Original article

Alleviation of carbendazim toxicity effect by *Moringa oleifera* oil and *Linum usitatissimum* L. oil on testes of male rats: Physiological, histological and *in silico* study

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ABSTRACT

Carbendazim (CBZ) is a widely used fungicide that is used to control the unwanted growth of fungi on fruits and vegetables. Sixty male rats were divided into six groups, each having ten. Group one served as control, animals belonging to group two were exposed to CBZ in the measure of 200 mg/kg body weight (BW). In the third and fourth groups, rats were administered 800 mg/kg BW of *Moringa oleifera* (moringa oil) and *Linum usitatissimum* L. (flaxseed oil), plus CBZ with the same dose given to group two. Groups five and six were administered with moringa and flaxseed oils respectively for six weeks. A marked decline was seen in oxidative stress markers, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and a rise in malondialdehyde (MDA) level in group two with severe histological disruptions. Moringa oil and flaxseed oil were used to alleviate these changes. In addition, a biocomputational molecular docking analysis of three proteins found in male rats was performed. In relation to CBZ (CID:10584007) the screened proteins namely testis-expressed protein (TX101_RAT), EPPI_RAT, and glutathione peroxidase 5 (GPX5_RAT) were docked, and their docking score were obtained (-5.9 kcal/mol), (-5.8 kcal/mol) and (-5.6 kcal/mol) respectively. By examining these interactions in 2D and 3D structures, a detailed understanding of the unique and specific binding affinity, hydrogen bonds, hydrophobic interactions, ionic bonds, and water bonds were obtained. Structure-based virtual screening (SBVS) molecular docking analysis showed that protein interaction with CBZ causes reproductive complications in protein expression and functions by hampering their normal function and blocking active sites.

1. Introduction

Currently, environmental pollution is clearly increasing and posing a threat to the environment. Pesticides are considered as environmental pollutants when used excessively. Pesticides seriously affect human and animal health. Experimental scientific investigations indicate that physiological, biochemical and histological alterations occur as a result of exposure to pesticides (Al-Attar, 2010; Al-Attar and Al-Taisan, 2010; Zari and Al-Attar, 2011; Al-Attar, 2015; Al-Attar et al., 2017; Abou Zaid et al., 2018; Al-Attar and Al-Saeed, 2021; Abu Zeid et al., 2022; Abo-mosallam et al., 2023).

Carbendazim (CBZ) is a fungicide that is used to prevent and control fungal diseases in crops. CBZ $C_9H_9N_3O_2$ or methyl-1H-benzimidazol-2-yl-carbamate (MBC) is applied in agriculture with a wide range of

applications. It works by inhibiting the growth and reproduction of fungi (McCarroll et al., 2002). However, it can also have toxic effects on both humans and the environment. In humans, exposure to CBZ can cause skin and eye irritation, as well as respiratory symptoms such as coughing and shortness of breath. It is also a possible human carcinogen and may increase the risk of cancer in the liver, kidney, pancreas, and testes. CBZ can also have negative effects on the environment. It can also harm beneficial insects, such as honeybees, and can target non-target plants. In addition, CBZ can persist in soil and water, which can lead to long-term negative effects on the ecosystem (Singh et al., 2016; Goyal et al., 2018) (see Table 1).

CBZ enhances reactive oxygen species (ROS) that ultimately leads to malfunction antioxidant enzymes including glutathione-S-transferase (GST), SOD, MDA, CAT, glutathione reductase (GSH-Rd) and γ-glutamyl

transpeptidase (GGT), responsible for hepatic oxidative stress and immune-suppressive endocrine disruptions. CBZ can interfere with antioxidant defense mechanisms, such as the activity of SOD and glutathione peroxidase (GPx). These enzymes protect cells from oxidative damage by neutralizing ROS. CBZ has been shown to reduce the activity of these enzymes, which can exacerbate oxidative stress (Jiang et al., 2015; Sharma et al., 2022; Ma et al., 2023). CBZ exposure leads to a decrease in testicular weight, degeneration of the seminiferous tubules, decreased sperm production and impaired spermatogenesis. These effects may lead to decreased fertility in male rats and induce histological disruptions in testicular tissues (Zari and Al-Attar, 2011; Abolaji et al., 2017; Patil et al., 2022; Hashim et al., 2023).

Medicinal plants are organic in nature and are highly compatible with the body system due to their natural phytochemical constituents and compatibility. The aim of the 21st century is to develop advanced screening, extraction, and purification techniques to develop natural drugs from medicinal plants. Over 80 % population of the world is relying on medicinal plants to treat different diseases (Shakya, 2016). With the advancement in drug discovery research, several contemporary techniques are being applied, such as molecular modelling, virtual screening, natural product libraries and database mining. Natural products will undoubtedly play a significant part in the development of new therapeutic medications in the future (Salmerón-Manzano et al., 2020; Najmi et al., 2022).

Moringa oleifera is a member of the family moringaceae having 13 species worldwide. It is a widely cultivated plant with high significance of nutritional, phytochemical and antioxidant properties (Liang et al., 2019). *M. oleifera* is known for its health benefits, which include antibacterial, anticancer, antihypertensive, antidiabetic, antioxidant, anti-inflammatory and cardioprotective activities. It is rich source of vitamins and minerals (Patil et al., 2022). Phytochemically screened food products of moringa are pharmacologically significant and greatly contribute to food products (Llorent-Martínez et al., 2023). *Linum usitatissimum* L. is generally called flaxseed. It is widely distributed with 14 genera and 230 species (Verma and Mishra, 2014). Flaxseeds have multiple nutritional constituents and phytochemicals to treat various illnesses. Its oil is rich in proteins, carbohydrates, minerals, vitamins, fibers, fatty acids, phenolic compounds, alpha-linolenic acid (ALA) and lignans. It can act as an anticancer, reduce antioxidant stress, improves cardiovascular health, cholesterol management, healthy hair and skin, control obesity, dissolve tumors, antidiabetic, nephropathy, hepatopathy, neuropathy, cure immune disorders and ultimately leads to a healthy life (Al-Saeed and Al-Attar, 2021; Shim et al., 2022).

Male reproductive proteins (MRPs) are important for fertilization and involved in immune responses. Additionally, act as phosphatases, protease inhibitors, proteases, protein kinases, signal transduction molecules, structural molecules and transporter proteins (Fung et al., 2004; Ness and Grainger, 2008). Testis-expressed protein TX101_RAT, gene name TEX101, primary accession number (PAN) (Q924B5) having 250 amino acids play important role in male fertility biomarkers.

TEX101 is a glycoprotein and crucial for spermatogenesis, epididymal maturation and sperm egg interaction (Cho, 2012; Schiza et al., 2014). GPX5_RAT epididymal secretory glutathione peroxidase is a mammalian epididymal protein with PAN (P30710) and 221 amino acids. GPX protein family greatly contributes to detoxification and helps in maintaining the integrity of membrane acrosomal structure for successful fertilization (Chabory et al., 2010; Noblanc et al., 2011). EPPI_RAT or EPPIN synonym name Spinlw1, PAN (D4A2Z2) with 134 amino acid sequence is an important rat protein. It is similar in function to TEX-101. EPPIN is found in the testicular and epididymal regions as well as in the brain of rats. It contributes to the epididymis's functionality (Bian et al., 2009; Silva et al., 2012). The purpose of this research is to highlight the potential health benefits of moringa oil and flaxseed oil against testicular toxicity caused by CBZ. The findings of this study include an assessment of antioxidant parameters level, biochemical, histological and in *silico* approaches.

2. Materials and methods

2.1. Animals groupings and treatment

Male albino rats of the Wistar strain (*Rattus norvegicus*), weighing 110–149 g, were utilized in the present study. The experimental animals were acclimated according to standard conditions (Zari and Al-Attar, 2011). King Abdulaziz University Animal Care and Use Committee (ACUC) guidelines include EU Directive2010/63/EU for practical research. Grouping (G) of 60 rats (n = 10) G1 (control), G2 (CBZ intoxicated 200 mg/kg BW), G3 (moringa oil 800 mg/kg BW + CBZ), G4 (flaxseed oil 800 mg/kg BW + CBZ), G5 (moringa oil 800 mg/kg BW) and G6 (flaxseed oil 800 mg/kg BW) for a duration of 6 weeks.

2.2. Analysis of blood serum

The rats were subjected to a 12-hour fasting period while being provided unrestricted access to water. They were then anesthetized using diethyl ether. A 23-gauge needle was utilized to puncture the orbital plexus veins of the rats, and the blood was collected in tubes without heparin. Subsequently, the samples were centrifuged at 2500 rpm for 15 min to separate the serum. The serum was carefully transferred to sterile tubes and stored at −80 °C until further analysis. (Chetwynd et al., 2017). Following the manufacturer's instructions, assay kits were used to measure GSH, SOD, CAT, and MDA levels.

2.3. Histopathological examination

Male rats from all groups were sacrificed to collect testes tissues, which were then processed. The processed samples were cut into thin sections 4–5 μm in size using a microtome. The prepared slides were subjected to hematoxylin and eosin (H&E) staining (Suvarna et al., 2018). Tissue sections of the testes from all experimental groups were

Table 1
Showing basic information about ligand molecule CBZ.

Ligand	Molecular formula	Molecular weight	Synonyms	IUPAC name	Hydrogen bonds donor count	Hydrogen bonds acceptor count	Ccanonical SMILES
Carbendazim (CBZ) PubChem CID (10584007)	$C_9H_9N_3O_2$	191.19 kg/kmol	Carbendazim-d4 291765-95-2methyl N-(4,5,6,7-tetradeuterio-1H-benzimidazol-2-yl) carbamateCarbamic acid, 1H-benzimidazol-2-yl-4,5,6,7-d4-, methyl ester (9CI)Carbendazim D4 (ring D4)	methyl *N*-(4,5,6,7-tetradeuterio-1*H*-benzimidazol-2-yl) carbamate	2	3	COC(=O)NC1=NC2=CC=CC=C2N1

then observed under a light microscope.

2.4. Statistical analysis

The statistical analysis was performed using SPSS software, version 22.0 for Windows. One-way analysis of variance (ANOVA) was conducted, followed by Dunnett's test for the calculation of statistical significance. The results were presented as mean $\pm$ standard deviation (S. D.). The P $\leq$ 0.05 level of probability was used as a criterion of significance.

2.5. Bioinformatics analysis

2.5.1. Ligand properties

The following information was retrieved from the PubChem server on 25 December 2022. (https://pubchem.ncbi.nlm.nih.gov/compound/Carbendazim-d4).

2.5.2. Toxicity prediction

Before clinical trials are started, in-silico toxicity evaluation is essential for docking analysis. The web based AdmetSAR 2.0 server (https://lmmd.ecust.edu.cn/admetsar2/) was used to record the compound's toxicity explained in (Table 2).

2.5.3. Proteins selection and preparation

Prior to conducting the docking analysis, the structural inconsistencies of the molecules were assessed and corrected. This involved addressing issues such as the absence of hydrogens, the removal of water molecules and ligands, and the proper orientation of different functional groups were evaluated and rectified. Out of the diverse variety of testicular proteins so far identified by Yang et al. (2018). In this study, three proteins from male rats, TX101_RAT, GPX5_RAT, and EPPI_RAT or EPPIN, were carefully screened and protein structures were obtained from one of the largest databases (https://www.rcsb.org/). Proteins' active sites for ligand interaction were identified using CASTp 3.0 web server (http://sts.bioe.uic.edu/castp/index.html).

2.5.4. Molecular docking

The docking procedure was conducted by converting the receptor and ligands files into the pdbqt format, utilizing AutoDockTools (v1.5.6, a vailable at https://autodock.scripps.edu, accessed on 25th December 2022). To perform molecular docking and calculate the binding affinity, AutoDock Vina, a virtual screening tool developed by PyRx (https://vina.scripps.edu, accessed on 25th December 2022), was employed. The outcomes of the molecular docking process were visualized using the BIOVIA Discovery Studio Visualizer.

3. Results

3.1. Assessment of biochemical oxidative markers

Significant decreases in serum GSH levels were observed in rats treated with CBZ (−49.2 %, P $\leq$ 0.000), moringa oil plus CBZ (−30.1 %, P $\leq$ 0.007), and flaxseed oil plus CBZ (−24.6 %, P $\leq$ 0.003), compared to control rats. However, there were no statistically significant differences in serum GSH levels between rats supplemented with moringa oil (group 5) or flaxseed oil (group 6) and control rats (Fig. 1). A noticeable decrease in serum SOD levels was observed in rats treated with CBZ (−61.1 %, P $\leq$ 0.000), moringa oil plus CBZ (−45.5 %, P $\leq$ 0.000), and flaxseed oil plus CBZ (−57.3 %, P $\leq$ 0.000), compared to control rats. Serum SOD levels in rats administered moringa oil (group 5) or flaxseed oil (group 6) did not show statistically significant differences compared to control rats (Fig. 2). The level of serum CAT exhibited a significant decline in rats exposed to CBZ (−49.0 %, P $\leq$ 0.000) compared to control rats. Similarly, rats treated with moringa oil plus CBZ (−30.4 %, P $\leq$ 0.000) and flaxseed oil plus CBZ (−38.7 %, P $\leq$ 0.001) showed statistically decreased levels of serum CAT. There were no statistically significant differences in serum CAT levels between rats supplemented with moringa oil (group 5) or flaxseed oil (group 6) and control rats (Fig. 3). Serum MDA levels were significantly increased in CBZ-treated rats (+87.7 %, P $\leq$ 0.001) compared to control rats. There were no obvious alterations in serum MDA levels in rats from groups 3, 4, 5 and 6 compared to control rats (Fig. 4).

3.2. Histological observations

Upon histopathological examination, the testes of the control rats showed a healthy structure. The seminiferous tubules were seen as rounded and oval structures, surrounded by a thin basal membrane. These tubules contained multiple layers of cells, including spermatogonia, primary spermatocytes, secondary spermtcytes, spermatids and mature spermatzoa. All of these cell types were intricately connected to Sertoli cells. The intertubular tissue present between the seminiferous tubules was observed to be composed of connective tissue, which held the tubules together and contained blood vessels. The intertubular tissue also housed Leydig (interstitial) cells which are responsible for endocrine secretion (Fig. 5). Similarly, the histopathological examination of the testes of CBZ plus moringa oil (Fig. 7), CBZ plus flaxseed oil (Fig. 8), rats treated with moringa oil (Fig. 9), and flaxseed oil (Fig. 10) treated rats showed normal structures. In rats of group 2, the testicular histopathological examination showed that the exposure to CBZ for six weeks caused severe structural alterations including the complete absence of all types of spermatocytes and the process of their formation (Fig. 6).

3.3. Findings of docking analysis

3.3.1. Proteins active site identification

The well-known online server CASTp 3.0 was utilized to find the protein's binding site from many amino acid residues in a certain area for selected proteins. The obtained data helped identify the complicated AS pocket. These active sites help in the determination of bindings interactions with ligand and selected proteins.

3.3.2. Grid preparation, active sites identification and molecular docking analysis for TEX101_RAT

To obtain high prediction-based accuracy for structure-based virtual screening methods, choosing the right dimensional grid box, active pockets identifications, and bonding strength are very crucial (Feinstein and Brylinski, 2015). During the procedure, the binding sites discovered

Table 2
Different toxicities induced by CBZ.

| Toxicity by CBZ	Hepatotoxicity	Nephrotoxicity	Reproductive	Micronuclear
Toxicities Consequences of toxicity in CBZ-exposed animals	Active Malfunction in liver enzymes, oxidative stress, formation of reactive metabolites that induce liver damage, and histological disruptions (Yossef et al., 2022).	Active The obvious increase in renal health biomarkers. Cause degeneration of glomeruli and Bowman's capsule (Nwozo et al., 2017).	Active Induce oxidative stress, endocrine disruption, loss of normal histological structure, and poor spermatogenesis (Zari and Al-Attar, 2011; Zhang et al., 2020).	Active Impaired cell division, CBZ binds with beta tubulins and blocks normal function (Zhou et al., 2016).
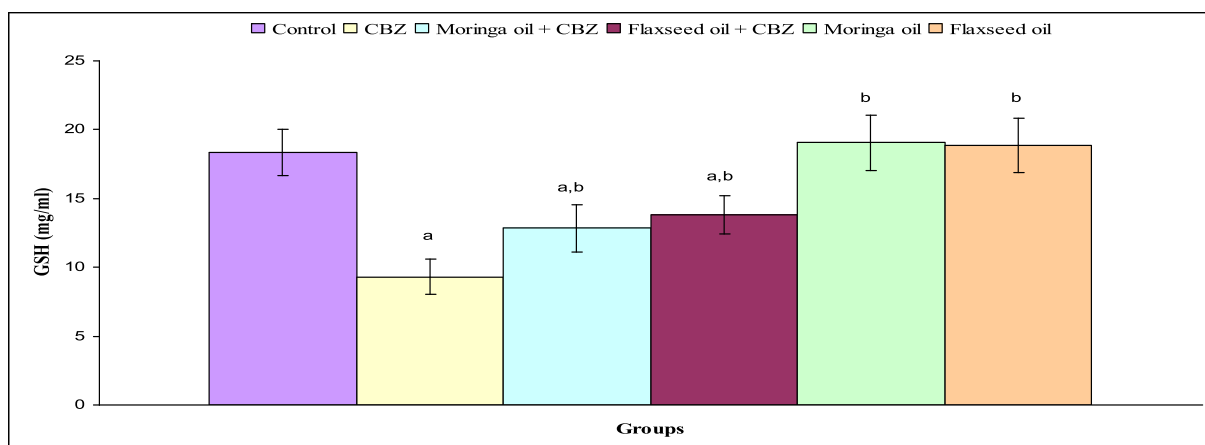
Fig. 1. Level of serum GSH in control (group 1), CBZ (group 2), moringa oil plus CBZ (group3), flaxseed oil plus CBZ (group4), moringa oil (group5) and flaxseed oil (group 6) treated rats. Significance levels shown for difference between control (group 1) and treated groups (2, 3, 4, 5, and 6) were indicated by [a]. Significance levels shown for difference between CBZ (group2) and groups 3,4,5 and 6 were indicated by [b].
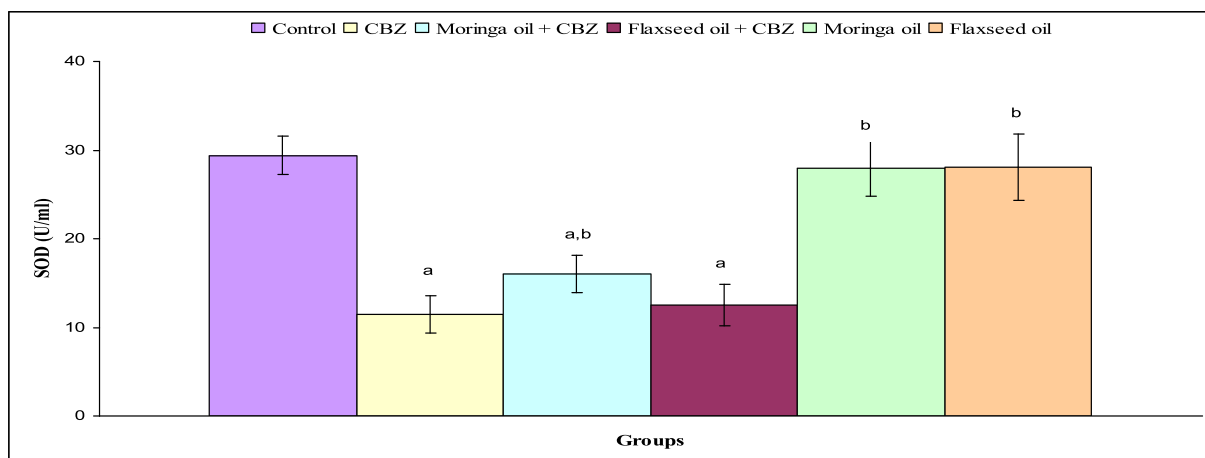

Fig. 2. Level of serum SOD in control (group 1), CBZ (group2), moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group4), moringa oil (group 5) and flaxseed oil (group 6) treated rats. Significance levels shown for difference between control (group 1) and treated groups (2, 3, 4, 5, and 6) were indicated by [a]. Significance levels shown for difference between CBZ (group 2) and groups 3, 4, 5 and 6 were indicated by [b].
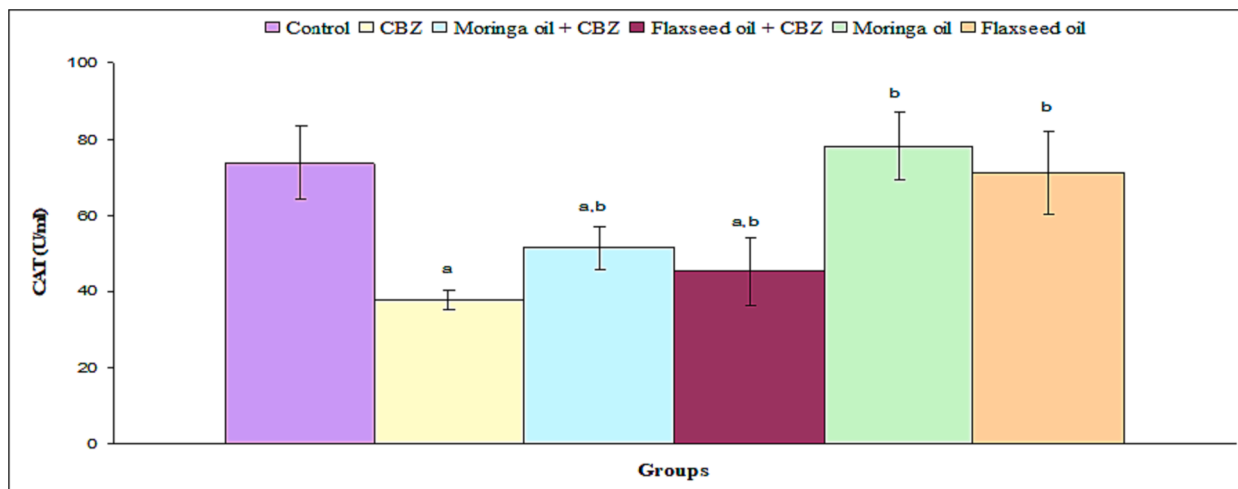

Fig. 3. Level of serum CAT in control (group 1), CBZ (group 2), moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group 4), moringa oil (group 5) and flaxseed oil (group 6) treated rats. Significance levels shown for difference between control (group 1) and treated groups (2, 3, 4, 5, and 6) were indicated by [a]. Significance levels shown for difference between CBZ (group 2) and groups 3, 4, 5 and 6 were indicated by [b].
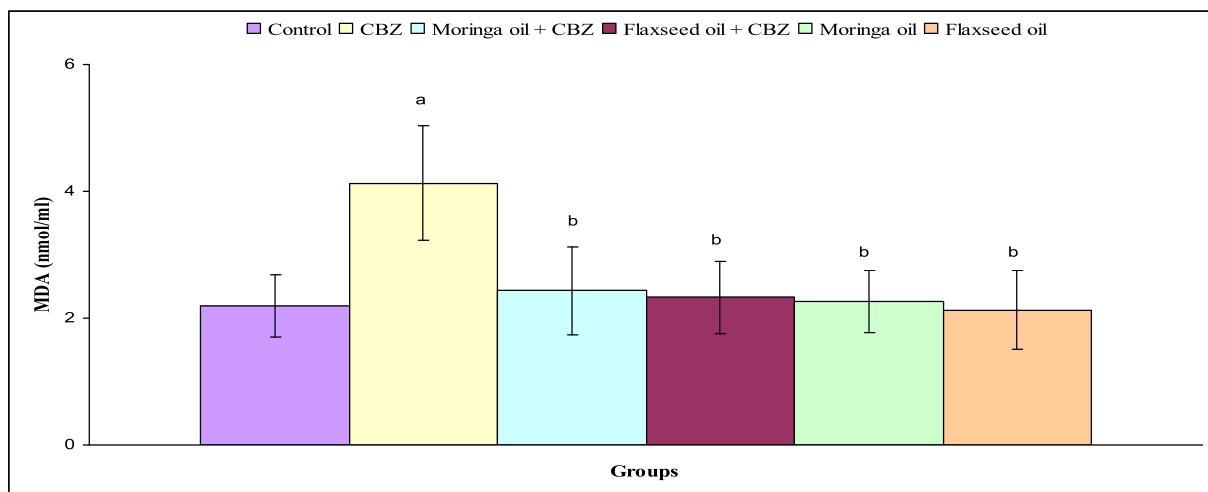
Fig. 4. Level of serum MDA in control (group 1), CBZ (group2), moringa oil plus CBZ (group 3), flaxseed oil plus CBZ (group 4), moringa oil (group 5) and flaxseed oil (group 6) treated rats. Significance levels shown for difference between control (group 1) and treated groups (2, 3, 4, 5 and 6) were indicated by [a]. Significance levels shown for difference between CBZ (group 2), and groups 3, 4, 5 and 6 were indicated by [b].
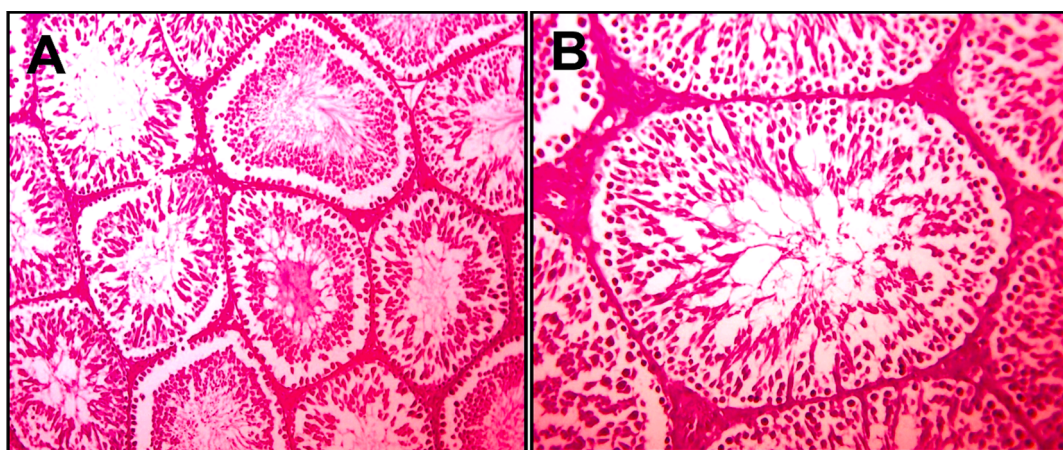

Fig.5. The histological section of testis of control rats showing regular seminiferous tubules structure with normal spermatogenesis process (H & E staining). X200 (A) and X400(B).
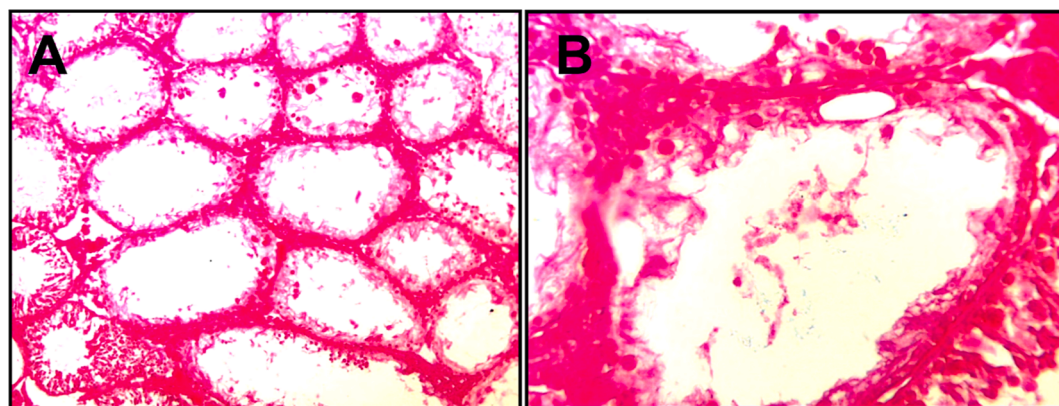

Fig. 6. The histological sections of testicular tissues of CBZ exposed rats with disrupted seminiferous tubules structure with an absence of spermatogenesis process (H & E staining). X200 (A) and X400 (B).

by the server were used to create a receptor grid using grid box dimensions X = −4.48, Y = 1.38, and Z = 5.88 in Angstrom (Å).This study determined all possible active sites that are responsible for binding are represented with ball shapes showing amino acid (AA) residues in (Fig. 11) and listed in (Table 3). Results obtained by the protein–ligand interactions between the chosen compound and the protein TEX101_-RAT based on the highest binding affinities after docking. The ligand-–protein complexes were visualized using the BIOVIA discovery studio
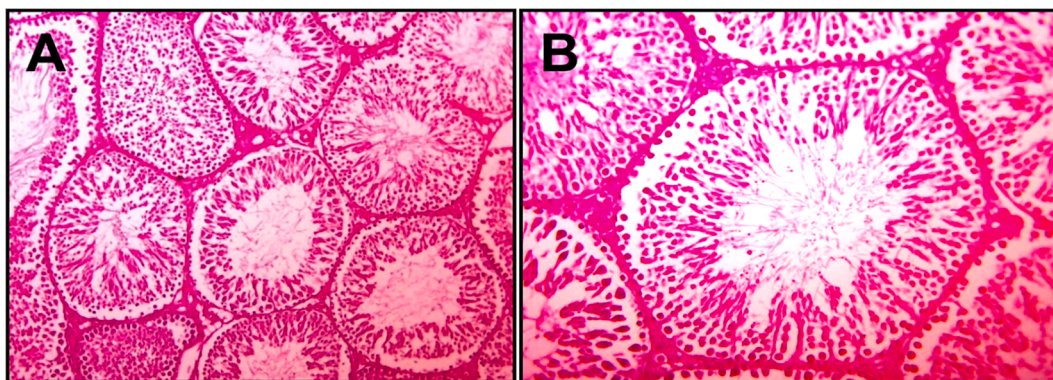
Fig. 7. The histological sections of testicular tissues of moringa oil plus CBZ treated rats showing regular seminiferous tubules structure with normal spermatogenesis process (H & E staining). X200 (A) and X400 (B).
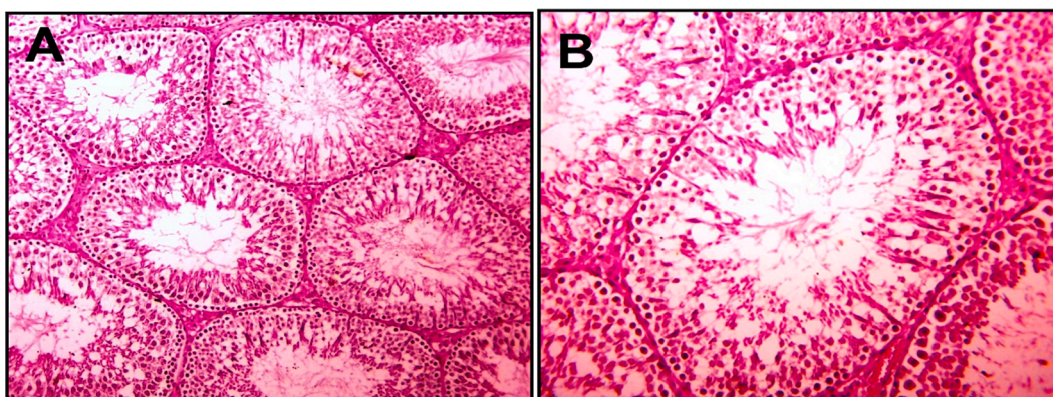

Fig. 8. The histological sections of testicular tissues of flaxseed oil plus CBZ treated rats exhibiting normal seminiferous tubules structure with normal spermatogenesis process (H & E staining). X200 (A) and X400 (B).
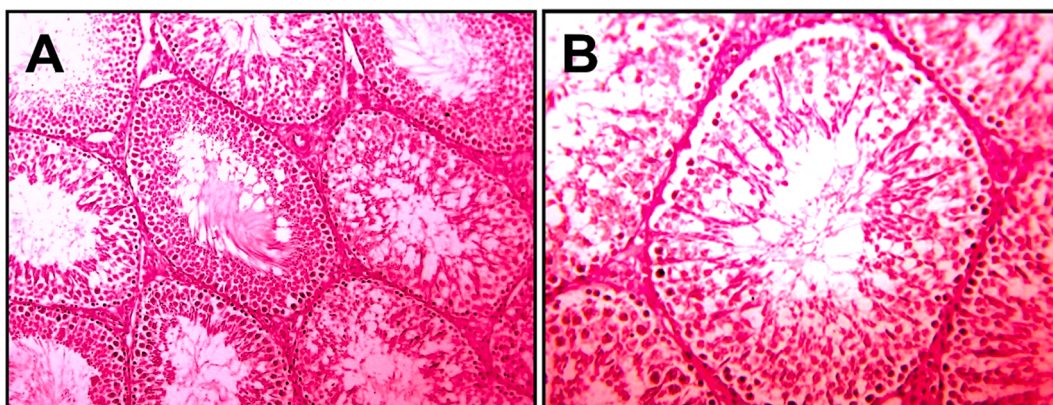

Fig. 9. The histological sections of testicular tissues of moringa oil treated rats showing normal seminiferous tubules structure with normal spermatogenesis process (H & E staining). X200 (A) and X400 (B).

visualzer. Compound (CID10584007) was observed to have unfavorable bumps, carbon-hydrogen bond, alkyl, Pi alkyl, and strong hydrogen bonding at ILE A:70 active site (Fig. 12).

3.3.3. Grid preparation, active sites identification and molecular docking analysis for GPX5_RAT

All possible active sites that are responsible for binding are represented with ball shapes showing AA residues in (Fig. 13) and given in (Table 3). Results obtained by the protein–ligand interactions between the compound and the protein GPX5_RAT are based on the highest binding affinities after docking. Binding sites discovered by the server were used to create a receptor grid with grid box dimensions $X = -4.41$, $Y = -6.52$, and $Z = -0.39$ in Angstrom (Å). Compound (CID10584007) was observed to have unfavorable bumps, unfavorable donor–donor, Pi-lone pair, Pi-Pi T-shaped, alkyl, Pi alkyl, and hydrogen bonding at LEU A: 134 active site at (Fig. 14).

3.3.4. Grid preparation, active sites identification and molecular docking analysis for EPPIN or EPPI_RAT

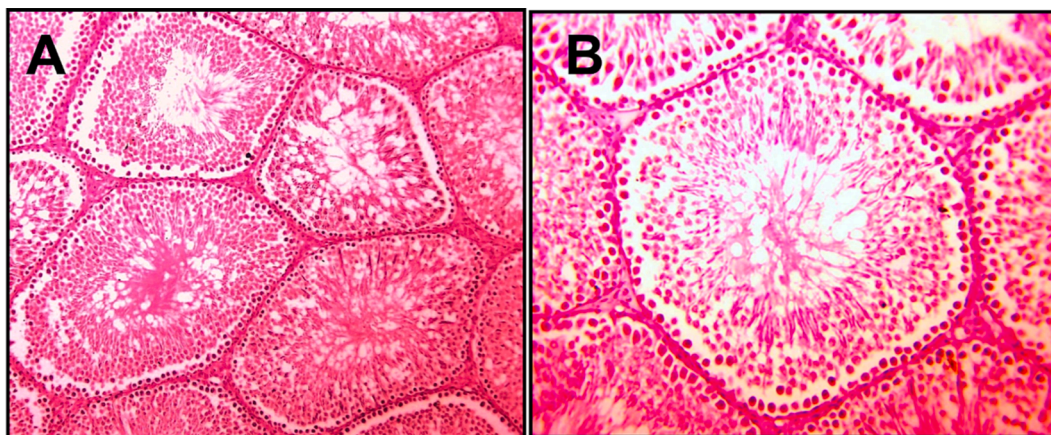The receptor grid was created by choosing the protein's active site

Fig 10. The histological sections of testicular tissues of flaxseed oil treated rats exhibiting typical seminiferous tubules structure with normal spermatogenesis process (H & E staining). X200 (A) and X400 (B).
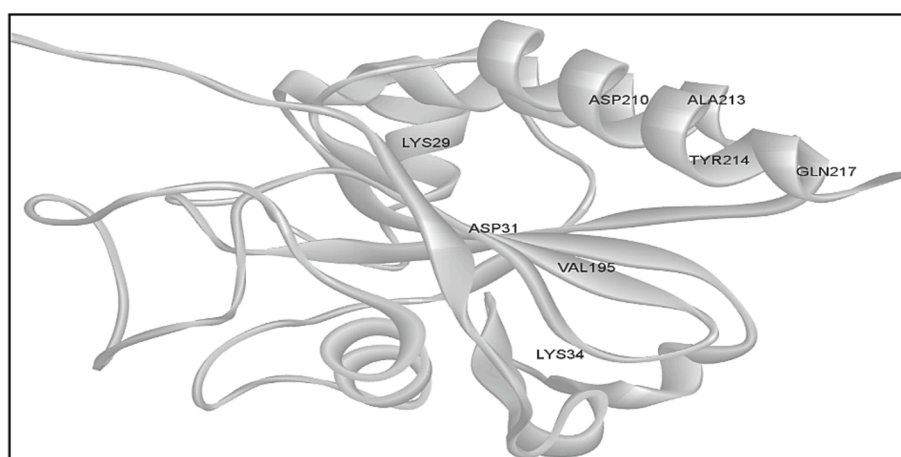

Fig. 11. Active sites of TEX_101 RAT protein.

Table 3
The binding affinity and active pockets of proteins.

Compound	Proteins	Binding affinity	Active pockets
PubChem CID (10584007)	TEX101_RAT	−5.9 kcl/mol	LYS 29, ASP 31, LYS 34, VAL 195, ASP 210, ALA 213, TYR 214, GLN 217.
	EPPI_RAT	−5.8 kcl/mol	PHE 29, PRO 30, ARG 31, ARG 32, CYS 33, PRO 34, ARG 35, PHE 36, PHE 63, LEU 70, ASN 71, PRO 72, GLN 73, GLN 74, ASP 75, ILE 76, CYS 77, PHE 117, ASN 125, ALA 126, CYS 127, GLU 128, LYS 129, ASN 132, SER 133.
	GPX5_RAT	−5.6 kcl/mol	ARG 24, LEU 25, GLU 26, MET 30, HIS 158, PRO 159, SER 160, GLU 161, THR 162.

using the PyRx program. The binding sites discovered by the server were used to create a receptor grid with grid box dimensions X = −3.47, Y = −5.55, and Z = 2.92 in Angstrom (Å). All possible active sites that are responsible for binding are represented in (Fig. 15) and cataloged in (Table 3). Results obtained by the protein–ligand interactions between the chosen compound and the protein GPX5_RAT based on the highest binding affinities after docking. Compound (CID10584007) was observed to have van der Val forces, unfavorable bumps, Unfavorable donor–donor, Carbon hydrogen bond, and Pi-cation (Fig. 16).

4. Discussion

The present study is the first experimental investigation to highlight the ameliorative capabilities of moringa and flaxseed oil to treat CBZ-induced testicular toxicity using different parameters including antioxidant stress markers, physiological and histopathological alterations, and *in silico* approach. CBZ exposure at a dose of 200 mg/kg BW induces a significant increase in MDA while the reduction in GSH, SOD, and CAT levels was recorded. In addition, histological alterations in testes tissues were seen as hypertrophy in seminiferous tubules, absences of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and poor spermatogenesis process was compared with previous experimental studies (Zari and Al-Attar, 2011; Catalgol and Alpertunga, 2013; Sharma et al., 2022).

M. oleifera improves SOD and CAT activities. Its extract proves to be effective for treating testicular toxicity, injuries, sperm count, and oxidative stress that was induced in rats by tramadol (Abd et al., 2020). *M. oleifera* leaves mitigate cyclophosphamide-induced toxicity by improving hormonal balance, modulating gene expresion, spermatozoa as well as spermatogenic cellular health (Nayak et al., 2020). The testicular damage caused by Pb acetate was significantly reduced due to *M. oleifera* extract's treatment. Its antioxidant, anti-inflammatory, and anti-apoptotic activity greatly contribute to reducing Pb toxicity. It is suggested as a helpful addition to adjuvant Pb-induced toxicity
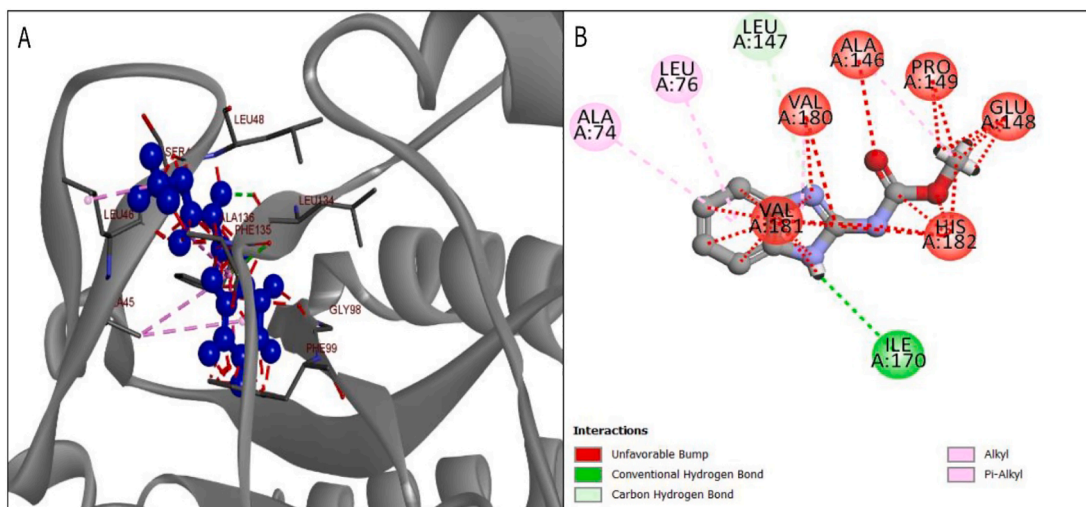
Fig. 12. The interaction between compound 10584007 (PubChem CID) and TEX_101 RAT protein 3D on right fig. A and 2D structure on left side fig. B represents complex protein–ligand interaction.
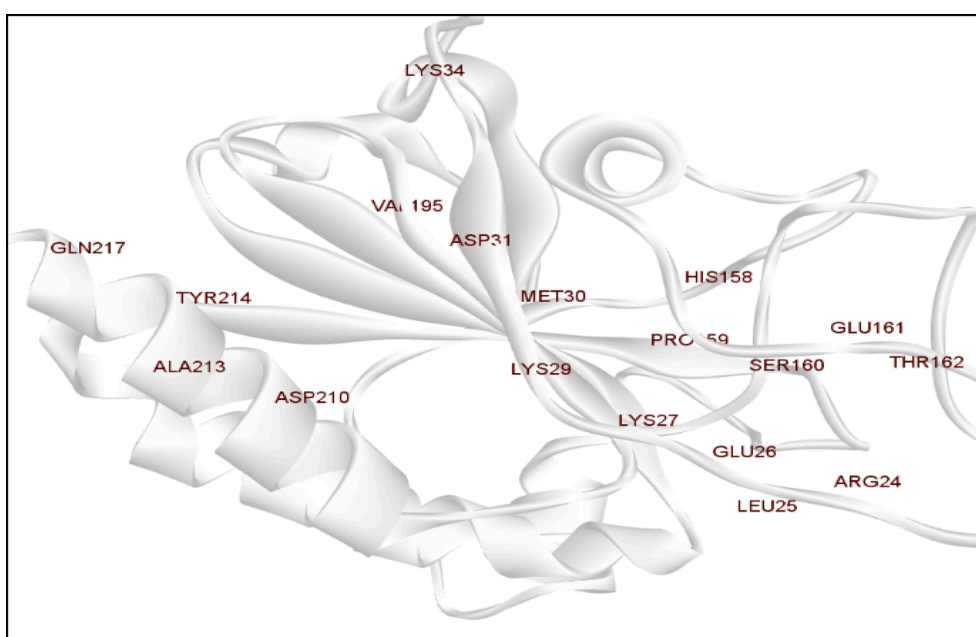

Fig. 13. Active pockets for GPX5_RAT protein.

(Albasher et al., 2021). Highly active antiretroviral therapy was used to induce testicular damage. *M. oleifera* extracts improve semen quality, hormone level, and testicular morphology (Ogunlade et al., 2022). Nano moringa can combat sodium fluoride toxicity by maintaining oxidant status, testosterone and dehydroepiandrosterone level, and histological alterations in testicular tissues (Abd-Allah and El-Rahman, 2023).

Flaxseed oil has shown potential of reducing oxidative stress, also as a treatment of hepatorenal toxicity, neuronal and testicular toxicity, cancer treatment by dissolving tumors and cholesterol and hormones balance. These therapeutic properties of flaxseed oil are attributed to the fact that it is rich in omega-3 fatty acids (Pilar et al., 2017). Lead acetate exposure caused significant oxidative damage in the testes of rats, leading to a decrease in testosterone levels, reduced antioxidant activity, and increased lipid peroxidation. Treatment with flaxseed oil prevented these effects (Moniem et al., 2010). Flaxseed oil supplementation on testicular toxicity induced by cadmium exposure in rats exhibited ameliorative results (Gupta et al., 2015). The study found that rats given

flaxseed oil had less testicular damage and reduced blood levels of markers of oxidative stress, and normal MDA level, compared to rats that did not receive flaxseed oil. Sperm parameters improvement in rats with diabetes-induced testicular toxicity was investigated by (Suresha et al., 2015). The authors found that flaxseed oil supplementation significantly improved sperm parameters compared to the control group. Flaxseed oil is a protective agent against thyroid function disturbance caused by exposure to diazinon in male rats (Al-attar and Al-Saeed, 2021). It was evident from the findings that rats in diazinon treatment had considerably higher serum levels of TSH and MDA. Moreover, the levels of T3, T4, GSH, SOD and CAT were significantly reduced. All these parameters were attenuated to normal levels due to the treatment of flaxseed oil and argan oil.

The results of this study proved the healing potential of moringa oil and flaxseed oil to treat CBZ-induced testicular toxicity. Moreover, the findings of the current study invigorate the use of moringa oil and flaxseed oil to treat testicular toxicity induced by CBZ. Various plants

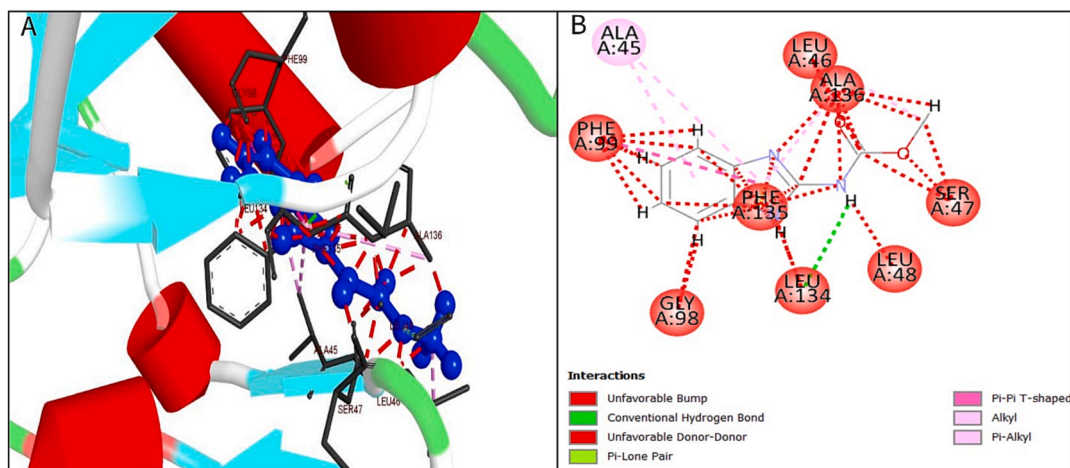**Fig. 14.** The interaction between compound 10584007 (PubChem CID) and GPX5_RAT protein 3D on right fig. A and 2D on the left side of fig. B represents protein–ligand interaction.
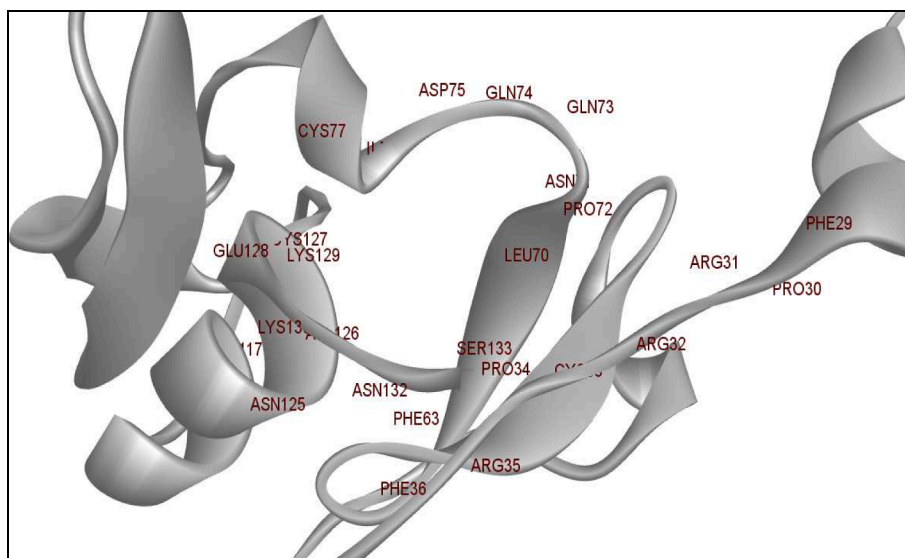

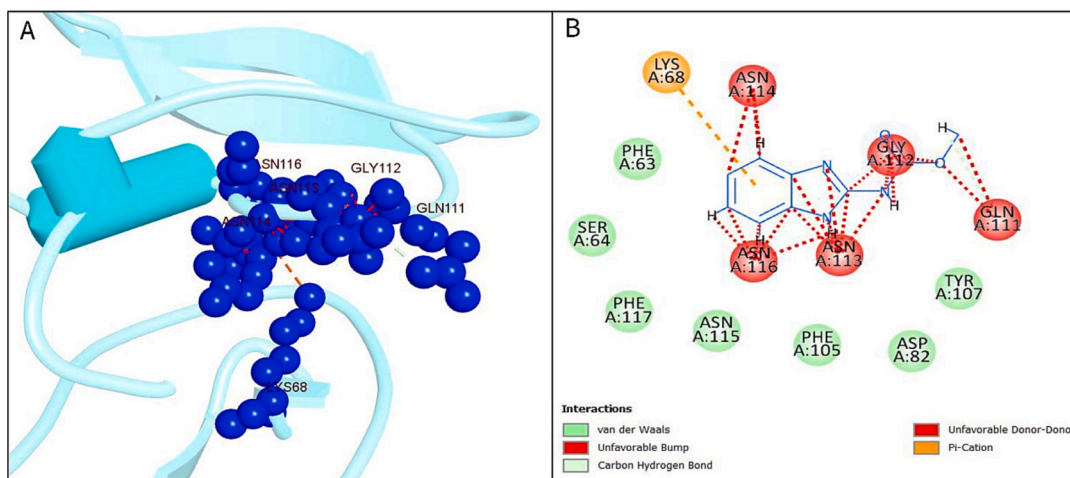**Fig. 15.** Active pockets for EPPI_RAT protein.

Fig. 16. The interaction between compound 10584007 (PubChem CID) and EPPI_RAT protein 3D on right fig. A and representing 2D complex left side fig. B protein–ligand interaction.

and their oils have been previously used to treat the toxicity of CBZ on animals, such as olive (*Olea europaea)* leaves Zari and Al-Attar, (2011), licorice (*Glycyrrhiza glabra*), Fenugreek (*Trigonella foenum-graecum Linn.*) seeds extract Lamfon (2012), *Gingo bilola* extract Mahboub and Lamfon, (2013), Ginger (*Zingiber officinale*) Salihu et al. (2016), Jaft internal layer of *Quercus brantii* Mirzaei et al. (2015), *Nigella sativa* oil Hashem et al. (2018), Quercetin Abou Zaid et al. (2018), *Nigella sativa* and *Foeniculum vulgar* seeds extracts Alghamdi, (2020), Vitamin C (Lalhriatpuia et al., 2021; Kiran, 2022), and banana peel (Abdel-Rahman et al., 2022).

According to a study on male reproductive output, the seminal plasma contains 3200 proteins (Batruch et al., 2012). In a study by Yang et al. (2018) about 107 testicular proteins and 263 long non-coding RNAs were identified. Protein-ligand docking requires several phases, including determining the active sites, the flexibility of the ligand, and the strength of the contact between the ligand and the protein (Zou et al., 2017; Leal et al., 2018). Benzimidazoles actively bind with amino acid residues and affect enzymatic activities. Benzimidazole interacts with β-tubulin proteins by making hydrogen bonds. β2-tubulin was docked with benzimidazoles (CBZ, benomyl, thiabendazole, and thiophanate-methyl). In this study, all selected ligands exhibit hydrogen bonds at different amino acid residues. β2-tubulin docking with CBZ exhibited the lowest binding energy (-5.72 kcal/mol) (Zhou et al., 2016; Vela-Corcía et al., 2018; Singh and Kaur, 2019). According to a study by Li et al. (2017), CBZ is a potent quencher and can attach to bovine serum albumin by altering their molecular shape as indicated by hydrophobic interactions that are the main contributors to bonding. Another molecular modeling study by Siddiqui et al. (2019) suggests that CBZ also shows static bonding with human serum albumin and stimulates alterations in overall structures that can be seen by hydrogen bonding, van der Waals, and pi-alkyl.

Sperm migration and male fertility are associated with TEX-101 protein in mice that contribute to successful oviduct fertilization reported by Li et al. (2013). TEX101 being a male protein is cell-specific and responsible for the normal functionality of active spermatozoa in spermatogenesis studied in mice by (Endo et al., 2016). Different proteomic measurement tools of human spermatozoa confirm that TEX-101 in combination with other functional chaperones (LY6K and ADAM29) plays a significant role in sperm production and maturation by ensuring its successful binding with oocyte (Schiza et al., 2019; Erbayram et al., 2021).

GPX5 is synthesized in the cauda and caput of the epididymis and affects oxidative/reductive activity by affecting spermatogenesis and sperm maturation events (Choi et al., 2009). GPX5 being androgen-regulated glutathione peroxidase has its potential role in protecting DNA from harmful mutations and reducing the chances of infertility in males by combating many stressors (Taylor et al., 2013; Li et al., 2018). GPX5 exhibits improved resistance to oxidative stress, as well as lower levels of lipid peroxidation and the downstream DNA lesion (Taylor et al., 2013). A study conducted by in-vitro experimentation in goat leads to the upregulation of androgen hormone specifically testosterone can increase GPX5 in epididymal epithelial cells which reduce oxidative stress and improve the proliferation activity of epididymal epithelial cells (Luan et al., 2019). Impaired DNA and oxidative stress are directly linked with GPX5 activity and the age of mice (Chu et al., 2020). According to a study, miR-542-3p targets GPX5 and decreases antioxidant capacity while increasing oxidative stress in caput epididymis epithelial cells of goats (Yang et al., 2022).

EPPIN regulates the development of sperm motility with reference to ejaculation (O'Rand et al., 2009). EPPIN also contributes to antimicrobial protection for sperm. Its downregulation in male mice can result in decreased sperm motility and affects ejaculation (Xu et al., 2021; Silva et al., 2021). The findings of a study by Torres et al. (2019) manifested that 2D and 3D structures obtained by using the BIOVIA discovery studio visualizer help in the determination of the binding interaction of ligands with proteins. The existence of hydrogen bonds, lipophilic or hydrophilic interactions, andionizable charges affect the binding affinity of the protein and ligand interactions. (Opo et al., 2022). Findings insinuate the motive that CBZ toxicity can be minimized by using moringa and flaxseed oil. Additionally, CBZ blocks protein's normal function and blocks their active sites by hampering normal protein functions and expression that leads to complications in the spermatogenic process confirmed by *in silico* approach.

5. Conclusion

Implications of this study provide future directions to remediate toxicity issues by using moringa oil and flaxseed oil by emphasizing importance of medicinal plants. In addition to in-vivo study, dashboards of the analysis showed that CBZ acts as an inhibitor against normal functions of testicular proteins, as determined by their binding affinity and scoring function, in the order of TEX101_RAT > EPPI_RAT > GPX5_RAT. This study suggests moringa oil and flaxseed oil could be used in treating toxicities induced by CBZ and other agrochemicals, drugs, and pollutants.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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