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Original Article

Alleviation potential activity of Cypermethrin by *Moringa oleifera* Lam. oil on testes and livers of male rats with response to affinity of specific physiological protein

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Abstract

The current study is to highlight the effect of natural phyto-product as Moringa oleifera Lam. oil and another artificial insecticide as cypermethrin on efficiency of two different organs of male rats related to different systems; testes and livers. The study utilized probit analysis to determine sub-lethal and lethal doses. Twenty-four male rats were divided into four experimental groups; G1: controlled group, G2 exposed to cypermethrin (CYP), G3 exposed to combination between Moringa oleifera Lam. oil and cypermethrin. G4 treated with moringa oil only. The biochemical analyses were performed as plasma glucose, total protein and albumin levels. ANOVA test besides histological features examined the parenchyma of both studied organs. Cypermethrin had detrimental effects on rats, leading to elevated serum glucose levels, reduced levels of total protein and albumin besides histopathological alterations observed in both studied organs. The molecular docking analysis of a specific testicular protein expressed the high affinity with Cypermethrin active bonds. The findings confirmed on the need of using natural products to overcome the spread of artificial chemicals in our environments.

Keywords: Insecticide, Medicinal plant, Edema, Hemorrhage, Histology, Toxicity, Anticancer

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Introduction

Nowadays, pesticides are used as global weapons against all dangerous harmful insects threaten human life all over the world. All specialized scientists try to find natural alternatives serving humans from one side and the environment from other side. They depend on active biological ingredients which can be able to coordinate and modify with alteration of pest genome that is obstructive against artificial ones (Abu Zeid et al., 2022). Other natural components are able to relieve the impact effect of artificial chemicals after exposure in the environments. The need of biological compounds becomes the demand of all people who are afraid of our planet (Al-Attar and Al-Saeed, 2021; Abomosallam et al., 2023).

Cypermethrin is a synthetic insecticide that used in a large scale from all farmers against many insects and moths facing most of crops, vegetables and fruits. The residues of this chemical keep in the environments causing the harmful effects on all types of living organisms especially wild animals and domestic ones (Giray et al., 2001).

Moringa oleifera Lam..is a plant species belonging to Moringaceae. It is cultivated all over the world. It is regarded as a natural biological relief on detrital histological and physiological effects resulting from direct or indirect chemical induction (Liang et al., 2019). Besides different fatty acids, there are other active chemical composition like sterols, tocopherols, alkaloids, flavonoids and phenolic acids that having anti-oxidant and anti-inflammatory properties (Patil et al., 2022).

Docking study is modern biological information which can interpret many biological problems related to biochemistry and gene expression. It can predict the interactions among enzymes and different legends and determine the consequences appearing morphologically, anatomically or physiologically. There are different types of proteins like primary, secondary and tertiary contributed to expression of testicular function and control testis properties referring to various embedding active sites at protein folding structures.

Molecular docking analysis helps to make precise combination between the ligand and the specific protein calculating the degree and affinities of binding energies (Yang et al., 2018).

The aim of this study is to illustrate the role of natural product like moringa oil to overcome the negative influence of artificial chemicals like Cypermethrin against potential activity for testes and livers of male rats besides determination of legend docking efficiency towards specific physiological proteins.

Material and Methods

Chemical and dose preparation

Cypermethrin (CYP) formulation (20% emulsifiable concentrate "EC") and *Moringa oleifera* Lam. oil (Herbarium sheet No. 1235; Voucher Number 1015) (Figure 1) were obtained from Egyptian Agricultural Pesticides Committee (APC) Dokki, Giza, Egypt and Natural Products Department of the National Center for Radiation Research and Technology Cairo, Egypt respectively.

Cypermethrin oral LD_{50} was processed according to the probit equation:

Total Dissolved Solids (TDS) (mg/l) = 640 x Electrical Conductivity (EC) (dS/m or mmho/cm or ms/cm) (Nagarjuna and Jacob, 2011).

Cypermethrin was added to negative control substance; this formulation relieves the irrigation of rat stomach during oral gavage (Hashim et al., 2023). According to probit analysis, the optimal dose was prepared by adding 4 ml of Cypermethrin to 750 ml of corn oil (145mg/kg bwt) and each treated rat was oral inserted with 2 ml of mixture daily for four weeks. On the other hand, 60 ml moringa oil was dissolved with 750 ml of corn oil and Cypermethrin and moringa oil was mixed to prepare another solution by adding half the quantity of prepared Cypermethrin with half quantity of prepared moringa oil solution (Manna et al., 2003).

Animals and experimental design

Twenty-four male Wister rats were kept under hygienic conditions, each weighing between 145 and 165 grams. The rats were kept in an environment with a 12-hour light/dark cycle, a temperature of 20 ± 4 °C, and room humidity of 60±10 %. They were fed standard rodent food and had access to water. After seven days (acclimatization period), the rats were randomly divided into four groups.

Group I: served as the control group (C) that received 2 ml distilled water.

Group II: was exposed to cypermethrin (CYP) solution alone representing the pesticide group (P).

Group III: received moringa oil solution only,

https://doi.org/10.35495/ajab.2024.160

representing the moringa group (M). **Group IV**: received a combination of cypermethrin and moringa oil, representing the pesticide and moringa oil group (PM).

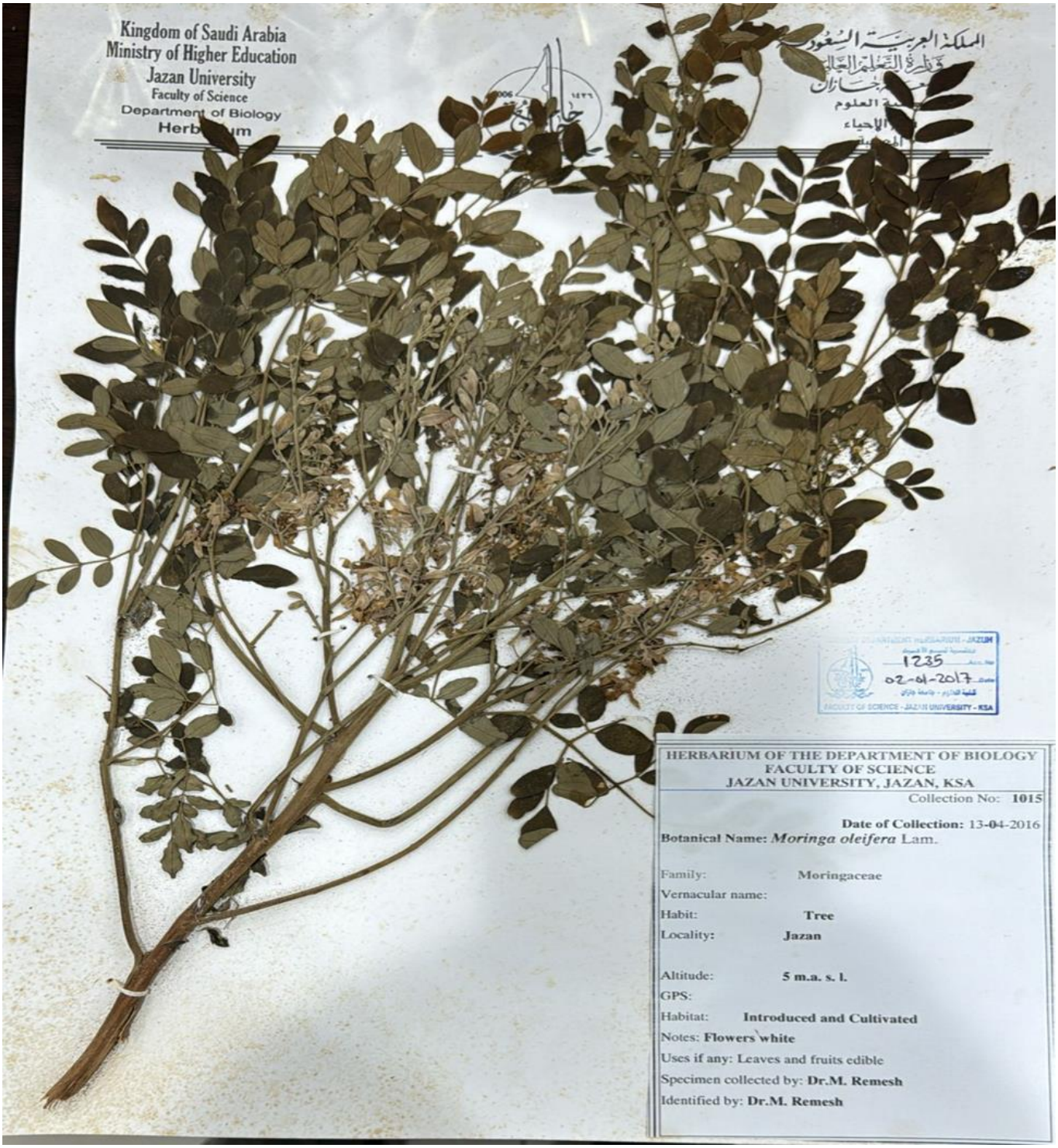

Figure-1. *Moringa oleifera* Lam. herbarium sheet

Blood sample collection

After four weeks, all the animals were euthanized while under light ether anesthesia. Once euthanized, blood samples were promptly obtained and divided into two portions. The first portion was collected in EDTA tubes for hematological analysis, while the second portion was collected in plain tubes to facilitate the separation of blood serum through centrifugation at a speed of 1059 xg for 15 minutes. The serum samples were then stored at a temperature of -20 °C until they were utilized for immunological analysis in the study.

Biochemical analysis

At the end of the experimental period, animals were fasted for 8–12 h before blood collection in order to cause no interference in the analysis of following:

- Plasma glucose: plasma glucose test was performed using Colorimetric Assay Kit (Cat. No. SKU: KBH4209, Krishgen Biosystems blood glucose kit, USA), according to the manufacturer's instructions.
- Serum Albumin: was performed using colorimetric assay kit (Ref. No.: 1001020, Bromocresol green colorimetric kit, Spain), according to the manufacturer's instructions.
- Serum total Protein: was performed using Colorimetric assay kit (Ref. No.: MD1001291 Biuret. Colorimetric kit, Spain), according to the manufacturer's instructions.

Each measurement was conducted using standard kits following the guidelines and procedures provided by the manufacturers. This ensures the reliability and reproducibility of the analytical results.

Histopathological study

The testicular and hepatic tissues obtained from different experimental groups were harvested and preserved in a 10% formalin solution. Afterwards, the tissues were embedded in paraffin wax and sliced using a rotary microtome. These resulting sections were then subjected to staining with hematoxylin and eosin (Bilinska et al., 2018). Finally, the stained tissue slices were observed and analyzed under a light compound microscope equipped with a digital camera.

Molecular docking analysis

Physical and chemical properties besides toxicity clinical analysis of Cypermethrin were determined as a ligand according to Chen et al., 2023. Through studying of 107 testicular proteins besides 263 long non-coding RNAs were identified and tested in docking analysis, TX101 was assessed and selected as a newest studied protein with high proper oriented docking combination. The docking procedure was processed by evaluating and rectifying the functional groups of both receptor and ligands and calculating binding affinity through the BIOVIA Discovery Studio Visualizer.

Statistical analysis

The findings were reported as the mean ± standard deviation (SD). The statistical analysis was carried out using SPSS software (Version 21), employing a one-way Analysis of Variance (ANOVA). Results with a p-value less than 0.05 were considered statistically significant (Areshi et al., 2023).

Results

Biochemical analysis

For blood glucose parameter, cypermethrin treated group (P) recorded the highest value (115.33 ± 4.50 mg/dl) while moringa group recorded the lowest one (68.57 ± 4.8 mg/dl) (at F test = 58.895, P>0.05). On the contrary, moringa oil treated group (M) recorded the highest value (6.1 ± 0.49 g/dl) while cypermethrin group (p) recorded (5.25 ± 0.34 g/dl) (at F test = 5.54, P>0.05) for total protein. Finally, group (MP) recorded higher value (2.63 ± 0.16 g/dl) than other groups except control one while group (P) recorded the lowest one (2.27 ± 0.19 g/dl) (at F test = 7.914, P>0.05) shown in Figure 2.

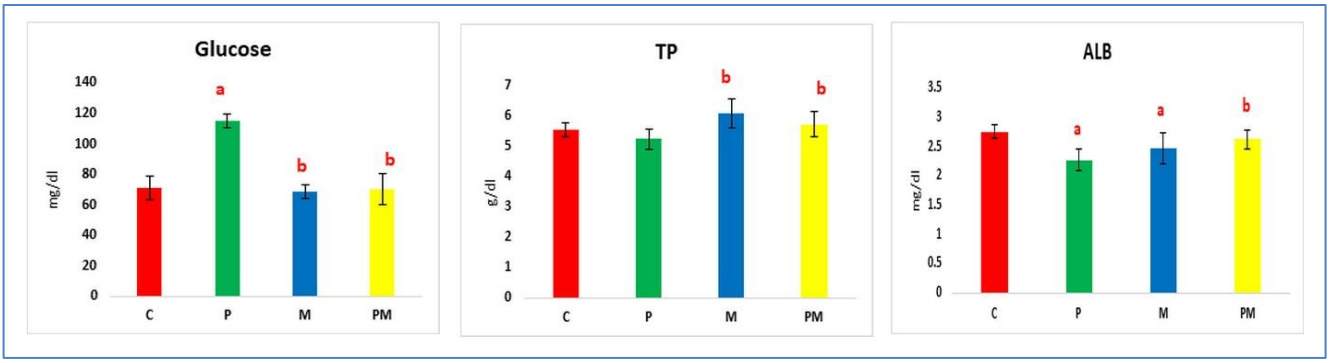

Figure-2. Serum glucose, total protein (TP) and albumin (ALB) conc. for control (c), Cypermethrin (P), moringa oil (M) and combination of cypermethrin and moringa oil group (PM) groups, a: significant compared to control (C) group b: significant compared to Pesticide (Cypermethrin) group. (p).

Histopathological criteria

Upon histological results, control and moringa oil groups exhibited no pathological alterations in testicular parenchyma. The testes were healthy and normal. The seminiferous tubules were rounded with normal lumen surrounded with a thin basal membrane accompanied with myofibroblasts. Multiple layers of cells were present inside the seminiferous tubules starting with spermatogonia followed by primary and secondary spermatocytes then spermatids and spermatozoa. Sertoli cells were more obvious in moringa oil treated group than control group intricately with spermatogonia. Normal appearance of intertubular tissues among seminiferous tubules was observed with blood capillaries with healthier histo-architecture in moringa oil treated group. Prominent Leydig cells were situated interstitially with normal size. On the contrary, the testes displayed such marked distortion of seminiferous tubules with large elongated atrophied lumen at cypermethrin treated group (P). Different stages of spermatocytes were sparse and disorganized with absence of spermatozoa. There was an increment of thickening and congestion for intertubular connective tissue with slightly edema. Furthermore, the healthy testicular view came back again in combination of cypermethrin and moringa oil group (PM). It revealed a similar description of control group with vacuolar degeneration as shown in Figure 3.

Similarly, liver tissues of control and moringa groups demonstrated distinct normal binucleated hepatocytes with intact cytoplasm and typical cuboidal epithelial cells disclosed as homogenous extended chords surrounding the clear well-defined central vein and portal vein with regular sinusoids. However, there were neither intact plates nor chords in liver parenchyma at cypermethrin group. Marked excess mononucleated infiltrative cells were present with areas of centrilobular congestion including definite edema and obvious hemorrhage. Plenty of steatosis appeared all over the section with slightly expression of pyknotic nuclei. Finally, appearance of typical healthy structure was presented at combination of cypermethrin and moringa oil group like previous first and second groups with leakage of hemorrhage as shown in Figures 4 & 5.

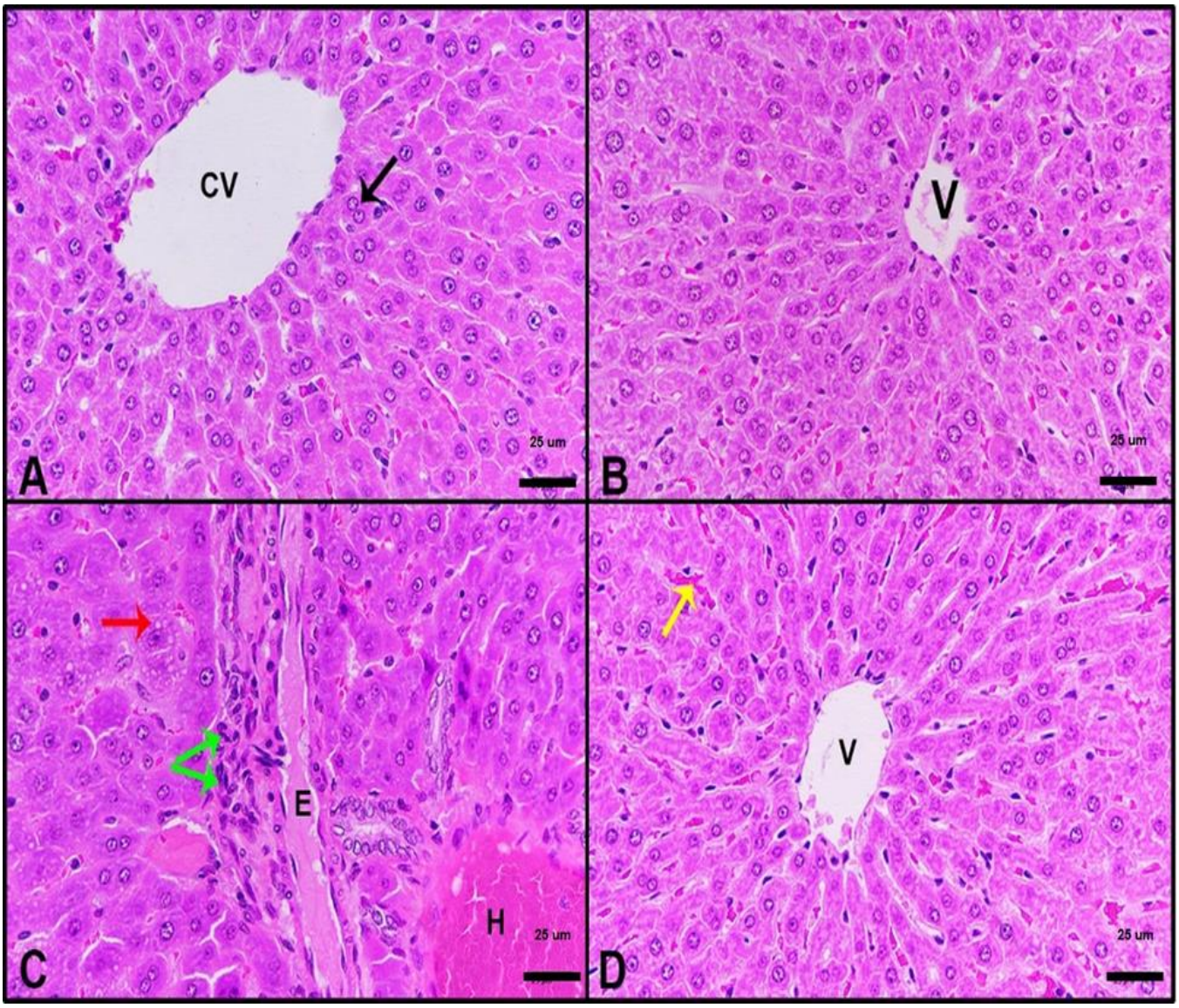

Figure-3. Hepatic tissue sections for control group (A), moringa oil treated group (B), cypermethrin treated group (C), combination of cypermethrin and moringa oil treated group (D) at (H&E, 400X), CV: central vein, V: portal vein, E: edema, H: hemorrhage, (black arrow): binucleated hepatocytes, (green arrow): infiltrative cells, (red arrow): steatosis, (yellow arrow): leakage of hemorrhage.

https://doi.org/10.35495/ajab.2024.160

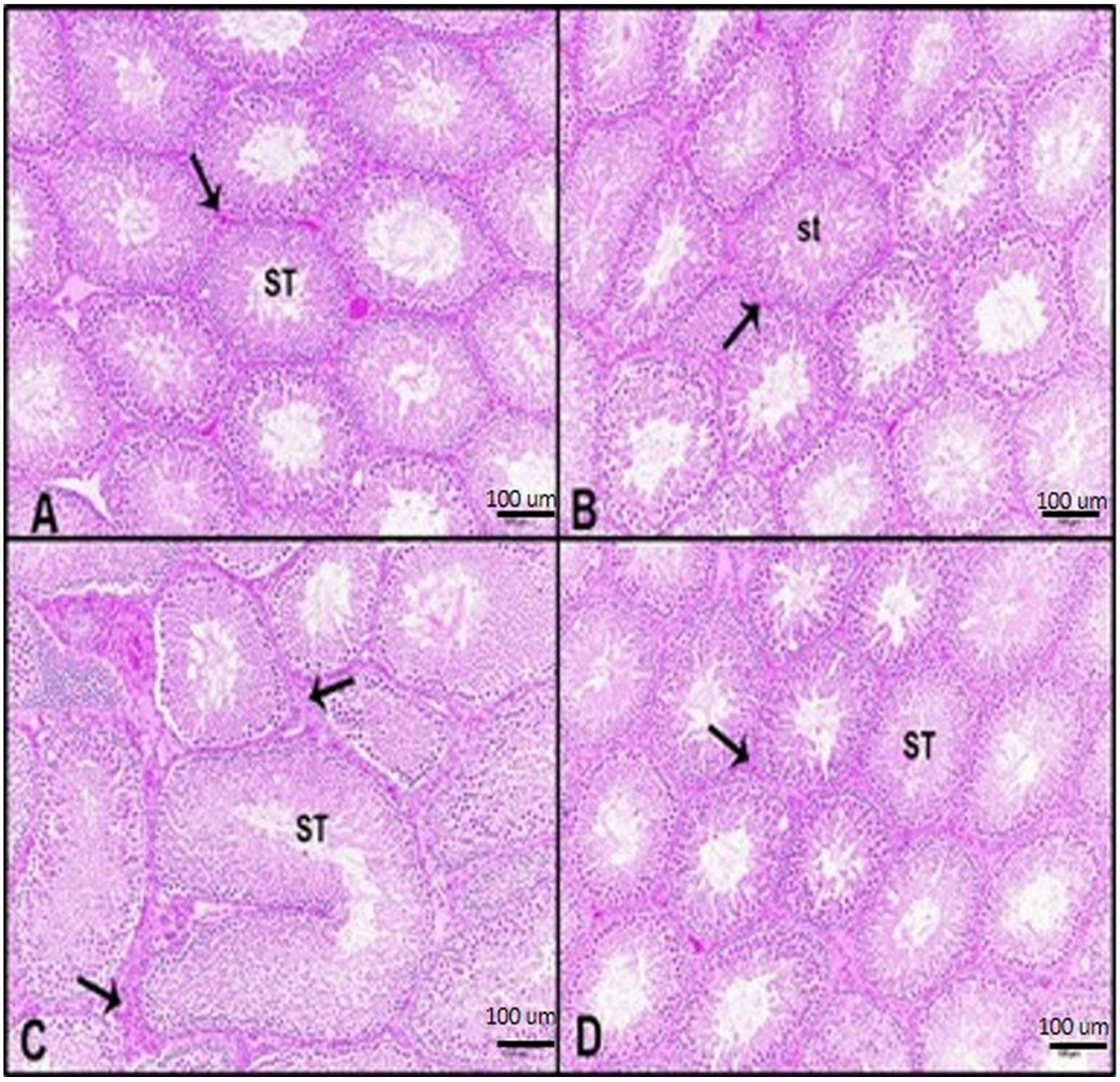

Figure-4. Testicular views for control group (A), moringa treated group (B), cypermethrin treated group (C), combination of cypermethrin and moringa oil treated group (D) at (a: H&E, 100X) ST: seminiferous tubes, spermatozoa, (black arrow).

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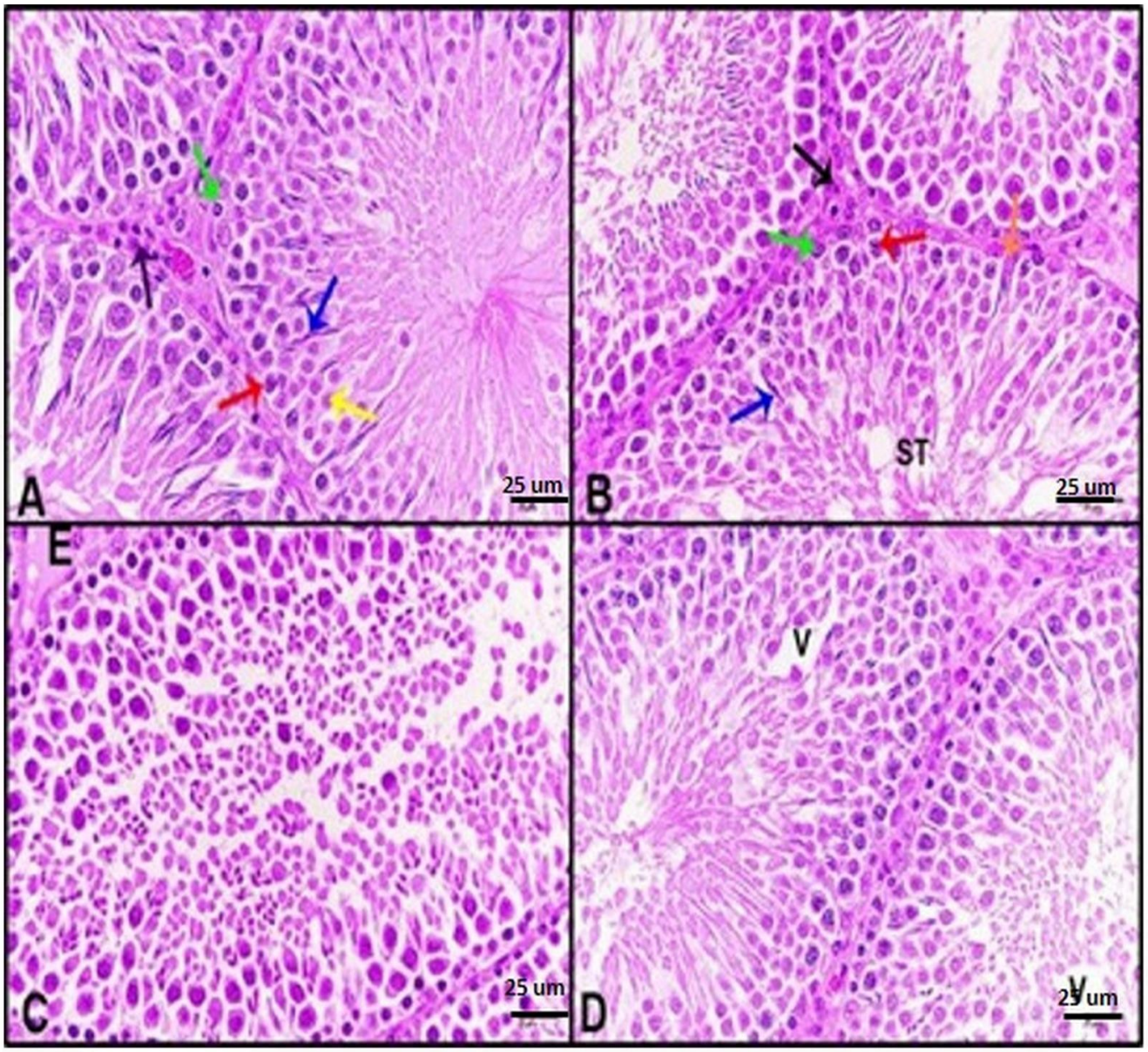

Figure-5. Testicular views for control group (A), moringa treated group (B), cypermethrin treated group (C), combination of cypermethrin and moringa oil treated group (D) at (b: H&E, 400X), ST: seminiferous tubes, V: vacuolar degeneration, (green arrow): spermatogonia, (red arrow): primary spermatocyte, (yellow arrow): secondary spermatocyte, (blue arrow): spermatozoa, (black arrow): Leydig cells, (orange arrow): Sertoli cell. (green arrow): spermatogonia, (red arrow): primary spermatocyte, (yellow arrow): secondary spermatocyte, (blue arrow): spermatozoa, (black arrow): Leydig cells, (orange arrow): Sertoli cell.

Molecular docking analysis

Cypermethrin as an artificial chemical substance has deteriorated effects on living organs so it can be regarded as a ligand that can interact with selected proteins at specific active sites. The nature of this substance was summarized in Table 1. Configuration and orientation of Cypermethrin structure can be identified according to Tavares et al., 2022 as shown in Figure 6. Docking of Cypermethrin with different physiological proteins related to testes was done.

Cypermethrin was classified as a carboxylic ester formed from condensation between 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclo propane carboxylic acid and the alcoholic hydroxyl group of hydroxy (3-

phenoxyphenyl) acetonitrile. Testis-expressed protein 101 (TEX101) had the high affinity to bind with the active functional group of Cypermetherin. This physiological protein is a glycosyl-phosphatidylinositol-anchored glycoprotein essential for sperm fertility and spermatogenesis. The protein molecule content as shown in Figure 7 was present in detail in Table 2. It comprises three entities: macromolecules, oligosaccharides and small molecules. Macromolecules were genetically expressed through two chains A and B with domain starting from position 120 bp ending at 195 bp. Chains A and B have 178 residues with 1306 atoms and 180 residues with 1323 atoms respectively as shown in Figure 8. Oligosaccharides are alpha-L-fucopyranose-(1-6)-2-acetamido-2-deoxy-beta-D-glucopyranose, 2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-[alpha-L-fucopyranose-(1-6)]2-acetamido-2-deoxy-beta-D-glucopyranose and glycosylation residues. Small molecules are query on SO_4. TEX101_RAT is able to interact with lymphocyte antigen 6 complex, locus K (Ly6k) as well as a disintegrin and metallopeptidase domain 3 (ADAM3) (Masutani et al., 2020).

Docking was conducted and visualized by using AutoDock Vina. The active sites which are responsible for binding procedure were represented by ball shapes showing Serine 31, Thyroxine 33, Phenylalanine 44, Asparagine 45 residues as shown in Figure 9. The outcome of docking was summarized in Table 3.

Table-1. Basic physical and chemical information of Cypermethrin ligand.

Ligand	IUPAC name	Molecular formula	Molecular weight	Hydrogen bond donor count	Hydrogen bond acceptor count	Therapeutic category
Cypermethrin	Cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	$C_{22}H_{19}Cl_2NO_3$	416.3 g/mol	0	4	Ectoparasticide

Table-2. Description of TEX101_RAT protein content.

Total structure weight (kDa)	No. non-hydrogen atoms	No. polymer monomers	No. non-polymer monomers
47.73	2885	358	64

Table-3. Docking energy report (kcl/mol) between Cypermethrin and TEX101_RAT protein.

Binding energy	Ligand energy	Intermolecular energy	Electrostatic energy	Torsional energy	Unbound energy
-5.12	-0.18	-7.21	0.06	2.09	-2.1

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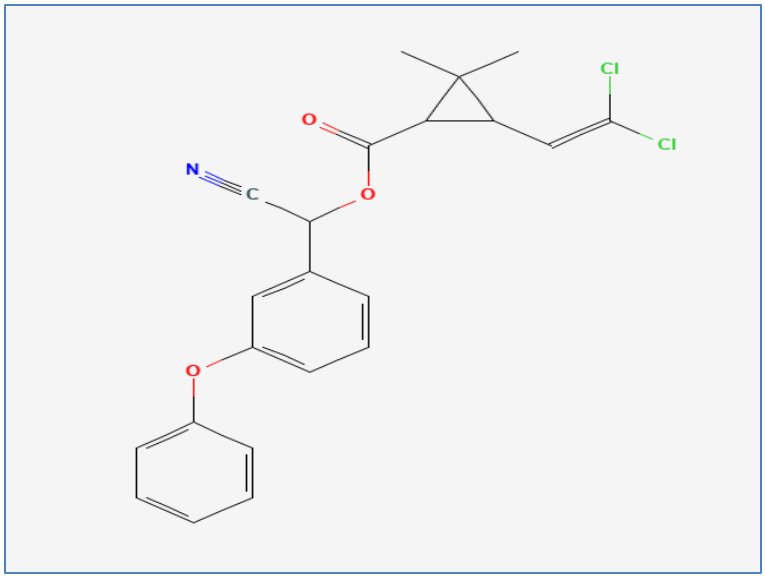

Figure-6. Cypermethrin structure

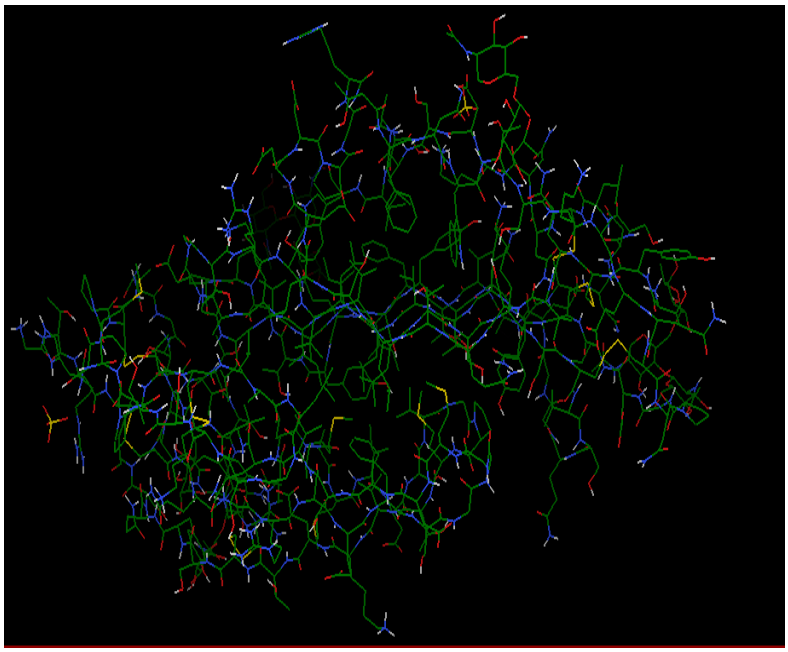

Figure-7. TEX101_RAT protein structure, red bonds; amino acids residues, green bonds; carbon-carbon bonds, blue bonds; polar bonds, while bonds; Sulphur Sulphur bonds

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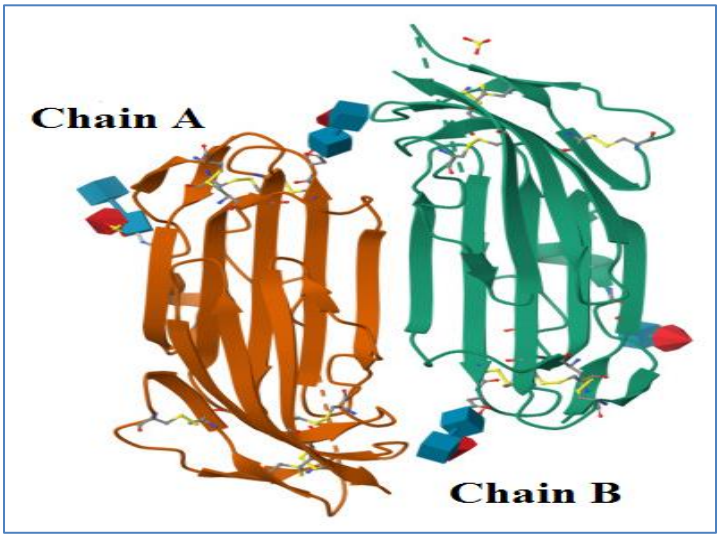

Figure-8. Chain A and chain B for TEX101_RAT protein expression; C: 800 & 808, N: 220 & 225, O: 267 & 270, S: 19 & 19 respectively.

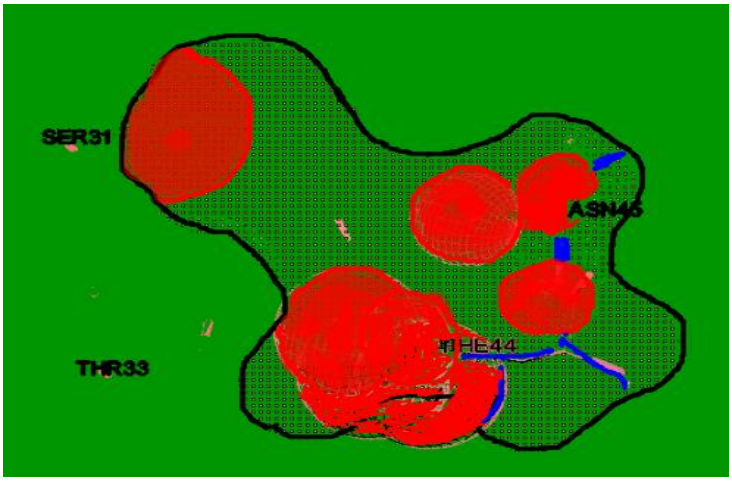

Figure-9. Docking between TEX101_RAT protein and Cypermethrin at Serine (SER31), Thyroxine (THR33), Phenylalanine (PHE44), Asparagine (ASN45) residues of active site.

Discussion

In recent years, researchers have been studying the bioactive compounds found in *Moringa oleifera* to better understand its potential benefits and biological effects. These studies aim to provide a more scientific foundation for its usage and to explore its properties.

The leaves of *Moringa oleifera* have traditionally been utilized as herbal medicine for their potential antidiabetic, antibacterial, and anti-inflammatory properties (Anudeep et al., 2016; EL-Shafey et al., 2018; Villarruel-López et al., 2018). Olayaki et al. (2015) observed that oral administration of extract of *M. oleifera* significantly reduces blood glucose concentration and inhibits weight loss in alloxan-

induced diabetic rats. In the present work more reduction in plasma glucose level in (G3) as compared to (G2) these data refereed to enhance the hepatic gluconeogenesis and glycogenolysis. Anwar and Bhanger (2003) highlighted the high resistance to oxidation of moringa oil due to the presence of high contents of tocopherols. Moreover, Al-Malki and Rabey (2015) reported that medication with *Moringa oleifera* improved glucose tolerance in diabetic patients. This finding supports the notion that moringa oil may have a positive effect on glucose levels, aligning with the task of exploring the impact of moringa oil on glucose levels in rats. This information is crucial as it indicates the potential impact of moringa oil on oxidative stress, which can be linked to its effects on glucose levels in rats. That was due to the medicinal value of moringa oil for having flavonoids (Glycosides), polyphenols, lycopene, carotenoids, sterols, vitamin E, tocopherols, vitamin C and phenolic acids. All these antioxidants, ant-inflammatory and detoxifying agents were able to promote glucose metabolism and overcome insulin resistance as anti-diabetic effect. Additionally, Kilany et al. (2020) discussed the anti-obesity potential of *Moringa oleifera* seed extract, which included restoring glucose levels towards normal values. moringa oil has been studied for its potential effects on liver function and serum protein levels in rats treated with cypermethrin.

Corn oil is commonly utilized as a vehicle in research for various reasons. Such as; many substances, particularly volatile oils and fat-soluble compounds, do not dissolve well in water. Corn oil can efficiently dissolve these substances, facilitating accurate dosing and ensuring that test subjects receive the correct amount of the active ingredient. Additionally, corn oil can dilute certain chemicals, which helps to reduce their toxicity and minimize the risk of harmful effects from high concentrations.

The present study showed a significant increase in serum albumin and total protein in (G3) and (G4) as compared to rats treated with Cypermethrin (G2). These data were consistent with the results obtained by Adeoye et al. (2022) who demonstrated the hepatoprotective effects of moringa seed oil in improving plasma protein levels and reducing elevated liver enzymes in rats. Increase in albumin levels, indicating enhanced hepatocyte function in detoxifying cypermethrin, has been observed in multiple studies (Manna et al., 2003; Mansour et al., 2022; Leone et al., 2016). Additionally, El-Hadary and Ramadan (2018) highlighted the protective impact of moringa leaf extract against diclofenac sodium-induced liver toxicity in rats, indicating its potential role in mitigating liver damage. Moreover, Sadek (2013) showed that *Moringa oleifera* leaf extract increased total protein and albumin levels in chromium-treated rats, suggesting its hepatoprotective properties. Liver histological changes induced by Cypermethrin that exhibited marked pathological signs such as congestion of veins with hemorrhage and edema, also presence of infiltrative cells and steatosis. Yavaşoğlu et al. (2006) demonstrated that cypermethrin, a pyrethroid insecticide, has been shown to induce liver damage in rats, as evidenced by histological alterations and biochemical changes. Saeed (2022) have reported adverse changes in liver tissues, including necrosis, congestion, vacuolization, and tissue degeneration in animals exposed to cypermethrin. Additionally, cypermethrin has been linked to alterations in nucleic acids and protein contents in the liver of freshwater fish (Kumar et al., 2007). The impact of cypermethrin on protein metabolism in fish liver, gills, and muscle tissues has also been investigated, highlighting its effects on nitrogen metabolism (Prashanth, 2007). On the other hand, (Adeoye et al., 2022) demonstrated that moringa oil has hepatoprotective effects in various studies. found that *Moringa oleifera* seed oil had a hepatoprotective effect in rats exposed to toxic substances, leading to an increase in plasma protein levels and a reduction in liver markers. Furthermore, moringa has been shown to attenuate hepatocarcinogenesis and modulate metabolic enzymes in liver tissues (Hussein et al., 2018). Studies have also indicated the ameliorating effects of moringa against liver and kidney injuries induced by toxic substances (Elgharabawy et al., 2019). The present study has provided evidence that the combined use of cypermethrin and moringa oil (G4) yields a plausible expectation that moringa oil can assist in reducing liver damage caused by cypermethrin. This is ascribed to the hepatoprotective properties of moringa oil and its capability to alleviate liver injuries. So, animals treated with moringa oil during exposure to cypermethrin posted obvious improvement that looked healthy. Furthermore, the histological alterations observed in the testes of rats treated with cypermethrin included deformation of seminiferous tubules, with some appearing atrophied and others elongated (Hashim et al., 2023). Additionally, there was thickening and congestion of the interstitial tissue,

along with sparse and disorganized spermatogenesis stages and absence of spermatozoa. However, the protective potential of moringa oil in CYP mediated deterioration and improved the intact texture and anatomy. The obtained results alignment with Joshi et al. (2010) who reported that histological examination of the testes in rats exposed to cypermethrin revealed various abnormalities, such as distorted seminiferous tubules, atrophy, elongation, thickened and congested interstitial tissue, and disrupted spermatogenesis with the absence of spermatozoa; meanwhile, the co-administration of cypermethrin and moringa oil resulted in a notable improvement in these histological changes, although vacuolar degeneration persisted. Also, these findings are consistent with the observed histological changes in the testes of *Yankasa rams* with cypermethrin (Simon, 2017). This improvement suggests a potential protective effect of moringa oil against some of the adverse effects induced by cypermethrin.

TEX-101_RAT protein is related to sperm migration as well as male fertility that facilities the oviduct fertilization by inducing cell-specific interaction invading through activating spermatogenesis mechanism. (Li et al., 2013; Endo et al., 2016). In human testes, TEX-101 plays a vital role in maturation of sperms and pavement of successful pollination and fertilization (Schiza et al., 2019; Erbayram et al., 2021). High affinity of Cypermethrin to TEX101_RAT confirmed on the damage signs of testicular tissues. These alterations may complete the symptoms of male infertility in addition to spreading testicular torsions besides intoxicant disorders. All disruptions appeared in selective biological organs induced by Cypermethrin evidenced on necessity of chemical elimination from the environment and providing of natural alternatives to decline the prolonged exposure of artificial constituents in our lives (El-Hak et al., 2022; Hashim et al., 2024).

Recently, separation of *Moringa oleifera* leaf extract exhibited presence of three phytochemical organic compounds with high percent peak adhering with pyridine (C_6H_{12}). These are 1-Propanol, 3,3′-oxybis-($C_6H_{14}O_3$), 1-propanamine, 3-propoxy- ($C_6H_{15}NO$) and 2-pentene, 2-methyl-(C_6H_{12}) with molecular weight 134.17, 117.19 and 84.16 g/mol respectively. Due to the same molecular weight between 2-pentene, 2-methyl- and pyridine let the three compounds to coordinate to play role in functional mechanism of liver performance (Chukwu et al., 2024).

Conclusion

Biochemical estimation can be chosen as vital indicators to the degree of toxicity of any artificial chemical composition. It can correlate with interpretation of histological analysis. Moringa oil is considered as a dietary supplement which should be added in meals of humans and domestic animals to remove the negative impact of artificial ingredients inserted to food. Implications of this study provide directions to remediate and prevent future illness or disorder coming from chemical exposure.

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Disclaimer: None.

Conflict of interest: None.

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Ethical Approval Statement: This study was ethically approved by Standing Committee for Scientific Research, Jazan University, Jazan, Saudi Arabia (HAPO-10-Z-001); Reference No.: REC-45/04/822, Date of decision: 24 October 2023.

Contribution of Authors

Khormi MA: Conceptualized study, designed research methodology, analyzed and interpreted data, wrote and edited manuscript, project administration, resource management and project supervision.

Alfattah MAAbo-Zaid MA, Abdalla SEB & El-Shabasy A: Designed research methodology, collected, analyzed and interpreted data, wrote original draft, reviewed and edited manuscript.

All authors read and approved the final draft of manuscript.

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