

# Immunoprotective Effect of *Cocos nucifera* Oil on Sheep Red Blood Cell-Induced Immunocompromised Rats

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## Abstract

The aim of this study is to investigate the immunoprotective effects of cold-pressed coconut oil in immunocompromised rat. Standard procedure was used to perform this research work with a modified method. The effect of immunomodulatory properties of coconut oil was evaluated after challenging the animals with 0.3 ml sheep red blood cells (SRBCs) intraperitoneally and further treated with graded doses (0.25, 0.5 and 1.0 ml/kg i.p) of the oil extract for 21 days. The hematological, CD<sub>4</sub>, CD<sub>8</sub> biochemical and histopathological analysis were evaluated. Result obtained from this study showed a significant increase in hematological indexes (WBC: 11.70; LYM: 9.9 GRAN: 8.0 and HGB: 15.57) across the treated groups, but majorly at lowest dose (0.25 ml/kg). More so a significant increase in CD<sub>4</sub> and CD<sub>8</sub> Count specifically at 0.25 ml/kg (7.763 and 7.830). Anti-oxidant property of the oil extract at 0.25 ml/kg had a significant reduction in malondialdehyde with an increased in antioxidant enzymes (SOD, CAT and GPx) when compared with untreated control. The body weight of the animals in the treated groups showed a significant increase at 0.25 ml/kg of the oil extract when compared with the untreated control. Liver function test (ALT, AST and ALP) showed no significant increase in the treatment groups when compared with the controls. The histopathological result reveals a normal physiological structure of the heart, lungs, spleen, liver and kidney in lowest dose of the oil extract. In conclusion, this study validated the ethnomedicinal property of the plant.

**Keywords:** Immunoprotective; *Cocos nucifera*; Sheep red blood cell; Immunocompromised.

## INTRODUCTION

Immune system is a versatile defense system that protect humans against invading pathogens and inhibit diseases, which can regenerate into variety of cells and molecules proficient in recognizing and eradicating foreign invaders (Richard *et al.*, 2003). Immunomodulation occurs when host defense mechanism is stimulated under impaired immune response conditions or when immunosuppressant is associated with such state includes; autoimmune disorders. Medicinal plants are the current known aspect in phytomedicine, involved in a wider range of herbal materials formulation into supplements. Immunomodulatory therapy with the help of medicinal plants serves as an alternative approach to conventional medicine against diseases. Hence, host defend mechanism is triggered under impaired immune feedback. Immune system is implicated in the investigation and pathological mechanisms of several diseases, with tremendous increased yearning for effective drugs for an improved immune system. To overwhelm the interval of synthetic immunomodulators, there is the need for the development of herbal immunomodulators (Z-ziza *et al.*, 2008). Immunomodulator is biological or synthetic substances,

which stimulate and modulate the components responsible for immune system booster including innate and adaptive immune response (Agarwal and Singh, 1999; Shivaprasad *et al.*, 2006). Modulation of immune system using herbal remedy as a therapeutic measure has brought in the need for scientific investigations.

Coconut (*Cocos nucifera* L.) belongs to the family 'Arecaceae' and subfamily 'Cocoidae'. It is a tropical tree species extending over large uniform zones. It is usually known as the "tree of life" because of its medicinal usefulness (Assa *et al.*, 2010). Oil from *Cocos nucifera* is the most well-known food product gotten from dried coconut pulp, called the copra (Shankar *et al.* 2013). Law *et al.* (2014) analyzed the significance of coconut oil for patients with breast cancer. Using coconut oil supplement has improved the general wellbeing of patients and reduce possible adverse effect associated with chemotherapy. Coconut oil is useful for weight loss due to the biological component of short and medium-chain fatty acids that help in eliminating obesity. It eases digestion and help in healthy functioning of thyroid and endocrine gland. It is known to increase body metabolic rate via pancreatic stress removal; thereby burning more energy and promoting and prevent various stomach and digestion-related

problems include; irritable bowel syndrome (Yousefi *et al.*, 2013). The saturated fats present in coconut oil have antimicrobial properties and help in dealing with various bacteria, fungi and parasites that can cause indigestion (Shilling *et al.*, 2013). Due to the presence of lauric acid, it plays a vital role in preventing diverse cardiovascular diseases (Vala *et al.*, 2014). *C. nucifera* oil has been reported to be an effective as a wound healing, immune booster and other therapeutic agents. (Nevin *et al.*, 2010).

## MATERIALS AND METHOD

### Collection and Preparation of Plant Materials

Fresh coconut seeds were procured from Egor Local government area of Benin City, Edo state, Nigeria. It was identified and authenticated by Dr. H. Akinnibosun in the Departmental herbarium unit of Plant Biology and Biotechnology, with a voucher number (UWH-X219). Cold extraction method was involved in the isolation of the coconut milk, allowed to stand for 20-24 hrs. Under favorable conditions (35-40°C, 75% relative humidity), the oil gets separated from the water and the protein. The airborne lactic acid bacteria, which can break the protein bonds, act on the coconut milk mixture for proper separation. The operation conditions and sanitary precautions were strictly followed. The top layer was floating curd. The curd also contained a considerable amount of trapped oil. The separated oil contained some adhering particles and was filtered using a sterilized filter paper.

### Experimental Animals

Thirty (30) Wistar male rats weighed between 150-315 g were obtained from the Animal House, of the Department of Biochemistry, University of Benin, Benin City. They were housed under disinfected and well-ventilated plastic cages in a standard laboratory condition (12 hours light/dark cycle: 23 ± 2°C) and fed using Bendel meal pelleted grower mesh. The food and water were given at *ad libitum*. The animals were handled in accordance to the ethical Laboratory animals' guide' from the approval of the Life Sciences ethical committee University of Benin, issued a voucher number LS21507.

### Preparation of Sheep Red Blood Cells (SRBC)

Fresh blood was collected from a healthy sheep sacrificed in the local slaughterhouse in a sterile bottle containing Elsevier's solution (1:1). Then it centrifuged at 500 g for 15 min at 4°C in cooling centrifugation apparatus to separate the red blood cells (RBCs). The sheep red blood cells (SRBC) were washed three times in normal saline. They were then kept under refrigeration for the immunization.

### Experimental Protocol

The animals were numbered, weighed and then divided into six groups with five animals in each: all groups will be immunized with 0.3 ml/kg SRBC for 7 and 14 days of the study. Group A serves as the normal control, group B was administered 0.3 ml/kg SRBC alone intraperitoneally (negative control), group C received 0.3 ml/kg SRBC and 5 mg/kg Azathioprine (standard drug p.o) (positive control), group D, E and F were treated with 0.3 ml/kg SRBC plus graded doses (0.25, 0.5 and 1.0 ml/kg of coconut oil p.o). After 14 days, the animals were sacrificed under mild chloroform anesthesia. Blood was collected via cardiac puncture and organ samples were isolated. The serum was separated for various biochemical tests include; Liver Function Tests (alkaline aminotransferase (ALT), aspartate aminotransferase (ALP), alanine phosphatase (ALP), gamma glutamine transferase (GGT), total bilirubin (TOT. BIL), conjugated bilirubin (CONG. BIL), unconjugated bilirubin (UB) and total protein (TP)); anti-oxidant Test. The estimation of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GPx).

### Hematological Indexes

The estimation of various (20) parameters was carried out. The White blood cell (WBC), Hemoglobin (HGB), Red blood cell (RBC), Platelet count (PLT) and lymphocytes. CD<sub>4</sub> and CD<sub>8</sub> count were carried out.

### Biochemical Assay

The blood sample for biochemical assays also collected via cardiac puncture were placed in lithium heparin sample bottles and centrifuged at 3000 revolutions per minute (rpm), the plasma was separated using Pasteur pipettes into clear labeled bottles. The samples were stored in deep freezer at -20°C until analyses were carried out (Bagul *et al.*, 2005). The blood plasma was used for the evaluation of the following biochemical parameters includes aspartate aminotransferase (AST), albumin (ALB), total protein (TP), total bilirubin (T BIL), direct bilirubin (D BIL), alanine aminotransferase (ALT) alkaline phosphate (ALP), Malondialdehyde (MDA), Catalase, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). The biochemical parameters were evaluated using commercial kits obtained from Randox Laboratories, UK.

### Histopathological analysis

Liver, kidney, heart, spleen and lungs were fixed in formal saline fluid. Affixed organs were utterly dehydrated with 99.9 % ethanol along with 70 % ethanol, and 96 % ethanol and washed using distilled water. 4 µm sections were prepared and stained in hematoxylin-eosin dye. Stained tissues were optical photomicroscope (Leica MC170 HD, Leica Biosystems,

Germany) viewed at x 400 magnification (Drury and Wallinton, 2013).

### Statistical Analysis

The data were calculated as mean  $\pm$  Standard Error of Mean. Significance differences of the mean value with respect to the control group was analyzed by one way ANOVA using Graph Pad Prism 8 Statistical Software.  $P < 0.05$  considered being significant.

## RESULTS AND DISCUSSION

Results obtained in Table 1, 2 and 3 showed a significant increase ( $p < 0.05$ ) at lowest dose of 0.25 ml of the treatment groups when compared with untreated control in the hematological parameters. The immune response is a biological process which solely depends on external protection and is differ for all members of vaccinated population (Okonkwo *et al.*, 2004; Yakubu *et al.*, 2007). The immune response is influenced by many genetic and environmental factors. Hematological parameters as one of the indexes in immune system understudied the White Blood Cell, Red Blood Cell, Lymphocyte, hemoglobin, Platelet and other. Result

from Table 1 showed a significant increase in the value of WBC counts across the treated groups but more on 0.5 ml/kg when compare with untreated control. Bone marrow being a flexible tissue is majorly responsible for blood cells regenerations to improve immunity. Lymphocyte and other key cells of the immune system are known to activate the production of several antibody polymorph with nuclear granulocyte to destroy antigen (Prescott, 1999). Lymphocyte indexes being one of the immune booster that protect the body against invaders showed a slight increase in the treated groups that received 0.25 and 0.5 ml/kg of coconut oil (9.90 and 8.20) when compared with untreated control. A significant increase in the level of granulocyte across the treated groups specifically at 0.25 ml/kg (8.0) when compared with the controls. There is a significant increase in the level of hemoglobin, hematocrit and platelet in the treated groups when compared with the control groups as shown in Tables 2-3. This present study agreed with the work of Oduola *et al.* (2005) that reported the leaf extract of *J. gossypifolia* on the biochemical and hematological analyses has no damaging effects.

**Table 1.** Effect of coconut oil on white blood and differentials in immunoprotective study.

Parameters	Control	Negative control	(5 ml/kg) Azathioprine	0.25 ml/kg coconut oil	0.5 ml/kg coconut oil	1.0 ml/kg coconut oil
WBC $10^3/\mu\text{L}$	11.73 $\pm$ 1.51 <sup>b</sup>	10.10 $\pm$ 0.17 <sup>a</sup>	9.30 $\pm$ 0.45 <sup>a</sup>	11.70 $\pm$ 1.25 <sup>b</sup>	9.90 $\pm$ 0.76 <sup>a</sup>	10.33 $\pm$ 1.56 <sup>a</sup>
LYM %	83.07 $\pm$ 3.53 <sup>a</sup>	84.43 $\pm$ 0.59 <sup>a</sup>	84.47 $\pm$ 1.68 <sup>a</sup>	84.03 $\pm$ 2.36 <sup>a</sup>	83.10 $\pm$ 1.10 <sup>a</sup>	85.17 $\pm$ 1.82 <sup>a</sup>
MID %	9.533 $\pm$ 1.82 <sup>b</sup>	8.67 $\pm$ 0.24 <sup>a</sup>	8.20 $\pm$ 0.60 <sup>a</sup>	8.83 $\pm$ 0.93 <sup>a</sup>	8.90 $\pm$ 0.47 <sup>a</sup>	8.10 $\pm$ 1.00 <sup>a</sup>
GRAN %	7.40 $\pm$ 1.70 <sup>a</sup>	6.90 $\pm$ 0.50 <sup>a</sup>	7.33 $\pm$ 1.13 <sup>a</sup>	7.13 $\pm$ 1.42 <sup>a</sup>	8.0 $\pm$ 0.67 <sup>b</sup>	6.73 $\pm$ 0.83 <sup>a</sup>
LYM $10^3/\mu\text{L}$	9.77 $\pm$ 1.39 <sup>b</sup>	8.53 $\pm$ 0.18 <sup>a</sup>	7.87 $\pm$ 0.41 <sup>a</sup>	9.90 $\pm$ 1.34 <sup>b</sup>	8.20 $\pm$ 0.50 <sup>a</sup>	8.80 $\pm$ 1.36 <sup>a</sup>
MID $10^3/\mu\text{L}$	1.10 $\pm$ 0.26 <sup>b</sup>	0.87 $\pm$ 0.03 <sup>a</sup>	0.77 $\pm$ 0.07 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>b</sup>	0.90 $\pm$ 0.10 <sup>a</sup>	0.83 $\pm$ 0.14 <sup>a</sup>
GRAN $10^3/\mu\text{L}$	0.87 $\pm$ 0.23 <sup>b</sup>	0.70 $\pm$ 0.00 <sup>a</sup>	0.67 $\pm$ 0.12 <sup>a</sup>	0.80 $\pm$ 0.10 <sup>b</sup>	0.80 $\pm$ 0.15 <sup>b</sup>	0.70 $\pm$ 0.11 <sup>a</sup>

The values were expressed in Mean  $\pm$  SEM and the significant difference was spotted as  $p$ -value  $< 0.05$ .

**Table 2.** Effect of coconut oil red blood cell and components in immunoprotective study.

Parameters	Control	Negative control	(5 ml/kg) Azathioprine	0.25 ml/kg coconut oil	0.5 ml/kg coconut oil	1.0 ml/kg coconut oil
RBC $10^6/\mu\text{L}$	6.36 $\pm$ 0.33 <sup>a</sup>	6.48 $\pm$ 0.44 <sup>a</sup>	6.0 $\pm$ 0.15 <sup>a</sup>	6.85 $\pm$ 0.25 <sup>a</sup>	6.71 $\pm$ 0.38 <sup>a</sup>	6.37 $\pm$ 0.27 <sup>a</sup>
HGB g/dl	13.60 $\pm$ 0.53 <sup>a</sup>	14.63 $\pm$ 0.89 <sup>a</sup>	13.83 $\pm$ 0.50 <sup>a</sup>	15.57 $\pm$ 0.67 <sup>b</sup>	15.33 $\pm$ 0.70 <sup>b</sup>	14.23 $\pm$ 0.62 <sup>a</sup>
HCT %	37.50 $\pm$ 1.47 <sup>a</sup>	40.83 $\pm$ 2.24 <sup>a</sup>	38.73 $\pm$ 1.31 <sup>a</sup>	42.37 $\pm$ 1.48 <sup>b</sup>	41.40 $\pm$ 1.68 <sup>a</sup>	38.70 $\pm$ 1.11 <sup>a</sup>
MCV $\mu\text{M}^3$	59.13 $\pm$ 0.84 <sup>a</sup>	63.20 $\pm$ 1.60 <sup>a</sup>	64.77 $\pm$ 0.64 <sup>a</sup>	61.93 $\pm$ 0.09 <sup>a</sup>	62.57 $\pm$ 0.37 <sup>a</sup>	60.97 $\pm$ 1.70 <sup>a</sup>
MCH pg	21.37 $\pm$ 0.34 <sup>a</sup>	22.57 $\pm$ 0.48 <sup>a</sup>	23.03 $\pm$ 0.35 <sup>a</sup>	22.67 $\pm$ 0.14 <sup>a</sup>	22.83 $\pm$ 0.35 <sup>a</sup>	22.30 $\pm$ 0.10 <sup>a</sup>
MCHC g/dl	36.20 $\pm$ 0.55 <sup>a</sup>	35.77 $\pm$ 0.23 <sup>a</sup>	35.67 $\pm$ 0.60 <sup>a</sup>	36.67 $\pm$ 0.29 <sup>a</sup>	37.00 $\pm$ 0.44 <sup>b</sup>	36.73 $\pm$ 1.17 <sup>a</sup>
RDWS $\mu\text{M}^3$	30.93 $\pm$ 1.23 <sup>a</sup>	32.20 $\pm$ 0.60 <sup>a</sup>	33.43 $\pm$ 1.07 <sup>a</sup>	31.57 $\pm$ 1.07 <sup>a</sup>	31.60 $\pm$ 0.00 <sup>a</sup>	32.83 $\pm$ 3.26 <sup>a</sup>
RDWC %	16.07 $\pm$ 0.42 <sup>a</sup>	15.80 $\pm$ 0.00 <sup>a</sup>	16.13 $\pm$ 0.43 <sup>a</sup>	15.70 $\pm$ 0.52 <sup>a</sup>	15.73 $\pm$ 0.24 <sup>a</sup>	16.50 $\pm$ 1.27 <sup>a</sup>

The values were expressed in Mean  $\pm$  SEM and the significant difference was spotted as  $p$ -value  $< 0.05$ .

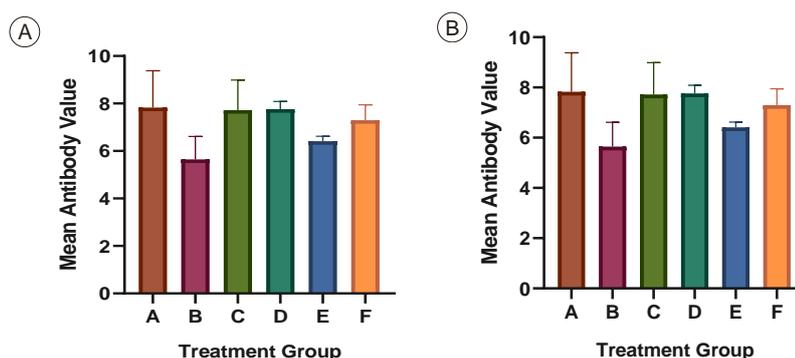
**Table 3.** Effect of coconut oil platelet and factors in immunoprotective study.

Parameters	Control	Negative control	(5 ml/kg) Azathioprine	0.25 ml/kg coconut oil	0.5 ml/kg coconut oil	1.0 ml/kg coconut oil
PLT $10^3/\mu\text{L}$	740.00±72.76 <sup>a</sup>	765.30±45.33 <sup>a</sup>	949.70±322.00 <sup>b</sup>	660.30±80.8 <sup>a</sup>	909.00±132.3 <sup>b</sup>	996.30±225.9 <sup>b</sup>
MPV $\mu\text{M}^3$	7.43±0.12 <sup>a</sup>	8.03±0.26 <sup>a</sup>	8.47±0.09 <sup>a</sup>	7.77±0.09 <sup>a</sup>	8.07±0.13 <sup>a</sup>	8.20±0.30 <sup>a</sup>
PDW %	9.37±0.33 <sup>a</sup>	10.47±0.70 <sup>a</sup>	11.17±0.44 <sup>a</sup>	9.57±0.07 <sup>a</sup>	10.40±0.59 <sup>a</sup>	10.80±0.67 <sup>a</sup>
PCT %	0.54±0.047 <sup>a</sup>	0.61±0.02 <sup>a</sup>	0.80±0.28 <sup>b</sup>	0.51±0.06 <sup>a</sup>	0.73±0.11 <sup>a</sup>	0.82±0.21 <sup>b</sup>
P-LCR %	3.83±1.93 <sup>a</sup>	8.30±3.30 <sup>a</sup>	12.80±1.12 <sup>b</sup>	5.03±0.27 <sup>a</sup>	8.03±2.43 <sup>a</sup>	12.10±3.16 <sup>b</sup>

The values were expressed in Mean ± SEM and the significant difference was spotted as *p*-value < 0.05.

Figure 2 showed an increase in the level of CD<sub>4</sub> count across the graded doses of the treated groups 0.25ml, 1.0ml extract when compared with untreated control. Figure 1 showed an increase in the level of CD<sub>8</sub> count across the graded doses of the treated groups 0.25ml, 1.0ml extract when compared with untreated control. CD<sub>4</sub> and CD<sub>8</sub> cell count provides an improved immune status against opportunistic infections, also it is regarded as a diagnostic decision-making, test

particularly with patients suffering from immunosuppressant and HIV disorders. Result from this study at graded doses of the extract-treated animals, significantly increase the level of CD<sub>4</sub> and CD<sub>8</sub> Count specifically at 0.25 ml/kg (7.763 and 7.830) when compared with untreated group (Figure 1 A and B). This concurred with the work of Oduola *et al.* (2005) on the effect of *Voacanga africana* leaves extract on serum lipid profile and hematological parameters.



**Key:** Group A: Normal control received water alone; Group B: Negative control received 0.3 ml/kg SRBC alone intraperitoneally; Group C: Positive control receive 0.3 ml/kg SRBC + 5 mg/kg standard drug (Azathioprine) orally; Group D: treated group receive 0.3 ml/kg SRBC + 0.25 ml/kg of coconut oil orally; Group E: treated group receive 0.3 ml/kg SRBC + 0.5 ml/kg of coconut oil orally; Group F: treated group receive 0.3 ml/kg SRBC + 1.0 ml/kg of coconut oil orally.

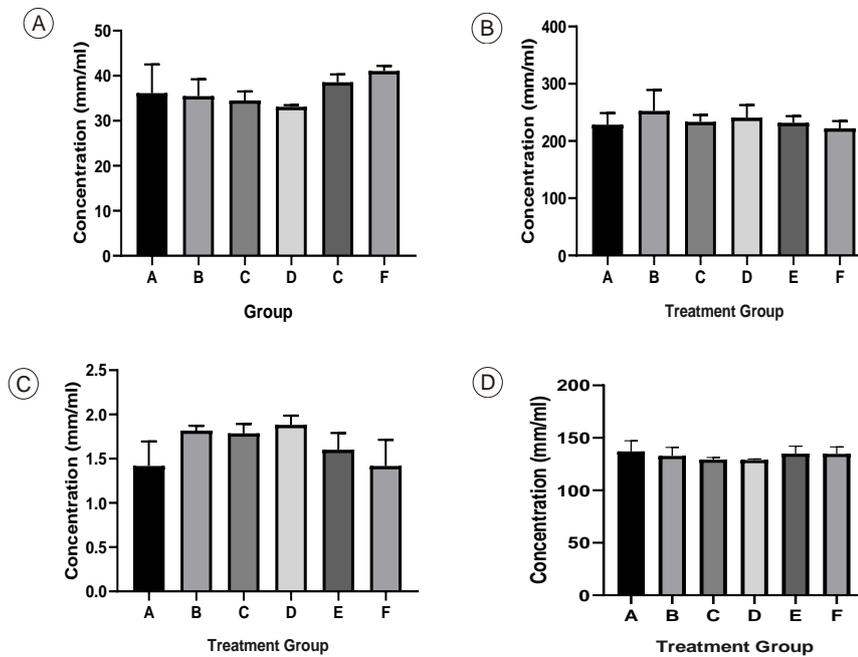
**Figure 1. (A and B)** Effect of Coconut oil extract on CD<sub>8</sub> and CD<sub>4</sub> Count.

Figure 2a showed the anti-oxidant test carried out to estimate the *in vivo* scavenging power of Malondialdehyde (MDA) of graded doses of coconut oil in Wistar rat. The anti-oxidant test carried out to estimate the *in vivo* scavenging power of Catalase Response on coconut oil extract in Wistar rat as shown in Figure 2b. Figure 2c elicited the anti-oxidant potential carried out to investigate the *in vivo* scavenging power of Superoxide Dismutase responses on coconut oil extract in Wistar rat. The results in Figure 2d exhibited anti-oxidant effect investigated in the *in vivo* scavenging power of Glutathione Peroxidase responses. Various antioxidant markers showed the scavenging effect caused by oxidation of lipid peroxidation. Hence, the level of malondialdehyde (MDA) in the blood was measured (Uchiyama *et al.*, 1978). *In vivo* MDA

antioxidant level elicited a significantly reduction at lowest dose (0.25 ml/kg) in *Cocos nucifera* oil extract (34.53) when compared with untreated control as shown in Figure 3. Antioxidant enzymes serves as delicate part associated with cellular defense against responsive oxygen species (ROS) with eventual oxidative stress (Nevin *et al.*, 2006). The determination of oxidative stress using the balance involved ROS generation includes; antioxidant protective systems like superoxide dismutase (SOD) and superoxide anion. Antioxidants enzymes are implicated in the removal of Reactive Oxygen Species such as GSH, SOD and CAT. Antioxidant enzymes such as Catalyst scavenging power significantly increased (*p* < 0.05) it scavenging capacity at 0.25 ml/kg (241.0) when compared with the control, however, in the activity of SOD, there was no significant

increase across the treated animals when compared to the control. Glutathione peroxidase activity, showed a significantly increased in its scavenging power at (129.0) of 0.25 ml/kg of the oil extract when compared with the control groups. Similarly, this study has

exhibited its biological effects on *Cocos nucifera* oil established to scavenge oxidative stress via boosting its antioxidant capacity, thereby scavenging free radicals and decreasing lipid peroxidation (Nevin *et al.*, 2006 and Van *et al.*, 2004).



**Key:** Group A: Normal control received water alone; Group B: Negative control received 0.3 ml/kg SRBC alone intraperitoneally; Group C: Positive control receive 0.3 ml/kg SRBC + 5 mg/kg standard drug (Azathioprine) orally; Group D: treated group receive 0.3 ml/kg SRBC + 0.25 ml/kg of coconut oil orally; Group E: treated group receive 0.3 ml/kg SRBC + 0.5 ml/kg of coconut oil orally; Group F: treated group receive 0.3 ml/kg SRBC + 1.0 ml/kg of coconut oil orally.

**Figure 2. (A, B, C and D)** Effect of *Cocos nucifera* on enzymatic (Malondialdehyde) and non-enzymatic (catalase, Superoxide Dismutase and Glutathione Peroxidase) effect on immune response.

Figure 7 showed the level of liver function tests (ALT, AST, ALP, GGT, Total bilirubin, Conjugated Bilirubin, Unconjugated, Bilirubin and Total protein) on the extract activity (Adeoye and Oyedepo, 2004). The eight liver function tests were found to be within normal range in all treated groups. Excessive secretion of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are raised in serum when malfunction of the liver occurs. The liver function test results elicited no significant increase in the graded doses of the oil extract (ALT, AST and ALP) specifically at 1 ml/kg of the treated groups at (15.28) when compared with the control. This is in line with the results showed in the findings of Adeosun *et al.* (2014) that reveal stem latex of *J. gossypifolia* as a protein precipitant for biochemical investigation with potential defensive measures. No significant difference at ( $p < 0.05$ ) on the conjugated bilirubin value when compared

with the control. 0.25 ml/kg of the oil extract showed a significant increase in the level of GGT, Total Bilirubin and Total Protein antibody level at 0.833, 0.053 and 13.57 when compared with the control groups. This agreed with the report of Igbe *et al.* (2013) and Magili and Bwatanglang (2018). Body weight of the animals in the treated groups showed significant increase specifically at 0.25 ml/kg of oil extract, when compared with the control groups (Kumar *et al.*, 2008). A slight significant increase in the body weight of the rats was revealed at weeks 1, 2, 3 and 4. Afterward treated with *C. nucifera oi* extract across graded doses of 0.25, .0.5 and 1.0 m/kg had a slightly significant difference in the body weight observed in dose dependent manner (Figure 3). Reduction in the body weight aids as an indicator and sensitive marker to mark toxicity. This is adhering to the work of Witthawaskul *et al.* (2003).

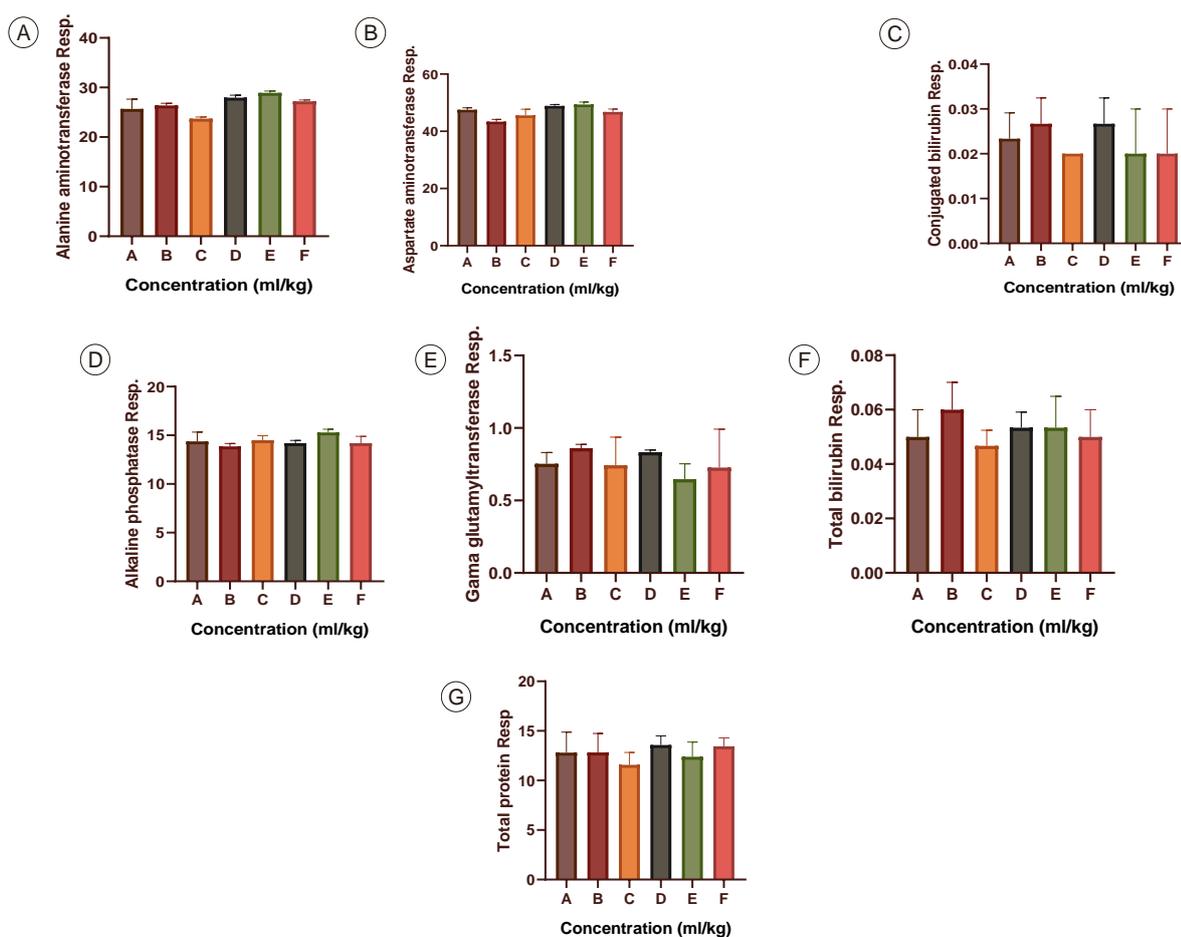


Figure 3. (A, B, C, D, E) Dose responses on the extract in various concentration of liver function test.

Group A: Normal control received water alone; Group B: Negative control received 0.3 ml/kg SRBC alone intraperitoneally; Group C: Positive control receive 0.3 ml/kg SRBC + 5 mg/kg standard drug (Azathioprine) orally; Group D: treated group receive

0.3 ml/kg SRBC + 0.25 ml/kg of coconut oil orally; Group E: treated group receive 0.3 ml/kg SRBC + 0.5 ml/kg of coconut oil orally; Group F: treated group receive 0.3 ml/kg SRBC + 1.0 ml/kg of coconut oil orally.

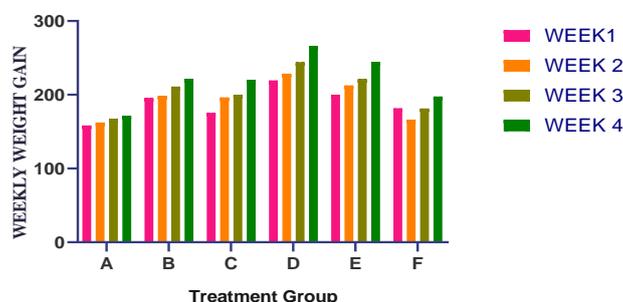


Figure 4. Dose responses on the extract in various concentration of the body weight.

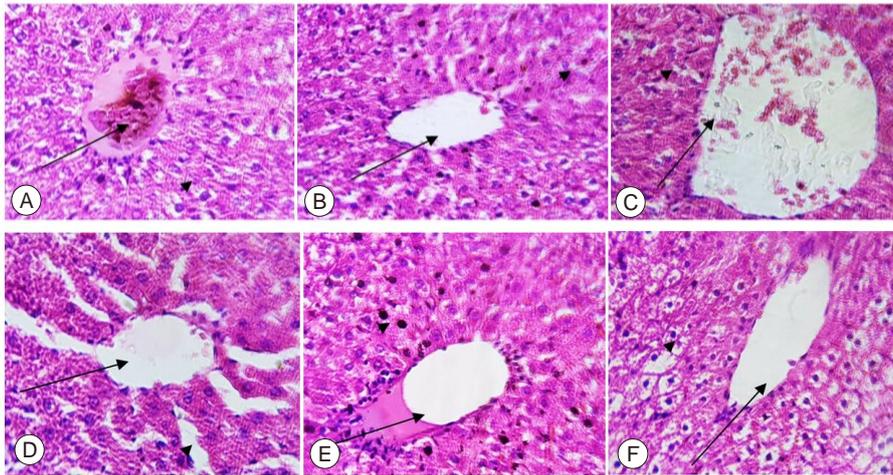
Group A: Normal control received water alone; Group B: Negative control received 0.3 ml/kg SRBC alone intraperitoneally; Group C: Positive control receive 0.3 ml/kg SRBC + 5 mg/kg standard drug (Azathioprine) orally; Group D: treated group receive

0.3 ml/kg SRBC + 0.25 ml/kg of coconut oil orally; Group E: treated group receive 0.3 ml/kg SRBC + 0.5 ml/kg of coconut oil orally; Group F: treated group

receive 0.3 ml/kg SRBC + 1.0 ml/kg of coconut oil orally.

The histopathological structure of the liver, kidney, heart, spleen and lungs (plate 1, 2, 3, 4 and 5) at graded doses (0.25ml, 0.5ml and 1.0 ml/kg) specifically 0.25 ml/kg of the extract revealed a distinct centriole and dilated sinusoidal, with no inflammatory cells and steatosis when compared with untreated control that

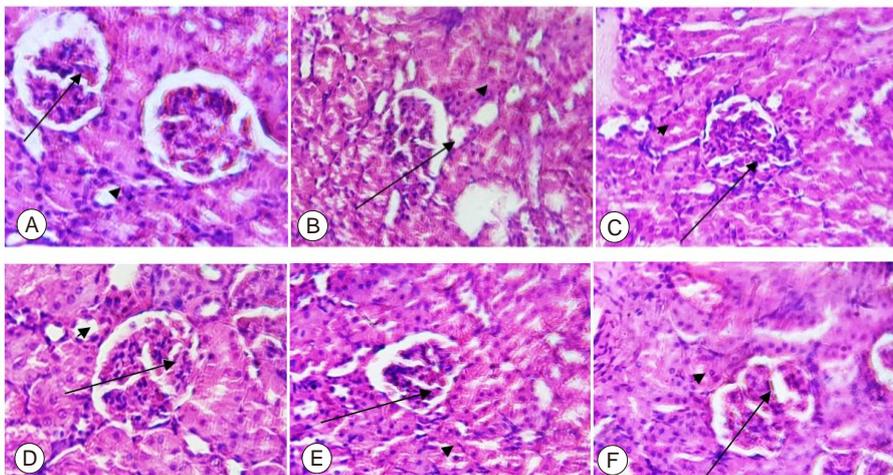
showed mild steatosis in hepatic cells This is in line with the findings of Eleazu *et al.* (2013) and Magili and Bwatanglang (2018) that worked on the toxicological profile of the aqueous leaves extract of *J. gossypifolia*.. The structure of renal cells reveals no necrosis in lowest doses (0.25 ml/kg) when compare with the untreated control with pronounced necrosis.



**Figure 5.** Effect of *Cocos nucifera* oil extract on histology of the Liver.

A= Normal control received water alone LIVER: reveals visible centriole (long arrow) with the hepatocytes revealing pyknotic nucleus (short arrow). B= 0.3 ml/kg SRBC alone LIVER: reveals centriole surrounded by mild inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow). C= 5 mg/kg standard drug (Azathioprine) LIVER: reveals centriole (long arrow) surrounded by hepatocytes that appear not so distinct. (Short arrow).

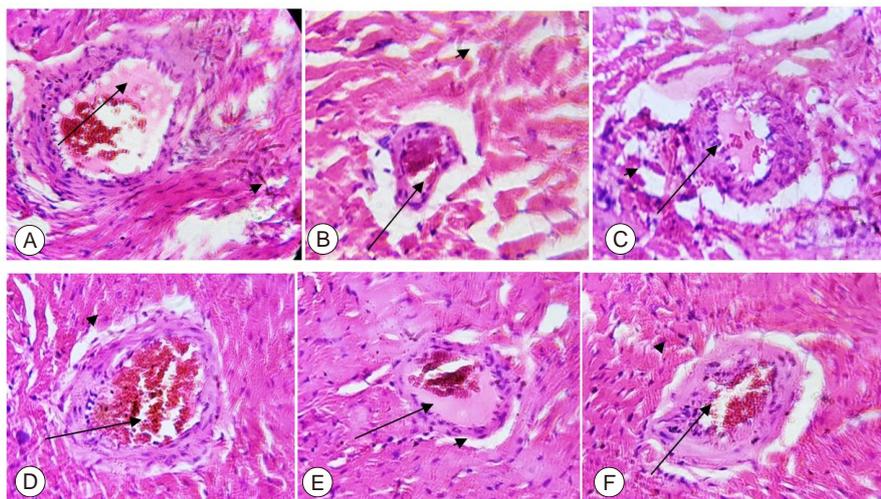
D= 0.25 ml/kg of coconut oil LIVER: reveals distinct centriole (long arrow) with the hepatocytes and dilated sinusoidal (short arrow). E= 0.5 ml/kg of coconut oil LIVER: reveals centriole surrounded by focal inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow). F= 1 ml/kg of coconut oil LIVER: reveals centriole (long arrow) surrounded by hepatocytes with hydropic fatty changes and revealing mild steatosis (short arrow).



**Figure 6.** Effect of *Cocos nucifera* oil extract on histology of the Kidney.

A= Normal control received water alone KIDNEY: reveals prominent renal corpuscle (long arrow) and interstitial space and tubules (short arrow). B= 0.3 ml/kg SRBC alone KIDNEY: reveals atrophied renal corpuscle (long arrow) and interstitial space with mononuclear cells (short arrow) and mild tubular necrosis. C= 5 mg/kg standard drug (Azathioprine) KIDNEY: reveals renal corpuscle appearing not so distinct with granulated nucleus (long arrow) and interstitial (short arrow) and

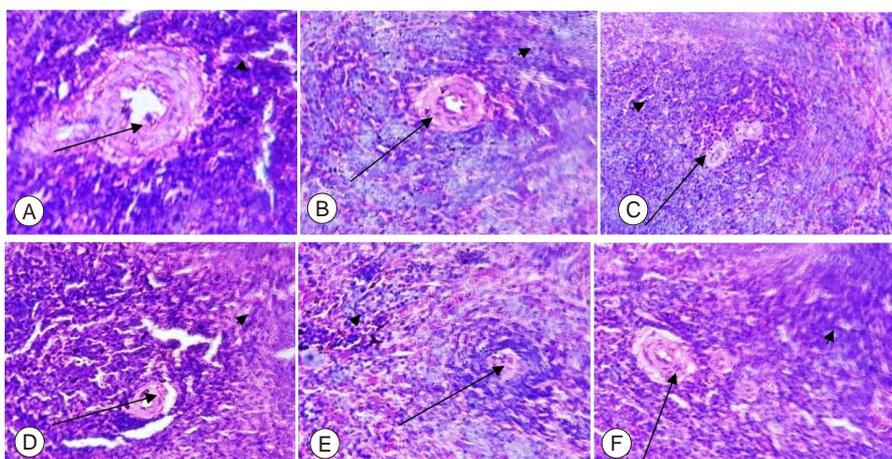
tubular necrosis. D= 0.25 ml/kg of coconut oil KIDNEY: reveals visible renal corpuscle (long arrow) and interstitial space (short arrow) and tubules. E= 0.5 ml/kg of coconut oil KIDNEY: reveals atrophied renal corpuscle (long arrow) and interstitial space with mononuclear cells (short arrow) and tubular necrosis. F= 1 ml/kg of coconut oil KIDNEY: reveals renal corpuscle appearing not so distinct (long arrow) and interstitial (short arrow) and tubular necrosis.



**Figure 7.** Effect of *Cocos nucifera* oil extract on histology of the heart.

A= Normal control received water alone Heart composed of bundles of myocardial fibres (short arrow), interstitial space and prominent coronary artery (long arrow). B= 0.3 ml/kg SRBC alone Heart composed of bundles of myocardial fibres (short arrow), interstitial space and atrophied coronary artery (long arrow). C= 5 mg/kg standard drug (Azathioprine) Heart composed of bundles of myocardial fibers (short arrow), interstitial space and congested coronary artery (long arrow). D=

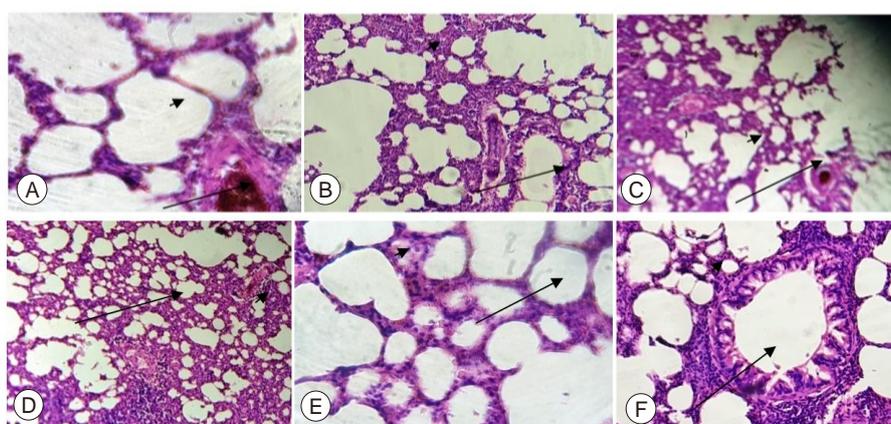
0.25 ml/kg of coconut oil Heart composed of bundles of myocardial fibers (short arrow), interstitial space and large prominent coronary artery (long arrow). E= 0.5 ml/kg of coconut oil Heart composed of tight bundles of myocardial fibers (short arrow), interstitial space and coronary artery (long arrow). F= 1 ml/kg of coconut oil Heart composed of bundles of myocardial fibers (short arrow), interstitial space and coronary artery (long arrow).



**Figure 8.** Effect of *Cocos nucifera* oil extract on histology of the spleen.

A= Normal control received water alone: spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear distinct. B= 0.3 ml/kg SRBC alone: spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear coarse. C= 5 mg/kg standard drug (Azathioprine): spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps

appear coarse. D= 0.25 ml/kg of coconut oil: spleen shows prominent lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps are prominent. E= 0.5 ml/kg of coconut oil: spleen shows lymphoid follicles (short arrow) with not so prominent eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps appeared coarse. F= 1 ml/kg of coconut oil: spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps are appeared coarse.



**Figure 9.** Effect of *Cocos nucifera* oil extract on histology of the lung.

A= Normal control received water alone: Lung reviewed prominent blood vessel (long arrow) and visible alveolar ring. The alveolus appears distinct (short arrow). B= 0.3 ml/kg SRBC alone: Lung reviewed prominent bronchiole (long arrow) and visible alveolar ring. The alveoli appears slightly congested (short arrow). C= 5 mg/kg standard drug (Azathioprine): Lung reviewed prominent blood vessels (long arrow) and visible alveolar ring. The alveoli appear slightly congested (short arrow). D = 0.25 ml/kg of coconut oil: Lung reviewed prominent alveolar sac (long arrow) and alveolar ring. The alveoli appear slightly congested. E= 0.5 ml/kg of coconut oil: Lung reviewed prominent alveolar sac (long arrow) and alveolar ring. The alveoli appear slightly congested. F= 1 ml/kg of coconut oil: Lung revealed prominent bronchiole (long arrow) and visible alveolar ring. The alveoli appear slightly congested (short arrow).

Differences between the hearts occurred in coronary artery which stated a large prominent coronary artery in the lowest dose when compared with untreated control that exhibited atrophied with congested coronary artery (Magili and Bwatanglang, 2018). The alveolus in the lungs showed a distinct structure in 0.25 ml/kg when

compared with untreated control that showed alveolus congestion. The extract feedback response triggered by cellular and humoral immune response. Hence, this study validated the ethnomedicinal property as immune booster.

## CONCLUSION

This study elicited a feedback response from the coconut extract, triggered by cellular and humoral immune response, and thereby boosted the immune physiological state of the body. This finding concurred with its ethnomedicinal property as an immunoprotectant.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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