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Impact of Virgin Coconut Oil and Carvedilol on Neurobehavior, Apoptotic and Inflammatory Brain Markers in Doxorubicin Treated Mice

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Abstract. One of the significant side effects of doxorubicin treatment for cancer patients is cognitive impairment, and the mechanisms underlying this impairment must be investigated to treat or prevent it. The current study investigates the impact of virgin coconut oil and carvedilol administration on neurobehaviour brain apoptotic and inflammatory markers in doxorubicin-treated mice. 32 male and 32 female mice were randomly assigned to four groups of 8 animals each, and the treatment lasted for twenty-eight days. Group 1 animals in both the male and female groups were the controls. In comparison, the Group 2 animals in both groups received doxorubicin dosage (3.75 mg/kg body weight) intraperitoneally weekly as a single dose on days 5, 12, 19 and 26 to make up for the desired amount (15 mg/kg body weight). Group 3 animals received doxorubicin and were orally treated with virgin coconut oil (5ml/kg body weight) for 28 days. Group 4 animals received doxorubicin and were treated with carvedilol (5 mg/kg body weight) weekly for three days (days 5-7 for four weeks). The histological analysis of the brain tissue was done by staining the tissues with haematoxylin and eosin. The data was analyzed using GraphPad Prism 9.0. Analysis of Variance (ANOVA) was used to compare between groups. All results were presented as mean±SEM. Both virgin coconut oil and carvedilol demonstrated ameliorative effects on neurobehaviour and apoptotic and inflammatory brain markers.

Keywords: Virgin Coconut Oil; Carvedilol; Cancer.

INTRODUCTION

Chemobrain, a term referring to cognitive impairment induced by chemotherapy, has gained recognition as a significant concern for cancer survivors, as acknowledged by the National Cancer Institute. It significantly diminishes the quality of life for these individuals and hinders their ability to return to their pre-cancer lifestyles [1–3]. Chemobrain manifests through various symptoms, including memory loss, reduced processing speed, difficulty concentrating, and language problems, often stemming from the compromised hippocampus and frontal circuit function [4].

Notably, Doxorubicin is frequently part of the chemotherapy regimens for breast cancer survivors who experience chemobrain [5]. Doxorubicin, an anthracycline topoisomerase II inhibitor, is an FDA-approved, highly effective cornerstone for treating various cancers [6, 7]. However, its clinical effectiveness is accompanied by multi-organ toxicity, including life-threatening cardiotoxicity, hepatotoxicity, nephrotoxicity, and troubling neurotoxicity, which limits its application [8–12]. Despite being unable to breach the blood-brain barrier (BBB), doxorubicin-induced chemofog is believed to result from cytokine-induced oxidative and nitrosative damage to brain tissues [13–15].

Given the increasing number of cancer survivors experiencing doxorubicin-induced chemobrain, there's an urgent need to address and even prevent this potentially life-threatening cognitive impairment without compromising Doxorubicin's anti-cancer effectiveness.

Doxorubicin triggers the production of reactive oxygen species (ROS), leading to oxidative damage in cellular and mitochondrial membranes and cellular macromolecules [17]. Furthermore, it induces inflammation in blood vessels and the heart by up-regulating NF-kB expression [18]. Programmed cell death, including apoptosis and autophagy, is also recognised as a significant contributor to the pathogenesis of doxorubicin-induced toxicity [17, 19, 20].

Several treatments are employed as cardioprotective agents against doxorubicin-induced cardiomyopathy, with β-blockers like carvedilol notable examples [21]. Carvedilol, also known as Coreg, exhibits antioxidant properties and inhibits the generation of oxygen radicals, which is attributed to its carbazole moiety [22]. Studies have confirmed that carvedilol protects against hepatoxicity and cardiotoxicity induced by Doxorubicin, as evidenced by biochemical and histopathological examinations of liver and cardiac tissues [23. The protective effects of carvedilol against doxorubicin-induced cardiotoxicity have been reported in various rodent models and human studies [24–27].

Modulating immune functions using medicinal plants and their derivatives has become an accepted therapeutic approach [28]. This has led to investigating several plant extracts and their immunomodulatory properties for potential benefits in mitigating the adverse effects of cancer chemotherapy [29]. Virgin coconut oil, in particular, has gained popularity in clinical research and its role as a functional food oil. Virgin coconut oil is derived from the fresh, mature kernel of the coconut palm (Cocos nucifera L.) and is rich in nutritional and medicinal value. It contains biologically active components like polyphenols, tocopherols, sterols, squalene, and medium-chain fatty acids [30]. These compounds have been implicated in providing beneficial effects such as binding prooxidant iron, scavenging reactive nitrogen, chlorine, and oxygen species, and possibly inhibiting cyclooxygenases and lipoxygenases [31]. Lauric acid, a 12-carbon medium-chain fatty acid in virgin coconut oil, has been associated with antihypertensive, antibacterial, antifungal, antiviral, and hypoglycemic properties [32]. Additionally, pure coconut oil has been clinically established as an effective moisturiser for patients with atopic dermatitis due to its anti-inflammatory and anti-infective properties and its skin barrier protective effects [33].

Justification of study. Neurotoxicity, cardiotoxicity and hepatotoxicity resulting from the use of the anthracycline doxorubicin have been reported in patients undergoing chemotherapy. Not only are patients vulnerable to chronic heart diseases that arise from doxorubicin use, but in extreme cases may lead to death. Carvedilol and virgin coconut oil have been reported to impact health positively, attenuating and preventing cardiovascular diseases and lowering cholesterol levels. Carvedilol possesses an antioxidant effect and inhibits oxygen radical generation. At the same time, virgin coconut oil contains bioactive components such as polyphenols, which positively impact cardiovascular function and neurological function, hence the need to study the ameliorative effect of carvedilol and virgin coconut oil following doxorubicin therapy.

This study investigates the impact of virgin coconut oil and carvedilol on neurobehaviour apoptotic and inflammatory brain markers in doxorubicin-treated mice.

Specific objectives

1. To evaluate the possible neuroprotective effect of carvedilol and virgin coconut oil in doxorubicin-treated mice.

2. To investigate the influence of Doxorubicin on brain inflammatory markers in mice.

3. To investigate the influence of Doxorubicin on brain apoptotic markers in mice.

4. To determine the impact of carvedilol and virgin coconut oil on brain apoptotic and inflammatory markers in doxorubicin-treated mice.

5. To compare how Doxorubicin, carvedilol and virgin coconut oil modulate cognition using the Y-maze test.

Significance of study. At the end of this study, it will Broaden our knowledge of the impact of carvedilol and virgin coconut oil in doxorubicin-treated mice, reduce the lack of information concerning the use of carvedilol and pure coconut oil in ameliorating neurotoxicity and add to other literature and improve learning.

Neurotoxicity

Neurotoxicity arises when harmful substances disrupt the nervous system's normal functioning, whether naturally occurring or human-made (neurotoxicants). This disruption can lead to malfunctions or even the death of neurons, the cells responsible for transmitting and processing signals in the brain and throughout the nervous system. The impact of anticancer therapies on the nervous system has been recognised for a considerable time. Both chemotherapy and radiotherapy can result in significant side effects affecting both the central and peripheral nervous systems, potentially shortening the duration of treatment.

It might be expected that the nervous system would be relatively shielded from the toxic effects of chemotherapy and radiotherapy due to protective mechanisms like the blood-brain barrier, blood-cerebrospinal fluid barrier, and blood-nerve barrier, as well as the limited capacity of neurons to regenerate. However, neurotoxicity ranks as the second most significant dose-limiting factor in cancer treatment, following only myelosuppression [34]. Nervous system toxicity can be attributed to various factors, including the treatment dosage, method of administration, interactions with other medications, the presence of pre-existing structural jumpy system conditions, and individual patient susceptibility, many of which remain poorly understood [35]. Toxicity can manifest directly through damage to neurons or glial cells or indirectly through alterations in the local microenvironment, such as vascular injuries [36].

Overview of the brain

The brain, a delicate organ weighing approximately three pounds, is enclosed within the protective cranium of the skull. It is surrounded by the meninges, comprising three layers: the outer, robust dura mater, the delicate and web-like middle arachnoid, and the innermost highly vascular pia mater, adorned with numerous blood vessels. The space between these layers is filled with cerebrospinal fluid, crucial for brain protection and function. The brain is not uniform; it consists of various parts and regions that collectively enable its remarkable functions, including regulating emotions, creativity, and learning [37].

The brain can be anatomically categorised into three main sections: the forebrain, the midbrain, and the hindbrain.

The forebrain comprises two significant components: the cerebrum and the diencephalon, with the cerebrum being the most substantial part [37]. The cerebrum is further divided into the left and right cerebral hemispheres. This prominent structure governs and initiates voluntary muscle contractions, processes sensory input from various sense organs, and facilitates complex mental activities such as thinking, reasoning, planning, and memory.

The diencephalon, positioned beneath the cerebrum, is another integral region of the forebrain and consists of two principal components: the thalamus and the hypothalamus. The thalamus acts as a relay station for sensory impulses, including those related to sensations of pain and pleasure, transmitting them to the cerebrum for further processing. Meanwhile, the hypothalamus, located below the thalamus, regulates motivated behaviours such as eating, drinking, and sexual activity.

The midbrain, a relatively small tubular segment, connects the forebrain to the hindbrain [38].

The hindbrain encompasses the cerebellum, pons, and medulla oblongata. The cerebellum, situated beneath the cerebrum, maintains body balance and coordinates muscular activities. The pons and medulla adjacent to the spinal cord are essential for controlling numerous involuntary bodily functions.

Anthracyclines

The introduction of anthracycline antineoplastic antibiotics has marked a significant advancement

in cancer medicine. Anthracyclines, a group of drugs derived from Streptomyces spp., treat various cancer types. This group encompasses Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mitoxantrone, and Valrubicin. The initial two anthracyclines, daunorubicin (also known as daunomycin or rubidomycin) and Doxorubicin (also known as adriamycin), were discovered in the 1960 from Streptomyces peucetius, an actinobacteria species [39].

While daunorubicin has shown high effectiveness against acute lymphoblastic and myeloblastic leukaemias, Doxorubicin exhibits a broader spectrum of anticancer activity, encompassing various solid tumours and haematological malignancies. The primary risk factor for anthracycline-associated cardiotoxicity is the cumulative dose, with other contributing factors including administration schedule, mediastinal radiotherapy, combination therapy, age (both very young and old), gender, ethnicity, hypertension, previous cardiovascular disease, chromosomal abnormalities, and liver disease [40]. Anthracycline-induced cardiotoxicity typically manifests as a reduction in ejection fraction (EF), becoming noticeable sometime after therapy.

Despite the extensive use of anthracyclines, the precise mechanism of their antineoplastic action remains a subject of debate. It seems to involve a combination of several tools, explaining the high efficacy of this class of anticancer drugs [8, 41]. Initially, the anticancer effect of anthracyclines was attributed to their ability to insert between base pairs of DNA strands, a process known as intercalation, which prevents the replication of rapidly dividing cancer cells [42]. However, more recent studies have suggested that intercalation may not play a significant role at clinically relevant anthracycline concentrations.

Doxorubicin. Doxorubicin is derived as a secondary metabolite from Streptomyces peucetius var caesius and is classified within the anthracycline family [43]. It has gained recognition as a highly effective antineoplastic agent employed in the treatment of various cancers, including breast cancer, solid tumours like Wilms' tumour, leukaemia, Hodgkin's disease, non-Hodgkin's lymphomas, and numerous other cancer types [44]. Nonetheless, the utilisation of Doxorubicin has been accompanied by adverse effects such as hematopoietic suppression, nausea, vomiting, extravasation, and alopecia. Among these side effects, cardiotoxicity remains the most concerning and feared complication associated with doxorubicin therapy.

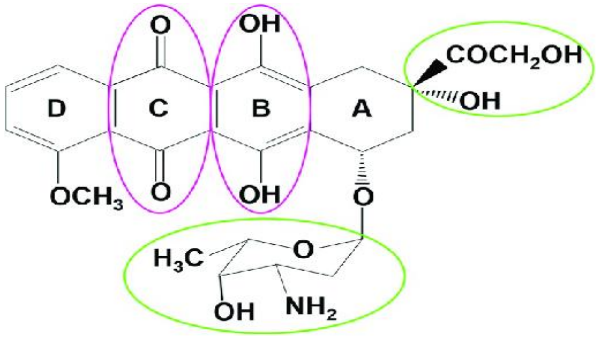


Figure 1 – Chemical Structure of Doxorubicin [45]

Doxorubicin stands out as one of the most potent antineoplastic drugs, either prescribed individually or as part of combination therapy, within its drug class. Among its class of compounds, it boasts the broadest spectrum of activity. Studies have attributed Doxorubicin's remarkable activity to its capacity to intercalate into a deoxyribonucleic acid (DNA) helical structure and covalently bind to proteins involved in DNA transcription and replication [46]. These interactions inhibit DNA, RNA, and even protein synthesis, ultimately leading to cell death.

Doxorubicin-Induced Neurotoxicity. In both clinical and academic settings, the relationship between chemotherapy and cognitive function has become a prominent topic of discussion [47, 48]. Recent studies involving women with breast cancer have highlighted changes in cognitive performance associated with chemotherapy. Several retrospective studies have investigated cognitive performance months to years after chemotherapy and have found long-term mental abnormalities linked to chemotherapy. The term "chemobrain" has been coined by the media to describe the cognitive decline experienced as a result of chemotherapy [49–54].

Cognitive functions in the brain encompass various aspects of healthy brain activity, including attentiveness, executive function, information processing speed, concentration, motor skills, language, visuospatial abilities, and learning and memory [55]. The term "chemobrain" was first introduced by [11] to describe cognitive function decline associated with chemotherapy. Subsequent studies have provided increasing evidence supporting the notion that chemotherapy can

lead to impairments in specific cognitive domains [10, 11, 49, 53–61].

A more recent study by [10] addressed some previously unexplored aspects, including changes in cognitive function over time, potential correlations between cognitive function and factors such as anxiety, fatigue, depression, haemoglobin levels, menopausal status, and self-perception of cognitive function. The primary findings of this study indicate that chemotherapy may hurt certain aspects of cognitive function, leading to decreases in total cognitive scores and visuospatial skills [10]. Another study on chemotherapy-induced cognitive impairments suggests that the adverse effects of chemotherapy on cognitive function may recover within one year after treatment cessation [61].

Although Doxorubicin does not typically penetrate the blood-brain barrier [14, 62], it can indirectly affect the brain. Research by [14] has provided direct biochemical evidence that doxorubicin-induced neurotoxicity is mediated by TNF-α, as elevated TNF-α levels have been observed in the cortex and hippocampus of doxorubicin-treated mice [13, 14].

Mechanism of action of Doxorubicin

While extensive basic and clinical research has been conducted for decades, the precise molecular mechanisms underlying doxorubicin-induced cardiotoxicity remain incompletely understood. Understanding these mechanisms is crucial for developing effective cardioprotective strategies.

Doxorubicin exerts its mechanism of action primarily by intercalating into DNA base pairs, leading to DNA strand breaks and inhibiting both DNA and RNA synthesis. Additionally, Doxorubicin inhibits topoisomerase II, resulting in DNA damage and the induction of apoptosis. When Doxorubicin interacts with iron, it generates free radicals, causing oxidative stress and further inhibiting DNA synthesis [63]. The cause of doxorubicin-induced cardiotoxicity is multifactorial, with interconnected mechanisms that include oxidative stress, iron accumulation, topoisomerase IIβ, inflammation, and apoptosis.

Oxidative stress arises from elevated intracellular levels of reactive oxygen species (ROS) and has long been considered a central mediator of doxorubicin-induced cardiotoxicity. The major types of ROS include the superoxide radical (O_2-), hydrogen peroxide (H_2O_2), and the hydroxyl free radical (HO) [64]. ROS are primarily generated through redox cycling in mitochondria [64]. However, they can also be produced outside mitochondria by prooxidant enzymes like NADPH oxidase and xanthine oxidases [65]. Under normal conditions, ROS function as signalling molecules and contribute to the cell's defence system. The body has an efficient antioxidant defence system to eliminate excess ROS and maintain physiological ROS levels [66]. However, an imbalance favouring ROS production over the antioxidant system leads to oxidative stress, resulting in harmful events such as DNA damage, mitochondrial dysfunction, disrupted cellular calcium homeostasis, apoptosis, impaired protein synthesis, and defects in protein and mitochondrial quality control [67]. Doxorubicin accumulates specifically in mitochondria after treatment, promoting ROS generation and impairing antioxidant enzyme activities, thus elevating ROS levels. Excessive ROS can damage mitochondria, creating a vicious cycle known as ROS-induced ROS release [68].

Doxorubicin-induced cardiotoxicity is associated with iron accumulation in the mitochondria following doxorubicin administration. Overexpression of ABCB8, a mitochondrial inner membrane protein involved in iron export, reduces mitochondrial iron accumulation and mitigates doxorubicin-induced cardiotoxicity [69]. Patients with doxorubicin-induced cardiotoxicity have been found to exhibit higher mitochondrial iron levels compared to patients with other cardiomyopathies or normal cardiac function [69].

Topoisomerase II (Top II) is an enzyme that creates double-strand breaks in DNA, crucial for controlling conformational changes and chromosome structure. Doxorubicin's anticancer activity involves the formation of the Top II-DOX-DNA ternary complex, also known as the cleavage complex [70]. In cardiomyocytes, where Doxorubicin targets Top II, increased DNA cleavage, particularly Top IIβ complexes, induces DNA damage and subsequent cell death [70].

Cardiac inflammation contributes to doxorubicin-induced toxicity, as doxorubicin treatment enhances the activity of nuclear factor kappa B (NF-kB), a vital component of the innate immune system, leading to elevated levels of pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α [71]. Doxorubicin-induced oxidative stress and damage-associated molecular pattern molecules

(DAMPs) activate toll-like receptors (TLRs), mainly TLR2, which, in turn, start NF-kB [71]. TLR9 may also play a role in cardiac inflammation in doxorubicin-induced cardiotoxicity [72].

Doxorubicin induces apoptosis, contributing to cardiotoxicity. Doxorubicin stimulates the generation of ROS and oxidative stress, which activate p53. The activated p53 signalling pathway promotes the apoptosis of cardiomyocytes [73, 74]. Calcium overload triggers mitochondrial dysfunction, leading to the rupture of the outer mitochondrial membrane and the release of cytochrome C, ultimately resulting in cardiomyocyte apoptosis [75].

Effects of Doxorubicin on apoptotic brain markers. Programmed cell death is a highly conserved and universal phenomenon in numerous biological processes, encompassing tissue regeneration in multicellular organisms and the removal of aged or damaged cells [76, 77]. As the role of mitochondrial dysfunction in prooxidant redox alterations gains recognition as a crucial event in various types of cell death, including apoptosis, our study sought to investigate the impact of in vivo doxorubicin-induced injury on the expression of pro- and antiapoptotic Bcl-2 family proteins, namely Bax and Bcl-2. The relative expression levels of these proteins within the mitochondrial outer membrane are believed to play a pivotal role in determining the cell's fate [78].

Recent research has shown that Doxorubicin induces apoptosis in the rat heart [79, 80], and these processes have been linked, at least partially, to mitochondria-mediated pathways [81, 82]. This underscores the significance of understanding the molecular mechanisms underlying doxorubicin-induced cell death, particularly within mitochondrial function and the delicate balance between pro- and antiapoptotic factors.

Effects of Doxorubicin on inflammatory brain markers. Cardiac tissue inflammation is a well-documented side effect associated with exposure to Doxorubicin, as observed in multiple animal studies [83–85]. Extensive research has provided compelling evidence that Doxorubicin instigates a series of inflammatory responses within the myocardium. This includes NF-B upregulation and the subsequent production of various pro-inflammatory cytokines, notably TNF-α [86, 87].

Recent studies have shed light on the progressive increase in pro-inflammatory cytokines within brain tissues, which is now recognised as a critical factor in the pathophysiology of doxorubicin-induced neurotoxicity [88].

Consistent with prior findings, our current investigations also underscore the pivotal role of inflammation in the development of doxorubicin-induced neurotoxicity. A significant increase in cardiac TNF-α and IL-1 levels was observed in the doxorubicin-exposed groups compared to the control groups [89].

The precise underlying mechanism driving this elevation in inflammatory markers remains to be fully elucidated. However, it is plausible that factors such as reduced tissue antioxidant capacity, heightened levels of ROS (reactive oxygen species), and ensuing lipid peroxidation may be contributing elements. Increased oxidative stress has been linked to the upregulation of inflammatory mediators, potentially inciting inflammatory responses through the activation of the NF-kB pathway, ultimately resulting in cytokine transactivation [85].

Agents Used in Ameliorating Dox-Induced Toxicity

Various drugs and interventions are employed to mitigate the cardiotoxic effects of doxorubicin-induced toxicity, primarily through antioxidant mechanisms or chelating agents. Here are some critical interventions and their respective products.

Dexrazoxane. Dexrazoxane hydrochloride is a cardioprotective agent and an antidote for extravasation effects [90, 91]. It operates by preventing the formation of anthracycline-iron complexes and free radicals. Dexrazoxane's conversion to an open-ring derivative, ADR-925, intracellularly chelates iron, reducing the generation of oxygen-free radicals and mitigating doxorubicin-induced cardiotoxicity [92].

Statins. Statins, typically used to manage hyperlipidemia and dyslipidemia, also impact the cardio-metabolic profile. Studies by [93] demonstrated that they can lower the cardiotoxic effects of Doxorubicin when administered concurrently with the lipid-lowering and antioxidant agent probucol. This combination increased antioxidant enzyme activity and decreased lipid peroxidation [94].

Calcium Channel Blockers. Calcium channel blockers offer protection through their antioxidant effects, which involve direct scavenging,

preservation of glutathione peroxidase enzyme activity, and inhibiting lipid peroxidation. Additionally, they reduce oxygen consumption and ischemic perfusion injury [95].

Beta-Blockers. Beta-blockers like carvedilol prevent doxorubicin-induced cardiotoxicity by reducing myocardial strain without interfering with Doxorubicin's therapeutic efficacy. They achieve this by avoiding calcium overloading independently of beta-adrenoceptors [95, 96].

Renin-Angiotensin System (RAS). Angiotensin-converting enzyme inhibition (ACEI) administered prophylactically has demonstrated partial cardioprotection in acute and chronic animal models of doxorubicin-mediated cardiotoxicity. ACEI can also prevent a decline in cardiac function in cancer patients receiving high-dose Doxorubicin [95].

Natural Products. Several natural products and medicinal plants have shown promise in offering cardio protection, providing alternatives to synthetic drugs with potentially toxic side effects. Some examples include:

Parkia biglobosa*.* This savanna tree has medicinal properties in traditional medicine, potentially protecting against cardiotoxicity through its antioxidant and anti-hyperlipidemic activities [97].

Vitexin*.* It may effectively ameliorate doxorubicin-induced cardiotoxicity by attenuating oxidative stress, reducing cardiac inflammatory cytokines, and inhibiting caspase-3 activation [98].

Melissa Officinalis*.* This plant has exhibited potent anti-tumour effects and may protect against cardiotoxicity by modulating oxidative stress, reducing inflammation, and preventing apoptosis in the heart [99].

Beet Root Juice. High in nitrates and antioxidants, beet root juice is considered a safe and natural chemo-preventive agent, especially when combined with Doxorubicin to provide cardio protection and chemoprevention [100].

This research, however, focuses on managing doxorubicin-induced cardiotoxicity using carvedilol and virgin coconut oil as interventions.

Virgin coconut oil

Virgin coconut oil (VCO) is derived from the fresh mature kernels of the coconut palm (*Cocos nucifera* L.). It is obtained without heat or chemical refining through mechanical or natural methods [101]. VCO is rich in biologically active compounds, including polyphenols, tocopherols, sterols, squalene, and medium-chain fatty acids, particularly lauric acid [30]. Beyond its role as a functional food oil, VCO has gained popularity for its therapeutic potential.

Research has unveiled several health benefits associated with virgin coconut oil. Studies have shown that VCO can effectively reduce oral microbial load, plaque, and gingival indices, indicating its potential as an oral health aid [102]. Additionally, VCO has demonstrated antihypertensive, antibacterial, and anti-inflammatory properties [103, 104]. Clinical research has revealed that VCO can alleviate symptoms of skin disorders by promoting skin relaxation, reducing cutaneous inflammation, and enhancing the skin's epidermal barrier function and hydration. These effects benefit individuals with atopic dermatitis [105].

Furthermore, VCO contains vitamins and polyphenols essential in its antioxidant and anticarcinogenic effects. These compounds are present in significant concentrations, contributing to the potential health advantages of VCO [106]. Studies have even linked VCO consumption to a reduced risk of atherosclerosis and its associated consequences [107, 108]. VCO has emerged as a versatile and promising natural product with various health-promoting properties.

Bioactive Components in Virgin Coconut Oil. Antioxidants such as phenolic compounds and phytosterols have been linked to a reduction in the risk of noncommunicable illnesses.

Phytosterols. Due to the lipophilic nature of phytosterols, they are more concentrated in virgin coconut oil. Because of their chemical nature, phytosterols have been shown to inhibit cholesterol absorption. Phytosterols compete with cholesterol for micelle mixing, inhibiting cholesterol absorption in the small intestine. Phytosterols help reduce inflammation among patients with autoimmune diseases such as rheumatoid arthritis and lupus.

Flavonoids and other polyphenols. Simple phenols, phenolic acids, hydroxycinnamic acid and its derivatives, and flavonoids are all phenolic chemicals. Phenolic chemicals have the potential to influence carcinogenesis through a variety of methods. These substances may scavenge carcinogens and free radicals. They could prevent the production of reactive oxygen species. Phenolic chemicals may potentially inhibit cellular growth by modulating protein kinase C activity. A

small number of phenolics may have antimutagenic effects.

Phospholipids. Phospholipids, the second most common type of lipid present in all living things after triglycerides, are the primary building blocks of life, with emulsifying and wetting properties that aid in the digestion and absorption of fatty foods. Lecithin, one of the most frequent phosphatides, is found in the brain, lungs and spleen.

Tocopherols. These are antioxidants that have a saturated phytyl side chain. The amount of tocopherols in coconut oil is lower than other vegetable oils.

Tocotrienols. A biologically active substance synonymous with tocopherols is collectively called tocols. Like in tocopherols, natural tocotrienols are also present in alpha, beta, gamma and delta tocotrienols.

Phytosterols. They are saturated phytosterols. It has been identified to have cholesterol-lowering activity. Phytosterols displace cholesterol from bile micelles.

Pharmacological potential of virgin coconut oil. *Cardio-protective Action.* Virgin coconut oil is now being researched as a test medicine in several research projects for its potential to treat various diseases. Authors [109] investigated the efficacy of VCO in the heated palm oil-induced lipid peroxidation of rat cardiac tissue, as virgin coconut oil dramatically reduced MDA levels that were elevated as the negative effect of the wild palm oil.

The efficiency of VCO as a cardioprotective agent in rats was demonstrated in rats with the same paradigm of heated palm oil-induced hypertension. VCO reduces blood pressure by decreasing AchE activity through an antioxidant mechanism [109].

Anti-cancer Effect. Nearly 17 cancer-related proteins have been demonstrated to be targeted by medium-chain fatty acids from VCO, such as lauric acid, caprylic acid, and myristic acid. The author [110], uncovered new molecular mechanisms via which lauric causes anti-proliferative and pro-apoptotic effects in both breast and endometrial cancer cells in a study including breast and endometrial cancer cells. According to the findings, lauric acid enhanced reactive oxygen species levels and induced apoptosis in breast and endometrial cancer cells, as evidenced by morphological alterations. A prospective analysis by [111] of breast cancer patients with an average age of 50.2 years, VCO treatment improved the quality of life in the VCO intervention group compared to the control group. The intervention group saw improvements in breast function and symptom ratings for body image, sexual function, breast problems, and systemic therapy side effects.

Neuroprotective effects. In the Alzheimer's disease model, VCO revealed a possible preventive impact for memory improvement, anti-excitotoxicity, and antioxidants [112]. VCO improved spatial memory, boosted acetylcholine and antioxidants, and decreased acetylcholinesterase, interferon-γ, IL-1β [113, 114].

Carvedilol

Carvedilol, also known as Coreg, is a multifaceted medication with properties that include being a β-adrenoreceptor antagonist, a vasodilator, a potent antihypertensive agent, and an antioxidant with anti-apoptotic capabilities [115, 116]. Its versatile pharmacological profile has made it a valuable tool in treating various medical conditions. Carvedilol has been employed in managing congestive heart failure, mild to moderate hypertension, and myocardial infarction [117, 118]. Particularly noteworthy is its role as a cardioprotective agent against doxorubicin-induced cardiotoxicity [119].

Carvedilol's antioxidative properties are linked to its ability to inhibit the generation of oxygen radicals, a trait associated with its carbazole moiety [22]. Furthermore, carvedilol aids in restoring calcium balance by enhancing sarco/endoplasmic reticulum Ca_2+-ATPase2a (SERCA2a) activity within muscle cells. In the context of doxorubicin-induced cardiotoxicity, carvedilol exhibits anti-inflammatory effects by significantly attenuating the tumour necrosis factor-alpha (TNF-α)/Nuclear factor-kappa B (NF-kB) pathway, leading to reduced expression of both cyclooxygenase 2 (COX2) and Interleukin-6 (IL-6) [120].

The protective attributes of carvedilol against doxorubicin-induced cardiotoxicity have been corroborated in both human and rodent models [24–27]. Histopathological and biochemical examinations of cardiac and hepatic tissues further support its efficacy in safeguarding against doxorubicin-induced tissue damage [121].

Carvedilol's ability to selectively block β1-adrenoreceptors enhances heart function and effectively treats heart failure [122]. While it exhibits moderate β1-adrenoreceptor selectivity and slight β2-adrenoreceptor selectivity, according to an investigation by [123], it is noteworthy that carvedilol's affinity for β2-adrenoreceptors allows it to accumulate in cardiac tissues [124].

Furthermore, carvedilol has demonstrated the capacity to prevent tissue injury and reduce β3-adrenoreceptor expression in the ventricles of diabetic rats subjected to myocardial infarction [125]. These multifaceted properties underline the potential therapeutic benefits of carvedilol across various cardiovascular and oxidative stress-related conditions.

Mechanism of action of carvedilol. At higher concentrations, it is a calcium channel antagonist; at relatively low concentrations, it is a competitive β-adrenoceptor antagonist and a vasodilator. Carvedilol prevents the heart and blood arteries from being affected by certain natural compounds in the body, such as epinephrine. This has the impact of lowering heart rate, blood pressure, and heart strain. It belongs to the group of alpha (α1), and beta (β1, β2) blockers or adrenergic antagonists [126, 127, 128, 129]. Its antihypertensive activity is portrayed by a decrease in peripheral vascular resistance, and this results from the vasodilator activity of the compound, with no reflex increased heart rate (tachycardia), resulting from β-adrenoceptor blockade. Also, carvedilol's antihypertensive activity is associated with the "renal sparing" effect, as the mean arterial blood pressure reduction does not trade off or compromise renal blood flow or urinary sodium excretion.

Carvedilol has cardioprotective effects in animal models of acute myocardial infarction and is considered in this regard than propranolol at comparable β-blocking doses. It also protects against neuronal damage in in-vitro and in-vivo brain ischemia models and has antiproliferative effects in vascular smooth muscle in vitro.

Low oral doses of 12.5 mg of carvedilol reduce healthy volunteers' resting and exercise blood pressure. In hypertensive patients, carvedilol dose-dependently reduced mean diastolic blood pressure. Carvedilol and other β-blockers have been reported to modulate cancer-associated pathways, such as COX-2 [130], AKT [26], PKC and PDGF signalling.

Also, it is obvious and evident that carvedilol is a potent cancer-preventative agent through investigations from previous preclinical studies [130, 131], and analyses by [130] supported this notion as well as extended this pharmacological property to other β-arrestin biased β-blockers. Studies by [131] explained that ERK inhibition may not be the only mechanism for β-blockers, as their studies revealed a novel anticancer agent for carvedilol commonly used for cardiovascular diseases.

MATERIALS AND METHODS

Materials

Materials used for this research include 32 male mice and 32 female mice, syringes, soaps/sanitisers, cages, mice feed, canula, hand gloves, towels, face mask

Equipment: Analytical weighing balance, digital weighing balance, refrigerator, water bath, incubator, centrifuge and spectrophotometer.

Samples: Blood Samples were collected via retroorbital puncture, heart and liver samples were collected from the sacrificed mice, and brain samples were collected from the skulls of the decapitated rats.

Purchase of drugs: Doxorubicin and carvedilol were purchased from Clanol Pharmacy in Abakaliki, Ebonyi state.

Reagents and chemicals: During the study, the following chemicals were used: 0.1 M phosphate-buffered saline, 10 % formalin, Distilled water, Normal saline, and 0.25 M sucrose buffer solution.

Methods

The ethical committee approved the protocol of this study by the rules and guidelines in experimenting at the Department of physiology, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State.

Procurement of Animals. Sixty-four mice (32 males and 32 females) were procured from the animal house of the department of physiology, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State and housed in the same facility. The animals were acclimatised for two weeks and fed with grower pellets and water ad libitum.

Experimental Design. The room temperature was maintained at 35±2. The mice were kept in a

controlled environment under standard conditions and humidity with alternating light and dark cycles, after which they were grouped randomly into four groups of 8 mice each, with the following treatment and administrations:

Table 1 – Animal grouping

Male mice	Female mice
Group 1 – Normal control	Group 1 – Normal control
Group 2 – Negative control (dox)	Group 2 – Negative control (dox)
Group 3 – dox + VCO	Group 3 – dox + VCO
Group 4 – dox + CARV	Group 4 – dox + CARV

The treatment for both genders is as follows:

1. Group 1 (Normal control): received normal saline (2 ml/kg b.w).

2. Group 2 (DOX): received Doxorubicin (3.75 mg/Kg b.w i.p) weekly as a single dose on days 5, 12, 19 and 26 only to make up 15 mg/kg b.w administered for 28 days and normal saline.

3. Group 3 (DOX + VCO): received virgin coconut oil (5ml/kg b.w, orally) daily + Doxorubicin as group 2.

4. Group 4 (DOX + CARV): received 5 mg/kg b.w of carvedilol weekly for three days (day 5-7 weekly, for four weeks) after DOX administration as group 2.

Carvedilol and virgin coconut oil were administered orally.

All administration lasted for 28 days (4 weeks).

Collection & extraction of Virgin coconut oil (Cold Pressed). Coconut kernel was obtained from a local market in Abakaliki. The coconut meat detached from its shell and blended with lukewarm water. The coconut milk was then strained using a cheese cloth, and the coconut milk was kept in a container and allowed to ferment for some days, such that the oil, the curd, and the water separated and the oil was carefully scooped from the top layer.

Neurobehavioural Assessment using Y-maze. This test measures an animal's capacity to recognise places they've previously explored and their ability to explore new areas. Y-maze was used in this study to assess the animals' ability to perform hippocampus-dependent tasks and their cognitive ability. The Y-maze was made of wood (dimensions 35 x 5 x 15 cm), with the three arms placed at 120. The arms and apparatus on the floor were painted brown for easy visualisation. There was provision of light from above to ensure equal distribution of sunlight. During the first trial (T_1), the mice were introduced into the base and allowed to go to a preferred arm (time taken was noted), after which the arm was blocked, and the mice were allowed to spend 30 seconds exploring that particular arm, then it was taken out. During the T_2 phase of the first trial, the mice were introduced into the base and allowed to move to an arm (Normally, there is expected to be an alternation), and the time taken was recorded as well. This procedure was done five times, and the time taken was recorded using a stopwatch (note: an animal was considered to enter an arm if half of its body was entered).

Animal Sacrifice. After the administration, which lasted for 28 days, the animals were sacrificed and decapitated. Blood samples were collected via the retroorbital puncture. Incisions were made through the skin to expose the organs needed for further analysis. The examples and tissues were taken to the central research laboratory at Ilorin, Kwara State, to assay for immunological, biochemical, and histopathological examination.

Histological Tissue Processing

Haematoxylin and Eosin procedure. Fixed tissues were put through the following slides to obtain micro thin drops for photomicrography

Dehydration. The tissues are dehydrated by passing through ascending grades of alcohol from 50%, 70%, 90%, absolute 1 and absolute 2 alcohols, with the tissues spending one hour in each alcohol.

Clearing. The dehydrated tissues were cleared in two changes of xylene for one hour each.

Infiltration. The cleared tissues were infiltrated with two wax changes at 60 °C for one hour each.

Embedding. The infiltrated tissues were embedded in the infiltrating medium and then cooled at room temperature to solidify.

Trimming. The embedded tissues were trimmed to reveal the tissue surface for microtomy.

Sectioning. Trimmed blocks were sectioned at 5 microns to obtain tissue ribbon.

Floating. Tissue ribbons were floated in the water bath at 40 °C.

Picking. Tissue ribbons were picked with glass slides and then dried on a hot-plated.

Clearing. Drops were cleared in 2 changes of xylene for one minute each.

Rehydratio.: Slips were passed to descending grades of alcohol and rinsed in water.

Hematoxylin. Slides were stained in hematoxylin for 20 minutes and then rinsed in tap water.

Differentiation. The slides were differentiated by dipping in 1% acid alcohol for 5 seconds and then rinsed in running tap water.

Eosin. The slides were stained in eosin for two minutes and then rinsed in tap water

Dehydration and clearing. The slides were upped through ascending grades of alcohol and cleared in xylene

Mounting. The slides were mounted in DPX.

Photomicrography. Slides were captured by a camera attached to the microscope and then analysed for histopathology.

Procedure for Analysis of Acetylcholinesterase

1. All reagents, working standards, and samples were prepared.

2. The number of wells to be used was determined using the assay manual, and all remaining wells were put back into the pouch and the Ziploc sealed while storing unused wells at 4 °C.

3. A standard of 100 µl and a sample of 100 µl were added per well. They were covered with adhesive strips and incubated for two hours at 37 °C. A plate layout was provided to record standards and samples assayed.

4. The liquid of each well was removed without washing.

5. Biotin-antibody of 100 µl (1x) were added to each well and covered with a new adhesive strip while incubating for 1 hour at 37 °C. (Biotin-antibody (1x) appeared cloudy. But was warmed up to room temperature and mixed gently until the solution seemed uniform.)

6. Each well was aspirated and washed, repeated twice for three washes. It was passed by filling each well with Wash Buffer (200 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer and allowed to stand for 2 minutes; complete removal of the liquid at each step is essential to good performance. After the last wash, any remaining wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.

7. HRP-avidin (1x) of 100 µl was added to each well. The microtiter plate was covered with a new adhesive strip and incubated for 1 hour at 37 °C.

8. The aspiration/wash process was repeated five times, as in step 6.

9. TMB Substrate of 90 µl was added to each well. Incubated for 15-30 minutes at 37°C and protected from light.

10. A stop Solution of 50 µl was added to each well, and the plate was tapped gently to ensure thorough mixing.

Data/Statistical Analysis

Results obtained were expressed as Mean±SEM (Standard error of the mean). One-way analysis of variance (ANOVA) was used to compare the mean differences between the control and other treatment groups in this study. P-value less than 0.05 ($P \leq 0.05$) was considered statistically significant. All the results were analysed using GraphPad version 9.0.

RESULTS AND DISCUSSION

Comparing initial final body weights and organ weight. Table 2 shows the effect of DOX, DOX + VCO, and DOX + CARV on body and organ weight in male mice. Compared to both control and DOX groups, the final body weight of male mice that received DOX + VCO and DOX + CARV increased, though it was insignificant ($P>0.05$). The liver weight in the control group was slightly increased in the DOX group, with the highest significant increase in DOX + VCO compared to both control and DOX ($p \leq 0.05$). Increased liver weight was also observed in the DOX + CARV group, compared to both control and DOX, but it was not considered statistically significant ($p>0.05$). The Heart weight increased in all groups when compared to the rule but decreased in the DOX + VCO and DOX +CARV groups when compared to the DOX group. The brain weight slightly increased compared to the control group and was almost constant in all treated groups.

Table 3 shows the effect of DOX, DOX + VCO, and DOX + CARV on body and organ weight in female mice.

Table 2 – Male Mice

Parameters, g	CONTROL (group 1)	DOX (group 2)	DOX + VCO (group 3)	DOX + CARVEDILOL (group 4)	P-value (significance)
Initial body weight	28.50 ± 4.173	32.75±0.478	34.75 ± 1.181	32.75± 0.853	0.290
Final body weight	29.00±2.915	29.75±1.250	34.25±0.946	30.25±0.478	0.172
Brain weight	0.41±0.033	0.43±0.023	0.43±0.007	0.43±0.018	0.907
Post hoc					
	½	1/3	¼	2/3	2/4
Brain weight	1.000	1.000	1.000	1.000	1.000
Initial body weight	1.000	0.421	1.000	1.000	1.000
Final body weight	1.000	0.279	1.000	0.488	1.000

Table 3 – Female Mice

Parameters, g	CONTROL (group 1)	DOX (group 2)	DOX + VCO (group 3)	DOX + CARVEDILOL (group 4)	P value
Initial weight	26.25±1.600	18.25±1.108	23.25±1.436	21.75±1.931	0.023
Final weight	28.00±2.041	22.75±2.428	22.75±1.250	23.00±1.080	0.156
Brain weight	0.42±0.018	0.41±0.021	0.41±0.025	0.50±0.016	0.023
Post hoc					
	½	1/3	¼	2/3	2/4
Initial weight	0.020*	1.000	0.373	0.248	1.000
Brain weight	1.000	1.000	0.128	1.000	0.042*
Final body weight	0.360	0.360	0.429	0.248	0.815

Compared to control, the final body weight of female mice decreased across all treated groups, though it was not considered statistically significant (p>0.05). The brain weight was slightly reduced in DOX and DOX + VCO compared to control but significantly increased in DOX + CARV compared to negative control, DOX (p≤0.05).

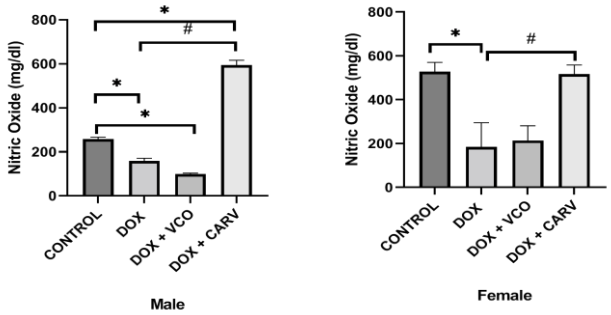


Figure 1 – Bar charts comparing the effects of DOX, virgin coconut oil and carvedilol on brain nitric oxide levels of doxorubicin-treated mice

Notes: * significant when compared to normal control (p≤0.05); # significant when compared to the DOX group (p≤0.05)

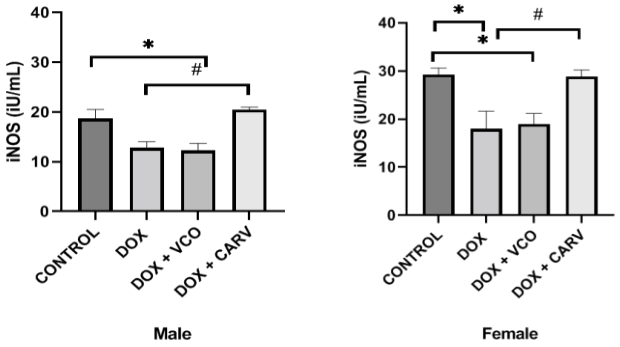


Figure 2 – Bar charts showing the effect of DOX, virgin coconut oil and carvedilol administration on brain level of inducible nitric oxide synthase (iNOS) in doxorubicin-treated mice

Notes: * significant when compared to normal control (p≤0.05); # significant when compared to negative control (p≤0.05)

In Figures 1–4 values are expressed as mean ± SEM (n=4).

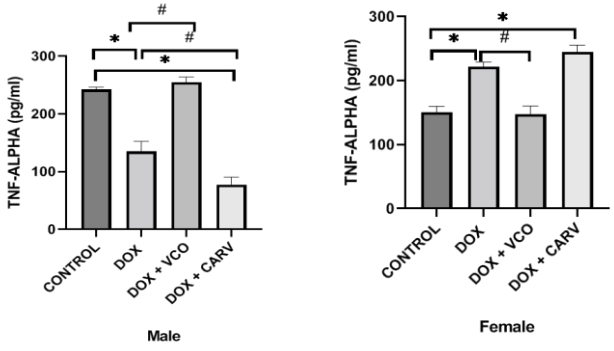


Figure 3 – Bar charts showing the effects of DOX, virgin coconut oil and carvedilol administration on TNF-alpha levels in the doxorubicin-treated mice

Notes: * significant when compared to normal control (p≤0.05); # significant when compared to negative control (p≤0.05).

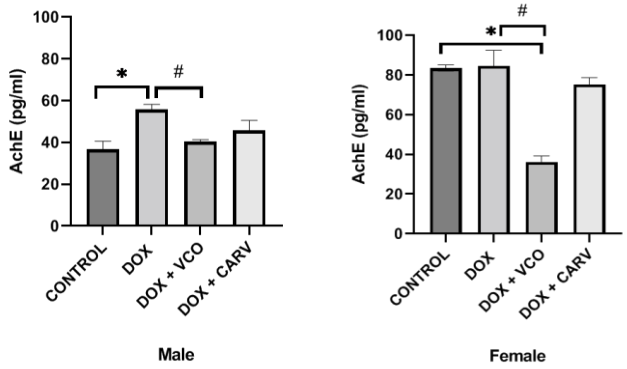


Figure 4 – Bar charts showing the effects of DOX, virgin coconut oil and carvedilol administration on hippocampal AchE activity in doxorubicin-treated mice

Notes: * significant when compared to normal control (p≤0.05). # significant when compared to negative control (p≤0.05).

Histological features of the cerebral cortex

Representative photomicrographs of the brain cortex showing a high-power magnification (x200) of the prefrontal cortex showing the external granular layer (Layer 3) with their constituent granular neurons and supporting cells (black arrows).

The photomicrographs present with general histomorphological characteristics, including cellular density, staining intensity, neutrophil intactness, morphological delineation and widespread cellular distribution across the brain area.

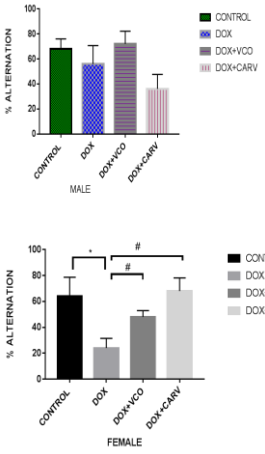


Figure 5 – Bar charts showing the impact of DOX, virgin coconut oil and carvedilol on % alternation in doxorubicin-treated mice

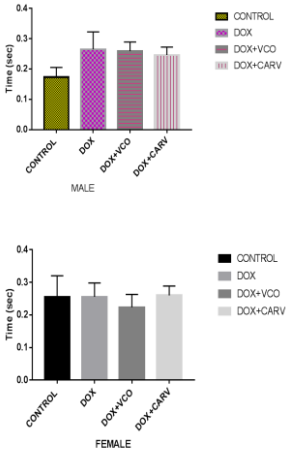
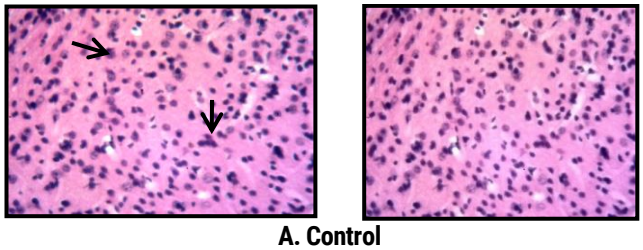


Figure 6 – Bar charts show the effect of DOX, virgin coconut oil, and carvedilol on the duration of time spent in the Y-maze in doxorubicin-treated mice

Notes: * significant when compared to normal control (p≤0.05). # significant when compared to negative control (p≤0.05).

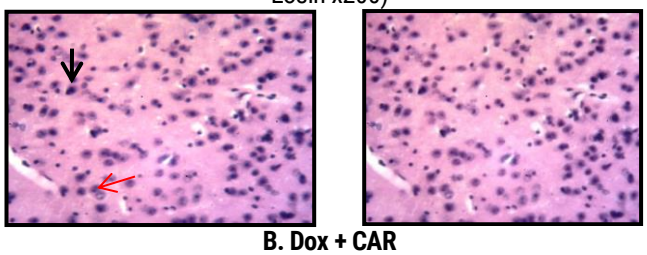
Normal and intact neuronal cells are depicted with a black arrowhead, while neuronal cells with perceived histopathological alteration are shown with red arrows. Two copies of each slide have been made available. The first is edited with hands, while the other is unedited and plain.

Doxorubicin is a pivotal development in cancer treatment. Alongside its cardiotoxic, nephrotoxic, and hepatotoxic effects, doxorubicin-induced cognitive impairment, colloquially known as chemobrain, is a well-documented affliction reported by cancer survivors, significantly impacting their overall quality of life. Currently, there remains an unmet need for adjunctive therapies to mitigate the toxicities induced by anticancer drugs during chemotherapy. Clinicians grapple with the challenge of administering cancer medications that bear substantial adverse effects [132]. The primary objective of this study was to explore the potential influence of virgin coconut oil and carvedilol on neurobehavior, apoptotic markers, and inflammatory responses in the brains of doxorubicin-treated mice.



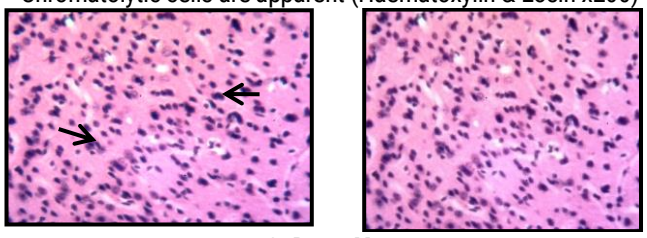
A. Control

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the coating can be appreciated. The staining intensity, cellular density and histomorphological delineation appear characteristically normal (Haematoxylin & Eosin x200)



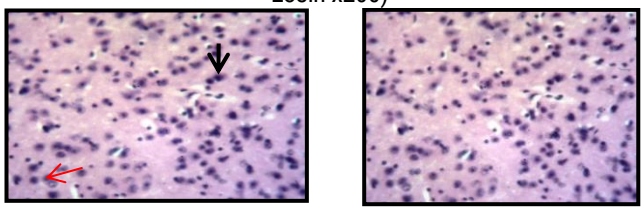
B. Dox + CAR

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. Chromatolytic cells are apparent (Haematoxylin & Eosin x200)



C. Dox + Vco

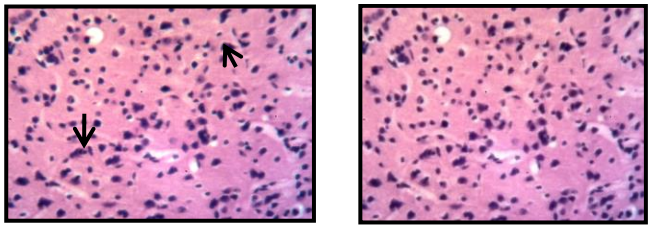
Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. The staining intensity, cellular density and histomorphological delineation appear characteristically normal (Haematoxylin & Eosin x200)



D. Dox

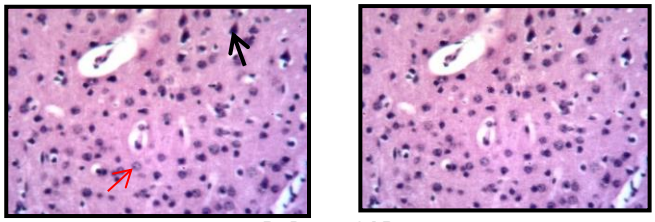
Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. Chromatolytic cells are apparent (Haematoxylin & Eosin x200)

Figure 7 – Male Photomicrographs



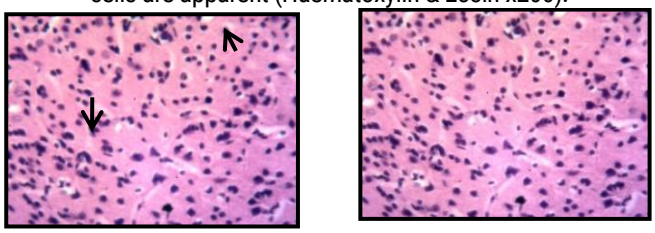
A. Control

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. The staining intensity, cellular density and histomorphological delineation appear characteristically normal (Haematoxylin & Eosin x200)



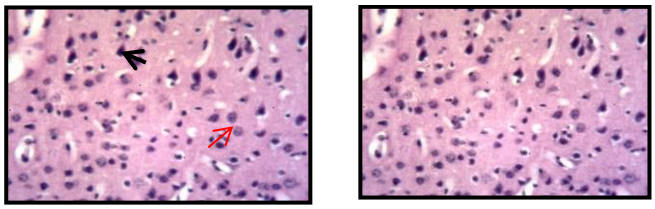
B. Dox + CAR

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. Chromatolytic cells are apparent (Haematoxylin & Eosin x200).



C. Dox + Vco

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the layer can be appreciated. The staining intensity, cellular density and histomorphological delineation appear characteristically normal (Haematoxylin & Eosin x200).



D. Dox

Representative photomicrographs of the brain of an experimental animal showing a panoramic view and a high-power magnification of the external pyramidal layer. The pyramidal cells (black arrow) in the coating can be appreciated. Chromatolytic cells are apparent (Haematoxylin & Eosin x200).

Figure 8 – Female Photomicrographs

Analysing the impact of DOX administration on the body weight of male mice, we observed that the control group exhibited a marginal increase in body weight compared to the negative control

(DOX) and the treated groups (DOX+VCO and DOX+CARV). Conversely, the latter groups slightly reduced their final body weight compared to their initial measurements, as detailed in Table 2. However, it's crucial to note that this variance did not attain statistical significance. Additionally, there were no noteworthy differences in brain weight compared to the control group.

Turning our attention to the initial and final body weights, as well as organ weights of female mice (as delineated in Table 3), we observed a modest increase in the body weight of all groups compared to their initial measurements, except the DOX + VCO group, which exhibited a minor decrease. Significantly, there was a notable disparity in initial body weight between the standard control and DOX groups, with a p-value below 0.05, indicating statistical significance. Among the organs, only the brain weight displayed a statistically significant increase in the DOX + CARV group compared to the DOX-treated group.

Nitric Oxide (NO) emerges as a pivotal messenger within various organ systems, particularly in the central nervous system (CNS). Extensive investigations have unveiled NO's dual role, encompassing both cytoprotective and cytotoxic effects within the CNS [133–135]. Alterations in NO levels are implicated in memory disorders, with reduced NO potentially contributing to the learning deficits observed in individuals with Alzheimer's disease and related neurodegenerative conditions [136]. Conversely, an excessive production of NO may trigger cell death within the nervous system. Turning our focus to the influence of DOX administration on Nitric Oxide levels, we noted a substantial decline in Nitric Oxide levels in both the male and female animal models employed. This contrasted with a prior study utilising a similar drug regimen [137]. In our investigation, the groups receiving carvedilol (DOX + CAR) exhibited an increase in Nitric Oxide levels, aligning with the findings of [138]. As previously elucidated, carvedilol, functioning as an adrenoreceptor blocker and a free radical scavenger, potentially exerts its effects via a NO-mediated mechanism. This implies that carvedilol promotes the generation of NO, known for its capacity to diminish sympathetic nerve activity [139, 140]. Nitric oxide serves as a critical mediator in endothelium-dependent vasorelaxation [14], and our results suggest that the antihypertensive attributes of carvedilol may owe their effectiveness to the stimulation of NO synthase, rather than direct adrenergic antagonism.

Interestingly, Nitric Oxide levels decreased in the male DOX + VCO group, while a slight increase was observed in the female group. Notably, despite virgin coconut oil's classification as a saturated natural oil, its phytochemical composition hints at potent natural antioxidants, potentially responsible for bolstering antioxidant defences in our study. These findings underscore Nitric Oxide's significance as a reliable marker for assessing brain injury.

Inducible nitric oxide synthase, referred to as iNOS or NOS_2, represents a high-output Ca_2+-dependent enzyme that can be induced by various stimuli, including cytokines, across different cells and organs. iNOS continues to generate nitric oxide (NO) after activation, persisting until the enzyme is degraded. Elevated NO levels produced by iNOS have been implicated in cardiac toxicity. Conversely, abnormal induction of iNOS has been linked to the pathogenesis of human disorders, such as neurodegenerative diseases, and pathophysiological conditions like inflammation.

In our investigation, the administration of DOX resulted in a reduction in brain iNOS levels in both male and female mice. Notably, the female group receiving DOX + VCO exhibited a statistically significant decrease compared to the standard control, possibly attributed to virgin coconut oil's ameliorative and inhibitory influence on iNOS levels, aligning with [142]. In contrast, the DOX+CAR group showed a significant increase in iNOS levels compared to the DOX group. The DOX and DOX+VCO groups displayed substantial decreases in iNOS levels compared to the standard control for male mice. Conversely, a significant increase in iNOS levels was observed in the group treated with DOX+CAR compared to the negative control. Consequently, the elevated iNOS levels in both male and female groups administered DOX+CAR led to increased NO production, according to [143], elucidating how carvedilol fosters NO display, a phenomenon known to reduce sympathetic nerve activity [139, 140]. However, it's worth noting that increased NO synthesis by inducible NO synthase (iNOS) may contribute to unfavourable inotropic effects and the progression of brain injury, as previously discussed by [144, 145].

Doxorubicin-induced cognitive impairment is predominantly attributed to cytokines [5]. TNF-alpha, a pro-inflammatory cytokine, is known for its involvement in apoptosis, cellular processes,

inflammation, cancer, and viral replication, among other functions [146]. TNF-alpha's role in compromising the blood-brain barrier leads to a continuous loop of inflammation between the peripheral and central nervous systems [147]. Furthermore, it can impede long-term potentiation in specific brain regions, such as the hippocampus's CA1 and dentate gyrus regions [148]. Recent studies have confirmed that DOX elevates TNF-alpha expression [149–152]. The upsurge in TNF-alpha levels aligns with the findings of [120, 153], indicating that anthracyclines elevate plasma TNF-alpha levels, instigating an inflammatory response in the brain.

Reactive oxygen species (ROS) have been implicated in the oxidative modification of apolipoprotein A1 (Apo-A1), potentially attributable to DOX's prooxidant activity [154]. Apo-A1 serves a multifaceted role in regulating the inflammatory response by suppressing the production of inflammatory cytokines, primarily TNF-α. It achieves this by promoting the production of tristetraprolin, an mRNA-destabilizing protein that interacts with TNF-α, leading to its degradation and thereby inhibiting TNF-α translation [155].

Our study reveals that TNF-alpha levels significantly increased in females in the DOX + CAR group compared to the standard control, contrasting with the findings of [156] that highlighted the significant impact of carvedilol in mitigating apoptosis and inflammation. In contrast, the group treated with DOX + VCO demonstrated a substantial decrease in TNF-alpha levels compared to the DOX-treated group, aligning with the study by [157], which illustrated that a polyphenolic fraction from virgin coconut oil effectively reduced inflammatory genes such as COX-2 and TNF-alpha. Among males, TNF-alpha levels notably decreased in the DOX + CAR group compared to both the standard control and negative control, consistent with the findings of [120, 158], where carvedilol significantly decreased TNF-alpha levels compared to the control group. Conversely, an increase in TNF-alpha levels was observed in the male group treated with DOX + VCO compared to the negative control, a significant finding.

Acetylcholinesterase (AchE) predominantly resides in cholinergic brain synapses, where it plays a crucial role in signal transduction and the regulation of acetylcholine (Ach) levels within cholinergic neurons [159]. In male mice, a noteworthy increase in AchE activity was observed in the DOX-treated group, corroborating the findings of [160, 161], but contrasting with a recent study by [162], which reported DOX-induced inhibition of cortical AchE activities.

In the female mice, the study revealed a slight increase in brain AchE activity in the negative control group compared to the standard control group but a significant decrease in the groups treated with DOX + VCO and DOX + CAR. In particular, the DOX + VCO group exhibited a significant decline in AchE activity compared to the negative control. Similar reductions were observed in the male group treated with DOX + VCO and DOX + CAR compared to the negative control. This aligns with previous research indicating that pre-treatment with VCO in male and female models administered DOX mitigated neurotoxicity by reducing AchE activity through an antioxidant mechanism [109].

Doxorubicin has been well-documented in preclinical and clinical studies for its impact on cognitive processes. Our investigation uncovered that weekly DOX therapy for four weeks led to impaired spatial memory in mice. This finding aligns with research indicating that DOX diminishes memory performance, even affecting novel location recognition in rats [59]. Furthermore, the administration of DOX in mice resulted in a specific impairment in the Y-maze task. While it was anticipated that VCO and CARV might offer protection against DOX-induced memory deficits through mechanisms like suppressing the metabolic stress response and reducing ROS-mediated stress, this study did not observe significant amelioration of DOX-induced memory impairment, especially in the male group. However, there was a notable improvement in the percentage of alternation in female mice in the DOX + VCO and DOX + CARV groups, which was considered statistically significant. This increase in % alternation in female mice may be linked to the protective effects of certain female hormones, such as estrogen.

It's important to note that both control groups of male and female mice exhibited higher alternation rates in less time, indicating increased activity compared to the treated groups. In the DOX group, there was a decrease in alternation, and the time taken for alternation in the Y-maze increased.

Estrogen, a complex gonadal hormone, has been extensively studied for its diverse neurobehav-

ioral effects in humans and animals. Evidence from basic science and clinical research suggests that estrogen can enhance cognitive function in older women and those with Alzheimer's disease. Estrogen has positively affected neuronal structure and function through estrogen receptors in various brain regions. These effects extend to multiple activities, including cognition, anxiety, body temperature regulation, eating, and sexual behaviour. While the efficacy of estrogen in enhancing awareness is not universally supported across all studies, there is a growing body of evidence from both basic science and epidemiological research supporting its neuroprotective properties. Any discrepancies in previous clinical research are likely due to methodological issues rather than the ineffectiveness of estrogen. However, this study did not include hormonal assays, making it challenging to draw definitive conclusions. It's important to note that the female mice in this study were ensured not to be pregnant during the administration period, with a two-week acclimatisation period to rule out pregnancy.

Hypogonadism, characterised by low testosterone levels, has been associated with cognitive decline. In male cancer survivors undergoing chemotherapy, low testosterone levels are a common hormonal issue. Previous research by [163] demonstrated a substantial reduction in testes in doxorubicin-treated male mice. Testosterone and its metabolites have been shown to protect hippocampal neurons and astroglial cells from damage due to glucose deprivation. Testosterone has also been found to protect motor neurons from dendritic atrophy resulting from the death of adjacent neurons.

Additionally, testosterone deficiency has been linked to increased susceptibility to oxidative damage in various brain regions under chronic stress. The hippocampus, where a high concentration of androgen receptors is found in CA1 pyramidal cells, is thought to mediate the impact of androgens on cognition. However, testosterone levels were not measured in this study, and the acute testicular shrinkage caused by DOX has the potential to impact the brain, necessitating further investigation in future studies.

Gender-based differences in DOX-induced toxicity have been observed in both clinical and preclinical studies. Although oxidative stress, energy factors, and estrogen's influence have all been proposed as contributing factors to these gender variations, the specific mechanisms remain unclear [163–166]. This research identified significant sexual dimorphism in doxorubicin sensitivity in mice, consistent with previous reports of gender differences in sensitivity to specific toxic effects of Doxorubicin in rats, such as impaired weight gain and nephropathy [167–169]. However, the underlying pathogenesis of gender-related differences in doxorubicin susceptibility remains an area of ongoing investigation. This study's findings underscore the importance of considering gender-specific responses when studying doxorubicin toxicity and related cognitive impacts.

Our research uncovered significant findings in the study of the impact of Doxorubicin, virgin coconut oil, and carvedilol on neurobehavior, apoptotic, and inflammatory brain markers in mice. These findings are instrumental in advancing our understanding of the complex interplay between chemotherapy-induced neurotoxicity and potential neuroprotective interventions:

1. Chemotherapy-Induced Cognitive Impairment: The administration of Doxorubicin successfully induced cognitive impairments in both male and female mice. This validates the widely recognised phenomenon of chemotherapy-related cognitive deficits, chemobrain.

2. Carvedilol's NO Modulation: Carvedilol, a synthetic compound, showed a notable effect by significantly increasing nitric oxide (NO) brain levels. This effect is critical as it promotes vasorelaxation of vascular tissues by inhibiting sympathetic activity. It suggests a potential mechanism through which carvedilol can exert neuroprotective effects.

3. Neurotoxicity Mechanisms: Our study elucidates the mechanisms behind doxorubicin-induced neurotoxicity, revealing its propensity to induce apoptosis and necrosis in healthy brain tissue. This insight sheds light on the underlying processes contributing to chemotherapy-related cognitive impairment.

4. Virgin Coconut Oil's iNOS Modulation: Virgin coconut oil, a natural intervention, demonstrated a dual role by ameliorating and inhibiting inducible nitric oxide synthase (iNOS) levels. This nuanced impact highlights the potential of virgin coconut oil in modulating neuroinflammation.

5. Gender-Based Resilience: Intriguingly, female mice exhibited greater resilience to neurotoxicity induced by Doxorubicin when compared to their male counterparts. This gender-based difference

underscores the importance of considering hormonal influences on cognitive function during chemotherapy.

Our research findings significantly contribute to the evolving field of chemotherapy-induced neurotoxicity and the exploration of potential neuroprotective strategies. This study advances our understanding in several key areas:

1. Comprehensive Neurotoxicity Assessment: We provide a comprehensive assessment of the neurotoxic effects of Doxorubicin, offering valuable insights into the multifaceted nature of chemotherapy-induced cognitive impairment. This comprehensive approach sets the stage for targeted interventions.

2. Natural and Synthetic Neuroprotection: Our study introduces a novel perspective by evaluating natural-based (virgin coconut oil) and synthetic-based (carvedilol) neuroprotective strategies. We explore their potential not only to mitigate neurotoxicity but also to enhance the therapeutic efficacy of Doxorubicin.

3. NO and iNOS Modulation: Our research contributes to understanding nitric oxide (NO) dynamics and inducible nitric oxide synthase (iNOS) modulation in the context of doxorubicin treatment. This knowledge highlights potential mechanisms through which these interventions exert their effects.

4. Inflammation Management: We elucidate the impact of Doxorubicin on pro-inflammatory cytokines, emphasising the potential of both interventions to mitigate inflammation. This knowledge is pivotal for developing strategies to safeguard the blood-brain barrier and mitigate inflammation-related cognitive impairments.

5. Gender-Based Considerations: Identifying gender-based differences in neurotoxicity response underscores the complexity of this phenomenon. Recognising the hormonal influences on cognitive function during chemotherapy is vital for personalised treatment approaches.

CONCLUSIONS

In conclusion, this research presents a promising avenue for addressing the challenging issue of chemotherapy-induced cognitive impairment, often referred to as chemo brain. Our study offers insights into Doxorubicin's neurobehavioral, apoptotic, and inflammatory effects and the potential neuroprotective roles of virgin coconut oil and carvedilol.

The multifaceted nature of chemotherapy-induced neurotoxicity necessitates a comprehensive approach, not only to prevent cognitive impairment but also to enhance the therapeutic effects of chemotherapy. Gender-based differences in neurotoxicity response underscore the importance of considering hormonal influences on cognitive function during chemotherapy.

While our study provides a foundation for developing neuroprotective strategies, it is imperative to conduct further research, including chronic trials and clinical studies, to validate the safety and effectiveness of virgin coconut oil and carvedilol in clinical settings. These efforts are essential for translating our findings into improved quality of life for cancer patients undergoing chemotherapy.

This study, conducted sub-acutely, offers valuable insights into the potential of virgin coconut oil and carvedilol as neuroprotective interventions. However, to further advance our understanding and application of these strategies, we recommend the following:

1. Chronic Administration Studies: Extend the duration of administration to encompass regular trials. This will enable a more comprehensive evaluation of the long-term effects of virgin coconut oil and carvedilol on doxorubicin-induced neurotoxicity.

2. Clinical Validation: Transition from animal models to clinical studies to validate the safety and effectiveness of virgin coconut oil and carvedilol in human cancer patients undergoing chemotherapy. This translational research is crucial for implementing these interventions in real-world medical practice.

3. Hormonal Influence Investigation: Conduct in-depth investigations into the hormonal influences on cognitive function during chemotherapy. Understanding the interplay between hormones and neurotoxicity can inform tailored treatment approaches for different patient profiles.

In summary, our research paves the way for potential interventions to alleviate chemobrain, but it also underscores the need for continued exploration and validation of these strategies. By addressing the multifaceted challenges of chemotherapy-induced cognitive impairment, we aim to enhance the quality of life for cancer patients on their journey to recovery.

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