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Original Research Article

Argan Oil Mitigates Oxidative Stress Induced by Chronic Alcohol Consumption in Rats: An *In Vivo* and *In Silico* Study with Computational ADMET and Molecular Docking Analysis

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ABSTRACT

The escalating issue of excessive alcohol consumption poses a significant health concern, leading to various disruptions across multiple physiological pathways. Argan oil (AO), derived from the fruit of "Argania spinosa L", an indigenous tree native to southwestern Morocco, is renowned for its diverse health and well-being advantages. In this study, we focused on the impact of alcohol on the oxidation-reduction system, a critical aspect frequently influenced by alcohol. To investigate the potential mitigating effects of argan oil on oxidative stress, we employed three distinct treatment groups of rats: (1) alcohol-treated (3 g/kg), (2) alcohol (3 g/kg) and argan oil-treated (10 ml/kg), and (3) water-treated (control). Following a one-month treatment period, the rats were sacrificed, and their organs were utilized for biochemical assays. Results revealed that argan oil treatment significantly mitigated oxidative stress markers, as evidenced by a reduction in malondialdehyde (MDA) levels and restoration of antioxidant enzyme activities, such as superoxide dismutase (SOD) and catalase (CAT), toward normal levels. At the molecular level, ADMET and docking analyses demonstrated favorable binding interactions between argan oil molecules and proteins implicated in oxidative stress pathways. These findings underscore the potential of argan oil to restore equilibrium in the oxidation-reduction system, offering neuroprotective and antioxidant effects. This study provides foundational insights into the protective effects of argan oil against alcohol-induced oxidative damage, highlighting its potential as a therapeutic agent.

***Keywords*:** Alcohol consumption, *Argania spinosa*, Molecular Docking, Pharmacokinetic parameters, Oxidative stress, Wistar rats

Introduction

In numerous nations, the misuse of alcohol emerges as a pressing public health issue. [1,2] Extensive scientific inquiry has been directed towards identifying remedies, encompassing interventions for psychological dependence and the array of health maladies stemming from heightened alcohol consumption.[3] Persistent and immoderate alcohol consumption can precipitate grave health consequences, spanning conditions such as hepatic cirrhosis, chronic pancreatic inflammation, and epileptic episodes.[4–9]

From a biochemical standpoint, within the adult population, the metabolic conversion of ethanol to acetaldehyde predominantly occurs via the alcohol dehydrogenase enzyme pathway.[7–11] Nevertheless, in specific demographic groups, including fetuses, the metabolic route is mediated by alternative enzymes, exemplified by CYP2E1, which has the propensity to generate deleterious free radicals and noxious byproducts during the ethanol catabolic process. [12–14] Despite recent advancements in research on treatments for the damages associated with excessive alcohol consumption, the medications currently available in the pharmaceutical market come with notable side effects.[15–18] In this context, exploring alternative strategies such as the administration of antioxidants appears relevant.

In recent years, the significance of antioxidant consumption and their potential to mitigate the risks of chronic diseases, including epilepsy, cancer, coronary ailments, strokes, diabetes, and arthritis, has been emphasised. [19–22] Argan oil (AO) has been specifically chosen for its rich chemical composition comprising polyunsaturated fatty acids (PUFAs), sterols, and antioxidants like polyphenols and tocopherols.[23,24] This unique composition grants AO preventive properties against various conditions, notably cardiovascular diseases, diabetes, and prostate cancer.[25–30] Originating from the seeds of *Argania spinosa* L, AO is consumed in its raw form in southwestern Morocco. Long revered in traditional medicine for its healing virtues, it is now

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acknowledged for its diverse pharmacological, cosmetic, bactericidal, and fungicidal properties.

This study, conducted in two parts, primarily aimed to examine the potential impact of AO pre-treatment on reducing oxidative stress induced by prolonged alcohol consumption in the hippocampal and prefrontal cortex regions of adult rats. The theoretical (first part) involved analysis through docking and ADMET, while the experimental (second part) aimed to evaluate the effect of argan oil on oxidative stress using biochemical assays.

Materials and Methods

GC-MS Volatile Composition Protocol

Using a gas chromatograph coupled with a mass spectrometer detector, qualitative and semi-quantitative analysis of the argan oil was conducted, as described by El Guerrouj et *al*. [31] The chemicals were identified and separated utilizing a Shimadzu GC system manufactured in Kyoto, Japan, in conjunction with an MS QP2010 equipment available from Fuji Scientific Instruments in Kyoto, Japan. The process of separation was carried out by employing a BPX5 capillary column that consisted of 5% phenyl and 95% dimethylpolysiloxane. The carrier gas utilized in this experiment was pure helium (99.99 percent). To ensure a consistent flow rate of 1.69 mL/min, the injection, interface temperatures, and ion source were all held constant at 250 °C. Programming the column heating system to increase at a rate of 10 °C per minute from 50 °C (for 1 minute) to 250 °C (for 1 minute), maintaining the temperature for 1 minute. The components of the sample were ionized using Electron Impact (EI) mode at an energy of 70 eV. The mass range that was analyzed varied between 40 and 300 m/z. Following that, 1 µL of each produced oil was injected with a fractionation ratio of 90:1. Each sample underwent three assessments, during which chemical identification was achieved by comparing retention durations to validated standards and mass spectrum fragmentation models derived from databases or NIST compounds.[32] The method of collecting and processing data was carried out using Laboratory Solutions (v2.5).

Experimental Protocol: Alcohol Intoxication, Pre-treatment (Argan oil) and Voluntary Intake Test

Argania spinosa (L) skeels fruits sourced from the Agadir region in southwest Morocco were used to extract virgin argan oil (AO) through cold pressing, voucher specimen number fruits HUMPOM164 of the fruits was deposited at the herbarium of the faculty of sciences, University Mohammed the First, Oujda, Morocco. This method was chosen to minimize the risk of auto-oxidation, given that the seeds were freshly harvested in the same year. The extracted AO was stored at a constant temperature of 25°C and protected from direct light in a tinted glass bottle. Its precise composition is detailed in Table 1.

Fifteen male Wistar rats were selected for this study, ensuring uniformity in age and weight. Only healthy animals, free from any signs of illness or complications, were included, weighing on average 45 ± 5 g and aged one month, were included in the study and divided into three groups of five: (1) a pretreated group receiving daily oral administration of AO, (2) a negative control group receiving daily oral administration of 0.9% NaCl saline solution, and (3) a positive control group receiving daily oral administration of the saline solution. AO administration was performed precisely, using a gavage needle adapted to the size of the rats to ensure accurate delivery of 10 ml per kg of body weight, administered daily between 10:00 and 12:00 for one month. Post-administration monitoring was performed to detect any reactions or side effects, and daily food intake and weight measurements were meticulously documented over a period of four weeks. Alcohol intoxication was induced over a two-week period, during which the rats received forced doses of alcohol through intraperitoneal injections of 3 g/kg three times a day. Following this period, a voluntary intake test was conducted: the rats were placed in individual cages with two bottles—one containing water and the other alcohol. This test measured the amount of alcohol consumed by each rat over four weeks to (The test is simple because directly after the two weeks of alcohol injections each rat will receive two bottles the first contains alcohol and the second water and this is where we can confirm if the rat has developed a preference for alcohol or not) compare consumption levels across the different groups and confirm the desire for consumption.[33–35]

Nitric Oxide Test

To assess nitrite levels in the control group (n = 5), AO plus alcohol group (n = 5), and alcohol group (n = 5), 10% (*w/v*) homogenates were subjected to centrifugation (800 × g, 10 min). The resulting supernatants were collected and quantification of nitric oxide production was performed using the Griess reaction. The microplate reader (Elisa Microplate Agilent YR06087, Santa Clara, CA, USA) was used, the measured values were expressed in nanomolar (nM) units.[36]

Catalase Activity Evaluation

The evaluation of catalase activity is based on measuring the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) by the enzyme catalase. The detailed protocol is as follows: For sample preparation, the tissue or cell culture to be analyzed should be homogenized in a phosphate buffer solution with a pH of 7.0. Then, centrifuge at 10,000 g for 10 minutes at 4°C to remove cell debris and collect the supernatant for enzymatic analysis.

For preparing the hydrogen peroxide solution, a fresh H_2O_2 solution at 30 mM in a phosphate buffer with a pH of 7.0 is required. During the enzymatic reaction, add 50 µL of the supernatant sample to 2.95 mL of the H_2O_2 solution in a spectrophotometry cuvette and mix quickly to start the reaction. The measurement of catalase activity is done by recording the decrease in absorbance at 240 nm at regular intervals (every 30 seconds) for 1 to 2 minutes using a spectrophotometer. The decrease in absorbance is due to the decomposition of H_2O_2. One unit of catalase activity is defined as the amount of enzyme needed to decompose 1 µmol of H_2O_2 per minute at 25°C and a pH of 7.0. For calculating catalase activity with Elisa microplate reader (Elisa Microplate Agilent YR06087, Santa Clara, CA, USA), this device features a large LCD touchscreen interface for intuitive operation. It supports 96-well plates, enabling multiple assays per plate, and includes adjustable plate shaking with customizable speed and duration settings, we used the molar extinction coefficient of H_2O_2 at 240 nm (ε = 43.6 $M^{-1}.cm^{-1}$) to calculate the concentration of remaining H_2O_2 at each time point. Catalase activity is expressed in units per milligram of protein (U/mg of protein).

The equation for the reaction catalyzed by catalase is:

$$2H_2O_2 - \text{Catalase} \rightarrow 2H_2O + O_2$$

This equation represents the decomposition of hydrogen peroxide into water and oxygen under the action of catalase.[36]

ADMET Analysis

The evaluation of ADME (absorption, distribution, metabolism, excretion, and toxicity) is crucial in obtaining pharmacokinetic properties, understanding the compound's nature, assessing its toxicity, and considering various parameters. To conduct this analysis, we employed Swiss ADME [37] and PkCSM. [38] This assessment is vital for the ongoing research as it provides an initial insight into molecules that may be unsuitable for further studies due to toxicity or those deemed essential after an initial passage. [39]

Ligands Preparation

To enhance and reduce the energy of compounds identified in Argan oil (AO) extracts, data sourced from the PubChem database were utilized. The LigPrep module within Maestro 12.8 (Schrodinger 2021-2) was employed to prepare ligands, with delta-Tocopherol (vitamin E) serving as the study's standard.[40] Energy minimization and optimization procedures adhered to the OPLS_2005 force field. Hydrogen atoms were introduced, and adjustments were implemented to mitigate salt and ionization effects at a pH of 7.00 ± 2.00.

Molecular Docking and Preparation of Target Protein

Molecular docking was performed using the X-ray crystal structures retrieved from the Protein Data Bank (PDB). Specifically, the structures

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of "STRUCTURE OF A NITRIC OXIDE SYNTHASE HEME PROTEIN FROM *BACILLUS SUBTILIS* WITH, TETRAHYDROFOLATE AND ARGININE BOUND" (PDBID: 1M7V) with an X-ray diffraction resolution of 1.95 Å, and "HUMAN ERYTHROCYTE CATALASE" (PDBID: 1DGF) with an X-ray diffraction resolution of 1.50 Å were utilized (see Figure 1).[40] The preparation of these protein structures involved the use of the Protein Preparation Wizard.[41] During this process, ligand and water atoms were removed, and non-polar hydrogens were merged. The active site was designated as the target center. To create an optimal docking environment, the central grid box dimensions were set to encompass all atoms of the ligand set, with 10 points allocated for each axis (x, y, and z). Energy minimization was carried out with default settings, constraining the root-mean-square deviation (RMSD) to 0.3 Å. The standard precision (SP) glide score was employed to predict binding, selecting anchored poses. The output docking scores were defined as affinity binding in Kcal/mol. Subsequently, the protein structure underwent further minimization using the OPLS_2005 force field. We employed BIOVIA Discovery Studio 2021 to visualize the protein-ligand complexes.[42]

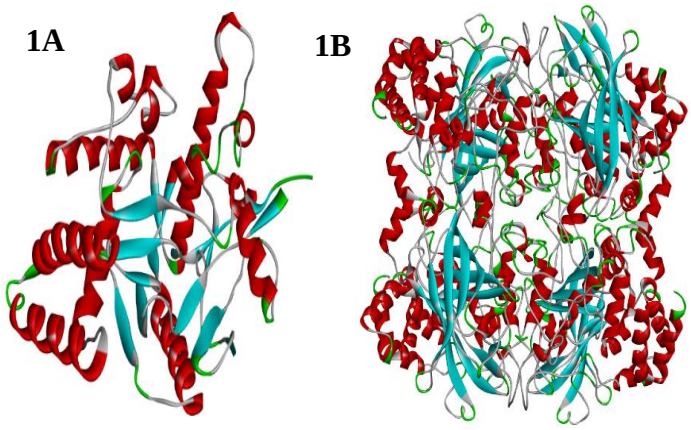

Figure 1: The Crystal structure of the proteins (1A) 1M7V, and (1B) 1DGF.

Statistical Analysis

The statistical analyses in this study were performed using GraphPad Prism software (Boston, MA, USA). One-way ANOVA was employed as the statistical analysis for each test. Significance was determined at $P < 0.05$. Variations among the distinct groups were assessed through a one-way analysis of variance.[43–45]

Results and Discussion

GC-MS Phytochemical Composition of A. spinosa Oil

The phytochemical analysis of Argan oil detailed in Table 1 identifies a diverse range of compounds, each characterized by distinct chemical formulas, molecular weights, retention times, and relative abundances. The primary findings reveal that Oleic Acid ($C_{18}H_{34}O_2$) is the most abundant component, with a retention time of 17.328 minutes and comprising 38.777% of the oil.

This high concentration underscores oleic acid's significance as a major constituent, contributing extensively to Argan oil's nutritional value and pharmacological benefits, including anti-inflammatory and heart-health-promoting properties. Squalene ($C_{30}H_{50}$) is the second most prevalent compound, with a retention time of 17.002 minutes and a percentage area of 37.389%. Known for its potent antioxidant effects, squalene's substantial presence supports its use in skincare formulations and therapeutic applications, enhancing the oil's protective and regenerative qualities. Another key compound identified is Trans-13-Octadecenoic Acid ($C_{18}H_{34}O_2$), which appears at a retention time of 17.334 minutes and accounts for 9.35 % of the oil. As an unsaturated fatty acid, *trans*-13-Octadecenoic Acid is beneficial for cardiovascular health, contributing to Argan oil's positive effects on cholesterol regulation and heart disease prevention (Figure 2). Oleamide ($C_{18}H_{35}NO$) was also detected, with a retention time of 17.457 minutes and a percentage area of 6.279%. This compound is known for its bioactive properties, including its potential role as a signaling molecule, influencing physiological processes such as sleep modulation and anti-inflammatory responses.

In addition to these major components, several other compounds were found in smaller quantities, including palmitic acid, ethyl nonadecanoate, *trans*-farnesol, farnesyl diphosphate, and hexadecenoic acid (Z-11), with percentage areas ranging from 0.091% to 4.164%. While present in lower concentrations, these compounds may still contribute to the overall bioactivity of Argan oil, enhancing its therapeutic properties through synergistic effects.

Overall, the detailed phytochemical profile of Argan oil highlights its complex composition, with each compound playing a role in its nutritional and therapeutic potential. The abundance of bioactive molecules such as oleic acid and squalene underpins Argan oil's widespread use in both traditional and modern health applications, validating its value as a natural product with diverse health benefits.

Voluntary Alcohol Intake

The test outcomes demonstrated a variation in the quantity of alcohol intake starting from the initial week in comparison to both the AO group and the control group. Notably, the second and third weeks were characterized by the most notable alcohol consumption during the test, particularly for the positive control (alcoholic) group, reaching 0.3 g/day. Their consumption significantly exceeded that of the other groups, namely T- and AO (Figure 3).

Table 1: GC-MS phytochemical profile of Argan's oil

No.	Compounds	Chemical formula / Molecular weight (g/mol)	Retention Time (min)	% Area
1	Palmitic acid	$C_{16}H_{32}O_2$ / 256.42	15.862	4.164
2	Ethyl nonadecanoate	$C_{21}H_{42}O_2$ / 326.56	16.077	0.091
3	*trans*-Farnesol	$C_{15}H_{26}O$ / 222.37	16.834	0.291
4	Farnesyl diphosphate	$C_{15}H_{28}O_7P_2$ / 382.33	16.873	0.346
5	Squalene	$C_{30}H_{50}$ / 410.72	17.002	37.389
6	Oleic acid	$C_{18}H_{34}O_2$ / 282.47	17.328	38.777
7	*trans*-13-Octadecenoic acid	$C_{18}H_{34}O_2$ / 282.47	17.334	9.356
8	Oleamide	$C_{18}H_{35}NO$ / 281.48	17.457	6.279
9	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$ / 254.41	18.042	0.125
	Total (%)			**96.818**

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Assessment of Nitrite Levels Catalase Activity in the Hippocampus and Prefrontal Cortex

The nitrite levels in the hippocampus of rats pre-treated with AO exhibited significant variations compared to the other groups. The impact of AO on nitrite concentrations in the prefrontal cortex and hippocampus is illustrated in Figures 4 (4A and 4B). A notable increase in nitrite levels was observed in the hippocampus and prefrontal cortex of the alcohol-treated group. Conversely, AO pre-treated rats showed a decrease in nitrite levels compared to the alcohol group. Furthermore, the control group animals (administered saline solution) did not exhibit any alterations in nitrite levels. Refer to Figure 4A and 4B for more details. The impact of AO on catalase activity in the hippocampus and prefrontal cortex of alcoholic rats was also investigated, Moreover, the impact of argan oil on catalase activity in the hippocampus and prefrontal cortex of alcoholic rats was examined in Figure 4C and 4D. Interestingly, the findings demonstrated that the group pre-treated with argan oil exhibited a notable reduction in catalase activity compared to the positive control group. This effect was consistently observed within both brain structures studied.

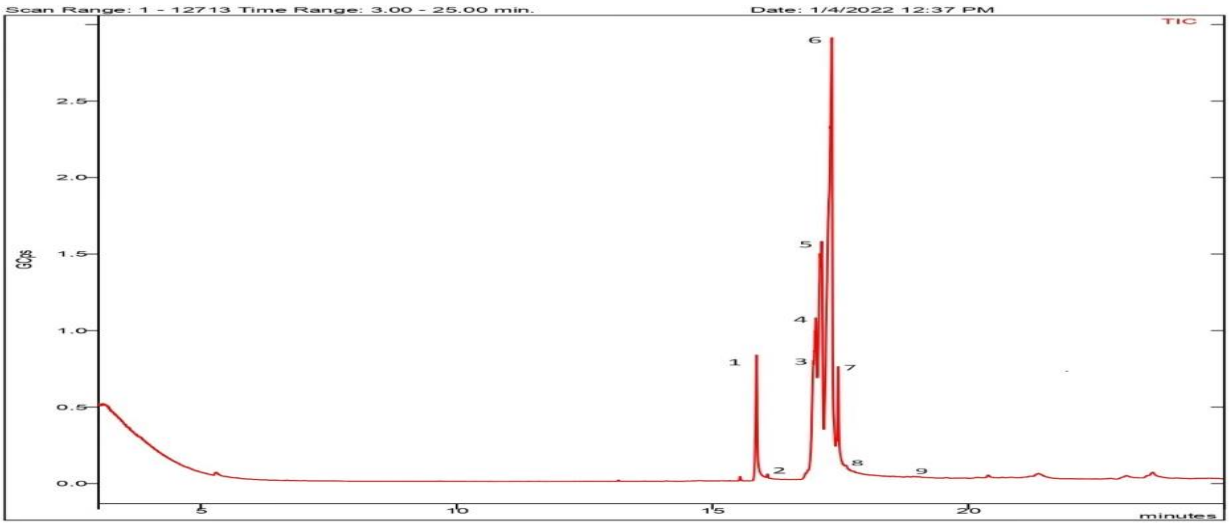

Figure 2: Phyochemical profile of Argan's oil using GC/MS analysis. (1) Palmitic acid (n-Hexadecanoic acid); (2) Ethyl nonadecanoate; (3) trans-Farnesol; (4) Farnesyl diphosphate; (5) Squalene; (6) Oleic acid; (7) trans-13-Octadecenoic acid; (8) Oleamide; (9) Hexadecenoic acid, Z-11-.

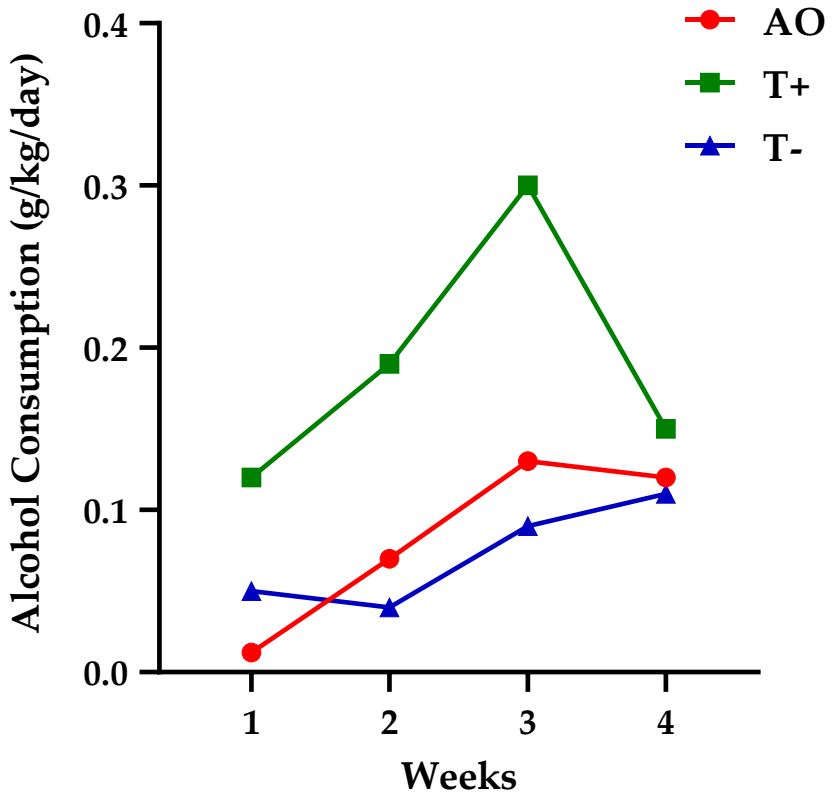

Figure 3: Alcohol consumption over four weeks of study period. Values are presented as Mean ± standard deviation (n=5 for each study group). AO: Alcohol + argan oil-treated group; T+: alcohol-treated group; and T-: saline water-treated group.

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ADMET profile

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) profile of a drug significantly influences its efficacy. However, assessing these characteristics in clinical research can be costly and challenging. Hence, the present study employed computational approaches to evaluate the ADMET properties and explore the potential of "*Argania spinosa*" oils for drug development.[46]

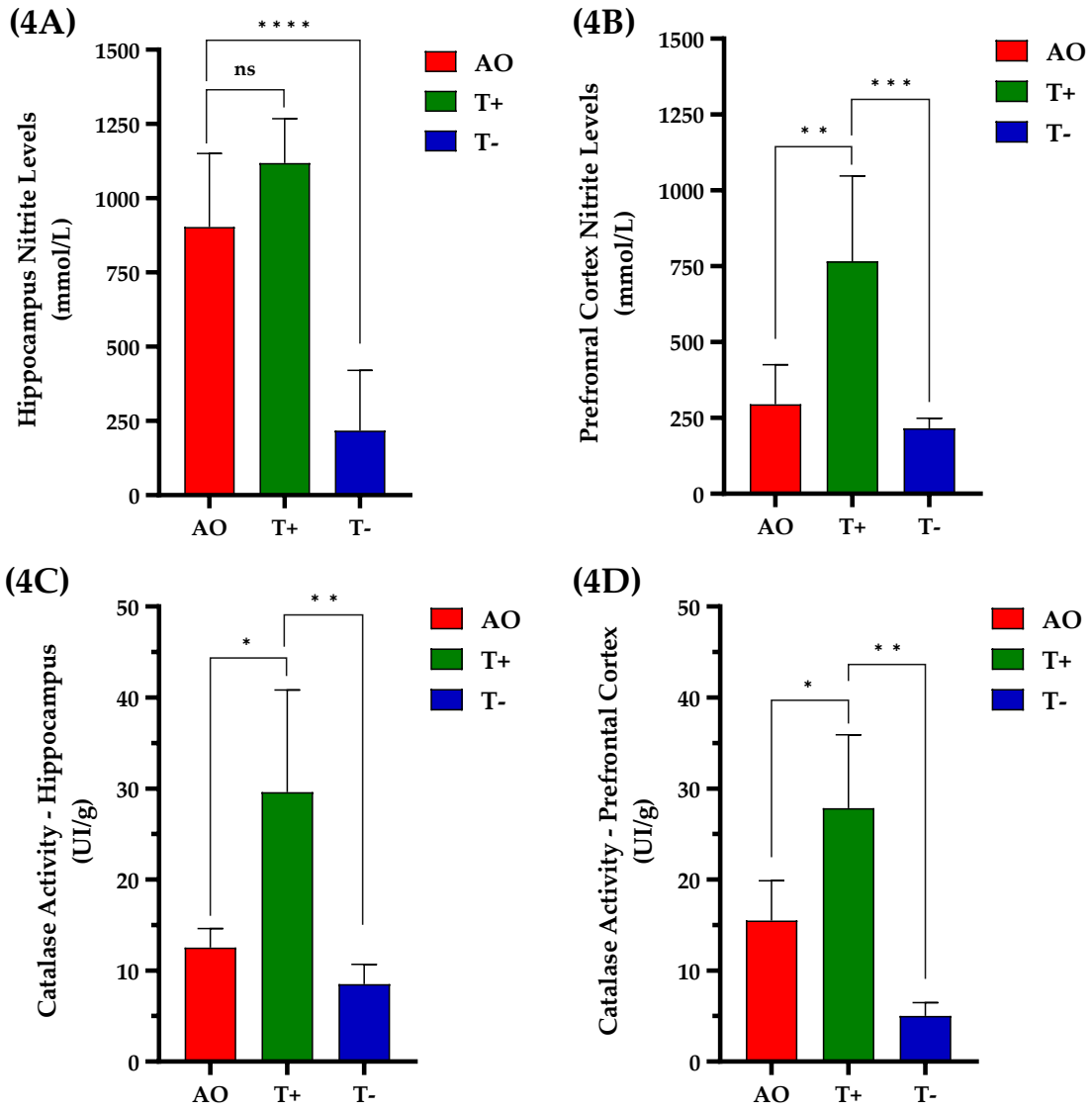

Figure 4: Oxidative stress markers of each treated group (n=6). Nitrite levels (4A, 4B), Catalase activity (4C, 4D). Values are presented as mean ± standard deviation. AO: Alcohol + argan oil-treated group; T+: alcohol-treated group; and T-: saline water-treated group.

Table 2 presents the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile of various compounds identified in argan oil (AO) extracts, offering essential insights into their pharmacokinetic properties and drug-likeness.[37,47] Regarding drug-likeness, all compounds comply with Lipinski's Rule of Five, indicating favorable physicochemical properties and potential oral bioavailability. The bioavailability scores of the compounds range from 0.55 to 0.85, with compounds 2, 3, 5, 6, and 8 displaying higher scores (0.85), suggesting superior oral absorption potential. In terms of water solubility, the Log s (ESOL) values vary significantly, ranging from -8.69 (compound 7) to -3.10 (compound 2). Lower values denote reduced solubility, posing potential challenges for formulation. This trend is corroborated by Log s (ali) and Log s (SILICO-IT) metrics, confirming compound 7's notably low solubility across different prediction methods.

Pharmacokinetically, compounds 3, 4, 5, 6, 8, and 9 demonstrate high gastrointestinal absorption, which is advantageous for oral administration. Compounds 3, 4, and 9 also exhibit the ability to permeate the blood-brain barrier (BBB), essential for targeting the central nervous system. None of the compounds are substrates of P-glycoprotein (Pgp), reducing the risk of efflux-mediated drug resistance. The cytochcome P450 enzyme inhibition profile reveals that most compounds are inhibitors of CYP1A2 and CYP2C9, indicating potential drug-drug interactions. Only compound 9 inhibits CYP2D6, suggesting a lower likelihood of interactions for the other compounds. Additionally, compounds 1 and 9 inhibit CYP3A4, a significant finding due to CYP3A4's role in metabolizing many drugs. The skin permeability values (LOGKp) range from -6.81 (compound 2) to -0.58 (compound 7), with lower values indicating reduced permeability.

From a medicinal chemistry perspective, no PAINS (Pan Assay Interference Compounds) alerts were triggered, implying a lower risk of false positives in biological assays. However, most compounds triggered one or two Brenk alerts, indicating the presence of potentially undesirable structural elements. None of the compounds meet lead-likeness criteria, which may affect their potential as lead compounds for drug development. Accessibility values range from 2.31 (compound 6) to 5.08 (compound 2), with lower values suggesting easier synthetic routes.

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The ADMET profiles indicate that several compounds have promising pharmacokinetic properties, particularly in terms of bioavailability and gastrointestinal absorption. However, concerns regarding water solubility, BBB permeability, and cytochrome P450 inhibition must be addressed. These findings are crucial for evaluating the feasibility of these compounds as potential drug candidates and guiding further optimization in drug development processes.

Figure 5 shows the "Boiled Egg Test" results provide a detailed assessment of the gastrointestinal absorption (GI) and blood-brain barrier (BBB) permeability of compounds identified in argan oil extracts, crucial for understanding their pharmacokinetic properties and potential as drug candidates. Compounds 3, 4, 5, 6, 8, and 9 show high GI absorption, indicating their potential effectiveness when administered orally, ensuring sufficient bioavailability and enhancing therapeutic efficacy. In contrast, compounds 1, 2, and 7 display low GI absorption, suggesting limited oral bioavailability and reduced therapeutic potential unless formulation strategies or alternative delivery methods are implemented.

Compounds 3, 4, 6, and 9 are predicted to cross the BBB, making them promising candidates for treating neurological disorders due to their ability to target the central nervous system (CNS). On the other hand, compounds 1, 2, 5, 7, and 8 are unlikely to cross the BBB, which restricts their use in CNS-targeted therapies but leaves them viable for peripheral targets where BBB permeability is not necessary.

An integrated analysis highlights that compound 3, 4, 6, and 9, with both high GI absorption and BBB permeability, are ideal candidates for oral drugs targeting CNS conditions, demonstrating a favorable pharmacokinetic profile for neurological applications. Compounds 5 and 8, despite high GI absorption, do not penetrate the BBB, making them suitable for peripheral applications but potentially requiring modification for CNS use. Compounds 1, 2, and 7, with low GI absorption and no BBB permeability, present significant challenges and may need optimized formulations or alternative administration routes to enhance their therapeutic potential.

The "Boiled Egg Test" emphasizes the importance of predictive modeling in drug development, aiding in the identification of compounds with desirable pharmacokinetic properties and guiding formulation strategies to maximize therapeutic efficacy. However, further experimentaland optimization are necessary to fully realize the therapeutic potential of these compounds.

Table 2: Drug-likeness, water solubility, pharmacokinetics, and medicinal chemistry parameters of the compounds identified in argan oil (AO).

Compounds		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Drug-Likeness	Lipinski's rule of five	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Bioavailability Score	0.55	0.56	0.85	0.55	0.85	0.85	0.55	0.85	0.55
Water Solubility	LogS (ESOL)	-6.79	-3.10	-4.69	-5.00	-5.41	-5.02	-8.69	-5.41	-4.17
	LogS (Ali)	-10.44	-5.00	-7.14	-7.71	-8.26	-7.77	-11.57	-8.26	-5.60
	LogS (SILICO-IT)	-8 .44	-1.45	-4.59	-5.61	-5.39	-5.33	-7.48	-5.39	-3.15
Pharmacokinetics	Gi absorption	Low	Low	High	High	High	High	Low	High	High
	BBB Permeability	No	No	Yes	Yes	No	Yes	No	No	Yes
	Pgp substrate	No	No	No	No	No	No	No	No	No
	CYP1A2 Inhibitor	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes
	CYP2C9 Inhibitor	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No
	CYP2D6 Inhibitor	No	No	No	No	No	No	No	No	Yes
	CYP3A4 Inhibitor	Yes	No	No	No	No	No	No	No	No
	Log Kp	-2.03	-6.81	-3.19	-3.05	-2.60	-2.77	-0.58	-3.81	-3.81
Medicinal Chemistry	PAINS	0	0	0	0	0	0	0	0	0
	Brenk (Number of alerts)	0	2	1	1	1	1	1	1	1

(1) Palmitic acid, (2) Ethyl nonadecanoate, (3) *trans*-Farnesol, (4) Farnesyl diphosphate, (5) Squalene, (6) Oleic acid, (7) *trans*-13-Octadecenoic acid, (8) Oleamide, (9) Hexadecenoic acid, Z-11-.

Molecular docking analysis

The results in Table 3 show the docking scores of selected compounds found in argan oil (AO) extracts with the target protein, measured in kcal/mol. These scores reflect the varying levels of affinity between the compounds and the protein. Ethyl nonanoate (-8.3 kcal/mol) and squalene (-8.1 kcal/mol) exhibit the highest docking scores, indicating a strong affinity for the protein. Farnesyl diphosphate (-7.2 kcal/mol) and trans-farnesol (-6.2 kcal/mol) show moderate scores, suggesting a reasonable affinity. Compounds like hexadecenoic acid Z-11- (-5.3 kcal/mol), oleamide (-5.2 kcal/mol), and the inhibitor N-nitroso-L-arginine (-5.2 kcal/mol) have lower docking scores, pointing to lesser affinity. In conclusion, ethyl nonanoate and squalene emerge as the most promising candidates for effective interaction with the target protein, making them interesting candidates for further investigation due to their strong binding affinity.

Table 3: The docking scores of the selected docked compounds identified in Argan oil (AO) extracts with the protein 1DGF-NO synthase, 1M7V-catalase.

Compounds	**Binding energy values (kcal/mol)**	
	1DGF	**17MV**
Ethyl Nona decanoate	-8.3	-8.9
Farnesyl diphosphate	-7.2	-9.1
Hexadecenoic acid, Z-11-	-5.3	-6.4
Oleamide	-5.2	-6.5
Oleic acid	-6	-6.4
Palmitic acid	-6.1	-6.2
Squalene	-8.1	-8.9
Trans-13-octadecenoic acid	-5.9	-6.4
Trans-farnesol	-6.2	-7.1
Inhibitor (N-nitroso-L-arginine)	-5.2	-4.7

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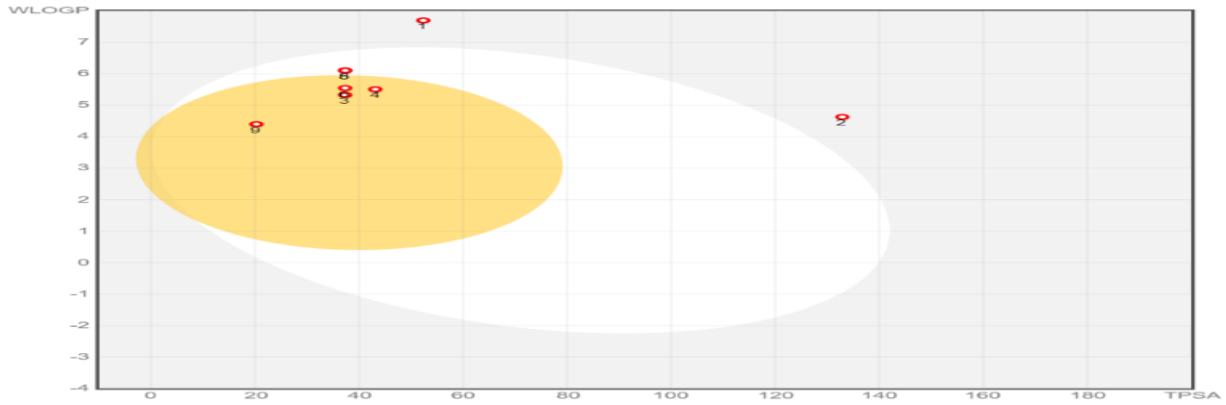

Figure 5: BOILED-EGG test results for the components of Argan oil. Compounds located in the yolk (yellow zone) are those with high intestinal absorption and high blood-brain barrier (BBB) permeability. In contrast, compounds in the white zone are absorbed in the intestine but do not penetrate the BBB. Red dots represent compounds that are P-glycoprotein substrates (-PGP). (1) Palmitic acid, (2) Ethyl nonadecanoate, (3) trans-Farnesol, (4) Farnesyl diphosphate, (5) Squalene, (6) Oleic acid, (7) trans-13-Octadecenoic acid, (8) Oleamide, (9) Hexadecenoic acid, Z-11-.

Analysis and Comparison of Docking Scores and Interactions

The docking scores for various compounds found in argan oil extracts, interacting with the proteins 1DGF (NO synthase) and 1M7V (catalase), are summarized in Table 2. These scores reflect the binding affinity between the compounds and the target proteins, where lower (more negative) scores indicate stronger binding interactions. Ethyl nona decanoate exhibits strong binding affinity to both proteins, with scores of -8.3 kcal/mol for 1DGF and -8.9 kcal/mol for 1M7V, showing a slightly stronger interaction with 1M7V. Similarly, farnesyl diphosphate displays a notable preference for 1M7V (-9.1 kcal/mol) over 1DGF (-7.2 kcal/mol), indicating a potential better accommodation in the 1M7V binding site. Hexadecenoic acid (Z-11) and oleamide both show moderate binding affinities for the proteins, with hexadecenoic acid scoring -5.3 kcal/mol for 1DGF and -6.4 kcal/mol for 1M7V, and oleamide scoring -5.2 kcal/mol for 1DGF and -6.5 kcal/mol for 1M7V. These results suggest a slightly better affinity for 1M7V in both cases.

Oleic acid also binds moderately to both proteins, with scores of -6.0 kcal/mol for 1DGF and -6.4 kcal/mol for 1M7V, indicating a slight preference for 1M7V. Palmitic acid, with scores of -6.1 kcal/mol for 1DGF and -6.2 kcal/mol for 1M7V, shows almost equal binding affinities to both proteins, with a marginal preference for 1M7V. Squalene stands out with strong binding affinities, particularly with 1M7V (-8.9 kcal/mol), compared to its score of -8.1 kcal/mol for 1DGF. Trans-13-octadecenoic acid and trans-farnesol exhibit moderate binding, with trans-13-octadecenoic acid scoring -5.9 kcal/mol for 1DGF and -6.4 kcal/mol for 1M7V, and trans-farnesol scoring -6.2 kcal/mol for 1DGF and -7.1 kcal/mol for 1M7V, both showing better affinity for 1M7V. The inhibitor N-nitroso-L-arginine, with scores of -5.2 kcal/mol for 1DGF and -4.7 kcal/mol for 1M7V, demonstrates relatively weak binding affinity for both proteins, with a slightly stronger affinity for 1DGF.

The docking scores reveal that most compounds have a stronger binding affinity with 1M7V (catalase) compared to 1DGF (NO synthase). This suggests that the active site of 1M7V may be more accommodating to the tested compounds, or that these compounds have a structural compatibility with the binding pocket of catalase. The notable exceptions are the inhibitor N-nitroso-L-arginine and ethyl nona decanoate, which show relatively similar binding affinities for both proteins. The compounds with the strongest binding affinities include ethyl nona decanoate and squalene, particularly with 1M7V. These interactions could be of interest for further exploration, especially in the context of modulating the activity of these enzymes. Farnesyl diphosphate, which participates in post-translational modifications, also shows significant binding, particularly with 1M7V, which may suggest potential regulatory effects.

Further experimental validation, such as biochemical assays and structural analysis, would be necessary to confirm these interactions and understand their physiological relevance. This analysis highlights potential candidates for modulating the activity of NO synthase and catalase, which could have implications in therapeutic development and the study of enzymatic regulation.

The figures (6, 7) presented in the document illustrate the interactions between NO synthase and several molecules, including ethyl nanodecenoate, squalene, and farnesyl diphosphate. These detailed examinations provide valuable insights into the nature and potential effects of these molecular associations.

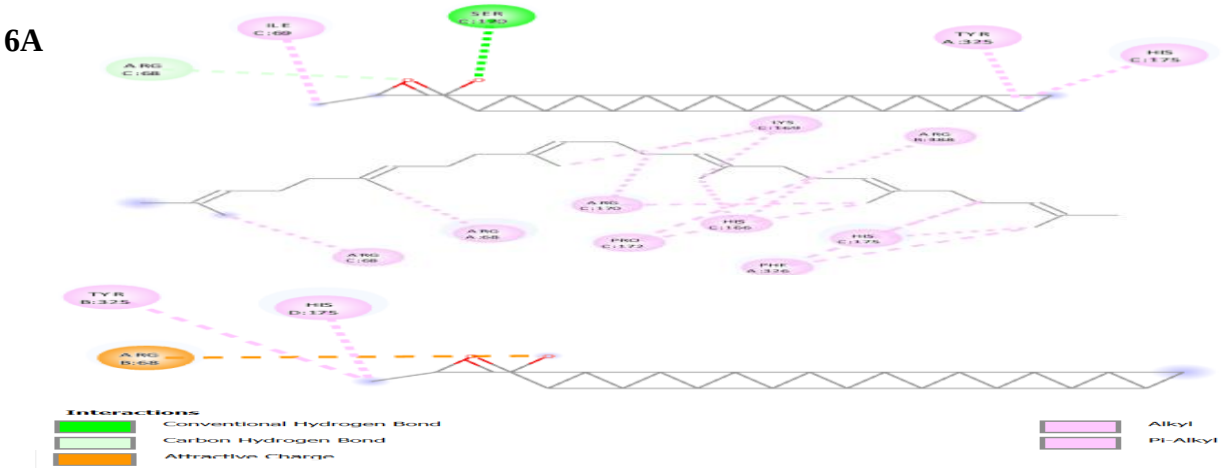

Figure 6: Catalase interactions with (7A, ethyl nanodecenoate; 7B, Squalen; 7C, pharnesyl diphosphate).

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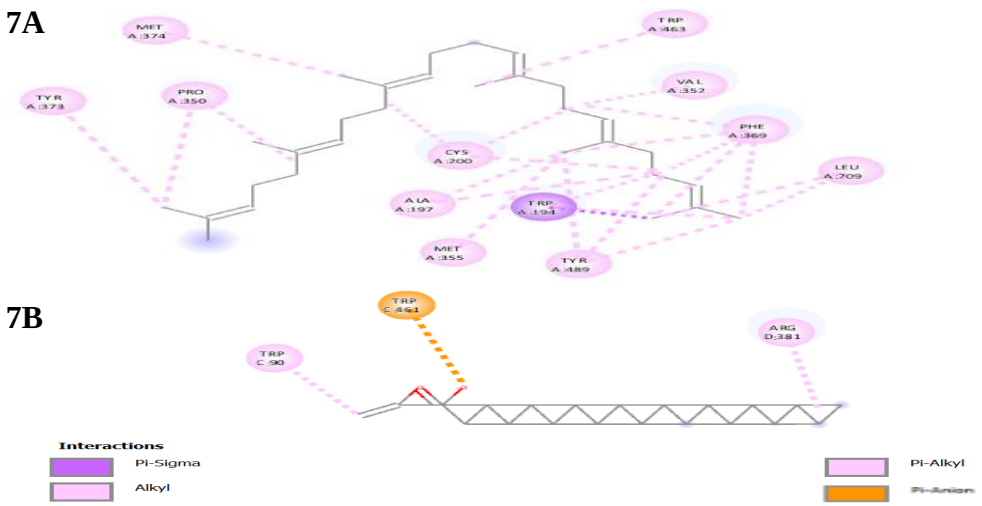

Figure 7: NO synthase Interactions with (7A) Ethyl nanodecenoate, and (7B) Squalene.

Ethyl nanodecenoate, an ester, is observed to interact with NO synthase, likely driven by hydrophobic forces due to its nonpolar nature. Such interactions may influence the positioning and orientation of NO synthase within the lipid membrane, potentially affecting its activity by altering the microenvironment around the enzyme. This could have implications for the regulation of nitric oxide production, as changes in membrane association can impact the enzyme's accessibility to substrates or cofactors.

Squalene, a triterpene, interacts with NO synthase, potentially through hydrophobic interactions as well. Given squalene's role as a precursor in sterol biosynthesis, its interaction with NO synthase could modulate the lipid environment, affecting membrane fluidity and, consequently, enzyme function. The presence of squalene might influence the structural dynamics of NO synthase, altering the enzyme's conformation and activity.

Farnesyl diphosphate, an intermediate in the biosynthesis of isoprenoids, interacts with NO synthase. This interaction may involve specific interactions, such as hydrogen bonds or ionic interactions, due to the presence of phosphate groups. Additionally, farnesyl diphosphate can participate in post-translational modifications, such as farnesylation, which could anchor NO synthase to specific membrane domains, thereby affecting its activity and localization.

The analysis suggests that NO synthase's interactions with various hydrophobic molecules, such as ethyl nanodecenoate and squalene, are primarily driven by hydrophobic forces. These interactions are significant because they can alter the enzyme's conformation and the lipid environment, potentially impacting its catalytic efficiency and stability. The interaction with farnesyl diphosphate highlights the possibility of post-translational modifications that could modulate the enzyme's function and localization within the cell.

Overall, these molecular interactions underscore the complexity of NO synthase regulation and the potential influence of lipid-soluble molecules on its activity. The modulation of NO synthase by hydrophobic molecules could be a critical factor in cellular signaling and homeostasis, affecting nitric oxide production and its downstream physiological effects. Further experimental studies, such as molecular dynamics simulations and mutagenesis, would be necessary to elucidate the precise nature of these interactions and their impact on NO synthase function.

Argan oil has commonly been utilized as a remedy for minor ailments such as eczema, skin inflammation, and various other issues. The beneficial effects of this oil can be attributed to its chemical richness, which is characterized by the presence of unsaturated fatty acids, sterols, and antioxidant compounds. Additionally, the oil is recognized for its *in vitro* activities, including cardioprotection and protection against cancer Therefore, the objective of this study was to assess the neuroprotective effect of argan oil against the detrimental effects induced by excessive alcohol consumption. Our findings revealed a significant reduction in alcohol consumption among subjects treated with argan oil, accompanied by a decrease in oxidative stress within two specific brain structures of the Wistar rat. It is widely acknowledged that under normal conditions, the brain generates free radicals which it can effectively manage. However, excessive alcohol consumption leads to an upsurge in radical production, consequently resulting in neuronal cell death. The richness of argan oil is a significant advantage, primarily due to its composition, which includes a high concentration of antioxidant molecules like tocopherols and various others. This may explain the observed decrease in free radical levels in the prefrontal cortex and hippocampus.

As evidenced by previous studies, there is a well-established correlation between excessive alcohol consumption and cell death.[48–50] Recent research indicates that this phenomenal on is associated with the induction of oxidative stress stemming from the generation of free radicals through NADPH oxidase activation. The subsequent involvement of the TRPM2 channel, allowing the influx of calcium ions, ultimately culminates in cellular demise, As illustrated in Figure 8, As depicted in Figure 8, this pathway elucidates one of the mechanisms through which cell death occurs, initiated by the activation of a cascade of reactions that commences with the activation of protein kinase C and culminates in the opening of TRPM2 channels. The subsequent entry of calcium ions leads to cell demise. Remarkably, the level of radicals plays a crucial role in regulating the activation of this cascade, thereby influencing cell fate. Motivated by this observation, we investigated the potential effect of argan oil on alcohol-induced oxidative stress. Our experimental results substantiated the presence of an antioxidative effect exerted by argan oil, which was observed in general oxidative stress reduction and in specific activities assessed by dosages. [50]

Our findings provide empirical evidence supporting the beneficial effect of argan oil on voluntary alcohol consumption in Wistar rats as illustrated in Figure "Alcohol Intake". The trajectory of alcohol craving in rats treated with argan oil closely resembled that of the negative control group, indicating a decrease in alcohol consumption. This effect aligns with the outcomes reported in recent studies conducted by Hicham, further corroborating the positive impact of argan oil on alcohol intake regulation. The administration of argan oil treatment has also been found to contribute to a reduction in oxidative stress, as manifested in this study by an elevation in catalase activity in the hippocampus and prefrontal cortex. This suggests an enhancement of antioxidant activity within the brain, resulting in a subsequent decrease in free radical levels and the preservation of cerebral cell viability. The ability of argan oil to combat oxidative stress is further supported by the observed decline in nitrite levels in both brain structures. These effects of argan oil can be attributed to its unique chemical composition. Additionally, the observed impact of argan oil in this particular case may be attributed to the initiation of treatment at an early age of three weeks, during a critical period of neural development.

Molecular docking studies were conducted to predict the binding interactions between proteins and compounds identified in Argan oil (AO). The active site of the HUMAN ERYTHROCYTE CATALASE protein (PDB: 1DGF) was targeted, and stable interactions were observed

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for all compounds except Omega-3, Omega-6, and Omega-9. Negative and low docking scores indicated robust and favorable binding interactions, with Spinasterol exhibiting the highest stability. For the protein (PDB: 1M7V), Gamma-tocopherol showed the highest stability. Despite promising *in silico* results, further research, including clinical trials with human subjects, is needed to assess the therapeutic efficacy, safety, and pharmacokinetics of these compounds.

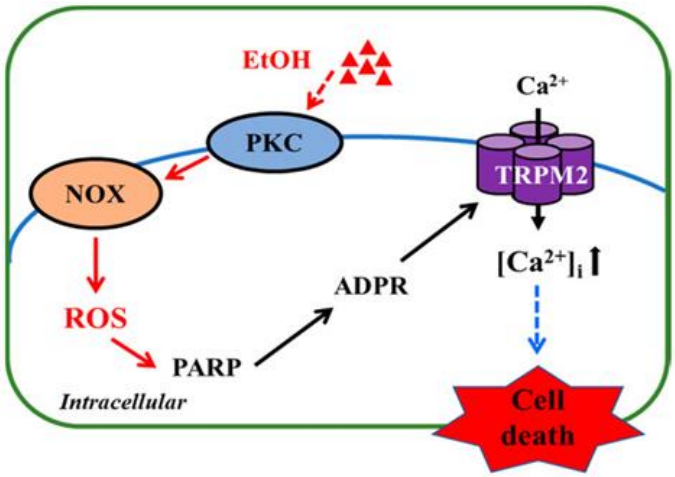

Figure 8: Mechanism of cell death (NOX- NADPH oxidase, PKC:protein kinase C, PARP: poly- (ADP-riboses) Polymérases, TRPM2: Transient receptor potential cation channel, subfamily M, member 2 .[55]

Conclusion

In summation, the research herein highlights the potential neuroprotective efficacy of argan oil against oxidative damage induced by prolonged alcohol exposure. The empirical evidence not only underscores a marked decrease in alcohol consumption and oxidative stress among the argan oil-administered rodent subjects but also accentuates the pivotal antioxidant properties inherent to argan oil. Predictive outcomes from molecular docking analyses offer a robust scientific framework for further exploration of the therapeutic potentials embedded within argan oil constituents, setting the stage for extensive future studies. The imperative progression to human clinical trials is essential for the corroboration of these preliminary findings and to comprehensively evaluate the therapeutic viability and safety profiles of argan oil compounds. This investigation significantly enriches the existing body of knowledge on natural therapeutic interventions for the mitigation and management of Alcohol-induced neurological disorders, thereby advancing the discourse on leveraging natural resources for the enhancement of human health and wellness.

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Availability of Data and Materials: The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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