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Original Research Article

Molecular mechanistic evaluation of spermatotoxic and mutagenic effects of Δ^9-tetrahydrocannabinol (THC) on the male reproductive system and genomic integrity

Edu N. Enyogor [a], Pius O. Adah [a], Bethel C. Ateb [a*], Henry B. Kogbara [a], Elizabeth U. Ateb [c], Gabriel I. Abu [a], Nsude L. Odinakachukwu [b], Akwagiobe P. Abuh [a], Adamu Muhammed [d]

[a] *Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria*

[b] *Department of Genetics and Biotechnology, State University of Medical Sciences, Enugu State, Nigeria*

[c] *University of Cross River State, Calabar, Nigeria*

[d] *Department of Biology, Nigerian Army University, Biu*

Abstract

Δ^9-tetrahydrocannabinol (THC), currently reported as the major psychoactive compound in *Cannabis sativa*, has become increasingly consumed by young adults and adolescents globally. Despite its recreational and medicinal potential, the molecular mechanism by which THC exerts its toxic effect on male fertility and genomic stability remains partially understood. This study seeks to address this gap by investigating the biochemical and genetic pathways affected by THC in male reproductive cells using an in-silico approach. The major target protein genes responsible for spermatogenesis and mutagenic genes were identified using Gene Card, and Network pharmacology was employed to reveal the main critical pathways affected. The docking result demonstrates high binding affinities with the spermatogenic and mutagenic-related genes, ranging from -6.2 to 8.2 kcal/mol. ADMET/Tox prediction of THC shows high intestinal absorption, high blood-brain barrier penetration, and active mitochondrial toxicity, revealing its adverse effect on male fertility and genomic stability when it comes into contact with genes responsible for regulating sperm development and DNA repair. These findings profile computational evidence revealing the toxic effect of THC on the male reproductive system and therefore underscore the need for further experimental validation and future awareness to save our male counterparts.

Keywords

Cannabis sativa, Δ^9-tetrahydrocannabinol, spermatotoxicity, mutagenicity, molecular docking, ADMET, network pharmacology.

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*Corresponding author

Email address: bethelateb@gmail.com (Bethel C. Ateb)

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1. Introduction

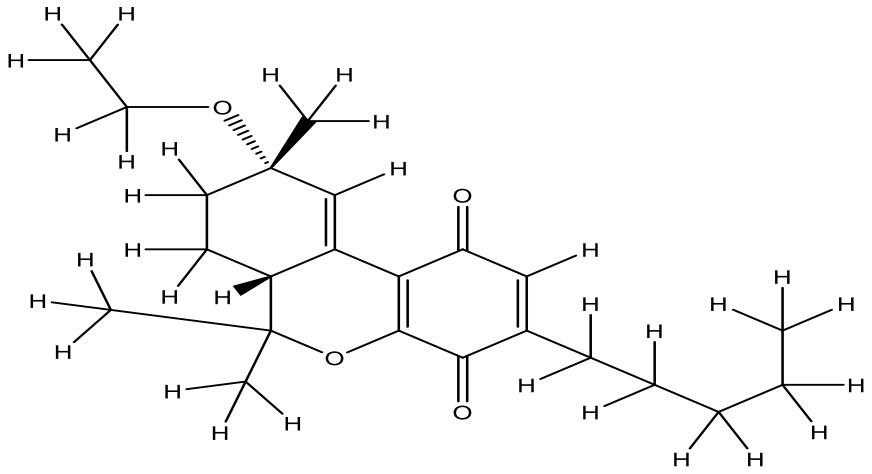
Cannabis (*Cannabis sativa*) is a plant with records of medicinal, industrial, and recreational use. It contains over 100 bioactive cannabinoids, with Δ^9-tetrahydrocannabinol (THC) documented as the major psychoactive compound accountable for most of its pharmacological effects **[1]**. Currently, they are raising global legalization and acceptance of cannabis, resulting in widespread use, particularly among adolescents and young adults **[2]**. Even though THC is used therapeutically to manage conditions such as chronic pain, nausea, and multiple sclerosis, emerging data have raised concerns regarding its impact on male reproductive health **[3]**. Research has shown that the human body contains a moderate number of endocannabinoids, which include the cannabinoid receptors CB1 and CB2, mainly found in various reproductive tissues, including the testes, seminal vesicles, and sperm cells. Therefore, having extra exogenous cannabinoids may pose a serious risk to reproductive processes **[4,5]**.

Men who consume cannabis frequently have been found to have low sperm counts, induce DNA disintegration, oxidative DNA damage, chromosomal aberrations, and micronucleus formation in exposed cells **[6,7]**. These effects are mostly expressed during spermatogenesis, a process requiring high-fidelity DNA replication and genome stability to ensure the transmission of intact genetic material to the next generation **[8]**. Sperm cells are particularly susceptible to oxidative stress because of their limited antioxidant defenses and high polyunsaturated fatty acid content **[7]**. When THC interacts with sperm mitochondria, it increases the generation of reactive oxygen species (ROS), which are known to damage DNA, lipids, and proteins **[8]**.

Despite these findings, themolecular mechanisms through which THC exerts its spermatotoxic and mutagenic effects remain in completely understood.

With the rapid developmentofstructural data and computational tools, in silico approaches such as gene identification, network pharmacology, molecular docking, and ADMET/Tox prediction have emerged as an essential technique in toxicology, paving the way for the computational evaluation of toxic compounds before further experimental validation.

This study comprehensively combines molecular docking, gene selection, network pharmacology, and ADMET/Tox prediction to investigate the molecular mechanisms through which THC exerts its spermatotoxic and mutagenic effects, to elucidate its impact on male reproductive function and genomic integrity, thereby contributing to the growing evidence supporting the toxic effects of THC.



(6a*R*,9*S*)-9-ethoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9-tetrahydro-1*H*-benzo[*c*]chromene-1,4(6*H*)-dione

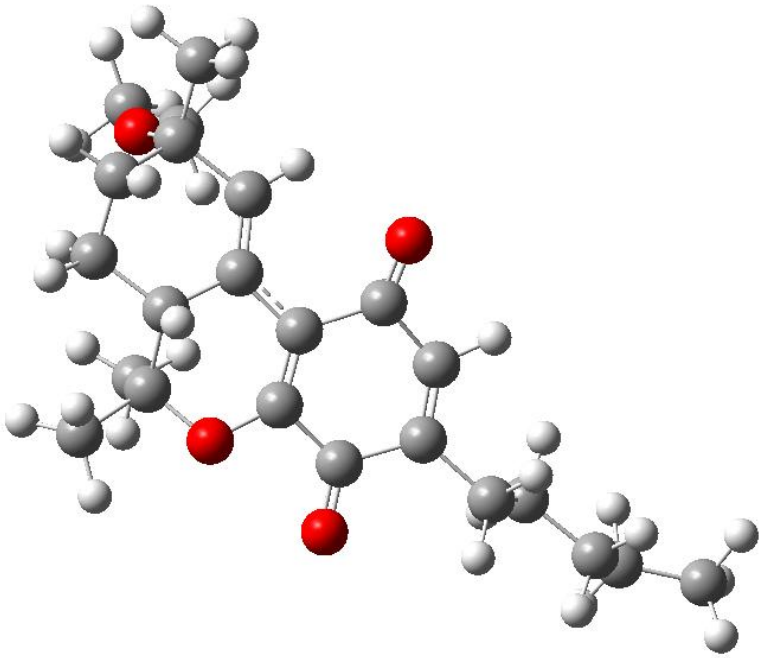


Figure 1. 3D structure of Δ^9-Tetrahydrocannabinol (THC)

2. Materials and Methods

2.1. Selection of Phytochemicals from *Cannabis sativa*

Δ^9-tetrahydrocannabinol (THC) was retrieved in SDF format from the reputable phytochemical databases PubChem **[9]**; https://pubchem.ncbi.nlm.nih.gov and energy minimization performed using the MMFF94 force field in Chem3D 15.1 **[10]**https://www.perkinelmer.com to obtain energetically stable conformations suitable for molecular docking studies.

2.2. Identification of target Genes and receptor preparation

A total of 49 genes associated with spermatogenesis and mutagenesis were identified through Gene Cards**[11]** https://www.genecards.org. The genes in **Tables 1 and 2** wereselected because of their protein-coding gene specificity associated with spermatogenesis and high GIFtS scores, which indicate their involvement in germ cell development, differentiation, and reproductive processes. The 3D structures of these genes were retrieved from the UniProt database in .pdb format **[12]** https://www.uniprot.org. Removal of water molecules and addition of polar hydrogens was carried out using BIO VIA Discovery Studio Visualizer **[13]**; https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/

Table 1.Spermatogenesis genes

Symbol	Description	Category	UniProt ID	GIFtS	GC id	Score
SPATA16	Spermatogenesis Associated 16	Protein Coding	Q9BXB7	45	GC03M172889	23.35
SOHLH1	Spermatogenesis And Oogenesis Specific Basic Helix-Loop-Helix 1	Protein Coding	Q5JUK2	45	GC09M135693	20.77
PATA22	Spermatogenesis Associated 22	Protein Coding	Q8NHS9	47	GC17M003440	18.85
SPATA6	S permatogenesis Associated 6	Protein Coding	Q9NWH7	46	GC01M048296	18.59
SPATA2	Spermatogenesis Associated 2	Protein Coding	Q9UM82	44	GC20M049903	18.28
SPATA9	Spermatogenesis Associated 9	Protein Coding	Q9BWV2	40	GC05M095652	17.76
SPATA25	Spermatogenesis Associated 25	Protein Coding	Q9BR10	37	GC20M045886	17.74
SPATC1L	Spermatogenesis And Centriole Associated 1 Like	Protein Coding	Q9H0A9	41	GC21M054956	16.78
GMCL1	Germ Cell-Less 1, Spermatogenesis Associated	Protein Coding	Q96IK5	43	GC02P069829	16.68
SPATA20	Spermatogenesis Associated 20	Protein Coding	Q8TB22	41	GC17P050543	16.35
SPATA32	Spermatogenesis Associated 32	Protein Coding	Q96LK8	34	GC17M045254	16.27
SPATA18	Spermatogenesis Associated 18	Protein Coding	Q8TC71	47	GC04P052051	16.24
SPATS2	Spermatogenesis Associated Serine Rich 2	Protein Coding	Q86XZ4	42	GC12P049366	16.16
SPATA4	Spermatogenesis Associated 4	Protein Coding	Q8NEY3	40	GC04M176184	16.10
M1AP	Meiosis 1 Associated Protein	Protein Coding	Q8TC57	44	GC02M074557	15.97
SPATA3	Spermatogenesis Associated 3	Protein Coding	Q8NHX4	41	GC02P231009	15.86
SOHLH2	Spermatogenesis And Oogenesis Specific Basic Helix-Loop-Helix 2	Protein Coding	Q9NX45	44	GC13M042958	15.57
AURKC	Aurora Kinase C	Protein Coding	Q9UQB9	60	GC19P153196	15.42
PROKR2	Prokineticin Receptor 2	Protein Coding	Q8NFJ6	53	GC20M010692	15.33
SPATA17	Spermatogenesis Associated 17	Protein Coding	Q96L03	44	GC01P217631	15.22
SPATA24	Spermatogenesis Associated 24	Protein Coding	Q86W54	35	GC05M139392	15.18
SPATA7	Spermatogenesis Associated 7	Protein Coding	Q9P0W8	48	GC14P088384	14.83
SPATA12	Spermatogenesis Associated 12	Protein Coding	Q7Z6I5	31	GC03P065196	14.76
SPATA33	Spermatogenesis Associated 33	Protein Coding	Q96N06	41	GC16P122954	14.71
SPATA6L	Spermatogenesis Associated 6 Like	Protein Coding	Q8N4H0	41	GC09M004553	14.70

Table 2.Mutagenesis genes

Symbol	Description	Category	UniProt ID	GIFtS	GC ID	Score
TP53	Tumor Protein P53	Protein Coding	P04637	66	GC17M007661	8.30
POLH	DNA Polymerase Eta	Protein Coding	Q9Y253	59	GC06P043576	6.23
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	Protein Coding	P00492	60	GC0XP134460	5.92
REV3L	REV3 Like, DNA Directed Polymerase Zeta Catalytic Subunit	Protein Coding	O60673	56	GC06M111299	5.70
REV1	REV1 DNA Directed Polymerase	Protein Coding	Q9UBZ9	54	GC02M102672	5.55
CFTR	CF Transmembrane Conductance Regulator	Protein Coding	P13569	66	GC07P117287	5.52
ACE	Angiotensin I Converting Enzyme	Protein Coding	P12821	64	GC17P063477	5.00
KCNQ1	Potassium Voltage-Gated Channel Subfamily Q Member 1	Protein Coding	P51787	62	GC11P002444	4.82
KCNH2	Potassium Voltage-Gated Channel Subfamily H Member 2	Protein Coding	Q12809	63	GC07M150944	4.76
BRCA1	BRCA1 DNA Repair Associated	Protein Coding	P38398	63	GC17M043044	4.74
EGFR	Epidermal Growth Factor Receptor	Protein Coding	P00533	68	GC07P055019	4.68
LPL	Lipoprotein Lipase	Protein Coding	P06858	63	GC08P019901	4.67
VWF	Von Willebrand Factor	Protein Coding	P04275	60	GC12M036869	4.55
RHO	Rhodopsin	Protein Coding	P08100	59	GC03P144186	4.48
UNG	Uracil DNA Glycosylase	Protein Coding	P13051	59	GC12P109097	4.47
SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase	Protein Coding	P12931	63	GC20P037344	4.30
CSNK2A1	Casein Kinase 2 Alpha 1	Protein Coding	P68400	63	GC20M000472	4.27
LDLR	Low Density Lipoprotein Receptor	Protein Coding	P01130	64	GC19P161380	4.25
SULT1A1	Sulfotransferase Family 1A Member 1	Protein Coding	P50225	55	GC16M053087	4.15
CD4	CD4 Molecule	Protein Coding	P01730	63	GC12P006786	4.12
PRKCA	Protein Kinase C Alpha	Protein Coding	P17252	65	GC17P066302	4.10
APP	Amyloid Beta Precursor Protein	Protein Coding	P05067	63	GC21M025880	4.07
APOBEC3B	Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 3B	Protein Coding	Q9UH17	50	GC22P038982	4.07
DNAH8	Dynein Axonemal Heavy Chain 8	Protein Coding	Q96JB1	49	GC06P190852	4.04

2.3. Network Pharmacology Analysis

To explore the interaction of THC with multiple biological pathways, a network pharmacology framework was established. Protein–protein interaction (PPI) networks were constructed using STRING **[13],** where all genes retrieved from Gene Cards were inputted. Cytoscape (v3.9.1) **[14]** https://cytoscape.org, integrated with NDExiQuery, was used to visualize the compound-target-pathway networks. This enabled the identification of key signaling and metabolic pathways potentially impacted by THC.

2.4. Molecular Docking

Molecular docking studies were conducted to evaluate the binding affinity and interaction profiles between THC and the selected genes. Docking was performed using AutoDock Vina 1.5.6 **[15]** (http://vina.scripps.edu) in triplicate to ensure reproducibility. Active-site docking was carried out for proteins with known ligand-binding residues, while blind docking was applied when binding sites were not clearly defined using BIOVIA **[13]**. Grid box parameters were set to encompass the entire active site or the protein surface (dimensions: X × Y × Z Å), depending on the docking approach. Validation was performed by redocking known ligands for each protein to confirm the reliability of the docking protocol. Genes with binding energies ≤ –6.0 kcal/mol were retained to include moderate binders that may have functional significance in the THC-targeted network, resulting in a final set of eight genes (four associated with spermatogenesis and four with mutagenesis). Docking interactions were visualized using PyMOL **[16]** for 3D representation and Discovery Studio Visualizer **[17]** for detailed 2D interaction analysis.

2.5. ADMET and Toxicity Prediction

Pharmacokinetic and toxicity profiles of THC were assessed using pkCSM **[18]** and ProTox-II **[19].** Key ADMET parameters evaluated included absorption (intestinal permeability, water solubility), distribution (blood–brain barrier penetration), metabolism (cytochrome P450 enzyme inhibition), excretion (biological half-life), and toxicity endpoints such as hepatotoxicity, mutagenicity via the Ames test, reproductive toxicity, and carcinogenicity. THC was input in SMILES format, and outcomes were analyzed comparatively to determine its safety profile.

2.6. QSAR Modeling

To predict the spermatotoxic and mutagenic potential of THC, Quantitative Structure Activity Relationship (QSAR) modeling was conducted using OCHEM **[20]** https://ochem.eu. THC was compared to known toxicants and mutagens obtained from curated training datasets. Molecular descriptors such as logP, molecular weight, polar surface area, and number of hydrogen bond donors and acceptors were calculated and analyzed.

3. Result

3.1. Network Pharmacology Analysis

The results shown in **Figs.2 and 3** enabled the mapping of key signaling pathways, including **PI3K/AKT**, **MAPK**, and **p53**, indicating that THC-targeted genes are involved in cell cycle control, apoptosis, DNA repair, and spermatogenesis progression**[21,22,23]**. SPATA6 and SPATA16 emerged as central proteins, linking reproductive signaling with general cellular stress responses. This is consistent with previous work by Sujitn et al. (2020), who found associations between cannabis metabolites and altered expression of reproductive pathway genes **[24].**

The involvement of p53 and RB signaling in the THC interaction network suggests potential impacts on DNA repair and cell cycle checkpoints **[25,26].** BRCA1, another key player affected, is central to homologous recombination repair. Disruption of such pathways by THC exposure could impair DNA repair fidelity, heightening mutagenic risk **[27].** Importantly, this THC-focused network outcome shows that THC specifically affects genes like SPATA6, SPATA16, and BRCA1, which could disrupt DNA repair and sperm development. This suggests a direct way that THC exposure might harm reproductive health, beyond the usual general stress responses.

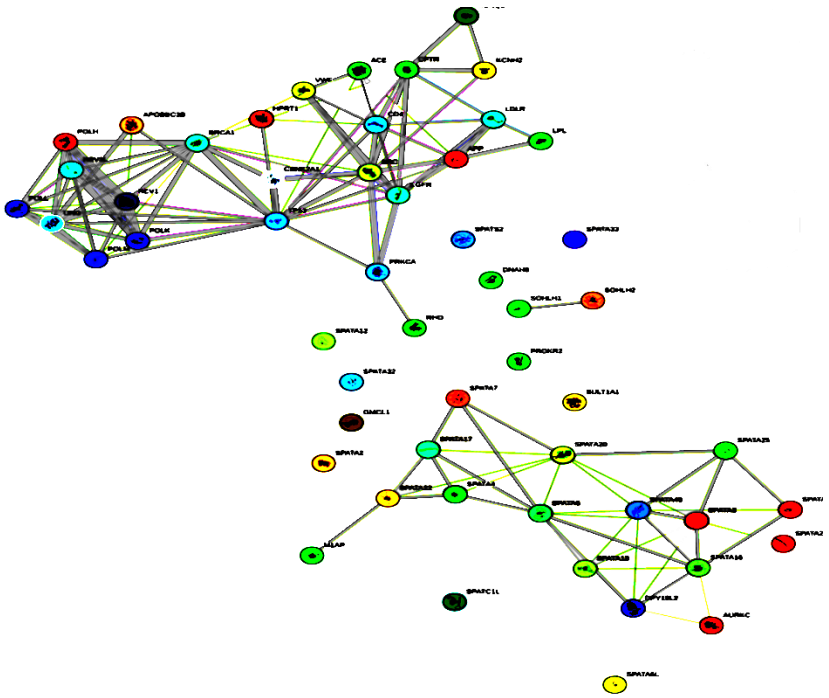


Figure 2. Protein-protein interaction

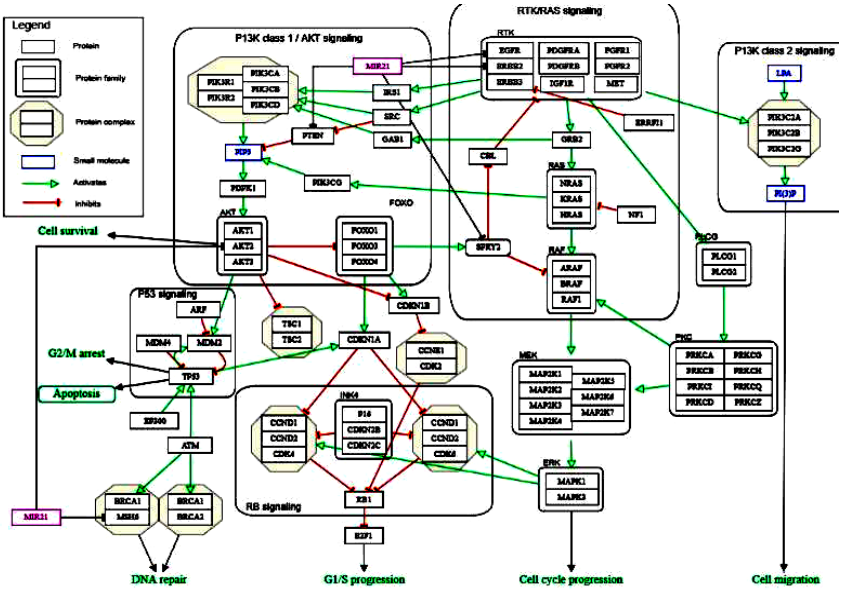


Figure 2. Signaling pathway map

3.2. Molecular Docking

The molecular docking results are in **Table3,** and the visualization in **Figs.3 and 4** reveal strong binding affinities between THC and several genes associated with spermatogenesis (Q8N4H0: –8.2 kcal/mol, Q86XZ4: –8.1 kcal/mol), suggesting a high likelihood of interaction. Notably, SPATA6-like (Q8N4H0) and SPATS2 (Q86XZ4) are critical regulators of sperm motility and germ cell development. Studies by Whan et al. (2006) and Hehemann et

al. (2015) have emphasized the importance of SPATA genes in the progression of meiosis and spermatid maturation. The high binding affinity observed suggests that THC could interfere with these functions, potentially impairing spermatogenesis **[28,29]**.

Similarly, the interaction of THC with mutagenesis-related proteins such as POLH (Q9Y253: –6.2 kcal/mol) and KCNQ1 (P51787: –6.0 kcal/mol) raises concern about DNA damage tolerance and genomic stability. POLH plays a pivotal role in translesion synthesis, enabling replication past DNA lesions **[30]**. Inhibitory binding of THC to this gene may result in replication stress and increased mutational load, consistent with earlier studies linking cannabis use to sperm DNA fragmentation **[31]**

Table 3. Binding affinities obtained from protein-ligand interaction

Protein–ligand interaction	Binding affinities
spermatogenesis genes	
Q8N4H0+THC	-8.2
Q8TB22+THC	-6.8
Q86XZ4+THC	-8.1
Q5JUK2+THC	-6.0
metagenes	
Q9Y253+THC	-6.2
P12821+THC	-6.3
P51787+THC	-6.0
Q12809+THC	-6.2

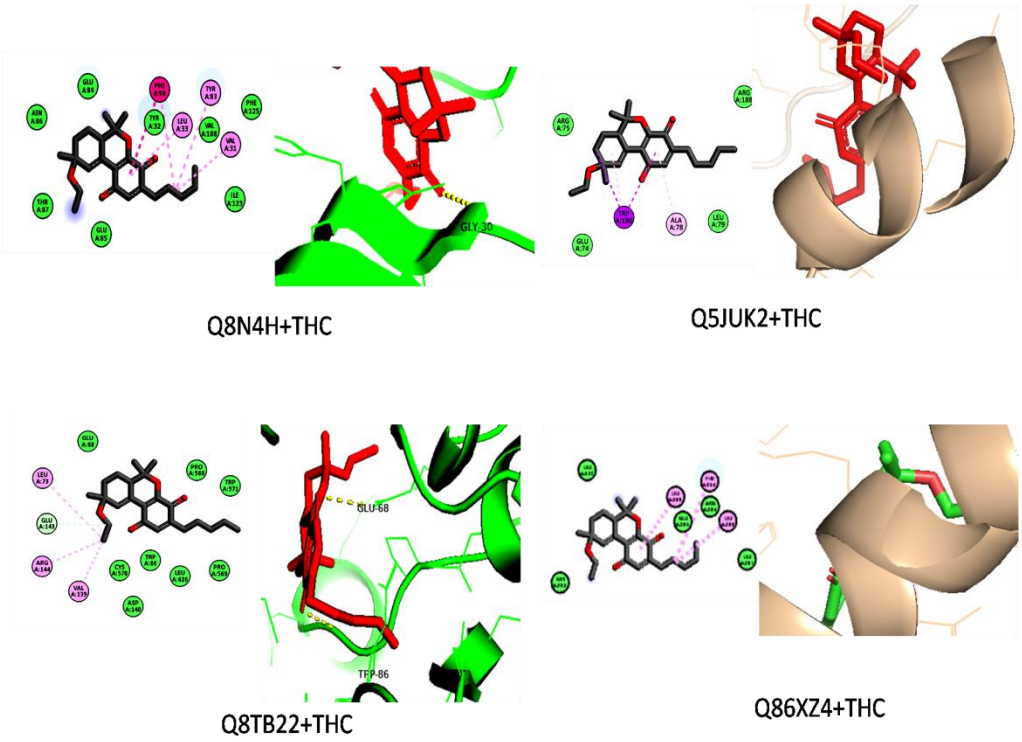


Figure 3. 2D and 3D visualization of spermatogenesis genes docked with THC.

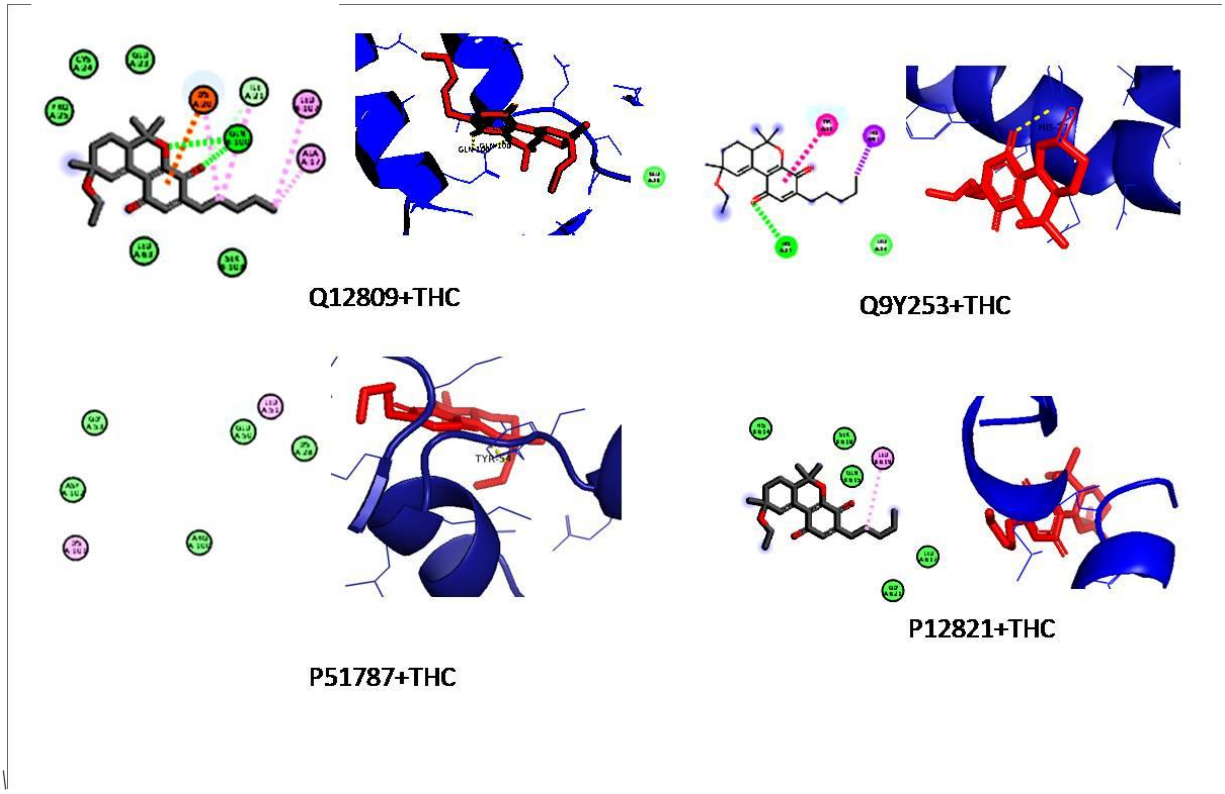


Figure 4. 2D and 3D visualization of mutagens docked with THC.

3.3. ADMET and Toxicity Prediction

ADMET predictions using pkCSM presented in **Table 4**revealed high intestinal absorption (95.3%) and moderate blood-brain barrier permeability (–0.236 log BB), indicating that THC readily enters systemic circulation and the Central nervous system. Although THC was not found to be hepatotoxic or mutagenic in the Ames test, its inhibition of CYP3A4 and interaction with reproductive proteins suggest potential for cumulative or reproductive-specific toxicity. These findings agree with Alsherbiny et al. (2018), who reported reproductive effects of cannabinoids despite low systemic toxicity **[32]**.

In **Table 5,** THC also showed activity in mitochondrial membrane potential disruption (active MMP), a hallmark of early apoptosis. This aligns with findings by Kuzma-Hunt et al. (2023), where THC exposure led to mitochondrial depolarization and reduced sperm motility in vitro studies **[33].** The predicted inactive androgenic or estrogenic receptor activation suggests that THC's reproductive effects are more likely due to cellular stress and gene interference than direct hormonal mimicry**[34]**.

Table 4. Shows the ADMET predicted values sourced from pkcsm

Model Name	Predicted Value
Absorption	
Water solubility	-5.844 log mol/L
Caco2 permeability	1.136 log Papp in 10^{-6} cm/s
Intestinal absorption (human)	95.308%
Skin Permeability	-3.014 log Kp
P-glycoprotein substrate	No

P-glycoprotein I inhibitor	Yes
P-glycoprotein II inhibitor	Yes
Disribution	
VDss (human)	0.419 log L/kg
Fraction unbound (human)	0.1 Fu
BBB permeability	-0.236 log BB
CNS permeability	-2.789 log PS
Metabolism	
CYP2D6 substrate	No
CYP3A4 substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Excretion	
Total Clearance	1.854 log ml/min/kg
Renal OCT2 substrate	No
Toxicity	
AMES toxicity	No
Max. tolerated dose (human)	-0.581log mg/kg/da
hERG I inhibitor	No
hERG II inhibitor	No
Oral Rat Acute Toxicity (LD50)	2.038 mol/kg
Oral Rat Chronic Toxicity (LOAEL)	1.681 log mg/kg_bw/day
Hepatotoxicity	No
Skin Sensitisation	No
T. Pyriformis toxicity	1.386 log ug/L
Minnow toxicity	-0.11 log Mm

Table 5. Toxicity prediction sourced from protox3.0

Classification	**Target**	**Prediction**
Organ toxicity	Neurotoxicity	Inactive
Toxicity endpoints	Mutagenicity	Inactive
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	Inactive
Tox21-Nuclear receptor signaling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Alpha (ER)	Inactive
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	**Active**
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	Inactive
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	Inactive
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	Inactive
Molecular Initiating Events	Constitutive androstane receptor (CAR)	Inactive

3.4.QSAR Modeling

The QSAR modeling result in **Table 6** confirmed that THC has acceptable drug-like properties (molecular weight: 273.24 g/mol, logP: 3.07), indicating it can readily diffuse into cells. But its LogP value (3.07) observed, reveals it lipophilic nature elucidating it potential to facilitates membrane permeability and access to reproductive tissues. However, this property also raises the possibility of bioaccumulation in lipid-rich compartments, which may influence long-term reproductive effects .The moderate number of hydrogen bond acceptors and donors supports its bioavailability and potential to interact with various proteins **[35]**. However, these same properties may also enable it to interact with unintended targets, including those involved in germ cell development and genomic maintenance, echoing.

Table 6. Physicochemical and Predicted Bioactivity Properties of the Compound

Name	Predicted values
Molecular weight	273.24
IC50	
Number of hydrogen bond acceptors	4
Number of hydrogen bond donors	2
Number of atoms	20
Number of bonds	21
Number of rotatable bonds	3
Molecular refractivity	74.15
Topological Polar Surface Area	103.35
octanol/water partition coefficient(logP)	3.07

Conclusion

This study investigated the molecular mechanisms through which THC exerts its spermatotoxic and mutagenic effects. The findings confirm the interaction of THC with key genes involved in spermatogenesis and mutagenesis, suggesting possible molecular mechanisms through which cannabis may impact male fertility and genomic stability, thereby supporting the hypothesis that THC exposure may pose a risk to male fertility and genomic integrity, primarily through disruption of key genes in spermatogenesis and mutagenesis pathways. While SPATA6 like exhibited the highest predicted binding affinity (–8.2 kcal/mol), docking is predictive and does not confirm biological inhibition. Protein conformational changes in vivo, metabolite activity, and reproductive tissue bioavailability could influence the actual effect of THC on these targets.

Although docking was performed on target proteins, the reported affinities provide relative insight into THC-protein interactions, with the limitation that specificity against control proteins was not assessed. While docking and network predictions are valuable. Future studies using in vivo models, gene knockdown, or fertility assays are needed to experimentally validate these computational predictions.

Author Contributions

Conceptualization and study design were led by Edu N. Enyogor. Pius O. Adah and Nsude L. Odinakachukwu.Bethel C. Ateb, Henry B. Kogbara, and Gabriel I. Abu conducted molecular docking and ADMET analysis. Elizabeth.U. Ateb,Akwagiobe P. Abuh, and Adamu Muhammed performed QSAR modeling and network pharmacology. All authors participated in manuscript drafting, critical review, and approved the final version.

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This research did not receive any funding.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

No ethical approval was required.

Data Availability Statement

All data analyzed during this study are included in this published article.

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