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Original Research Article

Virgin Coconut Oil Supplementation Ameliorates Diabetes and Atrazine-Induced Inflammation in Male Wistar Rats

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Abstract

We assessed the ameliorative effect of Virgin Coconut Oil (VCO) following atrazine-induced inflammation in rats. Adult male Wistar rats weighing 180 - 200 g body weight were separated into two major experimental groups. 35 rats in the test group were divided into five groups of 7 rats: Group 1,2 and 3 received 10 ml/kg body weight of distilled water, 10 ml/kg VCO and 123 mg/kg Atrazine (ATZ) respectively, group 4 was diabetic control and group 5 was diabetic group treated with 10 ml/kg of VCO for 2 weeks, after which the animals were sacrificed, and blood collected for analysis. 35 rats for the recovery group were also divided into 5 groups of 7 rats; group 1 and 2 received 10 ml/kg body weight of distilled water and 10 ml/kg of VCO; group 3,4 and 5 received 123 mg/kg of ATZ for 2 weeks. After the first 2 weeks, group 1,2,3 continued the initial treatment while the rats in group 4 and 5 were administered 10 ml/kg of VCO and 10 ml/kg of distilled water respectively. After 2 weeks all the animals were sacrificed and blood collected for analysis. C-reactive protein (CRP) and interleukin 6 (IL-6) were significantly ($p<0.05$) raised in VCO control, atrazine and diabetic untreated group when compared to normal control. Following recovery, CRP and IL-6 were significantly lowered in the VCO treated group when compared to ATZ group. ATZ toxicity resulted in increase in inflammatory markers but the withdrawal of ATZ significantly reversed some of these derangements; with more pronounced effect following VCO administration.

Keywords: Atrazine; C-reactive Protein; Diabetes; Interleukin 6; Virgin Coconut oil.

1. INTRODUCTION

Inflammation is a short-term adaptive response of the body elicited as a principal component of tissue repair to deal with injuries and microbial infections. It can be also increased in chronic kidney disease, fatty liver disease, and peripheral neuropathy (Badawi *et al*., 2010). The main role of inflammation is to remove the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process and initiate tissue repairs (Ferrero-Miliani *et al*., 2007). Inflammatory markers are the body changes that occur indicating the presence of inflammation, which includes: Increased Interleukin-6, C- reactive protein, increased erythrocyte sedimentation rate, increased plasma viscosity and Fibrinogen and ferritin (Kotulska *et al*., 2015) among others.

Inflammatory systems assume a key position in the pathogenesis of type 1 diabetes. People who progress to type 2 diabetes show characteristics of poor-quality inflammation, years ahead of disorder onset. This low-grade inflammatory response has been proposed to be associated with the pathogenetic procedures leading to type II diabetes (Kristiansen and Mandrup-Poulsen, 2005). Inflammation mediators, for example, interleukin-1, interleukin-6 (IL-6), interleukin-18, tumor necrosis factor-α (TNF-α) and certain chemokines have been hypothesized to be engaged with the occasions causing the two types of diabetes (Kristiansen and Mandrup-Poulsen, 2005; Zahng *et al*., 2009). The increased production of acute-phase reactant and pro-inflammatory proteins portrays the initial phases of type II diabetes and displays a steady increment in diabetes progress (Badawi *et al*., 2010). It is assessed that up to 25% of newly diagnosed diabetic patients already

possess signs of systemic inflammation at the time of diagnosis (Zahng *et al*., 2009), proposing that the inflammation is already present for a considerable period of years before the development of the predisposing factor (eg, overweight) to the disease (LeRoith, 2002).

IL-6 appears to epitomize a negative role in both major type of diabetes after adjusting for age, sex, waist-to-hip proportion, body mass index (BMI), sports, smoking, intake of alcohol and other factors; IL-6 emerges as an autonomous early indicator of type II diabetes mellitus before its clinical presentation (Spranger *et al*., 2003). Furthermore, IL-6 boosts the hepatic secretion of VLDL and hypertriglyceridemia (Sjoholm and Nystrom, 2006). These observations propose a connection between levels of IL-6, obesity, and inflammation in type II diabetes development (Yudkin *et al*., 2000), which shows that IL-6 can be considered as one of the major biomarkers for early type II diabetes discovery. In humans, several researches have proven that increased IL-6 concentration are related to a higher prevalence of type II diabetes not considering obesity (Wannamethee *et al*., 2007).

CRP constantly corresponds with different parameters applicable to diabetes, including lipogenesis, overweight or obesity and adiponectin levels. In this regard, CRP was demonstrated to be continually correlated with the occurence of diabetes in otherwise healthy individuals (Sattar et al, 2008). Research has evaluated that about 33% of T2DM cases can be related to raised serum CRP (Dehghan *et al*.., 2007). Though, the connection amid CRP and type II diabetes appears greater in females than males, but comparing forty-two males and females with high serum CRP (> 2.6 mg/L) and people with CRP less than 0.5 mg/L shows that the former has higher than two-fold risk for T2DM (Dehghan *et al.*, 2007); this observation establish that CRP is a conceivable biomarker for initial type II diabetes discovery. The first-rate mechanism via which CRP performs a vital position in diabetes is principally through its activity on the β-cell (Pfutzner *et al*., 2006).

Atrazine has been known to initiate inflammation therefore causing an increment in inflammatory markers. There are reports that atrazine induced inflammation in male rats and the higher the dose, the higher the severity of the inflammation. It has been observed to increase prevalence of prostate inflammation in male albino rats (Stanko *et al*., 2010). Atrazine cause inflammation through activation of NxRS reactions, distorting homeostasis of cyp and transcription of isoforms of cyp. It affects the metabolic actions of Nitric oxide, also activating inflammatory reaction and Endoplasmic Reticulum stress by initiating IREI/TRAF2 NF – KB mechanism (Li *et al*., 2018). Inflammation results in inflammatory responses which consist of increase of inflammatory markers. Consequently, CRP being an inflammatory marker increases (Kotulska *et al*., 2015). Hence, atrazine will increase CRP concentration (and different inflammatory makers) because it induces inflammation. Inflammation resulting from oxidative stress has been proposed to being the underlying mechanism (Liu *et al*., 2014).

VCO is dietary and medical nourishment of the customary regions growing coconut. VCO refers to unprocessed oil extracted out of dried, matured, and fresh white part, i.e., the kernel of a coconut fruit (*cocos nucifera*) either via natural or mechanized method, without or with the aid of low heat (Villarino *et al*., 2007). This keeps the significant biologically active contents of the oil, for example, the antioxidants and phenols. VCO is colourless oil at temperature above 30^{o}C and white at low temperature and solid form. It has a normal coconut smell and not soluble in water at room temperature. It is dominatingly made up of saturated fats (about 94%), with a good content (above 62%) of medium chain fatty acids having highest fatty acid content of 45 – 52% lauric acids. The lauric acid changes into monolaurin acid that is discovered to battle viral pathogens and shield the body from parasites (Bergsson *et al*., 1998). VCO has been found to have hypoglycemic actions, enhance insulin secretion and also ameliorate oxidative stress induced in type I Diabetes mellitus (DM) induced rats (Iranloye *et al*., 2013). VCO was also found to ameliorate high density lipoprotein levels in induced diabetes male rats (Akinnuga *et al*., 2014). VCO possess widespread therapeutic applications, one of which is inflammation. Research has reported that VCO showed an anti-inflammatory impact on acute and long-term inflammation through reduction of granuloma growth, serum alkaline phosphatase action and transudative weight (Intahphnak *et al*., 2010). Dietary polyphenols have been well discovered to be inflammatory modulators which disrupt the factors for pathogenesis and show valuable health benefits (Li *et al*., 2014). They activate non-direct pathway for discharge of anti-inflammation signaling molecules. This study was therefore designed to observe if VCO's effects on high density fats will be beneficial to the management of and prevention of diabetes and possibly atrazine induced inflammation in rats

2. MATERIALS AND METHODS

Experimental animals

Adult male albino Wistar rats (180-200g body weight) were purchased and kept at the animal house Unit of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar. The animals were kept in a well-ventilated space to acclimatize for two weeks. The animals were fed with rat chow and allowed drinking water *ad libitum*. After the acclimatization period, the animals were weighed, their fasting blood glucose level were measured and reassigned before the commencement of the experimental treatment. The cages were cleared and kept clean throughout the period of the experiment.

Experimental design and treatment of animals

The rats were randomly separated into two major experimental groups (the test and recovery groups) of 35 rats in each major group. Experiment for the test group lasted for two weeks while experiment for the recovery group lasted for four weeks.

Thirty-five (35) rats in the test group were randomly divided into five sub-groups (SG) of 7 rats per sub-group (n=7) and were fed by oral gavage and treated as follows: Group 1 served as normal control and received 10ml/kg body weight of distilled water, group 2 received 10ml/kg of VCO, group 3 received 123 mg/kg (20% of lethal dose) of ATZ, group 4 was diabetic group left untreated and group 5 was the diabetic group that were treated with 10ml/kg of VCO. Treatment in the test group lasted for 2 weeks, after which the animals were sacrificed, and blood collected for analysis. This is shown in Table 1.

During these 2 weeks' period, thirty-five rats for the recovery group were also divided into 5 sub-groups of 7 rats (n = 7) and were treated as follows: group1 served as normal control and received 10ml/kg body weight of distilled water, group 2 received 10 ml/kg of VCO, group 3, 4 and 5 received 123 mg/kg of ATZ. After 2 weeks, the animals were re-treated for recovery and were treated as follows: group 1 continued the initial treatment receiving 10 ml/kg body weight of distilled water, group 2 received 10 ml/kg of VCO, group 3 received 123 mg/kg of ATZ, group 4 was treated with 10 ml/kg of VCO and group 5 was given 10 ml/kg of distilled water. Treatment for recovery also lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis. The groupings are shown in Table 2.

EXPERIMENTAL GROUPING AND TREATMENT

Table 1: Test Group (2 Weeks)

GROUPS	TREATMENT
Normal Control + H_20 (SG 1)	10ml/kg of distilled water (H_2O)
Normal Control + VCO (SG 2)	10ml/kg of Virgin Coconut Oil (VCO)
Atrazine Treated (SG 3)	123mg/kg (20% of lethal dose) of Atrazine
Diabetic Control (SG 4)	10ml/kg of distilled water (H_2O)
Diabetes +VCO (SG 5)	10ml/kg of Virgin Coconut Oil (VCO)

Table 2: Recovery Group (4 Weeks)

GROUPS	TREATMENT (1st 2 weeks)	TREATMENT (2nd 2 weeks)
Normal Control + H_20 (SG 1)	10ml/kg of distilled water (H_2O)	10ml/kg of distilled water (H_2O)
Normal Control + VCO (SG 2)	10ml/kg of Virgin Coconut Oil (VCO)	10ml/kg of Virgin Coconut Oil (VCO)
Atrazine Treated (SG 3)	123mg/kg (20% of lethal dose) of Atrazine	123mg/kg (20% of lethal dose) of Atrazine
VCO after ATZ (SG 4)	123mg/kg (20% of lethal dose) of Atrazine	10ml/kg of Virgin Coconut Oil (VCO)
Untreated after ATZ (SG 5)	123mg/kg (20% of lethal dose) of Atrazine	10ml/kg of distilled water (H_2O)

Induction of Diabetes mellitus (DM)

150mg/kg body weight of alloxan monohydrate was injected intraperitoneally to induce diabetes mellitus (DM) (Etuk, 2010). The diabetic state was observed from about 48 hours by the symptoms of polyuria, polydypsia and glucosuria. After 72 hours, DM was confirmed with FBG concentration above 180 to 200mg/dL (Iranloye *et al.*, 2003) using a glucometer (ACCU-CHECK Active) and ACCU-CHECK compatible glucose test strips.

Preparation of Virgin Coconut Oil (VCO)

Matured dried coconut fruit (*cocos nucifera*) were harvested and identified by a botanist at the Department of Botany, University of Calabar. The method employed in extracting VCO was the modified wet extraction method of Nevin and Rajamohan, (2006). The hard white endosperm of the matured coconut was grated; 500ml H_2O was poured into the grated coconut and pressed via a muslin cloth to get coconut milk. The obtained milk was allowed to stay for around 18 hrs to encourage gravitational segregation into different segments. The demulsification process created three segments; water or aqueous segment at the lowest part, the coconut oil segment in the middle layer and cream or emulsion segment on top. The cream on the top segment was removed; the coconut oil was scooped and placed on low heat for about 5 mins for evaporation of moisture. The coconut oil gotten was then filtered with the aid of cotton wool, after which it was stored for further use at room temperature.

Evaluation of serum IL-6 concentration

RayBio Rat IL-6 ELISA kit (USA) was used for assay of IL-6 as employed by Rizzo *et al.*, (2012).

Assay Procedure

100µl standard solution, including 100µl test sample was separately pipetted into recommended wells with a covering, and incubated for about two and half hours at 18-25°C with delicate shaking. (The IL-6 available in the test sample should be binded with immobilized antibody present in each well). The solution

was then disposed, after which 300µl washing buffer was pipetted in all the wells and cleaned four times. When the final washing has been done, the plate was turned upside down and blotted on clean absorbent towels. Then, 100µl biotinylated anti-Rat IL-6 antibody will then be poured into all the wells and allowed to incubate for one hour at 18-25°C with light shaking. The resultant solution was then disposed of and the wells re-washed as before to remove unbound biotinylated antibody. After which, 100µl Horseradish Peroxidase-conjugated Streptavidin solution then was poured into each well and allowed to incubate for 45 minutes at 18-25°C with shaking gently. The resultant solution was disposed of, and then re-washed as before. Then, 100 µl of TMB One-Step Substrate Reagent was poured into the wells and allowed to incubate for 30minutes at room temperature in a dim space with delicate shaking. The resultant solution turns blue in relation to the amount of IL-6 bound. Finally, 50µl stop solution was poured to each well resulting in a change from blue to yellow colour and measured at 450nm intensity.

Evaluation of serum CRP concentration

The RayBio® Rat CRP (C Reactive Protein) ELISA kit (USA) was used as applied by Kordass *et al.*, (2016)

Assay Procedure

100µl standard solution, including 100µl test sample was separately pipetted into recommended wells with a covering, and incubated for about two and half hours at 18-25°C with delicate shaking. (The CRP available in the test sample should be binded with immobilized antibody present in each well). The solution was then disposed, after which 300µl washing buffer was pipetted in all the wells and cleaned four times. When the final washing has been done, the plate was turned upside down and blotted on clean absorbent towels. Then, 100µl biotinylated anti-Rat CRP antibody will then be poured into all the wells and allowed to incubate for one hour at 18-25°C with light shaking. The resultant solution was then disposed of and the wells re-washed as before to remove unbound biotinylated antibody. After which, 100µl Horseradish Peroxidase-conjugated Streptavidin solution then was poured into each well and allowed to incubate for 45 minutes at 18-25°C with shaking gently. The resultant solution was disposed of, and then re-washed as before. Then, 100 µl of TMB One-Step Substrate Reagent was poured into the wells and allowed to incubate for 30minutes at room temperature in a dim space with delicate shaking. The resultant solution turns blue in relation to the amount of CRP bound. Finally, 50µl stop solution was poured to each well resulting in a change from blue to yellow colour and measured at 450nm intensity.

Statistical Analysis

Windows SPSS package (SPSS 20.0) was used for the statistical analysis. One-way ANOVA were used to analyse the obtained data, after which it was subjected to Tukey's post hoc test. The obtained values were expressed as Mean ± Standard Error of Mean (Mean±SEM). Results with values of $P<0.05$ were accepted as significant**.**

3. RESULTS

IL-6 levels (pg/ml) in the test groups

Figure 1 showed that IL-6 levels was significantly ($p<0.05$) higher in group 2 (1.01±0.18 pg/ml) than group 1 (0.82±0.01 pg/ml). The IL-6 level in group 3 was significantly ($p<0.05$) lower than group 2 but significantly ($p<0.05$) higher than group 1. IL-6 levels in group 4 (0.95±0.02 pg/ml) was not significantly different from group 3 (0.90±0.08 pg/ml) but was significantly ($p<0.05$) lower than group 2 and significantly ($p<0.05$) higher than group 1. The IL-6 level in group 5 (95.99±0.37 pg/ml) was significantly ($p<0.05$) lower than all the other experimental groups.

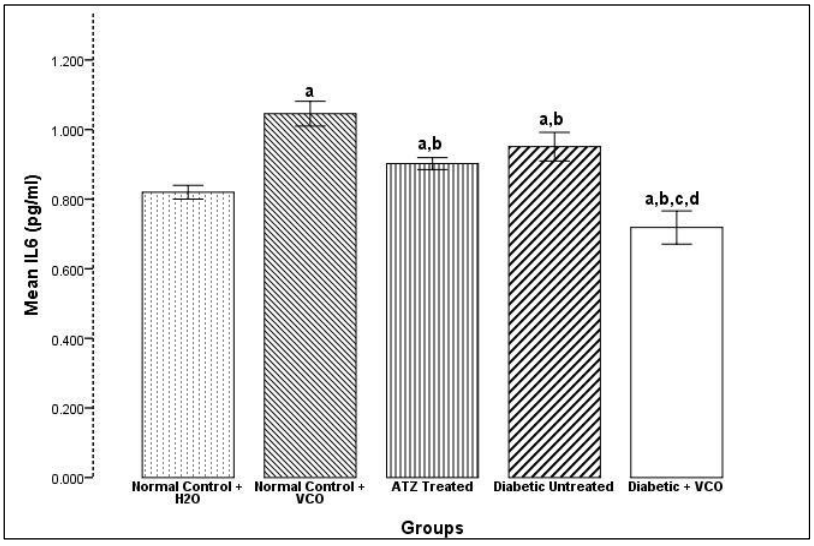

Fig 1: Comparison of IL-6 levels in the test groups

Values are mean ± SEM. n=7. a = p < 0.05 vs NC, b = p < 0.05 vs NC + VCO, c = p < 0.05 vs ATZ, d = p < 0.05 vs Diabetic control

IL-6 levels in normal control and ATZ recovery groups

The mean values for IL-6 in the recovery group are 0.83±0.15 pg/ml, 1.03±0.10 pg/ml, 0.91±0.11 pg/ml, 0.75±0.02 pg/ml, 0.92±0.12 pg/ml for groups 1, 2, 3, 4 and 5 respectively. IL-6 level in group 3 was significantly (p<0.05) lower than group 2 but significantly (p<0.05) higher than group 1. In group 4, the IL-6 level was significantly (p<0.05) lower than group 2 and 3 but there was no significant difference when compared with group 1. The IL-6 level in the group 4 was significantly (p<0.05) higher than the group 1 and 4, but was significantly (p<0.05) lower than the group 2 (Figure 2).

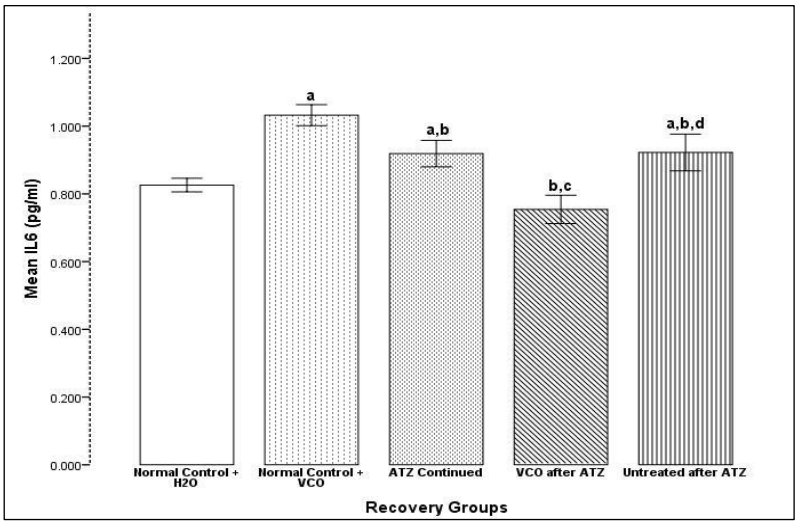

Fig 2: Comparison of IL-6 levels in the recovery groups

Values are mean ± SEM. n=7. a = p < 0.05 vs NC, b = p < 0.05 vs NC + VCO, c = p < 0.05 vs ATZ continued, d = p < 0.05 vs VCO after ATZ

CRP levels in the test groups

Figure 3 showed a significant (p<0.05) increase in CRP levels in group 2 (184.54±2.42 pg/ml) when compared with group 1 (171.85±1.51 pg/ml). There was a significant increase (p<0.05) in group 3 (213.76±2.17 pg/ml) when compared with groups 1 and 2. CRP levels significantly (p<0.05) increased in group 4 (220.20±1.77 pg/ml) when compared with groups 1 and 2. A significant (p<0.05) decrease in CRP levels was observed in group 5 (201.76±1.55 pg/ml) when compared with groups 3 and 4 but a significant increase was observed (p<0.05) when compared with groups 1 and 2.

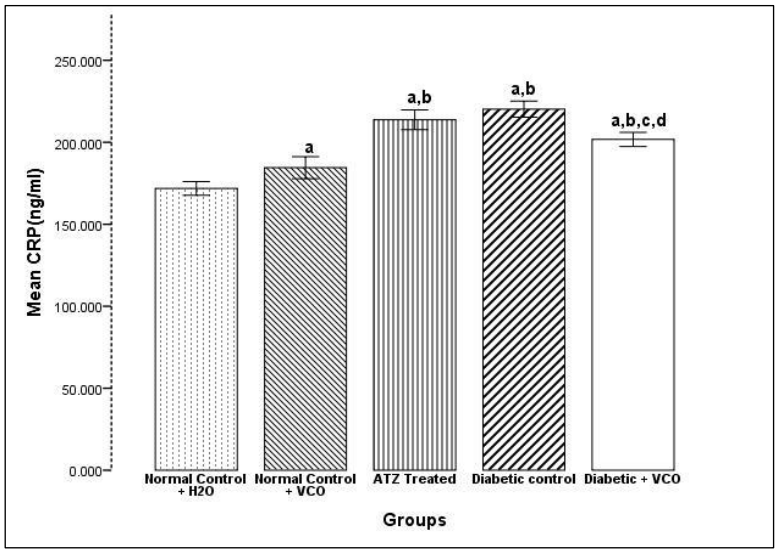

Fig 3: Comparison of CRP levels in the test groups

Values are mean ± SEM. n=7. a = p < 0.05 vs NC, b = p < 0.05 vs NC + VCO, c = p < 0.05 vs ATZ, d = p < 0.05 vs Diabetic control

3.4 CRP levels in the recovery groups

The results showed a significant ($p<0.05$) increase in CRP levels in group 3 (258.03±3.87 pg/ml) when compared with group 1 (173.07±1.15 pg/ml) and 2 (184.18±3.09 pg/ml) groups. Result showed a significant ($p<0.05$) reduction in CRP levels in group 4 (199.10±6.35 pg/ml) when compared with group 3 and a significant ($p<0.05$) increase when compared with group 1. In group 5 (236.55±2.84 pg/ml), the CRP levels increased significantly ($p<0.05$) when compared with groups 1, 2 and 4 but reduced significantly ($p<0.05$) when compared with group 3 (Figure 4).

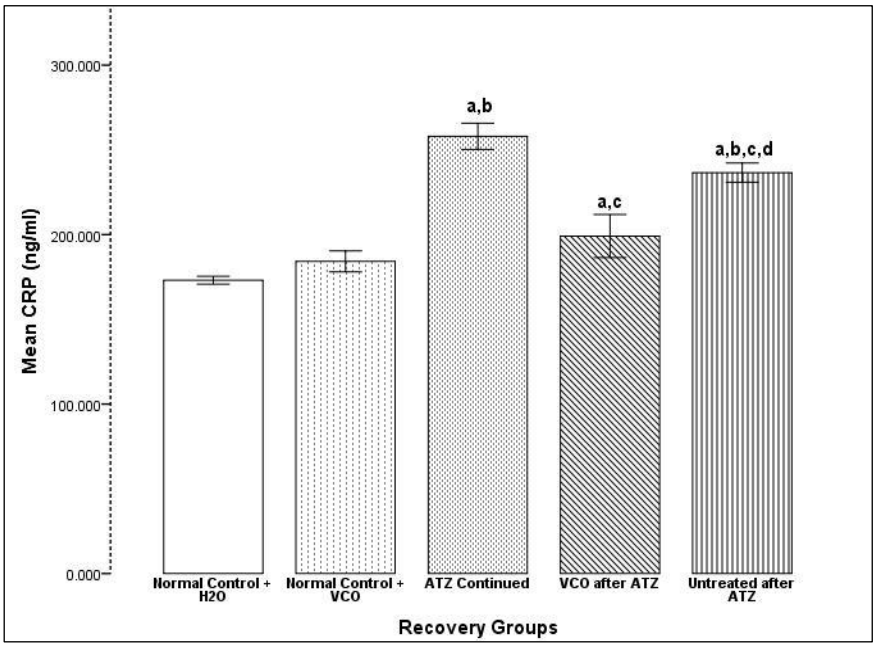

Fig 4: Comparison of CRP levels in the recovery groups

Values are mean ± SEM. n=7. a = p < 0.05 vs NC, b = p < 0.05 vs NC + VCO, c = p < 0.05 vs ATZ continued, d = p < 0.05 vs VCO after ATZ

4. DISCUSSION

Inflammation leads to elevated levels of inflammatory biomarkers (Kotulska *et al*., 2015). CRP is commonly connected with occurrence of diabetes in population or otherwise healthy persons (Sattar *et al*., 2008). Atrazine has also been observed to induce diabetes by causing inflammation giving rise to subsequent increase in inflammatory biomarkers (e.g. CRP)) (Stanko *et al*., 2010), the biomarkers then cause insulin resistance or may destroy the pancreas resulting in diabetes mellitus (Sattar *et al*., 2008). The result of this study indicated alteration in CRP levels of diabetic, atrazine and VCO treated rats compared to the normal rat groups. The results of the CRP levels in group 2 of the test was found to be significantly increased when compared to the CRP levels in group 1. The results might be unexpected because studies have shown the anti-inflammatory effect of VCO (Vysakh *et al*., 2014; Nair *et al*., 2016) but mostly not on normal animals but on acutely inflamed animal models. In a study by Haghikia *et al*., (2013), they added fatty acids ranging from C4 to C12 (from butyric acid to lauric acid) to naïve mouse T cells. Virgin Coconut Oil is about 45 – 52% rich in Lauric acid (Bergsson *et al*., 1998). They observed that as the length of hydrocarbon backbone increased, the number of T cells that differentiated into Th17 cells increased in a strikingly linear fashion. Th17 cells are meant to attack parasites and pathogenic bacteria but an abundance or imbalance T cells might offset inflammatory responses in the body (Kleinewietfeld and Hafler, 2014). Therefore, we could infer from our study that dose of VCO administered to group 2 probably offset and increased their Th17 cells therefore causing an increase in the CRP levels. The result of CRP levels in the group 3 (atrazine treated) was found to increase significantly when compared to the CRP levels in group 1 and 2; this showed that administration of atrazine increased the CRP levels. Atrazine is said to induce diabetes by inducing inflammation giving rise to subsequent increase in inflammatory biomarkers (e.g. CRP) (Stanko *et al*., 2010) the biomarkers then cause insulin resistance or may destroy the pancreas resulting in diabetes mellitus. Hence, atrazine increases CRP levels (and other inflammatory makers) as it induces inflammation (Liu *et al*., 2014). Inflammation attributable to oxidative disturbance has been proposed to be the underlying mechanism (Liu *et al*., 2014; Olatunbosun and Uka, 2021). In group 4, there was no significant increase in the CRP levels when compared with the CRP levels of group 3, but significant increase in the level of CRP was noticed when compared with group 1 and 2. This showed that in both diabetes mellitus and atrazine administration, CRP levels increased. In group 5, there was a significant reduction in the CRP

levels when compared with group 3 and 4 but significantly increased when compared with group 1 and 2. This showed that the administration of VCO to diabetic rat helps to reduce CRP level, if more time was given for the administration of VCO, the CRP levels of the diabetic rat could have been restored to normal irrespective of the fact that a significant increase was observed group 1 and 2. This supports the research by Intahphuak *et al.*, (2010) who reported that VCO reduces CRP levels. Its inhibitory role could be by reducing transudative weight, granuloma formation and serum alkaline phosphatase activity.

In the recovery group, the CRP level of group 3 was found to increase further when compared with the group 1 and 2, this supports the research by Stanko which stated that atrazine increases the CRP levels (Stanko *et al*., 2010). In group 4, the CRP level was found to be significantly reduced when compared with group 3, significantly increased when compared with group 1 and no significant increase when compared with group 2, this implies that VCO reduces CRP levels and capable of restoring the CRP levels to normal with time, this supports the research carried out by Intahphuak *et al*., (2010). In group 5, the CRP levels was found to increase significantly when compared to groups 1, 2 and 4 but reduced significantly when compared to group 3. This suggest that without administration of VCO or further exposure to atrazine, the rat may recover but the recovery will not be as effective as when treated with VCO. According to our results, we discovered that in normal rats, VCO could probably cause inflammation due to the increased levels of CRP but in both the alloxan induced diabetic rats and atrazine administered rats, which are inflammatory models; VCO showed the reported anti-inflammatory effects by reversing and reducing the CRP levels.

IL-6 is a proinflammatory marker which definitively cause development of type 2 DM and advancement of insulin resistance by initiating inflammation via regulation of cellular differentiation, multiplication, movement and cell death (Rehman *et al*., 2017). The result showed a significant increase in IL-6 concentration in group 2 compared to group 1. The result is in contrast with the study by Vysakh *et al.,* (2014) which showed that VCO possesses an anti-inflammatory effect thereby reducing inflammation and eventually inflammatory markers including IL-6. The increase in IL-6 levels could also be due to the increase in Th17 cells levels which could result in inflammation as also observed in the high levels of CRP. The IL-6 concentration in group 3 was significantly higher than group 1. Atrazine has been known to induce inflammation thereby causing increase in inflammatory markers. Report has also showed that atrazine causes inflammation in male rats and the inflammation severity was dose dependent. It has been recognized to increase incidence of prostrate inflammation in male wistar rats (Stanko *et al*., 2010). The results of this study also showed a significant increase in IL-6 in group 4 when compared to groups 1 and 2 but there no significant difference with group 3 and this could serve as an independent early predictor of type 2 diabetes mellitus proceeding its clinical onset (Spranger *et al*., 2003). In group 5, there was a significant decrease in IL-6 levels when compared to other experimental groups, hence, it seems that the anti-inflammatory action of VCO was only effective in the diabetic inflamed condition but in normal conditions it increased the level of IL-6. Famurewa *et al*., (2018) demonstrated that virgin coconut oil weakens Methotrexate stimulated pro-inflammation, because it considerably diminished NO, IL-6, and CRP in rodents that received methotrexate but pre-administered 5%-15% of virgin coconut oil. They ascribed the beneficial impact of VCO methotrexate-actuated oxidative stress to its ability to decrease MDA concentration, including boosting actions of SOD, GPx, CAT, and GSH level. Inflammation attributable to oxidative stress has been suggested to being the underlying mechanism of Atrazine (Liu *et al*., 2014). The antioxidative property of VCO against atrazine induced oxidative stress have been previously reported by Olatunbosun and colleagues, 2021, where they also observed an increase in SOD, CAT and GSH levels which could also be responsible for the anti-inflammatory effects observed in this report. VCO has also been reported to restore glucose and oestradiol metabolism (Titilope *et al*., 2021) and GLUT4 and insulin metabolism (Olatunbosun *et al*., 2021) in both diabetic and atrazine induced rats

Dietary polyphenols are documented to exhibit beneficial health effects by modulating inflammatory cascades and hence prevent development of diseases (Li *et al.*, 2014); they act by indirectly activating anti-inflammatory cytokines release (Maraldi *et al.,* 2014). These polyphenols can alter inflammation complex and thus drop the mediators of inflammation. However, Hamsi and colleagues had demonstrated that constantly heating VCO can make it loose its anti-inflammatory abilities (Hamsi *et al.,* 2015) The current beneficial health effects are in line with the previously reported antioxidant and anti-inflammatory effect of VCO against arthritis, cyclophosphamide, cardiovascular risk, insulin resistance and hepatic steatosis (Narayanankutty *et al*., 2017). Earlier report has also observed an anti-oxidative potency of VCO (Olatunbosun and Uka, 2021); therefore, we could attribute the anti-inflammatory effects in both alloxan induced diabetes and atrazine induction to polyphenols present in VCO.

The anti-inflammatory potency of VCO in both alloxan induced diabetes and atrazine induction could be attributed to the bioactive polyphenols in VCO which may modulate inflammatory networks resulting in a decrease in inflammatory mediators.

5. CONCLUSION

In conclusion, VCO supplementation caused an increase in inflammatory markers CRP and IL-6 in normal rats, suggesting that excess intake of VCO in normal condition could cause inflammation, therefore it is advised that it should be taken with caution in apparently normal conditions.

Ethical Approval

Maintenance and care of all animals were carried out in accordance with directives for animal experiments and the guide for the care and use of Laboratory Animals were strictly adhered to this study protocol was reviewed and approved by Faculty Animal Research Ethics Committee, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross Rivers State, with approval number [022PY30417]."

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