abc}	0.75±0.006^{abc}	0.69±0.012^{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
LDGPE= 300mg/kg *Goniopsis pelli*
HDGPE= 600mg/kg *Goniopsis pelli*

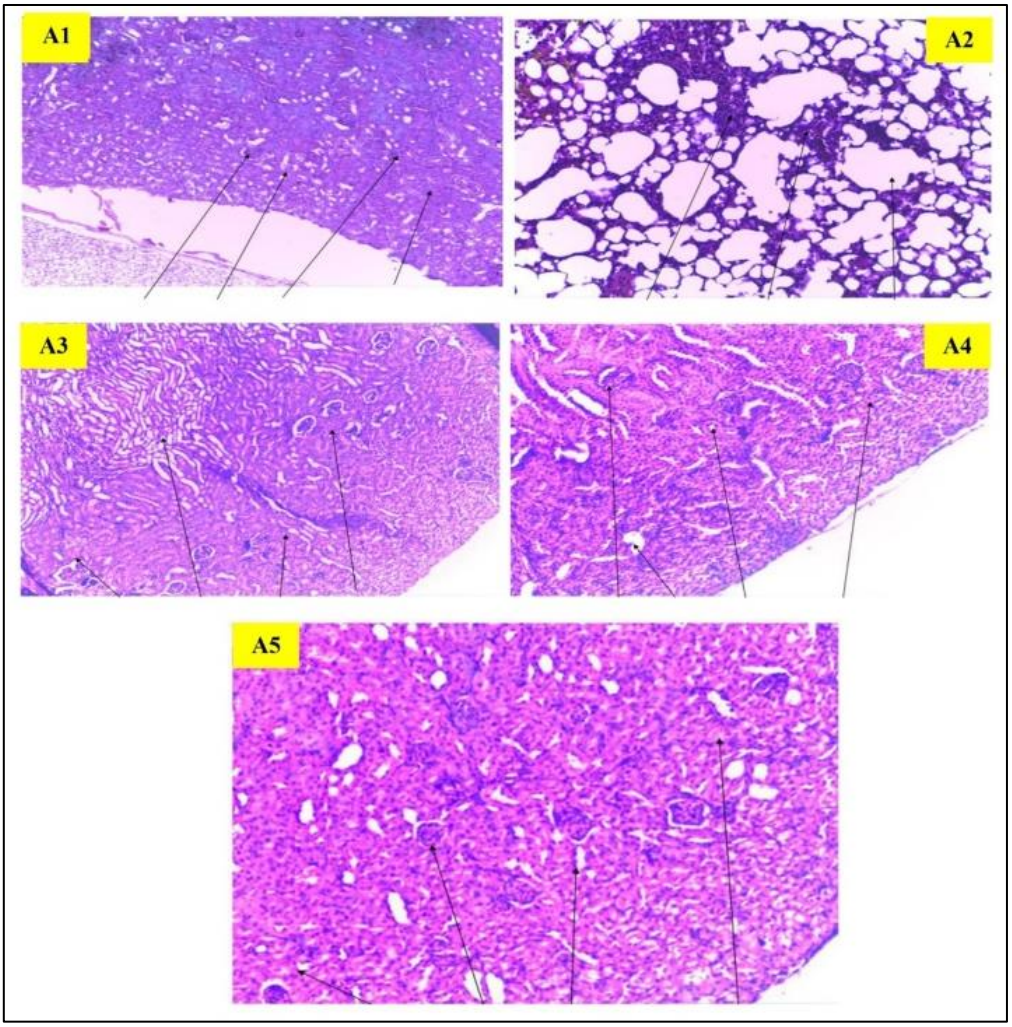

Figure 2: Photomicrograph of kidney tissue on day 6

(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 Histologicalalteration. (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 *Goniopsis pelli* showing no obvious Histologic alteration with clear glomeruli, renal tubules and blood vessels. (A5) Photomicrograph of *B. Pertussis* infected kidney treated with 600mg/kg *Goniopsis pelli* showing no obvious histological alteration. H & E stain; Magnification: x400

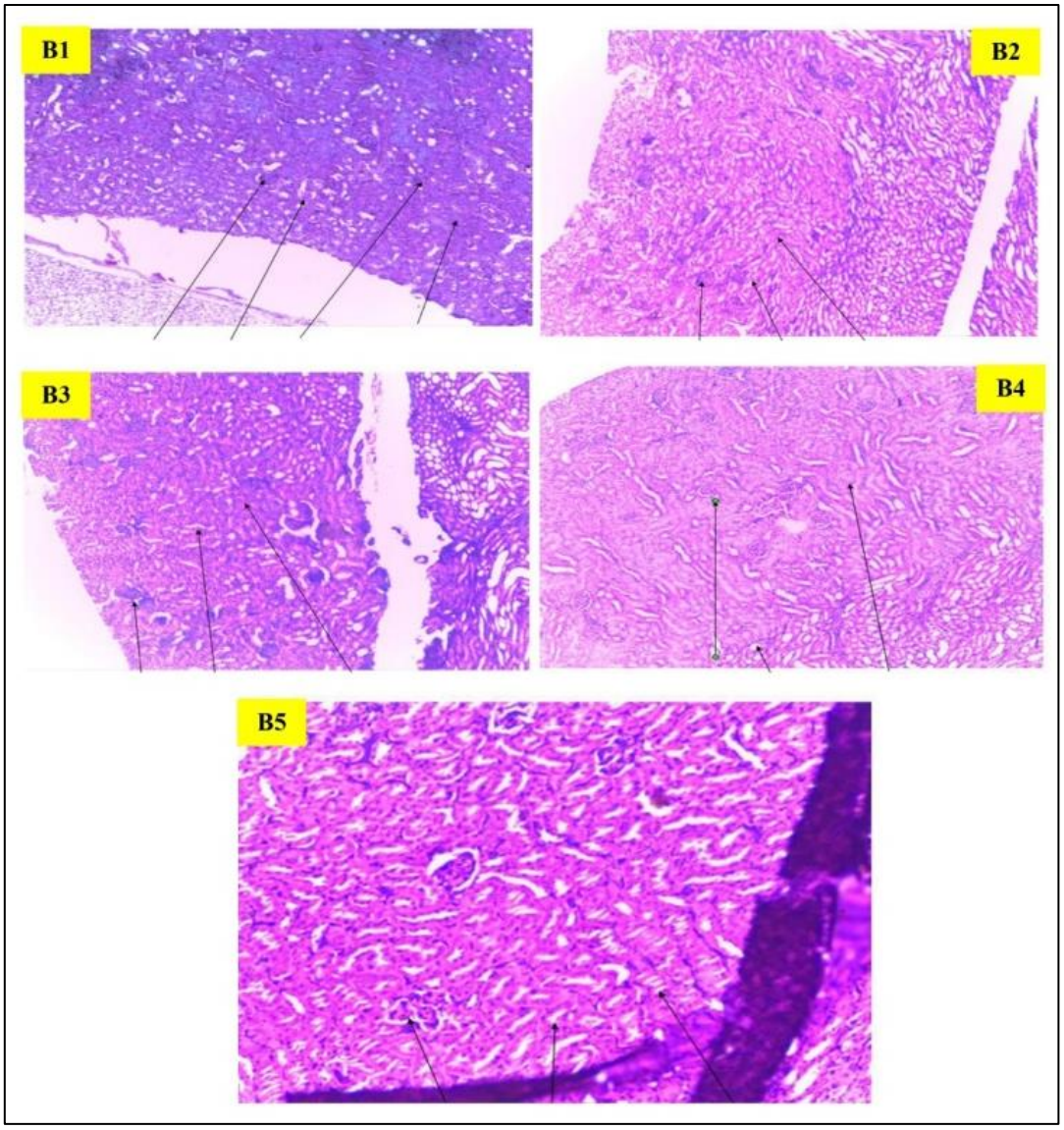

Figure 3: Photomicrograph of kidney tissue on day 12

(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 *Goniopsis pelli* showing no obvious histologic alteration showing clear glomeruli and renal tubules. (B5) Photomicrograph of *B. pertussis* infected kidney treated with 600mg/kg *Goniopsis pelli* showing no obvious histologic alteration with clear glomeruli and renal tubules.

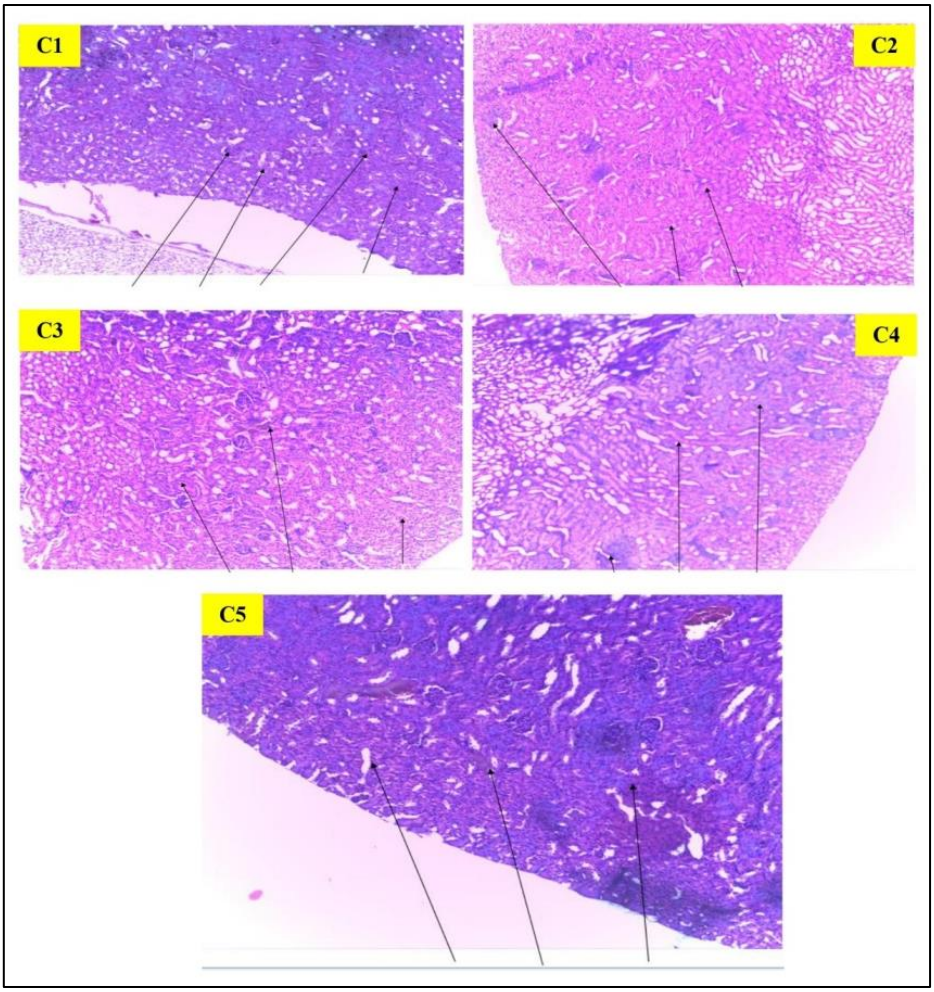

Figure 4: Photomicrograph of kidney tissue on day 18

(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 *Goniopsis pelli* showing no obvious histological alteration.(C5)Photomicrograph of *B. pertussis* infected kidney treated with 600mg/kg *Goniopsis pelli* showing no obvious histological alteration with clear glomeruli, renal tubules and blood vessels.

DISCUSSION

According to studies by Grunfeld *et al.*, (1992) and Sammalkorpi *et al.*, (1988), infection is characterized by an increase in triacylglycerol levels as well as an increase in VLDL and a decrease in HDL cholesterol levels. Elevated plasma triacylglycerol levels have been linked to either increased VLDL generation or slower VLDL clearance, according to Tiirola *et al.*, (2007) and Nonogaki *et al.*, (1995).

Studies in rats and mice revealed that even at low doses, bacteria lipopolysaccharide (LPS) can rapidly stimulate the production of VLDL by increasing adipose tissue lipolysis, increasing hepatic de novo fatty acid synthesis, and decreasing hepatic fatty acid oxidation. According to Nonogaki *et al.*, (1995) and Hardardottir *et al.*, (1997), these changes provide fatty acid substrate for esterification into triacylglycerols and assembly into VLDL particles in the liver. In another account, Xu & Nilsson, (1996) noted 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.*, (1997) and Sakaguchi & Sakaguchi, (1979) associated this effect to be responsible for the notable increase in VLDL-triacylglycerol. The increase in free fatty acids was linked to an increase in LDL-VLDL and plasma triacylglycerols, so Solomon *et al.*, (2012) came to the conclusion that this mechanism may also have been the cause of the increased plasma and erythrocyte free fatty acids seen in the experimental rats infected with *Salmonella*. Isirima, *et al.*, (2018) showed a rise in triacylglycerol levels and a decline in HDL cholesterol

in Wistar rats infected with *Salmonella typhi* bacteria to support these results.

This study demonstrated that receiving an infectious dose of *B. pertussis* resulted in a rise in total cholesterol, triglycerides, and low density lipoprotein cholesterol (LDL) concentrations and a significant ($p<0.05$) decrease in plasma levels of high density lipoprotein cholesterol (HDL). Treatment with erythromycin and GPE, however, reversed this tendency and gradually increased HDL levels while lowering LDL levels. These results support Solomon *et al.*, (2012) report that *B. pertussis* infection causes dyslipidemia, which is characterized by an increase in serum levels of triglycerides and cholesterol (hyper-triglyceridemia and cholesterogenesis), as well as Omeh *et al.*, (2015) report of a statistically significant ($p<0.05$) increase in LDL cholesterol and a decrease in HDL cholesterol. According to Ijeoma *et al.*, (2014), the reversal effects of GPE may be a result of an increase in LDL-cholesterol clearance, improved fatty acid oxidation, and a decrease in adipose tissue lipolysis, all of which point to a hypo-lipidemic and cardio-protective action of the extracts against degenerative disease. Infection with *B. pertussis* has no negative effects on the kidneys, and erythromycin and extract dosages have no negative effects either as observed from this study.

CONCLUSION

The administration of *Goniopsis pelli* extract to the infected animals exhibited beneficial effects, as it led to a decrease in LDL, Triglycerides, and Cholesterol levels, along with an increase in HDL. Conversely, *B. pertussis* vaccination in Wistar rats resulted in an unfavorable lipid profile, with an increase in LDL, Triglycerides, and Cholesterol, and a decrease in HDL. Notably, *B. pertussis* infection showed no adverse effects on the kidneys of Swiss mice. These findings suggest the potential therapeutic value of GPE in managing lipid profiles during infection. Further research is warranted to explore its long term implications.

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