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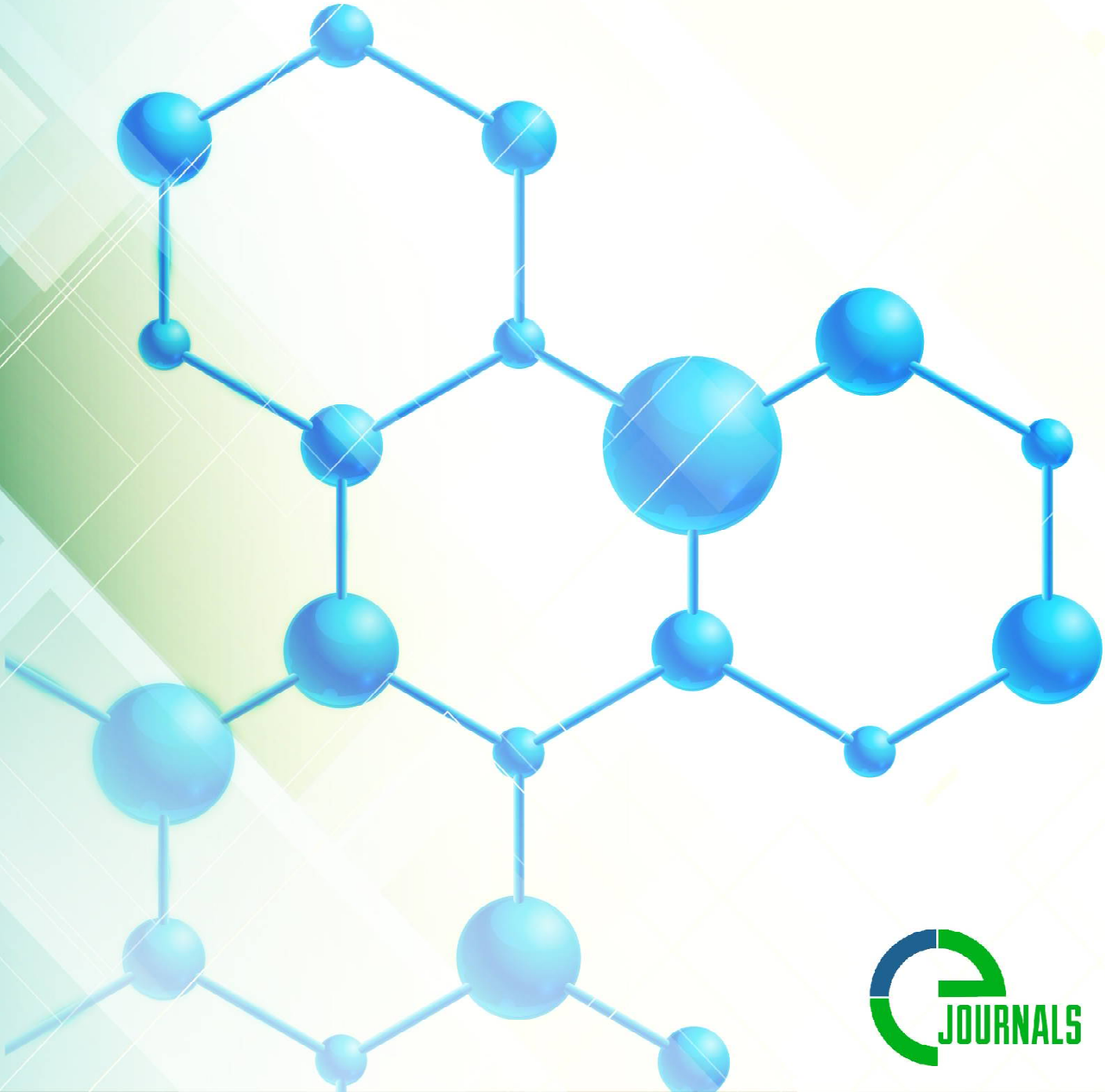

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PROTECTIVE ROLE OF VIRGIN COCONUT OIL AGAINST ADVERSE EFFECTS OF ATRAZINE TOXICITY ON GLUCOSE AND OESTRADIOL METABOLISM IN MALE WISTAR RATS

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Abstract.This study assessed protective role of Virgin Coconut Oil (VCO) following atrazine- induced glucose metabolic derangement in rats. Adult male albino wistar rats weighing 180-200 g body weight were used for the study. They were randomly separated into two major experimental groups (The test and recovery groups). Treatment in the test group lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis and treatment in the recovery group lasted for four weeks. In the test groups, the results of this study on glucose metabolism showed that Diabetes and Atrazine (ATZ) significantly increased ($p<0.05$) the fasting blood glucose level and reduced serum estradiol levels. In conclusion, Atrazine (ATZ) toxicity deranged glucose metabolism but the withdrawal of Atrazine (ATZ) significantly reversed some of these derangements; with more pronounced effect following Virgin Coconut Oil (VCO) administration.

Keywords: Virgin coconut oil; Atrazine; Glucose; Oestrogen; Diabetes; Environmental pollutants

Introduction.

Our health can be greatly affected by environmental stressors such as pollutants and chemicals that we are daily exposed to, and these have detrimental effects on the human body, which substantially contribute to major public health diseases [1]. Pollution is one of the most common risk factor for the increased rate of human diseases and has a link to several chronic ailments such as respiratory disease, cancers, cardiovascular diseases diabetes mellitus. In 2015, pollution killed over 9 million people in the world [2-7].

Diabetes mellitus is referred to a syndrome affecting the body's metabolism whose hallmark is excessive blood glucose or hyperglycemia which is due to a total reduction of insulin secretion or reduced insulin sensitivity in the tissues [8]. There is a high epidemiological and mortality rate of diabetes mellitus which has greatly affected our health, economic and social development [9]. By 2015, close to four hundred and fifiteeen million people have been reported to have diabetes worldwide and the rate in Nigeria is about 4 million. Diabetes mellitus was reported to cause about 105,091 deaths in Nigeria [9].

The most common risk factors for diabetes mellitus are genetics, diet, overweight or obesity and physical inactivity but it has been shown that these factors cannot completely account for the sudden increase in the prevalence of this disease; environmental pollutants are believed to be a major risk factor [10]. These pollutants can have a deleterious effect on a variety of biochemical and physiological processes and on structural organization within the cells [11]. Long-term exposure to environmental pollution is discovered to be linked to a greater risk of death in diabetic patient in comparison to patients without diabetes [12]. Additionally, people that have diabetes are more prone to dying or have an increased risk of being hospitalized due to heart or cardiovascular when they are

highly exposed to environmental pollution [13,14]. The assumption that environmental exposures are linked to metabolic disease has been shown by persistent organic pollutants and toxins that have consistently shown to be associated with Insulin Resistance (IR) and type II DM [15]. One of such endocrine disruptor of interest is Atrazine (ATZ) and the focus of the present study. It is one of the most commonly used herbicides worldwide and its use is on the increase in Nigeria [16]. Humans are exposed to ATZ in the air, water and food [17,18].

The use of Atrazine (ATZ) has been banned in Europe since 2005 because of some suspected detrimental effects on health and the environment [19].

It has been reported that substantial utilization of Atrazine (ATZ) might be related with the danger of obesity which is a pre- disposing risk factor for type II DM. Atrazine (ATZ) has been found to prompt overweight and insulin resistance in experimental rats by causing an impairment of the mitochondrial activity [20]. Atrazine (ATZ) has also been reported to cause inflammation by increasing inflammatory markers and chemicals which can incite diabetes mellitus [21]. Another report from showed a significant increase in fasting blood glucose levels in diabetic untreated and diabetic atrazine administered rats in comparison with normal rats [22]. In any case, no noteworthy difference in levels of plasma glucose was seen amid the diabetic rat administered Atrazine (ATZ) and diabetic untreatedrats demonstrating a conceivable connection among atrazine and diabetes mellitus [22].

It was initially accepted that oestrogens deleteriously affect metabolism of glucose, because elevated oral contraceptives dosages have incited intolerance of blood sugar in some females [23]. However, researches carried out on healthy and postmenopausal women affected by type II DM have demonstrated that utilization of oestrogen only, can decrease levels of blood sugar and boost sensitivity of insulin [24,25]. Additionally, report has shown the insulinotropic activity of oestrogen, as research in dogs and primates has demonstrated that oestrogens have the ability to ameliorate hyperglycemia after incomplete pancreatectomy [26]. Estrogens are main controllers of blood glucose homeostasis via metabolic homeostasis advancement, improvement of resistance to insulin and maintaining the function and survival of Я-cell. Loss of the primary circulating estrogen, 17-Esftroge n (E2) either through natural or surgically-induced menopause possesses impacts which surpass reproductive health. Lack of estrogen and the cellular activity disruption gives rise to a sudden decrease in metabolism, an increase in central obesity, lipid abnormalities, and worsening of the metabolic disorder.Collectively, all these progressions cause an increment in the danger of type II diabetes, nonalcoholic steatohepatitis, heart diseases and the factors aggravating it [27].

Virgin Coconut Oil (VCO) is dietary and medical nourishment of the customary regions growing coconut. Virgin Coconut Oil (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 [28]. 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 [29]. Other than lauric acid, short-chain fatty acids, like caprylic and capric caproic, acids are present in Virgin Coconut Oil (VCO), which have been reported to be anti-viral and anti-microbial [29]. Virgin Coconut Oil (VCO) has been found to have hypoglycaemic actions, enhance secretion of insulin and ameliorate oxidative strain in rats induced with non-insulin dependent diabetes [30].

Therefore, this research was designed to observe the effect of Atrazine on body weight, fasting blood glucose and oestrogen levels in experimental rats and any probable ameliorative effect of Virgin Coconut Oil (VCO).

Materials and Methods

Experimental animals

Matured albino male rats (180-200 g) Body Weight (BW) were purchased and maintained at the animal house Unit of the Department of Physiology, Faculty of Basic Medical Sciences, and University of Calabar. The animals were kept in a well-ventilated space and acclimatized for 2 weeks. The rats ate rat chow and drinking water freely. After acclimatization duration, they were weighed on an electronic weighing balance; their Fasting Blood Glucose (FBG) levels were measured and reassigned before commencing 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 of 7 rats per sub-group (n=7) and were oral gavaged and treated thus: Sub-Group (SG)1 served as normal control and received 10 ml/kg body weight of distilled water, Sub-Group (SG) 2 received 10 ml/kg of Virgin Coconut Oil (VCO), Sub- Group (SG) 3 received 123 mg/kg (20% of lethal dose) of Atrazine (ATZ), Sub-Group (SG) 4 was the diabetic control that were left untreated and Sub-Group (SG) 5 was the diabetic group that were treated with 10 ml/kg of Virgin Coconut Oil (VCO). Treatment in the test group lasted for 2 weeks, after which the animals were sacrificed and blood collected for analysis.

During these 2 weeks' period, thirty-five rats for the recovery group were also divided into 5 sub-groups of 7 rats per sub- group (n=7) and were treated as follows: (SG) 1 served as normal control and received 10 ml/kg body weight of distilled water, SG 2 received 10 ml/kg of Virgin Coconut Oil (VCO), SG 3, 4 and 5 received 123 mg/kg of ATZ. After 2 weeks, the animals were re-treated for recovery and were treated thus: (SG) 1 served as normal control and received 10 ml/kg body weight of distilled water, SG 2 received 10 ml/kg of Virgin Coconut Oil (VCO), SG 3 received 123 mg/kg of ATZ, SG 4 was treated with 10 ml/kg of Virgin Coconut Oil (VCO) and SG 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.

Induction of Diabetes Mellitus (DM)

150 mg/kg body weight of alloxan monohydrate was injected intraperitoneally to induce Diabetes Mellitus (DM) [31,32]. The diabetic state was observed from about 48 hours by the symptoms of polyuria, polydypsia and glucosuria. After 72 hours, DM was confirmed with Fasting Blood Glucose (FBG) concentration above 180 to 200 mg/dL using a glucometer (ACCU-CHECK Active) and ACCU-CHECK compatible glucose test strips [30].

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 Virgin Coconut Oil (VCO) was the modified wet extraction method of [33]. The hard white endosperm of the matured coconut was grated; 500 ml H2O was poured into the grated coconut and pressed via musflfin 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.

Determination of body weight

The body weight of the rats was determined before the commencement and at the end of the experimental treatment. This was done with the aid of an electronic weighing balance.

Determination of fasting blood glucose

Fiber Bragg Grating (FBG) was evaluated with the aid of ACCU- Check blood glucose meter with recommended blood glucose test strips. The tail tip was pricked and blood dropped gently on the test strip, after which the readings were noted. Fiber Bragg Grating (FBG) readings were taken before diabetes induction and 72 hours after induction; after which it was done weekly.

Estimation of serum oestrogen concentration

The Calbiotech, Inc (CBI) Oestrogen (E2) ELISA Kit (USA) for the quantitative evaluation of E2 concentration in rat serum and plasma was used as applied by [34].

Assay procedure

25 xl of the standard solution, control solution and test sample were separately pipetted to recommended wells and then 100 xl of oestrogen enzyme conjugate which is the working solution was added into each of the wells. A shaker was used to mix the combined solution very well for ten to twenty seconds, after which they were allowed to incubate at 18-25°C, room temperature for sixty minutes. At the incubation period, a specific quantity of Horseradish Peroxidase-labeled Estrogen competes with oestrogen endogenously available in standard solution, control solution or test sample for specific binding sites number of oestrogen antibody. As oestrogen concentration in each specimen increases, the concentrations of oestrogen peroxidase conjugate that is bound immunologically to the well gradually declined. The resultant solution of E2 peroxidase conjugate that which remain unbound was poured out from the wells and washed with 300 xL of wash buffer 3 times; the plate was then turned upside down and blotted on clean paper towels. Then, 100 microlitre of 3,3,5,5'-tetramethylbenzidine reagent was then poured into all the wells and mixed lightly for about ten seconds and incubated for 30 minutes at 18-25°C, resulting in development of blue color. The reactivity was halted by addition of 50 xl stop solution to all the wells and mixed lightly for 30 seconds for complete change of colour from blue to yellow. Finally, the absorbance was calculated at 450nm with the aid of a microplate reader within 15 minutes.

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 ë SEM). Results with values of $P<0.05$ were accepted as significant.

Results

Body weight changes in normal control, ATZ and diabetic groups

The initial body weight of all experimental groups were not significantly ($p<0.05$) different as shown in Table 1; but the final body weight of the Atrazine (ATZ) significantly ($p<0.05$) decreased when compared with the NC+H2O and NC+VCO. There was a significant ($p<0.05$) decrease in the final body weight of the diabetic untreated group when compared to the NC+H2O, NC+VCO and Atrazine (ATZ) groups. In the diabetic

group treated with VCO, the final body weight significantly ($p<0.05$) increased when compared to the diabetic untreated group.

Description

Vaccination is the management of a vaccine to assist the immune machine increase safety from a disease. Vaccines incorporate a microorganism or virus in a weakened, stay or killed state, or proteins or pollution from the organism. In stimulating the body's adaptive immunity, they assist save you illness from an infectious disease. When a sufficiently big percent of a populace has been vaccinated, herd immunity effects. Herd immunity protects folks who can be immune compromised and can't get a vaccine due to the fact even a weakened model might damage them. The effectiveness of vaccination has been extensively studied and verified.

Oestrogen activities in fat tissue, immune cells, skeletal muscle and liver are engaged in insulin tissue sensitivity, inflammation and accumulation of lipid.Oestrogen activities in pancreatic islet beta cells control insulin discharge, cellular nutrient homeostasis and survival. Oestrogen inadequacy elevates metabolic breakdown, which prompts metabolic disorder type II diabetes and obesity [63]. The result of this study showed no significant effect of Virgin Coconut Oil (VCO) on oestrogen level in normal rats showing that Virgin Coconut Oil (VCO) administration did not affect oestrogen levels in normal rats. There are conflicting reports about the oestrogenic potential of atrazine as an endocrine disruptor. Some reports suggest that atrazine is an environmental oestrogen while others have shown that atrazine could actually be an oestrogen antagonist [37-40,64]. Oestrogen level in the atrazine treated group decreased significantly comparable to the oestrogen levels in Co+H2O and Co+VCO groups. Several clinical investigations have documented the powerful defensive potential of estrogen against metabolic disorder and diabetes showing that the risk of diabetes increase as oestrogen level decreases [65,66]. This observation supports the regenerative toxicology research in rats sustained with atrazine diet at 500 ppm concentration; they observed a diminished oestrogenic response on vaginal cytology and oestrous cycling patterns following atrazine administration [67,68]. Documented that atrazine exposure to carp showed no impact on oestrogen-actuated generation of vitellogeni [69]. Found no evidence that atrazine effect had any naturally occurring oestrogen-mediated responses [70]. Hypothesized that triazines (atrazine, simazine and diaminochlorotriazine) might be able to express or modulate oestrogen activity using a rat uterine model system; they concluded that these triazines possess no oestrogen-like activity but possess a weak oestrogenic antagonist property [39]. From above reports, it seems that Atrazine (ATZ did not bind directly to oestrogen receptor and have no in vivo oestrogenic action, but was demonstrated to hinder estrogen-activated incorporation of 3H-thymidine and development of uterine invivo [39].Revealed that atrazine simazine, and cyanazine blocked estrogen-stimulated reaction in yeast cells, that is they have oestrogenic abilities [37]. However, did not uphold these findings; they reported a nor-inhibitory effect of atrazines on 17Я-oestrogen-induced trans-activation in yeast [71]. Also evaluated the potential of atrazine to trigger G Protein Oestrogen Receptor (GPER)-mediated signaling in cancer cells and cancer-associated fibroblasts and concluded that atrazine should be included among the environmental contaminants that may elicit oestrogenic activity through G Protein Oestrogen Receptor (GPER) mediated signalling [38]. This study therefore support that Atrazine (ATZ) might not possess an oestrogenic activity but might be an oestrogen antagonist. Various investigations have recommended that oestrogens have a significant influence on glucose homeostasis. They have shown that treatment with oestrogen reduces diabetic complications, and normalizes the endothelium role in diabetic condition [72,73]. Oestrogen receptors exist in islets of Langerhans (57 et al.) and the oestrogen effects in some functional part of

this islet are recorded [74,75]. In the alloxan induced diabetic untreated group, there was a significant increase in the oestrogen levels when compared with the oestrogen levels of the atrazine treated group, but significant decrease in the level of oestrogen was observed when compared with the normal control+VCO and normal control+H2O groups. This showed that oestrogen levels are reduced in diabetes mellitus. Hypogonadotropic hypogonadism, hypooestrogenism, menstrual irregularities, polycystic ovaries and early menopause have been described in T1DM women, which may be due to the relationshi p between decreased oestrogen levels and insulin action [76]. Clinical and experimental investigations have demonstrated a solid connection between estrogen inadequacy and metabolic disorder [77,78]. Women at their premenopausal age show more sensitivity to INL and lower occurrence of diabetes type II in relation to men at the same age. However, this beneficial effect vanishes in postmenopausal age with upset glucose homeostasis, which might be to an extent due to a decrease in plasma oestrogen [79]. In affirmation to this claim, clinical investigation on estrogen replacement treatment in women at their postmenopausal age showed a fall in INL resistance and a decrease in FBG concentration [80,81]. These reports further support the reduced level of oestrogen in the alloxan diabetic condition in this study. There was a significant increase in serum oestrogen level in diabetic group treated with Virgin Coconut Oil (VCO) when compared to the diabetic untreated group, though the level was significantly lower than the normal control groups. Osteoporosis one of the postmenopausal effects in elderly women is typically connected with estrogen inadequacy [82]. Worked on the impact of Virgin Coconut Oil (VCO)supplementation on bone loss in osteoporotic rat model; they reported that Virgin Coconut Oil (VCO) maintained structure of the bone, also prevented loss of the bone in oestrogen-deficient rodents [83]. They attributed these positive abilities of Virgin Coconut Oil (VCO) on bone micro-structure to its antioxidant ability. They deduced that the antioxidative properties of Atrazine, Virgin Coconut Oil (VCO) counteracted ROS-stimulated bone loss related to oestrogen inadequacy [83-85].

Conclusion

Therefore, in our oestrogen deficient diabetic rat model, Virgin Coconut Oil (VCO) was also effective in improving oestrogen level. It is suggested that if used for a longer period of time, the oestrogen level could possibly be restored to the normal level. These observations suggest that Virgin Coconut Oil (VCO) probably possess an oestrogen stimulating effect or uses another pathway for stimulating oestrogen synthesis and release which could be attributed to its polyphenolic constituent or lauric acid content.

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