

# ORIGINAL ARTICLE

## Protective Effect of Virgin Coconut Oil on Cyclophosphamide-induced Histological Changes in Lymphoid Tissues

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### ABSTRACT

**Introduction:** Virgin coconut oil (VCO) is known for its health and therapeutic benefits. However, the immunomodulatory effects of VCO have not been extensively investigated. **Objective:** The present study was devoted to examining the effects of VCO on cyclophosphamide (CY)-induced toxicity of lymphoid tissues. **Methods:** Thirty healthy male Wistar rats were sorted into 5 groups of 6 animals. The first control (NC) group was given distilled water via gavage at 5 ml/kg once daily. The second (CY) group received CY orally at 10 mg/kg/day for 4 weeks. Rats in the other three groups (CV5, CV10, and CV15) were given 10 mg/kg/day CY for 4 weeks, 5 m/kg/day, 10 ml/kg/day and 15 ml/kg/day VCO for 6 weeks, respectively. Rats were sacrificed at the end of 6<sup>th</sup> week; blood sample from the animals was collected for full blood count and biochemical analysis. The thymus and spleen of each animal was processed for histological examination. **Results:** The thymus and spleen showed marked reduction in lymphoid cellularity following daily administration of CY. The thymus also showed a marked reduction in the size of the medulla, and the white pulp areas of spleen had reduction in the follicle number and size. Supplementation with 10 ml/kg and 15 ml/kg VCO showed evidence of restoration of both the thymus and splenic lymphoid architecture. The total white cell counts, absolute lymphocyte counts and plasma globulin levels of the VCO groups were significantly increased compared to CY group. **Conclusion:** VCO displayed potential protective effects on CY-induced histological changes in lymphoid tissues.

**KEYWORDS:** Cyclophosphamide, Immunomodulatory, Spleen, Thymus, Virgin Coconut Oil.

### INTRODUCTION

The use of cyclophosphamide (CY) and other cytotoxic chemotherapeutic agents is associated with immunotoxicity that would increase the risk of immunosuppression-related complications in patients treated with these drugs.<sup>1,2</sup> The occurrence of these side effects cause reduced quality of life and treatment compliance.<sup>3</sup> Modulation of immune functions using medicinal

plants and their products has become an accepted therapeutic approach.<sup>4</sup> As a result, several plant extracts and their immunomodulatory properties are being researched for potential benefits in lessening or overcoming these adverse effects of cancer chemotherapy.<sup>5</sup>

The increasing popularity of VCO has led to new research in its clinical application apart from its role as functional food oil; it has been shown that the coconut oil is effective in reducing oral microbial load and decreasing plaque and gingival indices.<sup>6</sup> VCO has been shown to possess antihypertensive, antimicrobial and anti-inflammatory properties.<sup>7,8</sup> Additionally, VCO has been established clinically to be a potent moisturizer for patients with atopic dermatitis due to the anti-inflammatory and anti-

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infective activity as well as protector of the skin barrier.<sup>9</sup> Clinical studies also revealed that VCO improves the symptoms of skin disorders by soothing the skin, decreased cutaneous inflammation and increased the epidermal barrier function and hydration property by decreasing transepidermal water loss in atopic dermatitis condition.<sup>10</sup>

Currently, clinical trials are being conducted on cardiovascular protective, breast cancer quality of life supplementation and other therapeutic and health beneficial effects of virgin coconut oil (VCO) and other plant-derived antioxidants.<sup>11-13</sup> VCO is composed of about 92% saturated fats consisting of high level of medium-chain fatty acids i.e. lauric, caproic and caprylic acids.<sup>14</sup> It also contains noticeable amounts of vitamins and polyphenols; the key players for antioxidant and anti-carcinogenic properties.<sup>15</sup> The medium-chain fatty acid content has been linked to a reduction in the risks of atherosclerosis and its complications.<sup>16, 17</sup> VCO also possessed free radical reducing and scavenging properties that correlated positively with the content of polyphenols in VCO.<sup>15</sup>

However, the immunomodulatory property of VCO has yet to be extensively explored. Therefore, the present study was conducted to investigate the protective effects of VCO on the CY-induced immunotoxicity in the experimental rat model. In this setting, the probable immune-protection induced by VCO, is sought after by observation for the clinical signs of toxicity, studying the hematological and biochemical profiles and the histopathological changes in the lymphoid tissues of experimental rats treated with different volumes doses of VCO used to test for the dose response effect.

**MATERIALS AND METHODS**

**Virgin coconut oil**

VCO was purchased from the Malaysian Agricultural Research and Development Institute (MARDI) (Serdang, Selangor, Malaysia). It was produced through a cold pressed method using MARDI’s technology.<sup>18</sup> In brief, the coconut white meat was grated and mixed with a certain amount of high quality water. The soaked grated meat was then mechanically pressed to produce coconut milk. The coconut milk was placed in a sterile container and

left for 24 hours at room temperature following which the oil layer that partitions on top was scooped out and subjected to centrifugation. This step allows for sedimentation of fine particles and clarification of the oil.

**Animals**

Thirty adult male Wistar rats (age: 5-6 weeks) weighing approximately 100 - 200 gram were used. Each cage housed two rats under standard experimental conditions of 25°C with 12 hour-light and 12 hour-dark cycle each day. The rats were fed with rat chow (Gold Coin Sdn. Bhd., Malaysia) and distilled water given *ad libitum*. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC), International Islamic University Malaysia (IIUM). The experiment was conducted according to the Guidelines for the Care and Use of Laboratory Animals of the Kulliyyah of Medicine, IIUM.

**Experimental design**

The rats were divided into five groups of six animals each as described in Table 1. Cyclophosphamide (Merck Sdn. Bhd) at a dose of 10 mg/kg/day was given for 4 weeks while the VCO was given at different doses, namely 5 ml/kg/day, 10 ml/kg/day and 15 ml/kg/day for 6 weeks respectively. At the end of the treatment period, blood specimen from each animal was collected for hematological and biochemical analyses. All the rats were subsequently sacrificed and thymus and spleen were removed and fixed in 10% formalin saline for 72 hours at room temperature prior to processing, followed by haematoxylin and eosin (H&E) staining.

**Table 1:** Distribution of animals by experimental groups

Group	Definition	Treatment
NC	Normal control rats	Rat pellets and water only
CY	Cyclophosphamide	Rat pellets, water & CY 10 mg/kg/day
CV5	Cyclophosphamide & VCO 5	CY 10 mg/kg/day & VCO 5 mL/kg/day + pellets and water
CV10	Cyclophosphamide & VCO 10	CY 10 mg/kg/day & VCO 10 mL/kg/day + pellets and water
CV15	Cyclophosphamide & VCO 15	CY 10 mg/kg/day & VCO 15 mL/kg/day + pellets and water

### ***Hematological and biochemical analysis***

Blood specimens were analyzed for full blood count using the Advia 120 Hematology System. The serum obtained was analyzed for total protein, albumin, globulin, alanine aminotransferase, urea and creatinine levels (Thermo scientific Biochemical Analyzer).

### ***Statistical analysis***

Statistical analysis was performed using analysis of variance (ANOVA) available in the statistical programme SPSS version 20.0 to compare the hematological and biochemical parameters of the study groups. A value of  $p < 0.05$  was considered to be significant.

## **RESULTS**

### ***Histological changes in the thymus glands***

The control rats (NC group) showed normal histology of the thymus (Fig. 1a). Sections of the thymus of rats receiving only CY (CY group) showed depletion of lymphoid cell population in the medulla and cortex, shrinkage of the medulla, congestion of blood vessels, thickening of the capsule and trabeculae and increased number of macrophages (Fig. 1b). Rats in the group that received VCO at 5ml/kg/day (CV5 group) exhibited similar changes in the thymus as those in group CY (Fig. 1c). Animals that were given VCO at 10 ml/kg/day (CV10 group) showed an increase in thymic cellularity and improvement in the shrinkage of the medulla as compared to group CY (Fig. 1d).

The increase in lymphoid cellularity in the cortex and medulla was more apparent as compared to CV5 group with few macrophages were seen in the cortex. Thymus of rats receiving the highest dose of VCO (CV15 group) showed a similar thymic architecture to the control group; the medullary size was almost similar to that of group NC with significant restoration of lymphoid cellularity both in the medulla and cortex (Fig. 1e).

### ***Histological changes in the spleen***

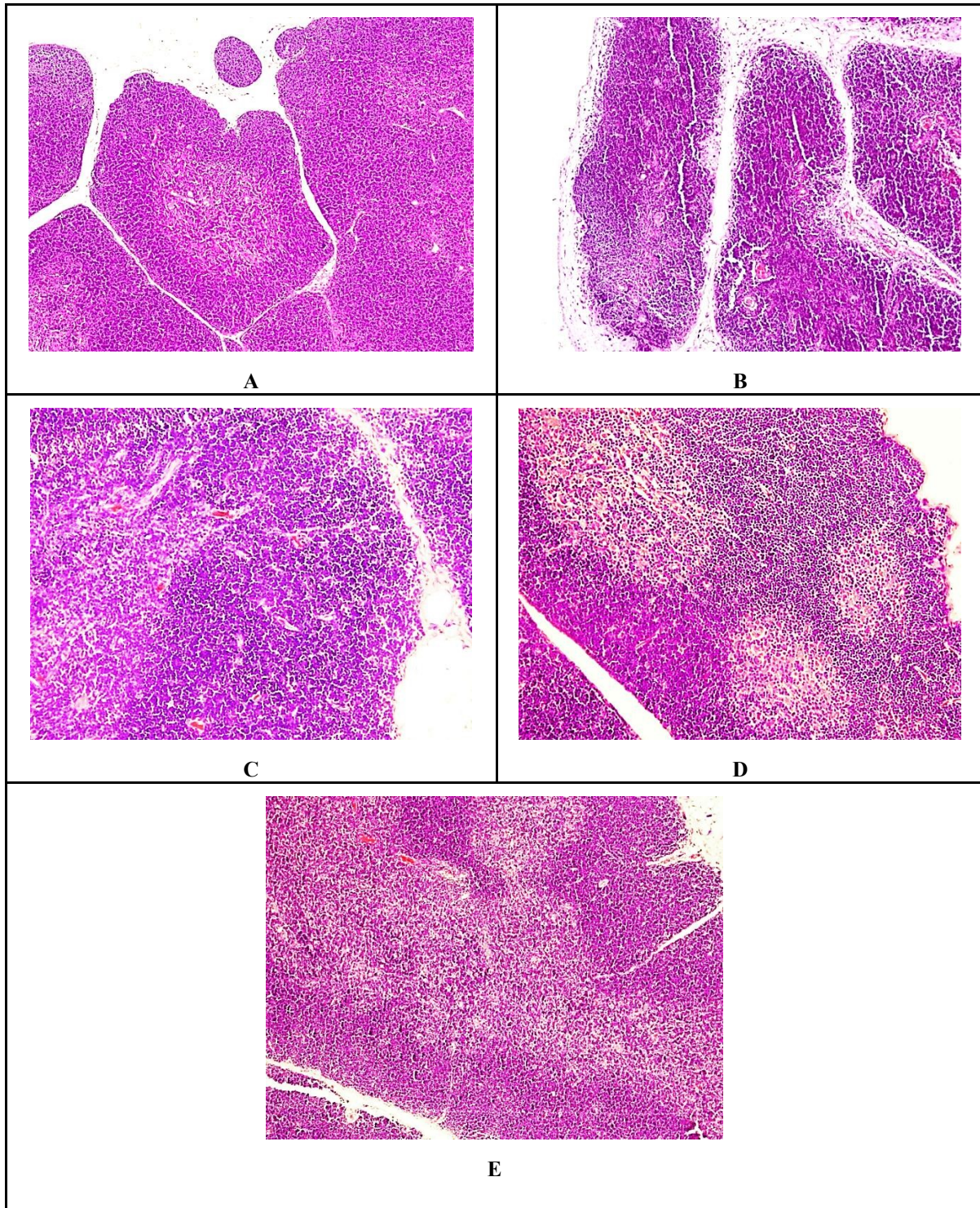
The spleen of the control group showed the normal architecture (Fig. 2a). Splenic sections of CY group

rats showed that the white pulp had an overall reduction in lymphoid cellularity, particularly in the follicles and the marginal zone with the size and number of follicles being markedly diminished, the germinal centers were barely seen. In the splenic red pulp, there was marked depletion of lymphocytes with congestion of venous sinuses (Fig. 2b). As for rats in the group that received VCO at 5ml/kg/day (CV5 group), the splenic sections also revealed marked depletion of lymphocytes in the follicles and marginal zones (Fig. 2c).

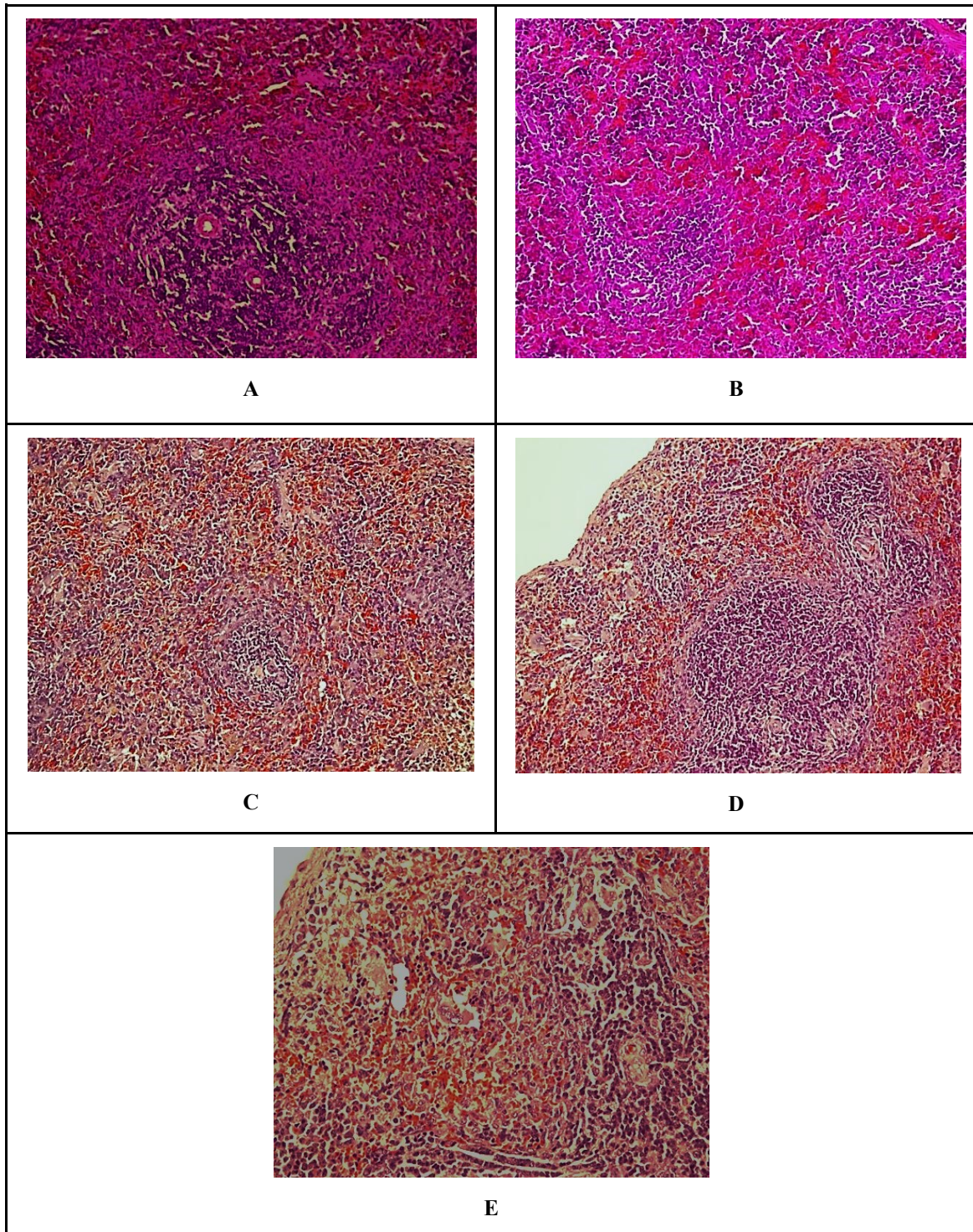
The red pulp showed congestion of sinusoids although it was less marked than in the CY group. On the other hand the histological features of the splenic section of CV10 group rats showed a marked increase in the cellularity of the white pulp as compared to CY and CV5 groups. The size of the follicles of the white pulp was also increased (Fig. 2d). There was evidence of germinal center regeneration as indicated by the presence of mitotic figures and follicular dendritic cells in the white pulp. The cellularity of the red pulp was also increased. Animals in the group that received VCO at 15ml/kg/day (CV15 group) exhibited restoration of lymphocytes in the white pulp and the marginal zone. The cellularity of the red pulp was also increased with less marked sinusoid congestion (Fig. 2e).

### ***Full blood count and biochemical analysis***

Results of full blood count are shown in Table 2. Both the total white cell count (TWCC) and the absolute lymphocyte count showed increasing trends in the groups receiving VCO. Groups CV10 and CV15 had significantly higher TWCC and lymphocyte count as compared to group CY. However, the values did not quite approach the normal control levels. The results of the biochemical parameters are shown in Table 3. All groups administered VCO had significantly higher globulin levels than group CY.



**Figure 1.** Haematoxylin and eosin stained sections of thymus. A. Normal thymic architecture of control group, x 100. B. A thymic section from a rat in group CY showed thickening of the capsule and cortex, congested blood vessels, decreased lymphoid cells population in the medulla, x 100. C. A thymic section of group CV5 rat showed no improvement in the thymus, x 200. D. Histologic section of thymus from group CV10 rat showed restoration of lymphocytes in the thymus, normal size of the medulla, cortex and capsule, x 100. E. Thymic section of rat from group CV15 showed normal thymic capsule, restoration of lymphocytes in the medulla and its size, x 100.



**Figure 2.** Haematoxylin and eosin stained sections of spleen. **A.** Group NC; Normal splenic architecture, x 200. **B.** Group CY; Reduced diameter of the splenic follicles, congested BV's, thickening of the central artery, hyalinization, splenic lymphocyte depletion, and degenerated cytoplasm was observed in the germinal center, x 200. **C.** Group CV5; Depletion of lymphocytes in the white pulp, thickening of the central artery and hyalinization in the marginal zone was observed. The red pulp contained focal hemorrhagic areas while the trabeculae appeared less thickened as compared to the CY group, x 200. **D.** Group CV10; Normal splenic capsule, restoration of lymphocytes in the white pulp, the marginal zone and the red pulp contain focal haemorrhagic areas, x 200. **E.** Group CV15; Restoration of lymphocytes in the white pulp and the marginal zone. The cellularity of the red pulp was also increased with less marked sinusoid congestion, x 200.

**Table 2.** Full blood count parameters of the different experimental animal groups.

Group	TWCC (x10 <sup>9</sup> /L)	Lymphocytes (x10 <sup>9</sup> /L)	Neutrophils (x10 <sup>9</sup> /L)	RBCC (x10 <sup>12</sup> /L)	Haemoglobin (g/L)	Platelets (x10 <sup>9</sup> /L)
NC	10.62 ± 1.24	6.82 ± 2.13	3.33 ± 1.81	8.56 ± 0.44	164.7 ± 2.73	747.33 ± 195
CY	3.32 ± 1.87*	0.83 ± 0.29*	2.28 ± 1.85	7.82 ± 0.29	151.3 ± 4.90	780.00 ± 124
CV5	4.98 ± 1.79*	1.97 ± 0.55*	2.33 ± 1.08	7.74 ± 0.18*	155.0 ± 5.60	598.16 ± 78
CV10	7.88 ± 2.60#	3.35 ± 1.26*#	3.65 ± 1.36	8.27 ± 0.29	160.0 ± 6.50	925.33 ± 256
CV15	8.22 ± 2.32#	3.95 ± 0.78*#	3.18 ± 1.37	7.32 ± 0.82*	149.2 ± 15.2	790.67 ± 298

Data presented as mean ± SD (n = 6). P < 0.05 is considered significant (one-way ANOVA followed by post-Hoc test Tukey HSD);

\*p < 0.05 compared to Group NC

#p < 0.05 compared to Group CY

TWCC - Total white cell count

RBCC - Red blood cell count

**Table 3.** Biochemical parameters of the different experimental animal groups.

Group	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	ALT (U/L)	Urea (mmol/L)	Creatinine (µmol/L)
NC	64.0 ± 3.0	37.3 ± 1.6	26.7 ± 1.9	67.67 ± 8.67	8.3 ± 0.6	25.5 ± 4.8
CY	60.0 ± 2.5	36.2 ± 2.1	23.8 ± 0.8*	66.00 ± 14.48	7.8 ± 0.5	30.3 ± 4.2
CV5	62.8 ± 2.0	36.5 ± 0.8	26.3 ± 1.5#	53.83 ± 8.04	7.0 ± 1.8	25.2 ± 7.0
CV10	60.2 ± 3.0	33.0 ± 1.4*	27.2 ± 1.8#	53.83 ± 9.02	6.5 ± 1.6	25.2 ± 5.0
CV15	63.8 ± 2.3	33.8 ± 1.7*	30.0 ± 1.3*#	68.83 ± 10.76	5.9 ± 1.1*	20.2 ± 3.4#

Data presented as mean ± SD (n = 6). P < 0.05 is considered significant (one-way ANOVA followed by post-Hoc test Tukey HSD);

\*p < 0.05 compared to Group NC

#p < 0.05 compared to Group CY

ALT - alanine aminotransferase

**Body weight gain and relative weight of thymus and spleen**

The body weight gain, relative thymus and spleen weight results are shown in Table 4. The groups that received VCO supplementation showed significant increments as compared to CY group. Although there were significant reduction in albumin levels and significant increase in globulin levels in the VCO

groups, especially the ones receiving the higher dose, however the levels were all within the normal range including the A/G ratio which did not go below the value of one. The level of Alanine Aminotransferase (ALT) as an indicator of liver cell injury and the levels of serum creatinine and blood urea as indicators of kidney function did not show any increase in all the VCO groups as compared to those of the normal control group.

**Table 4.** Weight gain and relative weight of thymus and spleen.

Group	Weight gain in 6 weeks (g)	Relative weight of thymus	Relative weight of spleen
NC	180.6 ± 24.3	0.135 ± 0.014	0.176 ± 0.004
CY	119.3 ± 20.8*	0.085 ± 0.014*	0.143 ± 0.008
CV5	119.3 ± 33.2*	0.089 ± 0.037*	0.124 ± 0.010
CV10	149.5 ± 43.3	0.092 ± 0.015*	0.146 ± 0.016
CV15	160.8 ± 16.7	0.105 ± 0.018	0.174 ± 0.017

Data presented as mean ± SD (n = 6). P < 0.05 is considered significant (one-way ANOVA followed by post-Hoc test Tukey HSD);

\*p < 0.05 compared to Group NC

#p < 0.05 compared to Group CY

## DISCUSSION

The current study revealed that VCO had protective effects against CY-induced histological changes in lymphoid tissues of Wistar rats. This was as evidenced by the restoration of the histological architecture and lymphocytic cellularity of the thymus and spleen of the groups receiving both CY and VCO as compared to the group receiving only CY. These histological findings were further supported by the significant increase in the TWCC, lymphocyte absolute counts and plasma globulin levels in the VCO groups as compared to group that received only CY.

Cyclophosphamide, is a known cytotoxic drug used in this study due to its documented efficacy in yielding immunotoxicity in healthy animals as shown by previous studies.<sup>19,20</sup> Clinical application of CY as an anticancer drug is often limited due to its harmful side effects.<sup>21</sup> CY-induced immunotoxic reactions often manifested as immunosuppression. This immunosuppressive effect has long been regarded as problematic when treating patients with malignancies.<sup>22</sup> Consequent to the immunocompromised state, the patients are susceptible to various infections which are associated with a higher rate of mortality.<sup>23</sup>

The depletion of lymphocytes population in the thymus of rats receiving only CY is consistent with previous observations that revealed decreased cellularity in the thymus of rats given CY orally at a dose of 10mg/kg/day for 30 days;<sup>19</sup> these changes are most probably attributed to the cytotoxic effects of CY, predominantly on the lymphocytes and medullary epithelial cells,<sup>24</sup> and to its immunotoxic effects which induces apoptosis, hypocellularity, and atrophy in the thymus.<sup>25</sup>

Reduction in lymphoid cellularity and alterations in the corticomedullary ratio, had been regarded as deleterious effect of xenobiotics on the thymus, and is considered as a highly sensitive indicator of immunotoxicant exposure.<sup>26</sup> The reduction in the lymphoid cellularity in spleen of CY treated rats is in agreement with previous studies that observed depletion of T lymphocytes in mice treated with a single dose of 4 mg,<sup>27</sup> and 200 mg/kg of CY,<sup>28</sup> and decreased cellularity in the spleen in rats treated daily with 10mg/kg CY for 30 days.<sup>19</sup>

Several studies have reported that naturally extracted remedies that possess high antioxidant property showed protective effects on CY-induced myelosuppression and cytotoxicity in lymphoid tissues.<sup>29,30</sup> As such, we assessed the protective effects of VCO against these effects; the VCO supplementation at 10 ml/kg and 15 ml/kg to the CY-treated rats were associated with improvement in the histologic architecture of both the thymus and spleen; this enhancement showed proportional favourable effects with the highest dose inducing the best response. The protective effects of the VCO against the CY induced injury could be closely linked to its contents of polyphenols as these had been identified as naturally occurring potent antioxidants.<sup>31</sup> Another factor that may contribute to the medium-chain fatty acid composition of VCO that have been shown to have immunomodulatory effects.<sup>32</sup> It has been demonstrated that lauric acid “the precursor of monolaurin” is an active compound in VCO,<sup>33</sup> which has been shown to modulate immune cell proliferation and the potential to hamper tumor growth.<sup>34</sup>

The present study has demonstrated a significant increase in TWCC; absolute lymphocyte count and plasma globulin levels in the CY/VCO treated groups. As CY administration is known to induce lymphocyte depletion,<sup>35,36</sup> the significant increment of TWCC and absolute lymphocytes count reflects restoration of leucopoiesis, specifically lymphopoiesis. Low levels of serum globulins following CY administration were also reported and may be indirectly linked to the immunological dysfunction.<sup>37</sup> The elevation of globulin levels in VCO treated rats of this study may be associated with medium-chain fatty acid content of the oil that has been shown to enhance antibody production through IL-6 induction as demonstrated in an animal study,<sup>38</sup> higher antioxidant activity,<sup>39</sup> or it may be due to other mechanisms which have not yet been explored.

As for the body weight gain, relative thymus and spleen weights, although the significant gains in then VCO groups as compared to CY groups were not observed, there were increasing trends observed with increasing doses of VCO. The reduction in body weight gain and lymphoid organ weight following treatment with CY was primarily shown to be due to systemic inflammation, degradation of structural proteins and apoptosis-induced activity of CY and its metabolite known as acrolein;<sup>40</sup> these

pathophysiological processes were believed to result in reduced appetite and increased fatigability, thus, leading to muscle mass loss. It has been shown that consumption of VCO assists in increasing the energy level as well as preserving physical function and improve quality of life among the breast cancer patients,<sup>41</sup> VCO improves fats and protein metabolism and has a positive effect on immune responses among HIV patients.<sup>42</sup> The ability of VCO to mitigate the weight loss in rats treated with CY may also be attributable to the medium-chain fatty acids and polyphenols contents that hold immunomodulatory and antioxidant properties respectively. Additionally, the coconut oil supplies approximately 8 calories per ml which may also be beneficial in this context.<sup>17,41</sup> It has been suggested that it is possible to prevent cancer by fatty acid supplementation and that VCO was shown to boost patients' energy and may have a place in supplementary treatment of cancer patients.<sup>43</sup> VCO also promotes the immune system response, completely abolishes the predictable immune reactions to endotoxin and decreases the production of pro-inflammatory cytokines.<sup>44</sup>

Cyclophosphamide did not cause significant deleterious effects on the neutrophil count, RBC and platelet count, albumin, alanine aminotransferase, urea and creatinine levels to indicate myelo-suppression and/or systemic hepatorenal toxic effects in our experimental animals; suggesting that CY at 10 mg/kg orally for 4 weeks is sufficient to induce lymphocytic depletion and expected subsequent immunosuppression without causing apparent systemic toxicity. Our finding concurs with previous studies that reported CY administration at low to moderate dosages resulted in depletion of lymphocytes prior to the myelo-suppression stage.<sup>35, 45</sup> Further immunohistochemical studies using CD3 and CD20 polyclonal antibodies for detection of T and B lymphocytes populations and to evaluate the immunoprotective effect of VCO against CY-induced histological changes in lymphoid tissues are in progress.

## CONCLUSION

In conclusion, this study demonstrated the immunoprotective effects of VCO against cytotoxicity induced by CY in lymphoid tissues as observed by significant improvement in the microscopic architecture and cellularity of thymus

and spleen, the peripheral TWCC and lymphocyte counts and plasma globulin level in a dose-dependent manner. These findings should encourage the conduction of further experiments and later clinical research on the immunoprotective effect of VCO intake in cancer subjects treated with cytotoxic drugs.

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