ISSN: 2475-6296

Research Article

Journal of Clinical & Experimental Immunology

Immunomodulatory Effects of Bio-Clean II Herbal Remedy on C-reactive protein, Corticosterone and Antiphospholipid antibodies in Rats exposed to Purified Bacterial Lipopolysaccharide

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Submitted: 24 May 2022; **Accepted**: 04 Jun 2022; **Published**: 19 Aug 2022

Citation: *Seyi Samson Enitan, Isaiah Nnanna Ibeh, Ayodele Ademola Adelakun, Richard Yomi Akele, Ayodeji Olusola Olayanju, Joy Nkechi Ashimole, Mirian Chiamaka Uchegbu, Chisara Sylvestina Okolo (2022) Immunomodulatory Effects of Bio-Clean II Herbal Remedy on C-reactive protein, Corticosterone and Antiphospholipid antibodies in Rats exposed to Purified Bacterial Lipopolysaccharide. Journal of Clinical & Experimental Immunology. 7(3): 476-484.*

Abstract

Background: *Bio-Clean II has been previously shown to fight viral infection, boost immunity, and possesses anti-inflammatory properties by regulating the serum level of inflammatory cytokines, as well as T-Helper 4 and Cytotoxic T-Lymphocytes in rats exposed to purified bacterial lipopolysaccharide (LPS).*

Aim: *The aim of this study is to assess the effects of Bio-Clean II on the C-reactive protein (CRP), corticosterone (CORT) and anti-phospholipid antibodies (aPLs) levels in rats exposed to bacterial lipopolysaccharide (LPS) using animal model.*

Materials and Methods: *A total of 36 male Wistar rats weighing 150g±50g (mean±SD) were purchased and randomly assigned to six (6) groups of 6 rats each. Group 1, 2, 3, 5 and 6 were induced with a single dose of 5mg/Kg of purified LPS® (E.coli 0127:B8, Sigma-Aldrich, St. Louis, USA), administered through intraperitoneal route using 1ml sterile needle and syringe, except for group 4 which served as the zero control (given water and food only throughout the experiment). Group 1 served as the inflammation control. Group 2 which served as the positive control received 50 mg diclofenac/kg [bid] and 500 mg ciprofloxacin/kg [bid] (positive control) in place of the Bio-Clean. Group 3 which served as the negative control received sterile phosphate buffer saline (PBS). While rats in group 5 and group 6 were treated orally with the herbal remedy "Bio-Clean II" for 7 days and 14 days, respectively. After which, the rats were killed and a cardiac blood specimen was taken from each rat and transferred to plain bottles to clot. Serum was obtained from the clotted blood by centrifugation. The serum levels of C-reactive protein, corticosterone and antiphospholipid antibodies were assayed using ELISA kits, supplied by Elabscience Biotechnology Inc, USA. Data generated were subjected to Statistical Package for Social Scientists-Version 20 (SPSS-20).*

Results: *The outcome of this results show that the serum level of C-reactive protein of the 7 days (1.05±0.06ng/ml) and 14 days (0.93±0.05ng/ml) Bio-Clean II treated rats was found to be significantly lower (p=0.002 and p=0.000, respectively) when compared to the inflammation control group (1.70±0.07ng/ml). Similarly, the serum level of anti-phospholipid antibodies of the 7 days (6.40±0.67 Uml) and 14 days (4.27±0.66 U/ml) Bio-Clean II treated rats was found to be significantly lower (p=0.02 and p=0.008, respectively) when compared to the inflammation control group (16.47±1.53 Uml). Meanwhile, the corticosterone level of the 7 days Bio-clean treated rats (9.40±1.30ng/mL) was found to be non-significantly lower (p=0.812) in comparison to the inflammation control (13.50±2.50ng/mL); while that of the 14 days Bio-clean II treated rats (6.80±1.00ng/mL) was significantly lower (p=0.026).*

Conclusion: *The outcome of this study underscores the anti-inflammatory potential of Bio-Clean II in the treatment of bacterial inflammatory diseases.*

Keywords: Antiphospholipid antibodies, Bio-Clean II, C-reactive protein, Corticosterone, LPS.

Introduction

Herbal remedies are medications of plant-origin used traditionally for the treatment of various ailments and diseases [1-3]. They have been found to modulate various components of innate and acquired immune system due to their antibacterial, antiviral and anti-inflammatory activities [4-7]. The herbal remedy "Bio-Clean II" for instance, has been shown to significantly increased CD4 cell count, but decreased CD8 cell count in rats treated with Bio-clean II (in a duration-dependent manner) when compared to the inflammation control [8]. In another study, we showed that Bio-Clean II reversed the leukocytosis, lymphocytosis and neutrophilia associated with LPS-induced inflammation [9]. In that study, the total white blood cell count (5.68±0.91X10^3/uL), absolute lymphocyte count (4.04±0.85X10^3/uL) and absolute neutrophil count of the 7days (not 14 days) Bio-Clean II treated rat was found to be significantly lower when compared to the inflammation control group: 12.52±1.04 X10^3/uL (p=0.018), 7.66±0.75X10^3/uL (p=0.004) and 4.40±0.81X10^3/uL (p= 0.027), respectively. In a recent study, we also found that Bio-Clean II modulated the levels of inflammatory cytokines including Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tissue Necrosis Factor-Alpha (TNF-α) and Interferon-Gamma (IFN-γ) in experimental lipopolysaccharide (LPS)-induced inflammation using animal model [10]. Many of the preventive and therapeutic effects of Bio-Clean II are directly or indirectly, attributable to its immunomodulatory properties [11-13] due to the presence of some important phytochemicals including resin, alkaloids, saponins, anthraquinones, tannin, and Cardiac Glycoside as reported in our previous studies [14].

Inflammation is one of the outcomes of bacterial infection [15]. Several bacterial components can trigger inflammation. The lipopolysaccharides (LPS) are characteristic components of the cell wall of Gram negative bacteria (absent in Gram positive bacteria). They are localized in the outer layer of the membrane and are in non-capsulated strains, exposed on the cell surface. They contribute to the integrity of the outer membrane, and protect the cell against the action of bile salts and lipophilic antibiotics [16]. These LPS are strong provokers of inflammation. They are recognized by immune cells as a pathogen-associated molecule through Toll-like receptor [15]. Exposure to bacterial lipopolysaccharide (LPS) has been related with inflammation of body tissues characterized by pain, redness, swelling, heat, and loss of function if the assaulting agent continues [17].

C-reactive protein (CRP) is an acute-phase protein synthesized by hepatocytes and is released into the bloodstream in response to pro-inflammatory cytokines during inflammatory/infectious processes [18]. It is known as a biomarker of acute inflammation, but many large-scale prospective studies demonstrate that CRP is also known to be associated with chronic inflammation [19]. It can increase up to 1000 fold at sites of infection or inflammation. CRP is not only a marker of infection and inflammation, but also has a protective role against bacterial infection. CRP is produced as a homopentameric protein, termed native CRP (nCRP), which can irreversibly dissociate at sites of inflammation and infection into five separate monomers, termed monomeric CRP (mCRP). In the presence of calcium, CRP binds polysaccharide of bacteria such as phosphocholine (PCh) on microorganisms and triggers the classical component pathway of innate immunity by activating C1q [20].

Corticosteroids, such as corticosterone, are a type of steroid hormone. They affect various mechanisms involved in inflammation. Corticosteroids have been used in the management of range of conditions, including: temporal arteritis, dermatitis, inflammatory bowel disease (IBD), systemic lupus, hepatitis, asthma, allergic reactions etc [21]. Corticosterone, unlike cortisol in humans, is the main glucocorticoid hormone in rodents like rats. It is involved in immune and stress responses amongst other functions [22, 23].

Anti-phospholipid (APL) antibodies are a heterogenous group of autoantibodies targeting different phospholipid binding protein antigens [24, 25]. Persistently high levels of APL antibodies, together with specific clinical manifestations, are required for the diagnosis of antiphospholipid syndrome (APS) [24]. It is a widely accepted view that pathogenesis of many autoimmune diseases is largely driven by inappropriate or inadequate immune responses toward bacterial agents [26, 27]. Similarly, a number of Gram-negative bacteria are recognized as being linked to aPL production. Despite this, transitory increase of bacteria-induced aPL autoantibodies was only occasionally associated with thrombotic events [28]. Many infections may be accompanied by increases in aPL and, in some, these increases may be accompanied by clinical manifestations of the APS [28, 29]. Infection-associated thrombosis is a type of inflammation resulting from the presence of microbial agents and their products in the blood vessels. This inflammation triggers the activation of platelets, which may accompany damage to the endothelium, resulting in fibrin deposition and thrombus formation. This process is often referred to as thrombo-inflammation [30].

To the best of our knowledge, this study is the first to investigate the effect of Bio-Clean-II on serum levels of C-reactive protein, corticosterone and antiphospholipid antibodies in experimental LPS-induced inflammation. Scarcity of data in this regard, necessitates this study.

Materials and Methods

Study Design

This is an analytic-experimental study using animal model.

Duration of study

The study lasted for a period of 2 months (April-June, 2021).

Study Area

The study was carried out at the Experimental Animal House, Babcock University, Ilishan-Remo, Ogun State; a Seventh-day Adventist Institution of higher learning. Ilishan-Remo community is one of the geopolitical wards in Ikenne Local Government Area

of Ogun state, situated in the tropical area of South-western part of Nigeria, coordinates: 7°29′00″N 2°53′00″E. Predominant occupations among the locals is trading, as well as farming and they are noted for the growing of the following agricultural products: rubber, cocoa, cashew nuts, plantain etc. Despite the availability of reliable medical service, the local populaces still rely on the use of herbs as medicines for both curative and prophylaxis purpose.

Reagents

Bio-Clean II (Figure 1) was procured from the manufacturer/ maker on demand. Purified lipopolysaccharide (Type 0127:B8 from *Escherichia coli* Sigma, St. Louis, MO) was purchased as lyophilized powder. For use in the experiments, the LPS (Figure 2) was reconstituted in sterile phosphate-buffered saline (PBS, pH 7.4) and administered as a single dose of 5 mg/Kg through the intraperitoneal route. Diclofenac and Ciprofloxacin were obtained from the Pharmacy Department of Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria and prepared in sterile distilled water for use in the exposures. All other reagents and kits used in this study were purchased from Elabscience (USA), unless specified otherwise.

Figure 1: Picture of Bio-Clean II (Photo Credit: Enitan, S. S.).

Figure 2: Picture of E. coli 0127:B8 purified lipopolysaccharide® (Photo Credit: Enitan, S. S.)

Experimental Animals

A total of 36 male Wistar rats weighing 150±50g (mean±SD) were procured from the University of Ibadan's small animal house and clinically checked upon arrival for any signs of infection or defect. The animals were housed separately in well-ventilated wire-bottom steel cages under hygienic conditions at 25±2oC and a relative humidity of 45–50% at the Experimental Animal House, Department of Animal Science, School of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo (Ogun State, Nigeria). The rats were indiscriminately divided into six groups of six rats each and provided a regular rat diet (10g/100g body weight) twice daily as well as free access to tap water. All rats were allowed to acclimate for 14 days prior to use in any experiment in the Animal House with a regular 12-hour light:dark cycle. All animal experimentations were carried out in compliance with the Institute for Laboratory Animal Research's Current Animal Care Regulations and Standards [31].

Animal Treatment

For the study, rats were randomly allocated into six groups (n = 6/group) and each provided a regular rat diet of standard rodent chow as well as ad libitum access to filtered tap water throughout the experimental period. The groups included rats to receive: only LPS (single intraperitoneal injection at 5 mg/kg; inflammation control); LPS and both 50 mg diclofenac/kg [bid] and 500 mg ciprofloxacin/kg [bid] (positive control); LPS and sterile phosphate-buffered saline (negative control), no treatments [water and feed only; zero control); LPS and Bio-clean II for 7 d; and, LPS and Bio-clean II for 14 d. All animals, including the controls were gavaged for 7 or 14 days, as the case may be. Therapy with Bio-clean II was initiated by day 2 post-LPS challenge. Before each treatment, the volume of Bio-clean II suspension to be delivered to each rat via intragastric tube was estimated based on body weight. The rats in those groups were given an appropriate volume of suspension (never to exceed 5 ml/kg) twice daily, every 12 hr (between 6.00-6.30 AM and PM) for the total number of days specified in the experimental protocol. The suspension was shaken gently before administration and administered. Each time, the rats were monitored for any changes in appearance, appetence,

etc. At Days 8 and 15 (24 hr after the respective final exposures for the 7 and 14-d regimens), the rats were euthanized by cervical dislocation as described by Ochei and Kolhatkar [32]. At necropsy, cardiac blood specimens were collected and serum prepared for later evaluation of C-reactive protein, corticosterone and anti-phospholipid antibodies levels in the rats.

Table 1: Experimental Pharmacological Protocol

Groups (n=6)	Treatments
G1	Received 5mg/Kg of purified LPS (Inflammation Control)
G2	Received 5mg/Kg of purified LPS + 50mg/Kg/bid Diclofenac + 500mg/kg/bid of Ciprofloxacin (Positive Control)
G3	Received 5mg/Kg of purified LPS + sterile PBS (Negative control)
G4	Received water and food only (Zero Control)
G5	Received 5mg/Kg of purified LPS + Bio-clean II for 7 days
G6	Received 5mg/Kg of purified LPS + Bio-clean II for 14 days

C - reactive protein Assay

Serum C-reactive protein (CRP) was estimated using the Sandwich enzyme linked immunosorbent assay (*sELISA*) as described by Eckersal and Ehiaghe et al. [33, 34]. C-reactive protein (PTX1) two-site ELISA Kit obtained from Elabscience Biotechnology Inc., USA with a sensitivity of 0.1 ng/ml and range of 0.16-10ng/ml was used for the assay. This kit recognizes Rat CRP in samples. Protocol was according to manufacturer's instructions. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat CRP. Samples (or Standard are added to the micro ELISA plate wells and combined with the specific antibody. The biotinylated detection antibody specific for Rat CRP and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components were washed away and the TMB substrate solution added to each well. Only those wells that contain Rat CRP, biotinylated detection antibody and Avide HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm using the KC-100 microplate reader (BMG LABTECH, Germany). The OD value is proportional to the concentration of Rat CRP. The concentration of Rat CRP in the samples was calculated by comparing the OD of the samples to the standard curve. Data was analyzed using the MILLIPLEX™ analyst software.

Corticosterone Assay

Serum corticosterone (CORT) was determined by the Sandwich enzyme linked immunosorbent assay (*sELISA*) as described by Rød *et al* [35], using rat Corticosterone ELISA Kits purchased from Elabscience (USA) with a sensitivity of 1.93 ng/ml and detection range of 3.12-200 ng/ml according to the manufacturer's protocol This kit recognizes Rat CORT in samples. The level of CORT was calculated using standard curve and reported in ng/mL. The plate was read at 450nm using the KC-100 microplate reader (BMG LABTECH, Germany). Data was analyzed using the MILLIPLEX™ analyst software. The ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with CORT. During the reaction, CORT in the sample or standard competes with a fixed amount of CORT on the solid phase supporter for sites on the Biotinylated Detection Ab specific to CORT. Excess conjugate and unbound sample or standard are washed away, and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of CORT in tested samples can be calculated by comparing the OD of the samples to the standard curve. This kit recognizes Rat CORT in samples. No significant cross reactivity or interference between Rat CORT and analogues was observed.

Antiphospholipid Antibodies Assay

The Antiphospholipid antibodies (aPLs) were assayed by Immunometric Enzyme Immunoassay using the rabbit anti-Phospholipid Screen IgG/IgM ELISA kit supplied by Elabscience Biotechnology Inc. USA as described by Nalli *et al.* and Olayanju *et al.* [36, 37]. The ELISA plate washer and ELISA reader was operated as instructed in the user's operational manual. The plate was read at 450nm using the KC-100 microplate reader (BMG LABTECH, Germany). Data was also analyzed using the MILLIPLEX™ analyst software.

Data Analyses

Data for the serum level CRP, CORT and aPLs were presented as means of 6 rats using tables and charts. Data was analyzed using one-way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-20.0 (Statistical packages for social Scientists – version 20.0) statistical program. P values<0.05 were considered significant [38].

Results

This present study assessed the effects of Bio-Clean II on the serum level of C-reactive protein (CRP), Corticosterone (CORT) and antiphospholipid antibodies (apLs) in experimental LPS-induced inflammation using animal model.

Effects on serum level C-reactive protein

The effect of Bio-Clean II on serum level of C - reactive protein in rats exposed to purified bacterial lipopolysaccharide is presented using a bar chart (Figure 3). The serum level of C-reactive protein (CRP) of the 7 days (Group 5: 1.05±0.06ng/ml) and 14 days (Group 6: 0.93±0.05ng/ml) Bio-Clean II treated rats was found to

be significantly lower (*p=0.002* and *p=0.000*) when compared to the inflammation control group (Group1: 1.70±0.07ng/ml). Also, on the other hand, it was found to be non-significantly higher (p=0.384 and p=0.997 respectively) when compared to positive control group (Group2:0.86±0.04ng/ml), but on the other hand, it was found to be non-significantly lower (*p=0.631 and p=0.098*, respectively) when compared to the negative control group (Group 3: 1.25±0.07ng/ml).

Furthermore, the serum level of C-reactive protein (CRP) of the 7 days Bio-Clean treated rats (Group5: 1.05±0.06ng/ml) was also observed to be non-significantly higher (*p=0.797*) when compared to zero control group (Group 4: 0.92±0.03ng/ml); but that of the 14 days (Group 6: 0.93±0.05ng/ml) was found to be non-significantly lower (*p=1.000*). In addition, the serum level CRP of 7 days Bio-Clean II treated rats (Group 5: 1.05±0.06ng/ml) was found to be non-significantly higher (p=0.888) when compared to 14 days Bio-Clean (Group 6: 0.93±0.05ng/ml). Meanwhile, the serum level of CRP of positive control (Group 2: 0.86±0.04ng/ml) was found to be significantly lower (*p=0.039*) than of the negative control (Group 3: 1.25±0.07ng/ml).

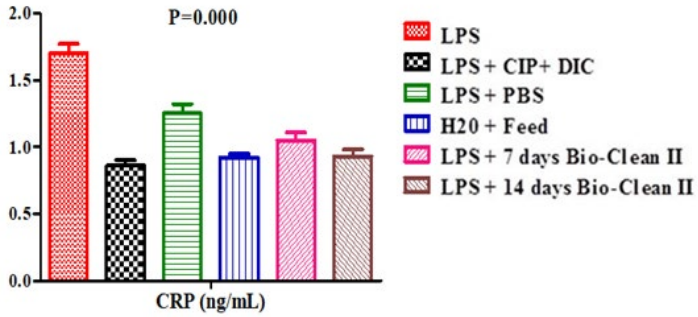

Figure 3: Effect of Bio-Clean II on serum level of C - reactive protein in rats exposed to purified bacterial lipopolysaccharide.
Keys: CRP = C - reactive protein, LPS = Lipopolysaccharide, CIP = Ciprofloxacin, DIC = Diclofenac, PBS = Phosphate Buffered Saline, H20 = Water.

Effect on Serum Corticosterone

The serum Corticosterone (CORT) level of the test and control rats posttreatment with Bio-clean II is represented using a bar chart in Figure 4. The CORT level of the 7 days Bio-clean treated rats (Group 5: 9.40±1.30ng/mL) was found to be non-significantly lower (*p=0.812*) in comparison to the inflammation control (Group 1: 13.50±2.50ng/mL); while that of the 14 days Bio-clean II treated rats (Group 6: 6.80±1.00ng/mL) was significantly lower (p=0.026). In comparison to the positive control (Group 2: 7.25±2.20ng/mL), the CORT level of 7 days (Group 5: 9.40±1.30ng/mL) and 14 days (Group 6: 6.80±1.00ng/mL) Bio-clean treated rats were found to be non-significantly higher and lower, *p=0.177* and *p=1.000*, respectively. Still, in comparison to the negative control (Group 3: 12.60±1.45ng/mL), the CORT level of the 7 days Bio-clean treated rats (Group 5: 9.40±1.30ng/mL) was found to be non-significantly lower, but that of the 14 days Bio-clean treated rats (Group 6: 6.80±1.00ng/mL) was significantly lower (*p=0.000*).

Furthermore; there was no significant difference (*p=1.000*) between the CORT level of the 7 days Bio-clean II treated rats (Group 5: 9.40±1.30ng/mL) and that of the Zero control (Group 4: 9.45±1.00ng/mL), but that of the 14 days (6.80±1.00ng/mL) was significantly lower (*P=0.048*). Meanwhile, the CORT level of the 14 days Bio-clean treated rats (Group 6: 6.80±1.00ng/mL) when compared with that of the 7 days Bio-clean treated rats (Group 5: 9.40±1.30ng/mL) was found to be significantly lower (*p=0.005*). Lastly, the CORT level of the positive control (Group 2: 7.25±2.20ng/mL) when compared with that of the negative control (Group 3: 12.60±1.45ng/mL) was found to be significantly lower (p=0.015). Also the CORT level of the inflammation control (Group 1: 13.50±2.50ng/mL) was found to be significantly (P=0.031) elevated than of the zero control (Group 4: 9.45±1.00ng/mL).

Effect on Anti-Phospholipid Antibodies

The serum level of anti-phospholipid antibodies (aPLs) of the test and control rats, post-treatment with Bio-Clean II is presented using a bar chart (Figure 5). The serum level of aPLs of the 7 days (Group 5: 6.40±0.67 U/mL) and 14 days (Group 6:4.27±0.66 U/mL) Bio-clean II treated rats was found to be significantly lower (*p=0.02* and *p=0.008*, respectively) when compared to the inflammation control group (16.47±1.53U/mL). It was also found to be non-significantly lower (*p=0.990* and *p=0.0.241*, respectively) when compared to the positive control group (Group 2: 7.82±0.96 U/mL), but on the other hand, it was found to be significantly lower (*p=0.001* and *p= 0.000*, respectively) when compared to the negative control group (Group 3: 17.51±1.10 U/mL). Furthermore, the aPLs serum level of the 7 days Bio-clean II treated rats (Group 5: 6.40±0.67 U/mL) and 14 days (Group 6:4.27±0.66 U/mL) was also found to be non-significantly lower (p=1.000 and p=0.174, respectively) when compared with the zero control group (Group 4: 7.09±0.58 U/mL). The serum levels of the aPLs of the 14 days Bio-clean II treated rats (Group 6:4.27±0.66 U/mL) when compared with that of the 7 days Bio-clean II treated rats (Group 5: 6.40±0.67 U/mL) was found to be non-significantly lower (p=0.562). Meanwhile the anti-phospholipid antibodies serum levels of the positive control (Group 2: 7.82±0.96 U/mL) was found to be significantly lower (p=0.003) than the negative control (Group 3: 17.51±1.10 U/mL).

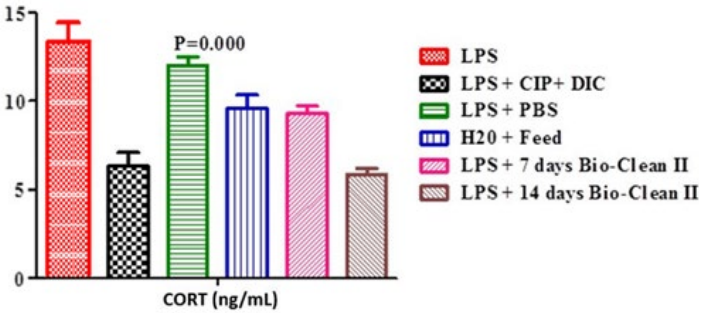

Figure 4: A bar graph showing the effect of Bio-Clean II on serum level of corticosterone in rats exposed to purified bacterial lipopolysaccharide.

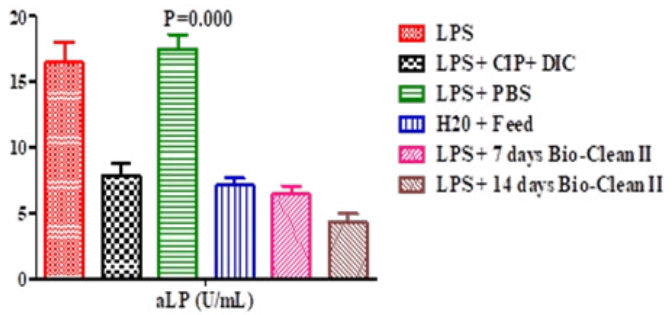

Figure 5: A bar graph showing the effect of Bio-Clean II on serum level of anti-phospholipid antibody in rats exposed to purified bacterial lipopolysaccharide.

Keys:*aLP=Anti-PhospholipidAntibody,LPS=Lipopolysaccharide, CIP = Ciprofloxacin, DIC=Diclofenac, PBS=Phosphate Buffered Saline, H_20=Water.*

Discussion

Lipopolysaccharide is a potent trigger of inflammatory response [39]. Inflammation is the body's way of protecting the tissues if they have been injured or have an infection. There are several markers of inflammation. This current study is the first to evaluate the effect of Bio-Clean II on serum level of CRP, CORT and aLPs in rats exposed to purified bacterial lipopolysaccharide. In the inflammation control, the serum level of CRP was significantly higher when compared to other groups. Physical examination of the rats in the inflammation control group, revealed the presence of swelling and redness especially in the feet of the rats. A reduction in body weight of the rats was also observed after exposure to bacterial LPS. Furthermore, decrease in the serum level CRP was observed in the positive control group administered with Ciprofloxacin (a potent broad spectrum antibiotic) and Diclofenac (Anti-inflammatory agent) combined, with a concomitant loss in body weight. This adverse effect must be noted in clinical practice. The Negative control which received LPS and PBS, also experience elevated serum CRP and decrease in body weight. PBS contains no antibiotic or anti-inflammatory agent, hence the elevated serum level CRP observed.

In this study, the serum level CRP was significantly high in the inflammation control group (1.70±0.07ng/ml) when compared with 7 days (1.05±0.06ng/ml) and 14 days (0.93±0.05ng/ml) Bio-Clean II treated rats, as well as the zero control rats (0.92±0.03ng/ml). The outcome of this study is consistent with the work of Suryadinata *et al*., who evaluated the effect of 30 days treatment with lime peel extracts (*Citrus aurantifolia swingle*) against C-reactive protein levels in *Alloxan*-induced wistar rats [40]. In their study, CRP value was reported to be high in the alloxan induced rats (>12mg/mL) when compared to the non-induced rats (<6mg/mL) and lime peel extract treated rats (<12mg/mL). This study is also consistent with the work of Ben *et al*., who reported a significant (*P<0.01*) high serum level of CRP in untreated diabetic group (95.67±5.6ng/ml) when compared to the non-diabetic control group (11.0±0.3 ng/ml) [41]. Meanwhile, the serum levels of C-reactive protein reduced significantly (*P<0.05*) to 54.33±3.2 ng/ml and 47±0.53 ng/ml in the *Terminalia catappa* extract treated diabetic group and insulin treated group, respectively, when compared to the untreated diabetic group (95.67±5.6ng/ml). Furthermore, the outcome of this study is also in agreement with the work of Dimitrov et al., in which the serum CRP concentration was significantly (*p<0.0001*) elevated in the obesity induced rats (963.41 µg/ml) fed with a high-fat diet, when compared to the control group (649.34 µg/ml) fed with standard rodent food [42].

On the other hand, the outcome of this current study differs from that of Kalsait *et al*., who investigated the effect of 120 days High Fat Diet (HFD) on CRP level in Wistar albino rats [43]. The outcome of their study shows that high fat diet for 120 days (3.37±0.15mg/dL) did not increase C-reactive protein level when compared to the control group (3.02±0.06mg/dL). Whereas in our study, rats exposed to LPS had significant high level of serum CRP.

Furthermore, in this study, the serum Corticosterone level of the Bio-clean II treated rats was significantly reduced in a duration dependent manner when compared to the inflammation control post-exposure to LPS. This agrees with the study of Hwang et al., in which treatment with Mox (a traditional therapeutic procedure used in Oriental Medicine for treating infectious diseases, ulcerative colitis, rheumatoid arthritis, cancer and pain) was found to significantly reduce high cortisol levels in a dose-dependent manner in mice [44]. It is also in agreement with the work of Lim et al. in which *Lindera obtusiloba* extracts, at concentrations that were not affected by cell viability, significantly decreased luciferase activity in response to cortisol in a concentration-dependent manner [45]. We also found the outcome of this study to be in harmony with the work of Rabei in which Dextromethorphan (a synthetic opioid analogue), was reported to induce a significant decrease in the levels of cortisol in rats during the experimental time, with the exception of the last period of treatment in the 3rd group, where, the level of cortisol production gradually return to normal level as the control group [46].

In addition, the serum Corticosterone level of the LPS-induced rats in this study was significantly elevated when compared to the Positive control, Zero control and the Bio-clean II treated rats. This is consistent with the work of Walker *et al.*, Wang *et al* and Kirsten *et al.* in which acute LPS challenge resulted in a significant elevated corticosterone levels when compared with saline-treated rats [47-49]. Vakharia and Hinson also reported a significant increase in cortisol secretion by human H295R cells post exposure to LPS [50]. The effects of LPS on cortisol were however, attenuated in the presence of both indomethacin and a specific COX-2 inhibitor. The outcome of this study also agrees with the work of Gong *et al*. in which cortisol and corticosterone were elevated to the highest level within 3 minutes and 40 minutes exposure time of heat stress, respectively. It is also in accordance with the work of Ehiaghe *et al.*, in which Cortisol level was significantly elevated in rats subjected to strenuous exercise compared to the control group kept in solitary confinement [51, 52].

Similarly in this current study, the anti-phospholipid antibodies (aPLs) levels were found to be significantly reduced in Bio-clean II treated in a duration-dependent manner. This is comparable with the outcome of previous related studies in human subjects. For instance, Takakuwa *et al*. treated twelve (12) patients suffering from recurrent abortion, who had shown positive antiphospholipid antibodies with a Japanese modified traditional Chinese herbal medicine *Sairei-To* (*Chan ling-Tang*) [53]. The patients had experienced a total of 27 spontaneous abortions in their previous pregnancies and had no other pregnancy history except for one patient. The patients were treated with 9.0 g of *Sairei-To* per day before their next pregnancy. The positive value of antiphospholipid antibodies returned to negative in 9 patients out of 12 patients through the treatment. Out of 12 patients, in 10 patients, their new pregnancy continued uneventfully and delivered an offspring with a success rate of 83.3%.

Still, in another similar study, Takakuwa *et al.* investigated the effect of *Sairei-To* combine with low dose aspirin and prednisolone on four recurrent reproductive failure women who are positive for anti-phospholipid antibodies [54]. The clinical courses of their new pregnancies in conjunction with the dynamic changes in their APL titers shows that the Chinese herbal remedy in combination with conventional standard drugs has the potential to resolve anti-phospholipid syndrome in women with recurrent reproductive failure.

Likewise; the outcome of this study agrees with that of Olayanju et al. [37], in which the mean serum antiphospholipid antibody level was significantly ($P<0.001$) higher in HIV positive Patients (11.83±7.36u/ml) compared to the control group - HIV negative patients (7.30±3.95u/ml). But also disagrees with the same in which no significant differences ($P>0.05$) between the aPLs level of HIV group on ART (11.44±7.74 u/ml) and those not on ART (12.00±7.24 u/ml) was observed.

Conclusion

The outcome of this current study further underscores the therapeutic potential of the herbal remedy, with special focus on its immunomodulatory role. LPS-induced inflammation was resolved post-treatment with Bio-Clean II in a duration-dependent manner. This was evident by significant reduction in the serum level of the CRP, CORT and aLP when compared with the inflammation control group. The outcome of this study further gives credence to the therapeutic potential of the herbal remedy as acclaimed by the maker/vendor. However, more studies will be required to understand the molecular mechanism behind this effect.

Ethical Approval

Ethical approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC) with ethical approval registration number: BUHREC 508/21.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content of this manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, [Enitan S. S.],

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