Trop J Nat Prod Res, March 2025; 9(3): 1263 – 1269 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https ://www.tjnpr.org

Original Research Article

Dietary Virgin Coconut Exerted a Neuroprotective Effect via Upregulation of Nuclear Erythroid Factor and Downregulation Of Nuclear Factor KB Expression In Acrylamide-Induced Neurotoxicity: Role Of BDNF And AchE Pathways

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ARTICLE INFO

Article history:
Received 22 November 2024
Revised 23 November 2024
Accepted 10 December 2024
Published online 01 March 2025

ABSTRACT

Acrylamide (AA), a widespread environmental neurotoxicant found in heat-processed foods, poses significant risks to neurological health. This study aimed to investigate the neuro-modulatory effects of virgin coconut oil (VCO) against AA-induced neurotoxicity in a rat model. Twenty male Wistar rats (180-250 g) were randomly divided into four groups: Control, AA (10 mg/kg), AA + 5% VCO, and AA + 10% VCO, and treated for 56 days. AA was administered orally at 10 mg/kg, with VCO incorporated into the diet at 5 g and 10 g per 100 g of normal feed. Behavioural tests demonstrated that AA exposure led to significant anxiety-like behaviour and memory deficits. Biochemical analyses revealed AA decreased serum superoxide dismutase (SOD) and catalase (CAT) activities while increasing malondialdehyde (MDA) and interleukin-1*Beta* (IL-1*β*) levels. Molecular assessments showed AA downregulated hippocampal brain-derived neurotrophic factor (BDNF) and nuclear factor erythroid 2-related factor 2 (Nrf2) and upregulated nuclear factor-*κB* (NF-*κB*) gene expression. Histological examination confirmed reduced dentate gyrus cellularity in the AA group. VCO supplementation, particularly at 5%, significantly attenuated these AA-induced alterations by restoring antioxidant enzyme activities, reducing oxidative stress and inflammation, and modulating key neuroprotective signalling pathways. These findings suggest dietary VCO supplementation can effectively mitigate AA-induced neurotoxicity through coordinated antioxidant, anti-inflammatory, and neurotrophic mechanisms.

Keywords: Neurotoxicity, Neuroprotection, Elevated Plus Maze, Y-maze, Memory Impairment

Introduction

Acrylamide is a chemical compound formed during high-temperature cooking of carbohydrate-rich foods and is also present in tobacco products. It can be found in various foods and water, posing significant health risks, particularly neurological toxicity.[1,2] Reports of neurotoxicity with AA associated with chronic exposure and impacts are reported on the central nervous system comprising brain and spinal cord tissue and peripheral nervous system.[3] Therefore, oxidative stress and inflammation, and alteration of neuronal development and survival coupled with deranged cholinergic transmission are the causes that contribute to the neurotoxicity of AA.[4,5]

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Citation:Ajibare J.A, Akintoye O.O, Oriowo A.O, Asuku O.A, Oriyomi A.I, Adedara F J, Ayoola M.A, Adejumo M.O,. Dietary virgin Coconut exerted a neuroprotective effect via upregulation of nuclear erythroid factor and downregulation of nuclear factor KB expression in acrylamide-induced neurotoxicity: Role of BDNF and AchE pathways. Trop J Nat Prod Res. 2025; 9(3): 1263 – 1269 https://doi.org/10.26538/tjnpr/v9i3.50

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Previous studies, which explained the effects of AA exposure, have demonstrated raised intracellular oxidative signalling, and lower antioxidant enzymes. Besides, AA exposure also caused increased cytokine IL-1*β* and Tumor Necrosis Factor-*Alpha* (TNF-*α*).[1] Therefore, these oxido-inflammatory improvements, when coupled with the mechanism of AChE inhibition and cholinergic neurotransmission dysfunction, could be considered valid for the neurotoxicity of AA, which leads to the deterioration of cognitive function.[6]

In contrast, Virgin coconut oil (VCO), a non-chemically refined coconut oil extracted from fresh coconut, has been claimed to possess various health benefits, mainly as an antioxidant and anti-inflammatory.[7] All these effects are associated with phenolic compounds, isoflavones, Medium-chain fatty acids, and flavonoids present in VCO.[8] Studies have identified several molecular targets that could be involved in the neuromodulatory mechanism of natural products, including activation of Nuclear factor erythroid 2-related factor 2 (Nrf2) / Nuclear factor-*kappa B* (NF-κB), modulation of brain-derived neurotrophic factor (BDNF) and acetylcholinesterase (AChE).[9–11] These pathways are involved in the modulation of antioxidant responses, pro-inflammatory cytokine production, cholinergic neurotransmission, and neuronal survival. They, hence, may offer promising targets for neuromodulatory interventions against AA-induced cognitive impairment. Even though a few reports have investigated the antioxidant efficacies of various natural products in

counteracting the detrimental effects of AA neurotoxicity,[12,13] the possibility of VCO as a neuroprotectant in this context has not been researched yet. As the demand for VCO increases and findings show that VCO can provide health benefits, examining its efficacy on AA-induced neurotoxicity is both timely and necessary.[14,15] Therefore, based on the roles of the aforementioned molecular targets and the plausible relevance of VCO in cognitive impairment induced by AA in male Wistar rats, the present investigation aimed to determine the neuromodulatory potential of VCO against AA-induced brain damage and the possible molecular mechanisms involved.

Materials and Methods

Drugs

Acrylamide was purchased from (LobalChemie Pvt Ltd, Mumbai, India), while fresh and matured coconut were purchased from Ado-Ekiti market, Ekiti, Nigeria, respectively.

Animals

Twenty (20) male Wistar (180 - 210 g) rats were purchased from the animal breeding centre, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. They were housed in groups of 5 per cage (45 x 34 x 18 cm) with access to standard rat chow and water *ad libitum*. The animals were allowed to acclimate for 14 days before the start of the experiment in a 12-hour light/dark cycle at room temperature. The Institutional Ethical Committee approved the use of Animals in this research with Ethical Number: EKSU/P100/2022/010/045.

Preparation of Virgin Coconut Oil

Fresh and mature coconut oil was used based on earlier protocols to obtain virgin coconut oil (VCO).[7,14] Grated coconut pulp was manually pressed, fermented (48 hrs, 25 ℃ room temperature), centrifuged at 3000 rpm for 15 minutes, and the isolated VCO band was collected. 5% and 10% VCO-enriched diets were constituted by thoroughly mixing the freshly prepared VCO into the normal rat chow at 5 g and 10 g per 95 g and 90 g of chow, respectively.

Experimental Design

Rats were randomly assigned to one of the four groups (n=5 per group):
Group 1 (Control) administered a daily dose of vehicle 1 mL/kg of distilled water;

Group 2 (AA) given oral acrylamide (10 mg/kg)[1]

Group 3 (AA + 5 VCO) acrylamide (10 mg/kg) + 5% VCO diet[16,17]

Group 4 (AA+10 VCO) acrylamide (10 mg/kg) + 10% VCO diet, for eight weeks[16]

Behavioural Tests

The animals were subjected to the Elevated Plus Maze test on day 56, followed by the Y-Maze test on day 57, with a 24-hour interval between the tests, to evade any lag from the previous test. Before the commencement of each test, the animals were acclimatised to the testing chamber for 20 to 30 minutes. Tests were carried out at low light (dark) conditions at the appropriate room temperature. More so, after each test session with a certain animal, the experimental chamber was cleaned with 70% ethanol and allowed to air dry before continuing to the next animal.[7]

Elevated Plus Maze

An elevated plus maze (EPM) made from dark-painted plywood was used to measure the rats' anxiety levels. The height of the maze's four (4) arms above the plane surface was 80 cm. There were two opposing closed arms (with a wall height of 50 cm) and two opposing open arms (45 x 10 cm). The animals were placed in the maze's centre, facing an open arm, and allowed 5 minutes to explore the maze. A blind observer was tasked with counting how many times the animal entered the arms and how long it remained in the closed or open arms. It was determined that the rat entered when all four limbs had fully entered a specific arm, according to [7]

$$\text{Percentage of time spent in open arm} = \frac{Total\ time\ on\ open\ arm}{300}\ x\ 100$$

$$\text{Percentage entrance} = \frac{Total\ open\ arms\ entries}{Total\ number\ of\ entries\ into\ both\ arma}\ x100$$

This formula covered the closed arms;

The anxiety index (AI) was calculated as

$$\frac{1-[(time\ in\ open\ arms\ divided\ by\ total\ time)+(open\ arm\ entries\ ivided\ by\ total\ entries)]}{2}$$

Y maze

Short-term memory was assessed using the Y-Maze test on day 57 utilising Y-Maze Test[7], a three-arm, smooth plywood structure of 25 x 10 x 75 cm, with arms spaced 120 degrees apart from one another. Each rat was initially positioned in the middle of the maze and given six (6) minutes to investigate the arms of the three (A, B, and C). The initial arms entry sequence was recorded with the help of a blind observer. The rats are usually expected to move to a relatively new arm rather than return to the arm already being explored. Any rat exhibiting a rate of greater spontaneous alternation (SAP) (visit pattern of arms A, B, and C sequentially without repetition) demonstrates a stronger short-term memory capacity. An arm entry was defined as all four animal limbs within a specific arm. The maze was cleaned using cotton wool soaked in a 20 % ethanol solution between tests to avoid olfactory cues before introducing the next rat.
The percentage spontaneous alternation (% SAP) was determined as;

$$\frac{Number\ of\ SAP}{Total\ number\ of\ arm\ entries-2}\ x\ 100$$

Biochemical Analysis

The rats were given intraperitoneal xylazine/ketamine (10/50 mg/kg) 24 hours after the last day of treatment to induce anaesthesia.[16] Blood samples were obtained from the retro-orbital sinus. The blood samples were kept in plain bottles and centrifuged for 15 minutes at 3000 revolutions per minute. After that, they were placed in a freezer at 20°C for biochemical analysis according to the manufacturer's instructions. The rat brain was dissected and kept in appropriate solutions for histology and gene expression.

Estimation of Superoxide Dismutase (SOD)

The serum samples were homogenised in phosphate buffer (pH 7.8) and centrifuged to obtain a clear supernatant for spectrophotometric analysis, as previously reported.[7] The absorbance of the supernatant was measured at 560 nm using a spectrophotometer.

Estimation of Catalase (CAT) Activity

The activity of the enzyme catalase was measured using a colourimetric assay. The absorbance of the yellow colour produced, corresponding to the amount of H_2O_2 broken down by catalase, was measured at 405 nm using a spectrophotometer, as previously documented.[16]

Estimation of Malondialdehyde (MDA)

According to [18], the amount of MDA in the serum samples was estimated using thiobarbituric acid reactive compounds. The absorbance of the coloured (red) pigment obtained from the spectrophotometric analysis was measured at 532 nm.

*Measurement of Interleukin 1β (*IL-1β)

The rat serum sample was diluted 1:10 with the sample diluent provided in the Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog No. E-EL-R0012). IL-1β was subsequently measured according to the earlier method.[16]

Gas Chromatography Flame Ionisation Detector (GC-FID)

Procedure

The gas chromatography flame ionisation detector GC-FID is as described by and modified[19]; using a Hewlett Packard Gas Chromatograph with a flame ionisation detector and a Hewlett Packard 7683 series injector, the gas chromatography-flame ionisation detector (GC-FID) procedure was performed. The GC utilised a fused silica capillary column-HP-5MS (30 x 0.25 mm) with a film thickness of 1.0 m and helium gas (99.999%) as the carrier gas at a constant flow rate of 22 cm/s. One micron of virgin coconut oil was injected. The silylation of fatty acids and acylglycerols with Bis trimethylsilyl trifluoroacetamide (BSTFA) and Trimethylchlorosilane (TMCS) produces volatile and thermally stable silyl derivatives for analysis. Individual GC-FID internal standard solutions were prepared using n-tetradecane dissolved in pyridine.

Table 1. Primer sequence for RT-PCR

Genes	Forward Primer	Reverse Primer
Nrf2	GTCAGCTACTCCCAGGTTGC	CAGGGCAAGCGACTGAAATG
NF-Kb	CCACTGTCAACAGCAGATGG	TTCTTCTCACTGGAGGCACC
BDNF	GTCAGATTTTGGAGCGGAGC	CTCACCTGGTGGAACCGGA
AChE	ACGTGAGCCTGAACCTGAAG	CTCGTCCAGCGTGTCTGTG
ACTIN	CACCCGCCACCAGTTCG	CCCACGATGGAGGGGAAGA

The standard mixture stock solution of respective fatty acids was prepared at concentrations of 5, 5, 10, and 10 mg/mL, respectively, and diluted with a 5 mg/mL internal standard solution to generate a dilution series used as a standard curve. For GC-FID analysis, virgin coconut oil samples were separately dissolved in the internal standard solution and adjusted with pyridine to attain final concentrations of 10 and 100 mg/mL. The silylation reaction was conducted at 70°C for 30 minutes with excess silylation reagent, BSTFA-TMCS (99:1). The silyl derivatives were analysed with a DB-5HT capillary column-equipped GC-FID. The method was validated regarding linearity, LOD, LOQ, precision, and accuracy. Six concentration levels of the standard mixture solutions were utilised to evaluate linearity.

Gene Expression

Tissue samples of the hippocampus were homogenised to get the hippocampal homogenate. Total RNA from the homogenate was isolated using an RNA isolation commercial kit as per the manufacturer's protocol. Spectrophotometric and electrophoretic analyses of RNA were done using the NanoDrop spectrophotometer and agarose gel, respectively. Amplification of RNA was done using reverse transcription to obtain complementary DNA (cDNA) using 1 µg of total RNA per sample and a commercial reverse transcription kit according to the manufacturer's recommendations. Polymerase chain reaction (PCR) was performed to determine the expression of the target genes. The cDNA obtained during the reverse transcription step was used as a template for the PCR assays. Consequently, specific forward and reverse primer sets were used for the target genes of interest and the internal control gene, β- Actin.[20]

Statistical Analysis

The obtained data was analysed using Graph Pad Prism, Version 9.0. One-way analysis of variance (ANOVA) was utilised. The p-value was set at $p<0.05$ and adjusted for multiple comparisons using the Bonferroni correction. A typical snapshot of the samples that were taken and the gel image was produced with Image-J. The relative control expression of β-Actin or a particular gene is displayed in each bar graph.

Results and Discussion

This study aims to determine the neuromodulatory action of virgin coconut oil (VCO) against acrylamide (AA)-induced neurotoxicity. The present study affirms that VCO supplementation alleviated AA-induced cognitive impairment, increased anxiety, oxidative stress, and neuro-inflammation, as well as upregulated the antioxidant defence system and cholinergic activity. The GC-FID result showed the presence of P-HydroxyBenzoic Acid, *Alpha*-Tocophenol, Gallic Acid, Caffeic Acid, Salicylic Acid, Syringic Acid Catechin, Epicatechin, Epigallocatechin, β-Sitosterol, Lauric Acid, Stigmasterol, Ergosterol, Myristic Acid, Ferulic Acid, Campesterol which are phytochemicals present in virgin coconut oil. Figure 1 shows that The AA group spent less time in the open arm (A) and more time in the closed arm (B) of the Y-maze and administration of 5% VCO and 10% VCO abated acrylamide-induced anxiety. As demonstrated in A and B, anxiety successfully decreased in VCO-treated groups but more significantly in the AA + 5% VCO group.

The pattern was consistent for both the open and closed arm entrances (C & D). In E, compared to the control group, the AA group's anxiety index was significantly higher. This study also provided evidence that any modulations in the rat's behaviour elicited with AA could be categorised as anxiogenic-like, as depicted by the elevated plus-maze test.[21] It was noted that the extent of the influence of VCO on cognitive deficit and anxiety is not dose-related since AA + 5% VCO proved to yield better results than AA + 10% VCO. In Figure 2, the AA group exhibited a significant decrease in their performance on the spontaneous alternation tests (A and B) compared to the control group, indicating impaired short-term memory. However, the treatment significantly reversed the observed decline in spontaneous alternation performance. The results demonstrated that AA exposure caused a notable detrimental effect on spatial memory performance. This aligns with previous studies showing that AA negatively affects learning and memory.[22] This study demonstrated that AA + 5% and AA + 10% VCO supplementation could reverse this cognitive impairment. Our study's observed cognitive enhancement effects of virgin coconut oil (VCO) corroborate previous research findings.[22,23] The AA group had significantly lower levels of superoxide dismutase (SOD) compared to the control group, as shown in Figure 3A. However, the 5% VCO treatment group exhibited a significant increase in SOD levels. Similarly, the catalase levels were considerably lower in the AA group compared to the control group, as depicted in Figure 3B. Figure 3C demonstrates that the 10% VCO treatment group significantly reduced the elevated malondialdehyde (MDA) levels observed in the AA group. As illustrated in Figure 3D, Nrf2 gene expression was significantly downregulated in the AA group compared to the control group. Conversely, Nrf2 expression was significantly upregulated in the AA + 5% VCO and AA + 10% VCO treatment groups.

One key finding was increased nuclear factor erythroid 2-related factor 2 (NRF- 2) in all VCO-treated groups. Since Nrf2 is responsible for modulating the antioxidant system *in vivo*[24], this upregulation may be behind the neuroprotective effect. This observation agrees with the previous findings that have highlighted the role of Nrf2 in mitigating oxidative stress disorders.[25] In line with this, this study showed that AA exposure significantly reduced the antioxidant enzymes in tandem with earlier studies [26], while VCO supplementation increased the activities of SOD and CAT, which supported the previous studies [27.] revealing the antioxidant effects of VCO. The groups that received VCO also had reduced MDA levels, indicating a low lipid

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

peroxidation level. This decrease, alongside the increase in the activities of antioxidant enzymes, confers an antioxidant effect on VCO.[8] Taken together, these results support the antioxidative activity

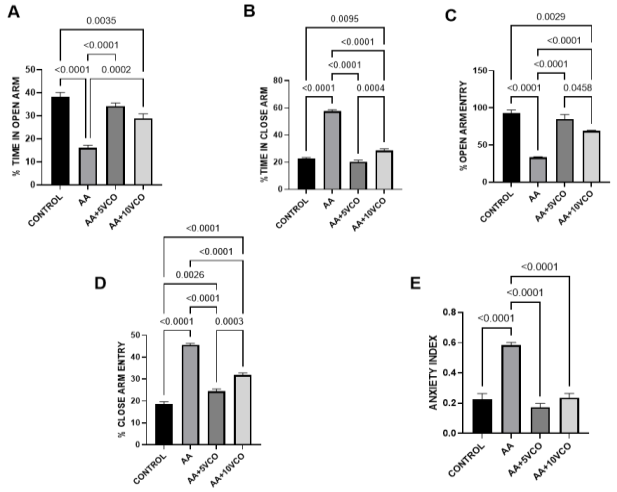

Figure 1. Virgin Coconut Oil abates acrylamide-induced anxiety-like behaviour in rats.
Data expressed are mean ± SEM, n = 5. Data were analysed by one-way ANOVA followed by Tukey's multiple post hoc test. $p < 0.05$. AA- Acrylamide, VCO- Virgin Coconut Oil.

of VCO as a neuromodulatory agent against AA-induced oxidative stress.[7] In Figure 4A, the VCO treatment groups (5% and 10%) exhibited a significant decrease in the elevated IL-1β levels observed in the AA group. As shown in Figure 4B, the NF-κB gene was significantly upregulated in the AA group. This upregulation was reversed and downregulated in the VCO treatment groups, with the 10% VCO treatment group showing peak reduction in the NF-κB expression. In the context of the present study, the relations between oxidative stress and inflammation are clearly expressed regarding the results. Our study showed that AA promoted inflammation because IL-1β level and NF-κB expression were upregulated. This concurs with earlier works identifying that AA increases the production of inflammatory mediators.[28] By reducing these inflammatory markers, it can be asserted that VCO supplementation possesses anti-inflammatory properties attributable to VCO's polyphenol compounds, which are known to be anti-inflammatory.[29] Taken together, VCO reduces the AA-induced oxidative stress, which is expressed by upregulating the activity of Nrf2, SOD, and CAT, as well as by decreasing the MDA level, while on the other hand potently inhibiting the inflammatory cascades by downregulating NF-κB and significantly reducing its downstream IL-1β. It is very plausible that these two activities of VCO, which suppress oxidative stress and inflammation, could be crucial underpinning mechanisms for its neuroprotective properties.[30]

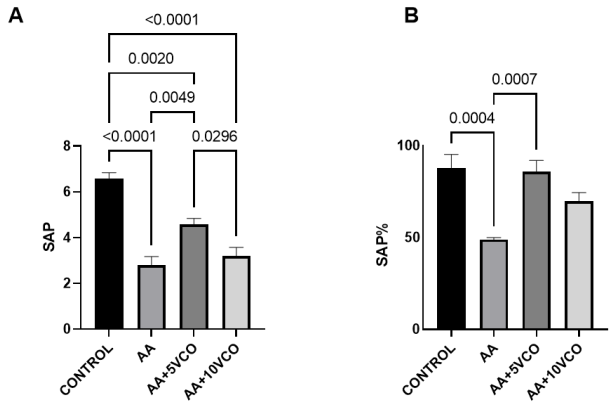

Figure 2. Dose-dependent modulatory effect of VCO on Acrylamide-induced memory impairment.
Data expressed are mean ± SEM, n = 5. Data were analysed by one-way ANOVA followed by Tukey's multiple post hoc test. $p < 0.05$. AA (Acrylamide), VCO (Virgin Coconut Oil), SAP (Spontaneous Alternation Performance).

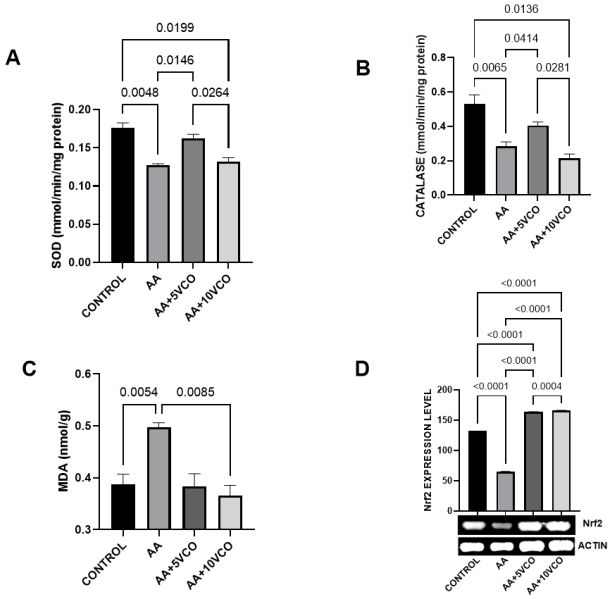

Figure 3. Virgin coconut oil mitigates acrylamide-induced oxidative stress.
The figure presents quantified band values from each sample, expressed as mean±SEM (n=5) for all four groups. The gel image represents pooled samples, while bar graphs show control-normalised relative expression (Nrf-2 to β-actin ratio).AA (Acrylamide), VCO (Virgin Coconut Oil), SOD (Superoxide dismutase), and MDA (Malondialdehyde).

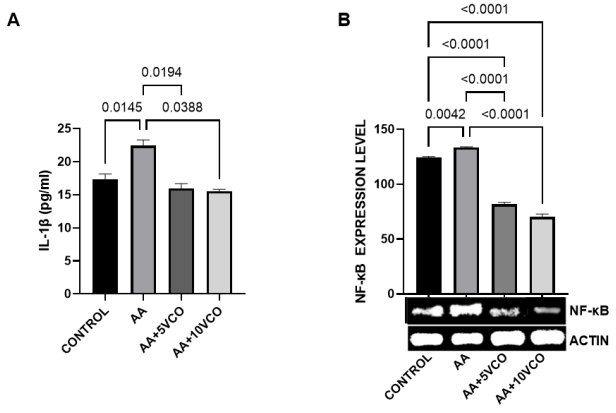

Figure 4. Virgin coconut oil reverses acrylamide-induced inflammation in rats
The figure presents quantified band intensities for NF-κB across all groups, normalised to β-actin expression, and expressed as mean ± SEM (n=5). The accompanying gel image provides a visual representation of the pooled samples, highlighting the differences in NF-κB expression among the experimental groups. AA (Acrylamide), VCO (Virgin Coconut Oil), IL-1β (Interleukin 1-*beta)*, NF-κB (Nuclear Factor *Kappa B).*

Figure 5 shows the dentate gyrus's form and cell distribution depicted in the hippocampal histoarchitecture. The dentate gyrus's normal architecture was shown in the control group. The distribution of dentate gyrus (DG) cells was sparse in the AA group, and there was a decrease in DG cells when compared to the control group. Comparing the AA + 5% VCO group to the AA group, there was a considerable increase in the number of DG cells with a minor loss of DG cells. When compared to the AA group, AA + 10% VCO exhibits a normal DG cell and a considerable increase in DG cell count. Our histological examinations corroborated the behavioural and biochemical assessments. Acrylamide administration reduced the number of cells in the dentate gyrus, indicative of impaired neurogenesis.[31] This structural change aligns with the observed cognitive deficits and decreased BDNF levels. At the same time, VCO supplementation demonstrated the potential to mitigate these detrimental effects of acrylamide. VCO's modulatory action may be multifaceted, encompassing its antioxidant and anti-inflammatory activities, enhancement of BDNF levels, and cholinergic function.[32] The antioxidant compounds in VCO, such as polyphenols and tocotrienols, may help neutralise oxidative stress and create a neuromodulatory environment.[33] Additionally, the medium-chain fatty acids in VCO could reduce inflammation and support neuronal membrane integrity.[34]

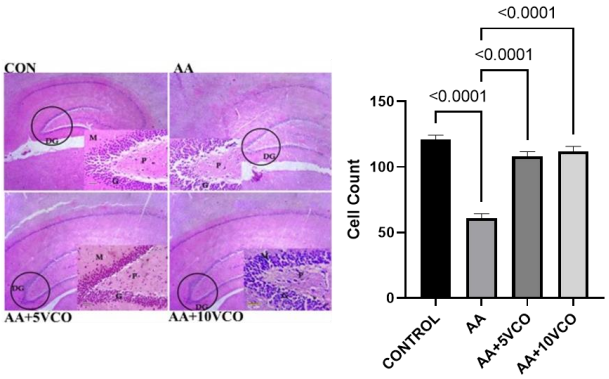

Figure 5. Virgin Coconut Oil increased the DG cells in the rat brain H&E-stained, (Mag. x800). (VCO: virgin coconut oil; DG: dentate gyrus; G: granular layer; P: pyramidal layer; M: molecular layer; CON: control).

Acetylcholinesterase (AChE) is the enzyme that degrades acetylcholine and, not surprisingly, is linked with cognition.[35] Acetylcholine is a neurotransmitter that regulates learning, memory, and cognition.[36] In Figure 6, the AA group showed significantly higher AChE gene expression than the control group. VCO exerted a dose-dependent reversal in AchE expression in the AA group. Peak reduction in AChE gene expression was observed in the 5% VCO treatment group compared to the 10 % VCO group. The AA group displayed a significantly lower BDNF gene expression than the control group. The VCO treatment groups, especially the 5% dose, effectively restored the BDNF gene expression to normal levels. It may be postulated that by increasing AChE activity, the concentration of ACh available for synaptic transmission at the level of nerve terminals is decreased and ultimately negatively influence cognitive performance. The findings of the present work elucidated that the acrylamide-treated group showed a highly significant upregulation of the AChE gene. However, VCO supplementation exhibited a dose-dependent effect, reducing the activity of AChE acrylamide-treated animals. This change in cholinergic function may underlie the cognitive-enhancing effect of VCO.[37]

Furthermore, the active components of VCO could explain the observed AChE-lowering effect of VCO. For instance, the Medium-Chain Fatty Acids (MCFAs) present in VCO, especially lauric acid, have been determined to have neuromodulatory effects.[38] These MCFAs may interact with cellular membranes and proteins, impacting the enzyme's activities, such as AChE.[39] In addition, other polyphenolic compounds found in VCO include caffeic acid and ferulic acid, which possess acetylcholinesterase inhibitory effects.[8] VCO is known to possess antioxidant properties, mainly because of its tocopherols and tocotrienols (vitamin E) contents,[40] which have the potential to directly affect AChE activity, as changes in the enzyme's function could be caused by oxidative stress. The multiple ways of VCO in regulating AChE may imply that the bioactive constituents of this product exert synergistic actions in cognitive improvement.[33]

BDNF plays a crucial role in neuronal survival, growth, and synaptic plasticity, and its depletion could profoundly affect cognitive function.[41,42] Our study showed a significant reduction in brain-derived neurotrophic factor (BDNF) levels in acrylamide-treated rats. This decrease may be attributed to elevated levels of pro-inflammatory proteins induced by acrylamide exposure. Notably, supplementation with 5% virgin coconut oil (VCO) led to a significant upregulation of the BDNF gene.[43] This finding suggests that VCO's cognitive-enhancing properties may be partly mediated through its ability to restore BDNF levels.[44] The mechanism behind this effect could involve VCO's anti-inflammatory properties due to the presence of lauric acid, which is said to possess anti-inflammatory and antioxidant activity, which may create a more favourable environment for BDNF production and signaling.[44,45] The increased BDNF levels with VCO supplementation may promote neurogenesis and synaptic plasticity, potentially explaining the improved cell count in the dentate gyrus.[43] Furthermore, VCO's ability to modulate cholinergic function, as evidenced by its effects on acetylcholinesterase activity, may work synergistically with BDNF enhancement to enhance cognitive processes.[46]

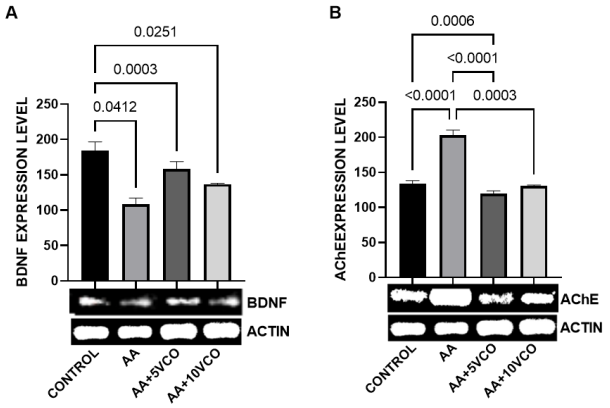

Figure 6. Virgin coconut oil reverses acrylamide-induced impaired acetylcholinesterase activity.

The accompanying gel image visually represents the pooled samples, highlighting the differences in NF-κB expression among the experimental groups. AA (Acrylamide), VCO (Virgin Coconut Oil), BDNF (Brain-derived Neurotrophic Factor), AChE (Acetylcholinesterase).

Conclusion

Collectively, these findings highlight the neuromodulatory potential of VCO against acrylamide-induced neurotoxicity. It suggests that a complex interplay of mechanisms involving antioxidant defence, anti-inflammatory action, neurotrophic support, and neurotransmitter modulation may be involved in this neuromodulatory effect. In conclusion, this study has revealed that VCO supplementation may have a neuroprotective effect against AA-induced neurotoxicity by upregulating NrF2, downregulation of NFKB, and modulation of BDNF and AchE.

Conflict of Interest

The authors declare they have no financial or non-financial conflict of interest.

Authors' Declaration

The authors declare that the work presented in this article are original neither is it being considered for publication in any journal. The authors declare that any liability for claims relating to the content of this article will be borne by them.

References

1. Ajibare AJ, Odetayo AF, Akintoye OO, Olayaki LA. Zinc ameliorates acrylamide-induced oxidative stress and apoptosis in testicular cells via Nrf2/HO-1/NfkB and Bax/Bcl2 signaling pathway. Redox Rep. 2024;29(1):2341537
2. Asuku AO, Ayinla MT, Ajibare AJ, Adeyemo MB, Adeyemo RO. Heavy Metals and Emerging Contaminants in Foods and Food Products Associated with Neurotoxicity. In: Emerging Contaminants in Food and Food Products. CRC Press; 2024:236-250.
3. Lindeman B, Johansson Y, Andreassen M, Husøy T, Dirven H, Hofer T, Knutsen HK, Caspersen IH, Vejrup K, Paulsen RE, Alexander J. Does the food processing contaminant acrylamide cause developmental neurotoxicity? A review and identification of knowledge gaps. Reprod Toxicol. 2021;101:93-114
4. Dan P, Senthilkumar S, Narayanan Nampoothri VP, Swaminathan A, Mohideen SS. Drosophila melanogaster as a Model to Study Acrylamide Induced Toxicity and the Effects of Phytochemicals. Environ Biotechnol. 2021;4:201-224.
5. Zhao M, Deng L, Lu X, Fan L, Zhu Y, Zhao L. The involvement of oxidative stress, neuronal lesions, neurotransmission impairment, and neuroinflammation in acrylamide-induced neurotoxicity in C57/BL6 mice. Environ Sci Pollut Res. 2022;29(27):41151-41167.
6. Kopańska M, Łagowska A, Kuduk B, Banaś-Ząbczyk A. Acrylamide neurotoxicity as a possible factor responsible for inflammation in the cholinergic nervous system. Int J Mol Sci. 2022;23(4):2030.
7. Akintoye OO, Ajibare AJ, Omotuyi IO. Virgin coconut oil reverses behavioral phenotypes of letrozole-model of PCOS in Wistar rats via modulation of NRF2 upregulation. J Taibah Univ Med Sci. 2023;18(4):831-841.
8. Zeng YQ, He JT, Hu BY, Li W, Deng J, Lin QL, Fang Y. Virgin coconut oil: A comprehensive review of antioxidant activity and mechanisms contributed by phenolic compounds. Crit Rev Food Sci Nutr. 2024;64(4):1052-1075.
9. Kelsey NA, Wilkins HM, Linseman DA. Nutraceutical antioxidants as novel neuroprotective agents. Molecules. 2010;15(11):7792-7814
10. Sharifi-Rad M, Lankatillake C, Dias DA, Docea AO, Mahomoodally MF, Lobine D, Chazot PL, Kurt B, Boyunegmez Tumer T, Catarina Moreira A, Sharopov F. Impact of natural compounds on neurodegenerative disorders: from preclinical to pharmacotherapeutics. J Clin Med. 2020;9(4):1061.
11. Uddin MS, Mamun AA, Rahman MM, Jeandet P, Alexiou A, Behl T, Sarwar MS, Sobarzo-Sánchez E, Ashraf GM, Sayed AA, Albadrani GM. Natural Products for Neurodegeneration: Regulating Neurotrophic Signals. Oxid Med Cell Longev. 2021;2021(1):8820406.
12. Mehri S, Dadesh Q, Tabeshpour J, Hassani FV, Karimi G, Hosseinzadeh H. Evaluation of the neuroprotective effect of silymarin on acrylamide-induced neurotoxicity. Jundishapur J Nat Pharm Prod. 2016;11(4). https://doi.org/10.17795/jjnpp-37644
13. Farouk SM, Gad FA, Almeer R, Abdel-Daim MM, Emam MA. Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brains. Biomed Pharmacother. 2021;138:111458.
14. Famurewa AC, Folawiyo AM, Enohnyaket EB, Azubuike-Osu SO, Abi I, Obaje SG, Famurewa OA. The beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats. Integr Med Res. 2018;7(3):257-263.
15. Karandeep K, Navnidhi C, Poorva S, Garg MK, Anil P. Coconut meal: Nutraceutical importance and food industry application. Foods Raw Mater. 2019;7(2):419-427.
16. Ajibare AJ, Akintoye OO, Folawiyo MA, Babalola KT, Omotuyi OI, Oladun BT, Aransi-Ola KT, Odetayo AF, Olayaki LA. Therapeutic potential of virgin coconut oil in mitigating sodium benzoate-model of male infertility: Role of Nrf2/Hmox-1/NF-kB signalling pathway. Iran J Basic Med Sci. 2024;27(5):54317.
17. Famurewa AC, Ekeleme-Egedigwe CA, Nwali SC, Agbo NN, Obi JN, Ezechukwu GC Dietary Supplementation with Virgin Coconut Oil Improves Lipid Profile and Hepatic Antioxidant Status and Has Potential Benefits on Cardiovascular Risk Indices in Normal Rats. J Diet Suppl. 2018; 15, 330–342.
18. Ajibare AJ, Odetayo AF, Akintoye OO, Oladotun AJ, Hamed MA. Zinc abates sodium benzoate-induced testicular dysfunction via upregulation of Nrf2/ HO-1/ Nf-κB signaling and androgen receptor gene. JTEMB. 2024; 86, 127505.
19. Danish M, Nizami M. Complete fatty acid analysis data of flaxseed oil using GC-FID method. Data Br. 2019; 23: 103845.
20. Omotuyi OI, Nash O, Enejoh OA, Oribamise EI, Adelakun NS. *Chromolaena odorata* flavonoids attenuate experimental nephropathy: Involvement of pro-inflammatory genes downregulation. Toxicol Rep. 2020; 7, 1421–1427.
21. Lee S, Park HR, Lee JY, Cho JH, Song HM, Kim AH, Lee W, Lee Y, Chang SC, Kim HS, Lee J. Learning, memory deficits, and impaired neuronal maturation attributed to acrylamide. J Toxicol Environ Health A. 2018; 81, 254–265.
22. John J, Sapa NK & Shenoy RR. Virgin Coconut Oil Ameliorates Colchicine Induced Cognitive Dysfunction- A Preclinical Study. Pharm Sci. 2020; 26, 1–12.
23. Young L, Smith B, Webber-Waugh A, Thaxter K. Virgin Coconut Oil Improved Discriminative Learning and Working Memory in Aging Cycling and Non-Cycling Female Sprague-Dawley Rats Supporting Its Beneficial Effect in Retarding Age-Related Cognitive Decline. Adv Aging Res. 2021;10(5):97-112.
24. Ajibare AJ, Akintoye OO, Famurewa AC, Folawiyo MA, Bamisi OD, Asuku AO, Oyegbola OE, Akintayo CO, Olofinbiyi BA, Omotuyi OI. Synergistic Action of Virgin Coconut Oil and Clomiphene in Reversing Endocrine Dysregulation in Letrozole-Model of Polycystic Ovarian Syndrome in Rats: Role of Nrf2/HMOX-1 Pathway. J Med Food. 2023; 26, 683–691.
25. Mizumura K, Maruoka S, Shimizu T, Gon Y. Role of Nrf2 in the pathogenesis of respiratory diseases. Respiratory investigation. 2020;58(1):28-35.
26. Cong, B., Liu, C., Wang, L. & Chai, Y. The Impact on Antioxidant Enzyme Activity and Related Gene Expression Following Adult Zebrafish (*Danio rerio*) Exposure to Dimethyl Phthalate. Animals 2020;10(4):717. https://doi.org/10.3390/ani10040717
27. Famurewa AC, Maduagwuna EK, Folawiyo AM, Besong EE, Eteudo AN, Famurewa OA, Ejezie FE. Anioxidant, anti-inflammatory, and antiapoptotic effects of virgin coconut oil against antibiotic drug gentamicin-induced nephrotoxicity via the suppression of oxidative stress and modulation of iNOS/NF-κB/caspase-3 signalling pathway in Wistar rats. J Food Biochem. 2020; 44(1):e13100.
28. Sayed S, Alotaibi SS, El-Shehawi AM, Hassan MM, Shukry M, Alkafafy M, Soliman MM. The Anti-Inflammatory, Antiapoptotic, and Antioxidant Effects of a Pomegranate-Peel Extract against Acrylamide-Induced Hepatotoxicity in Rats. Life. 2022; 12(2), 224. https://doi.org/10.3390/life12020224
29. Illam, S. P., Narayanankutty, A., Kandiyil, S. P. & Raghavamenon, A. C. Variations in natural polyphenols determine the anti-inflammatory potential of virgin coconut oils. J Food Sci. 2021; 86, 1620–1628.
30. Famurewa, A. C., Maduagwuna, E. K., Aloke, C., Azubuike-Osu, S. O. & Narayanankutty, A. Virgin coconut oil ameliorates arsenic hepatorenal toxicity and NO-mediated inflammation through suppression of oxidative stress in rats. Thai J Pharm Sci. 2022; 46, 167–172.

31. Ündağ İ & Dönmez HH. Protective Effect of Nigella Sativa Oil on Hippocampus in Acrylamide-Induced Toxicity in Rats. Pak Vet J. (2023) doi:10.29261/pakvetj/2023.046.
32. Bhat BA, Almilaibary A, Mir RA, Aljarallah BM, Mir WR, Ahmad F, Mir MA. Natural Therapeutics in Aid of Treating Alzheimer's Disease: A Green Gateway Toward Ending Quest for Treating Neurological Disorders. Front Neurosci. 2022;16:884345.
33. Rahim NS, Lim SM, Mani V, Hazalin NA, Majeed AB, Ramasamy K. Virgin Coconut Oil-Induced Neuroprotection in Lipopolysaccharide-Challenged Rats is Mediated, in Part, Through Cholinergic, Antioxidative and Anti-Inflammatory Pathways. J Diet Suppl. 2021; 18, 655–681.
34. Sandupama P, Munasinghe D & Jayasinghe M. Coconut oil as a therapeutic treatment for Alzheimer's disease: a review. J Future Foods. 2022; 2, 41–52.
35. Moss, D. E. Improving Anti-Neurodegenerative Benefits of Acetylcholinesterase Inhibitors in Alzheimer's Disease: Are Irreversible Inhibitors the Future? Int J Mol Sci. 2020; 21(10), 3438
36. Teleanu RI, Niculescu AG, Roza E, Vladâcenco O, Grumezescu AM, Teleanu DM. Neurotransmitters—Key Factors in Neurological and Neurodegenerative Disorders of the Central Nervous System. Int J Mol Sci. 2022; 23, 5954.
37. Chang YC, Lee HL, Yang W, Hsieh ML, Liu CC, Lee TY, Huang JY, Nong JY, Li FA, Chuang HL, Ding ZZ. A common East-Asian ALDH2 mutation causes metabolic disorders and the therapeutic effect of ALDH2 activators. Nat Commun. 2023; 14(1), 5971.
38. Silalahi, J. Nutritional Values and Health Protective Properties Of Coconut Oil. Indones J Pharm Clin Res. 2020; 3, 1–12.
39. Baptista LC, Sun Y, Carter CS, Buford TW. Crosstalk Between the Gut Microbiome and Bioactive Lipids: Therapeutic Targets in Cognitive Frailty. Front Nutr. 2020; 7. https://doi.org/10.3389/fnut.2020.00017
40. Sundrasegaran S & Mah SH. Extraction Methods of Virgin Coconut Oil and Palm-pressed Mesocarp Oil and their Phytonutrients. eFood 2020; 1, 381–391.
41. Pisani A, Paciello F, Del Vecchio V, Malesci R, De Corso E, Cantone E, Fetoni AR. The role of BDNF as a biomarker in cognitive and sensory neurodegeneration. J Pers Med. 2023;13(4): 652.
42. Khotimah H, Prima WE, Weningtyas A, Aninditha D, Alita SN, Kalsum U, Nandar SK, Rahayu M, Handayani D. DNeuroprotective Activity and Antioxidant Effect of *Salacca zalacca* Peel Ethanol Extract on High Glucose Induced Zebrafish (*Danio rerio*) Embryo. Trop J Nat Prod Res. 2021;5, 2079–2084.
43. Edem EE, Ihaza BE, Fafure AA, Ishola AO, Nebo KE, Enye LA, Akinluyi ET. Virgin coconut oil abrogates depression-associated cognitive deficits by modulating hippocampal antioxidant balance, GABAergic and glutamatergic receptors in mice. Drug Metab Pers Ther. 2022; 37, 177–190.
44. Mansouri E, Asghari S, Nikooei P, Yaseri M, Vasheghani-Farahani A, Hosseinzadeh-Attar MJ. Effects of virgin coconut oil consumption on serum brain-derived neurotrophic factor levels and oxidative stress biomarkers in adults with metabolic syndrome: a randomised clinical trial. Nutr Neurosci. 2024; 27, 487–498
45. Hariyadi DM, Sudarma S, Shandra NM, Rosita N. Formulation of Peel-Off Mask Containing Natural Antibacterial: Study on Poly Vinyl Alcohol (PVA) and Virgin Coconut Oil (VCO) Contents. Trop J Prod Nat Res. 2021;5(3):514-8.).
46. Ortega DR, Esquivel DF, Ayala TB, Pineda B, Manzo SG, Quino JM, Mora PC, de la Cruz VP. Cognitive impairment induced by lead exposure during lifespan: mechanisms of lead neurotoxicity. Toxics. 2021;9(2):23. doi: 10.3390/toxics9020023