Research Article

In vivo and *in silico* Evaluation of Flavonoid and Glucosinolate as Aromatase Inhibitor for Assessing Sexual Performance against Cadmium Induced Infertility in Male Wistar Rats

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ABSTRACT

Aim: The purpose of this research was to explore the effectiveness of Indole-3-carbinol and Resveratrol as aromatase inhibitors and their effects on sexual performance in male wistar rats against cadmium-induced infertility. **Materials and Methods:** Male Wistar rats were exposed to cadmium (2 mg/kg) to induce infertility. The rats were separated into different groups, control, cadmium-exposed and sildenafil citrate (5 mg/kg), test includes flavonoid (Resveratrol 20 mg/kg) and glucosinolate (Indole-3-carbinol 100 mg/kg) for 28 days of treatment. Sexual performance parameters, such as number of mounts, mounting frequency, mount latency, intromission frequency, were evaluated at different time intervals. For the *In silico* evaluation, Autodock (4.0.) software was used for molecular docking investigation, to determine the binding affinities of Sildenafil citrate, Indole-3-carbinol and Resveratrol compounds with aromatase protein (3EQM), which is a targeted enzyme. **Results:** The results showed that treatment with Indole-3-carbinol and Resveratrol significantly improved sexual behavioural parameters in male Wistar rats. The number of mounts, mounting frequency and intromission frequency increased, while mount latency decreased in the combined treatment group compared to the cadmium-exposed group. Furthermore, the *in silico* analysis revealed favorable binding affinities and docking scores for Sildenafil citrate (-10.5 Kcal/mol), Indole-3-carbinol (-4.7 Kcal/mol) and resveratrol (-6.25 Kcal/mol) with the aromatase enzyme (3EQM). **Conclusion:** So, the current study evaluated that these compounds may act as potent aromatase inhibitors through synergistic effect, thus reducing the conversion of androgens to estrogens and potentially improving sexual performance with optimal maintenance of Gonadotropins.

Keywords: Aromatase, Cadmium toxicity, Gonadotropins, Infertility, *in silico*, Molecular docking, Sexual performance.

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INTRODUCTION

Cadmium, a toxic heavy metal, is a significant concern due to its detrimental effects on human health, particularly in male fertility. Exposure to cadmium leads to a decline in semen quality parameters, increased risk of infertility and disrupted hormonal balance in the male reproductive system. The mechanisms underlying cadmium-induced infertility in males are multifaceted, including oxidative stress, DNA damage and apoptosis in germ cells. Cadmium also disrupts the blood-testis barrier, compromising the testicular microenvironment and impairing spermatogenesis. The general population is also at risk of cadmium-induced male infertility, emphasizing the need for public health interventions and regulatory measures.[1-4] Cadmium poisoning also affects the aromatase enzyme, potentially affecting

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male infertility. This enzyme turns androgens into estrogens, disrupting the balance in the male reproductive system.[5] Indole-3-carbinol positively impacts aromatase activity, potentially improving sperm production by decreasing oestrogen levels and increasing androgen levels, promoting optimal sperm development and maturation.[6] Indole-3-carbinol and resveratrol, is a plant origin which was found in cruciferous vegetables and grapes, have potential as therapeutic agents for treating reproductive toxicity. Indole-3-carbinol has anti-inflammatory and antioxidant properties, protecting against testicular damage, improving sperm quality and restoring fertility. Resveratrol, a natural polyphenol, has potent antioxidant and anti-inflammatory effects, preserving testicular function and enhancing sperm parameters.[7-12]

The purpose of the study was to find out how Indole-3-carbinol, resveratrol and sildenafil citrate affected the activity of the aromatase enzyme and whether they may lessen the negative effects of cadmium exposure on male rats' sexual function and fertility The researchers sought to offer important insights into the causes and prospective treatment options for treating male infertility brought on by cadmium poisoning by using both experimental and computational methodologies.

In males, testosterone acts as a "pro-hormone" that is metabolized into estradiol in the brain, where aromatase expression occurs in the brain, prostate and testes, influencing male behavior**.** The following discoveries further backed up this judgement: I) Male usual sexual behaviour is inhibited by inhibiting aromatase activity and II) The direct delivery of oestrogens right away into the brain can initiate male characteristic sexual behavior in castrated animals.[13-17] Most vertebrates' gender-specific brain development is dependent on local oestradiol synthesis in the brain.[18] The existence of aromatase in the brain emphasizes the importance of local estrogen production in not only controlling sexual processes, but also in other neural processes such as behavior, memory, cognition and injury recovery.[19] Testicular testosterone regulates gonadotropin secretion in males.[20] In primates, inhibiting aromatase activity causes a large rise in both LH and FSH.[21] Early studies revealed that oestradiol is just as efficient as testosterone in masculinizing rat behaviour.[22] Accordingly, Aromatase and oestrogen receptors are abundant in the brain, particularly in rats.[23] Furthermore, testosterone alone cannot cure defective male copulatory behaviour, whereas a combination of dihydrotestosterone and oestradiol nearly entirely copulatory behaviour has been restored.[24] To sustain male sexual behaviour, species differences and neuronal aromatisation are essential.[23] In fact, it has been demonstrated that adequate Testosterone: Oestradiol (T: E) ratios are necessary for ideal prostate function, whereas too much T:E ratios cause prostate expansion and hyperplasia.[13,14] Both Leydig and Sertoli cells in humans generate oestrogens *In vitro*.[25] Aromatase activity has also been detected in spermatogenic cells from various animals, which includes humans.[26] Several studies have clearly established a Lower sperm concentration and motility have been associated with aromatase polymorphisms.[27] Decreased sperm motility is common in aromatase deficient men and in aromatase knockout mice.[28] Oestrogens are essential for epididymal activity and sperm maturation.[26] Consequently, Aromatase distribution in masculine gonads plays an essential role to sustaining the high amounts of oestradiol required for most effective sperm maturation, sperm motility, spermiogenesis and possibly acrosomal response.[29,30]

MATERIALS AND METHODS

Chemicals

Indole-3-carbinol (*purity*≥98%), Resveratrol (*purity*≥99%), Cadmium chloride ($CdCl_2$, ≥99% purity) and Dimethyl sulfoxide (*DMSO*, ≥99.9% purity) were purchased from Sigma-Aldrich. The use of standard chemicals adds credibility to the study and allows for meaningful comparisons and interpretation of the findings.

Experimental animals

This study used male albino wistar rats that were 10 weeks old and weighed between 170 and 200 g. The animals were obtained from the Animal House, Kerala Veterinary and Animal Sciences, Mannuthy, Kerala. Reg.No.328/GO/Re/S/01/CPCSEA and housed in Department of Pharmacology at Nandha College of Pharmacy, Erode. The rats were acclimatized for 7 days in the animal house of the Department of Pharmacology, Nandha college of Pharmacy and exposed to 12/12 hr light/dark cycle. The institutional animal ethics committee authorised this study protocol and experimental animals had unlimited access to food and water. All experimental techniques and protocols employed in this investigation were in conformity with the recommendations of IAEC Proposal No: Reg No: 688 /PO/Re/S/02/CPCSEA.

Experimental design and study protocol

In silico docking study

To ascertain the impact of the molecular interaction pattern of Indole-3-carbinol and resveratrol with the

human placental aromatase protein (3EQM), *In silico* docking study was carried out. The objective of this study was to evaluate the possible therapeutic efficacy of Indole-3-carbinol and resveratrol in the treatment of male infertility caused by cadmium toxicity. The targeted protein, aromatase (3EQM), with a resolution of 2.90 Å, is the three-dimensional structure of human placental aromatase cytochrome P450 in complex with androstenedione. This structure was selected for investigation from the RCSB Protein Data Bank (http://www.rcsb.org/pdb).

Ligand preparation

The ligands of, Indole-3-carbinol (https:// pubchem.ncbi.nlm.nih.gov/compound/ 3712) and resveratrol, (https:// pubchem.ncbi.nlm.nih.gov/ compound/445154) as well as Sildenafil citrate as standard compound (https://pubchem.ncbi.nlm.nih.gov/compound/ 135413523) in 3D PDB format were transferred from the database of PubChem and formatted in PDBQT format using software (BIOVIA Discovery Studio Visualizer 2021) and torsion, ionisation, degree of freedom and stereochemical variation are further processed for molecular docking in Autodock.

Protein structure preparation for docking

The chosen aromatase protein structure (3EQM) was prepared for docking by using the Autodock (4.0.) The protein structure for the 3EQM protein, only enzyme in vertebrates known to catalyse the biosynthesis of all oestrogens from androgens; was retrieved as PDB format with the following specifications: Resolution: 2.90, R-Value Observed: 0.215R-Value Work: 0.214 and R-Value Free: 0.244. The protein structure was improved in the Autodock (4.0.) tool by removing the water atoms and adding polar hydrogen atoms. Molecular docking was carried out using the software programmes Autodock (4.0.)[31-33]

In vivo experimental study

The rats used in this study included 36 male Wistar albino rats and 54 female Wistar albino rats. Rats were assigned randomly into 6 groups (n=6): control group (vehicle: 0.5 ml of 2% DMSO in distilled water), $CdCl_2$ prescribed group (2 mg/kg body weight in 2% DMSO), $CdCl_2$+sildenafil citrate (5 mg/kg), $CdCl_2$+Indole-3-carbinol prescribed group (100 mg/kg body weight in 2% DMSO), $CdCl_2$+Resveratrol prescribed group (20 mg/kg body weight in 2% DMSO), $CdCl_2$+Indole-3-carbinol+Resveratrol treated group (similar dosage as in $CdCl_2$, I3C and RES treated groups, respectively. The doses for sildenafil citrate,[34] Indole-3-carbinol, resveratrol[35] and $CdCl_2$ were selected from previous studies, respectively. At this level, cadmium chloride was demonstrated to significantly increase oxidative[36] stress, as was seen in our prior study on female Wistar rats.[37] For $CdCl_2$, I3C and RES, our chosen solvent (2% DMSO in distilled water) was well tolerated. Cadmium chloride, Indole-3-carbinol and resveratrol were given one time a day for three weeks (between 8 a.m. and 9 p.m.). After three weeks of treatment, rats in the control, $CdCl_2$, $CdCl_2$+sildenafil citrate, $CdCl_2$+I3C and $CdCl_2$+RES, $CdCl_2$+I3C+RES, groups were euthanized. After the rats were sentenced to death, male sexual and reproductive function parameters were measured.

Mating behaviour study

According to protocol, experiments on mating behaviour were conducted in a separate space with low red lighting. For the study, healthy male albino rats with active sex lives and female rats with predictable oestrus cycles (vaginal smear collection) were chosen. For 10 min prior to introducing a primed female, the male rats were positioned in a rectangular transparent glass chamber to acclimate to the room's surroundings. After introducing the primed female into the chamber with a female to male ratio of 2:1. Female wistar rats were used to evaluate the sexual behaviours of male rats, during the first and third weeks following the start of the treatment. The following parameters of mating behaviour were noted:

(a) Mount Frequency (MF) is the number of mounts that happen without an intromission from the time the female gets introduced until ejaculation;
(b) Intromission Frequency (IF) is the number of intromissions that occur from the time the female is introduced until ejaculation;
(c) Mount Latency (ML) is the amount of time between the male's initial mount and the female's entrance;
(d) Intromission Latency (IL): The time between the female's introduction and the male's first introduction (characterized by pelvic thrusting and leaping dismount);
(e) Ejaculation Latency (EL) refers to the time between the first intromission and ejaculation.

It is characterized by longer, deeper pelvic thrusting and slow dismount followed by inactivity. At the first and third weeks of medication administration, the values of the observed parameters were measured and compared to the control.[38,39]

Estimation of cadmium concentration in serum, testis and epididymis

The technique of Atomic Absorption Spectrophotometry (AAS), as previously reported, was used to determine the quantity of Cd in the blood, epididymis and testis at the end of the three-week treatment period.[40]

Semen analysis

Each rat's left vas deferens and epididymis were removed for semen analysis. Using the techniques outlined in a prior study, viability, epididymal sperm pH, motility, count and morphology were evaluated. Samples from the vas deferens were utilised to assess sperm motility and the cauda epididymis was used to assess sperm count, viability and morphology.[41,42]

Evaluation of serum reproductive hormone levels

Blood was drawn from each animal's retroorbital venous plexus after the procedure was completed. Blood samples have been processed at 2500 rpm in a tabletop centrifuge for duration of 10 min. The concentrations of testosterone, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) have been determined using various serum samples. A radioimmunoassay kit from the Board of Radiation and Isotope Technology in Mumbai, India, was implemented to measure serum FSH, a microplate Chemiluminescence Immunoassay (CLIA) kit was used to assess FSH concentration and a double antibody ELISA kit from the Eiagen Testosterone kit in Italy was used to quantify testosterone.[43]

Histological studies

The testis underwent a histological investigation using the Haematoxylin and Eosin (H&E) method. Briefly, the animals' right testicle has been extracted, fixed in Bouin's solution, dried and then placed within paraffin blocks. Blocks of sectioned tissue were stained with H&E and examined under a light microscope. Sections of the testicular tissue were examined.[44,45]

RESULTS

In silico docking study

Aromatase inhibitors are commonly used in the treatment of hormonal receptor-positive breast carcinoma. By blocking the enzyme aromatase, which converts androgens into estrogens. However, there is emerging interest in exploring the potential use of aromatase inhibitors as aphrodisiacs due to their ability to modulate estrogen levels, which can impact sexual function. I3C and resveratrol are natural compounds that have been suggested to possess aphrodisiac properties. In this research, we used docking simulations to look into the potential interaction between a protein aromatase inhibitor and a ligand such as sildenafil citrate, I3C and resveratrol (Table 1). Docking simulations were carried out using molecular docking software, which predicts the binding orientation and affinity between a ligand. I3C and resveratrol are two natural compounds that adhere to the Lipinski rule without violations. The Lipinski rule is a set of guidelines used in drug discovery to assess the drug-likeness and oral bioavailability of potential compounds. The docking results demonstrated that the aromatase inhibitor formed hydrogen bonds and hydrophobic interactions with I3C. These interactions were primarily mediated by specific amino acid residues in the active site of the aromatase enzyme. Similarly, docking simulations between the aromatase inhibitor and resveratrol revealed favorable binding interactions. Resveratrol exhibited a different binding mode compared to I3C, with distinct hydrogen bonding and hydrophobic interactions. These interactions indicate the potential for modulating aromatase activity, supporting the hypothesis that resveratrol may possess aphrodisiac properties through estrogen modulation. Significantly, sildenafil citrate created hydrogen bonds with known important amino acid residues for aromatase's catalytic mechanism. Additionally, hydrophobic interactions were observed between sildenafil citrate and hydrophobic residues lining the binding site of aromatase. These interactions contribute to the stabilization of the ligand within the active site. The docking scores indicated a favorable binding affinity between sildenafil citrate and aromatase, suggesting a potential inhibitory effect on the enzyme's activity Figure 1A.

Table 1: Docking scores and Inhibitory Constants for Interacting Residues Calculated by Autodock (4.0.)

Ligand	Hydrogen bond	Number of rotational bonds	Docking score (Kcal/mol)	Inhibitory constant (*Ki*) (Nm)
Indole-3-carbinol **CID: 3712**	2	1	-4.7	359.15
Resveratrol **CID: 445154**	4	2	-6.25	26.18
Sildenafil citrate **CID: 135413523**	0	7	-10.05	43.31

Effect of cadmium chloride, Indole-3-carbinol, resveratrol on mating behaviour of rats

According to a study on mating behaviour, continuous treatment of I3C and resveratrol for three weeks resulted in substantial ($^{*}p<0.05$), significant ($^{***}p<0.001$) reductions in mount and intromission latencies when compared

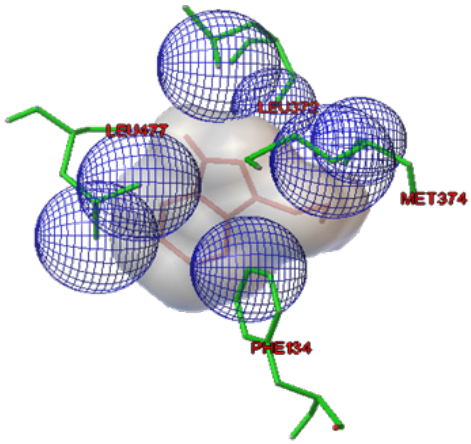
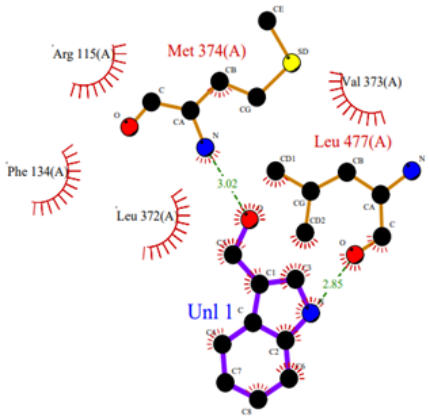
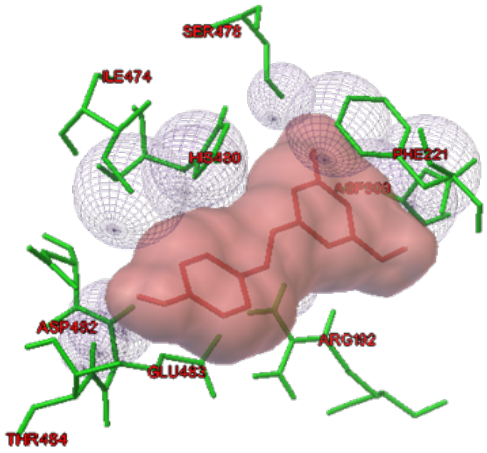
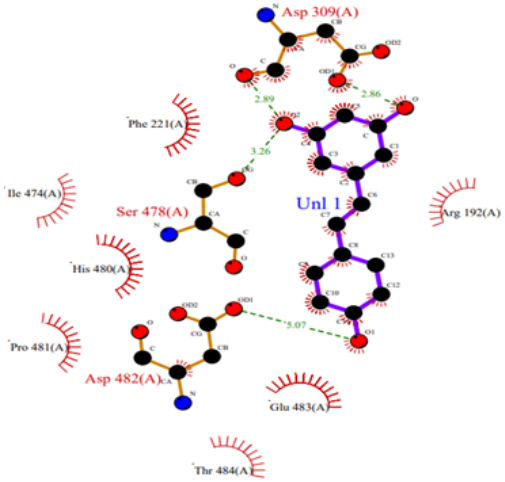
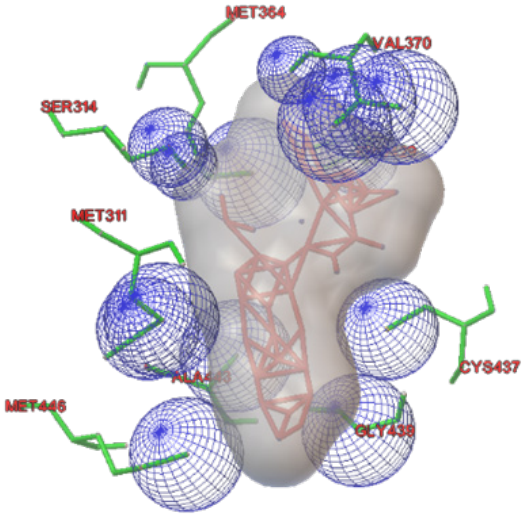
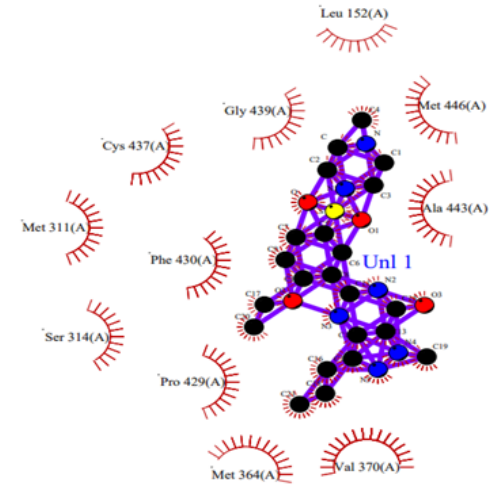
3D image of interacting proteins	2D image of interacting proteins
	
Protein-ligand interaction of 3EQM and Indole-3-carbinol.	
	
Protein-ligand interaction of 3EQM and Resveratrol.	
	
Protein-ligand interaction of 3EQM and Sildenafil citrate.	

Figure 1A: 3D and 2D image (Ligplot) of docked compound with 3EQM aromatase protein.

Table 2: Effects of mating behaviour on rats on 1 and 3 week of drug treatment.

Parameters	Control		Negative control ($CdCl_2$)		Standard		$CdCl_2$+I3C		$CdCl_2$+RES		$CdCl_2$+I3C+RES	
	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks
ML	10.26± 1.26	10.63± 1.14	15.61± 1.02*	25.12± 2.21*	2.03± 2.04***	1.91± 1.14***	4.12± 1.54*	3.01± 1.04*	4.54± 1.45*	4.03± 2.12*	3.45± 1.41**	3.12± 2.12**
IL	9.93± 1.45	10.97± 2.21	11.09± 2.14*	14.43± 0.54*	2.78± 1.45***	1.46± 2.12***	5.01± 1.65*	4.11± 2.01*	5.12± 2.01*	5.03± 1.04*	3.52± 0.58**	3.05± 1.45**
EL	226± 1.04	229± 1.50	259± 2.01*	298± 0.84*	143± 2.21***	129± 0.54***	183± 0.58*	172± 2.12*	178± 1.21*	169± 2.01*	155.21± 1.28**	140.15± 2.12**
MF	70.48± 2.12	68.23± 0.98	42.58± 1.04*	35.83± 1.54*	197± 2.02***	207± 2.54***	75.19± 2.54*	81.43± 0.41*	70.19± 2.13*	79.23± 1.45*	220.14± 1.58**	211.62± 1.01**
IF	77.41± 0.84	79.31± 1.14	58.23± 5.01*	46.05± 1.04*	196± 4.10***	219± 1.45***	159.03± 0.98*	169.11± 2.11*	121.35± 0.81*	155.13± 2.01*	215.26± 0.48**	231.54± 1.04**

EJ for ejaculation delay, IL for intromission latency, ML stands for mount latency, MF for mount frequency and IF for intromission frequency. Values are mean SEM, n=6 and ***p<0.001, **p<0.01, *p<0.05 vs the negative control group *p<0.001.

to the negative control (*p<0.001). In comparison to the negative control, it also considerably shortens the ejaculatory latency (*p<0.05, ***p<0.001). Finally, compared to the negative control (*p<0.001), there was a significantly (*p<0.05), (***p<0.001) higher proportion of mounting frequency and intromission frequency. However, the number of times of mounting and intromission as well as other sexual reactions was greatly enhanced by the combination of Indole-3-carbinol (100 mg/kg) and resveratrol (20 mg/kg). According to Table 2, all of the dosages of I3C and resveratrol were followed by a dose-dependent effect on the mating behaviour of male rats at intervals of the first and third weeks. The indices of sexual behaviour increased overall and the findings were statistically significant.

Analyzing the levels of cadmium in the serum, epididymis and testis

Cd concentrations in the blood, testicles and epididymis increased in the ($CdCl_2$) negative group, while in comparison to the control. (Figure 1A) demonstrates that the standard deviation of the serum Cd content was considerably (***p<0.001) lower in sildenafil group and (*p<0.05) in $CdCl_2$+I3C, $CdCl_2$+RES and (**p<0.01) in $CdCl_2$+I3C +RES and compared to the negative control group (p<0.001) which showed higher concentration of serum Cd. The testicular levels of Cd were significantly decreased (***p<0.001) in the standard group and (*p<0.05) $CdCl_2$+I3C, $CdCl_2$+RES and (**p<0.01) in $CdCl_2$+I3C +RES, in comparison with the negative group (*p<0.001) (Figure 1B). Additionally, there was a notable reduction in Cd levels in the epididymis (***p<0.001) in the standard group and (*p<0.05) $CdCl_2$+I3C, $CdCl_2$+RES and (**P<0.01) in $CdCl_2$+I3C +RES, in contrast to the negative group (*p<0.001) (Figure 1C).

Semen analysis

The group's average semen pH was the same. While in comparison to the negative control, ESN and the percentage of viable sperm were considerably higher in Standard, $CdCl_2$+I3C, $CdCl_2$+RES and $CdCl_2$+I3C+RES, respectively (***p<0.001, *p<0.05 and **p<0.01) (Table 3). Sperm motility was dramatically reduced (*p<0.001) in the negative control group in contrast to the control, but significantly enhanced (*p<0.05) in the standard, $CdCl_2$+I3C and $CdCl_2$+RES when compared to the negative group (*p<0.01). In comparison with the negative control group, it was considerably greater (**p<0.01) in the $CdCl_2$+I3C+RES group (Table 3). In comparison to the control, the negative control considerably (*p<0.001) increased the proportion of spermatozoa with aberrant morphology. The percentage of spermatozoa with aberrant morphology considerably decreased (***p<0.001) in the standard group and (*p<0.05) in the $CdCl_2$+I3C and $CdCl_2$+RES groups. When compared to the negative control (**p<0.01), the proportion of spermatozoa with aberrant morphology dropped considerably (**p<0.01) in $CdCl_2$+I3C+RES (Table 3).

Effect of $CdCl_2$, Indole-3-carbinol, resveratrol on serum testosterone, FSH and LH in male wistar rats

Compared to the control, the negative group's (*p<0.001) serum FSH levels fell (Figure 2A). When compared to the negative control group, the serum FSH concentration increased considerably (***p<0.001) in the standard group, (**p<0.01) in the $CdCl_2$+I3C+RES group and (*p<0.05) in the $CdCl_2$+RES and $CdCl_2$+I3C group, respectively.

Contrary to the negative control (*p<0.001), the $CdCl_2$+I3C+RES (**p<0.01) and standard group had

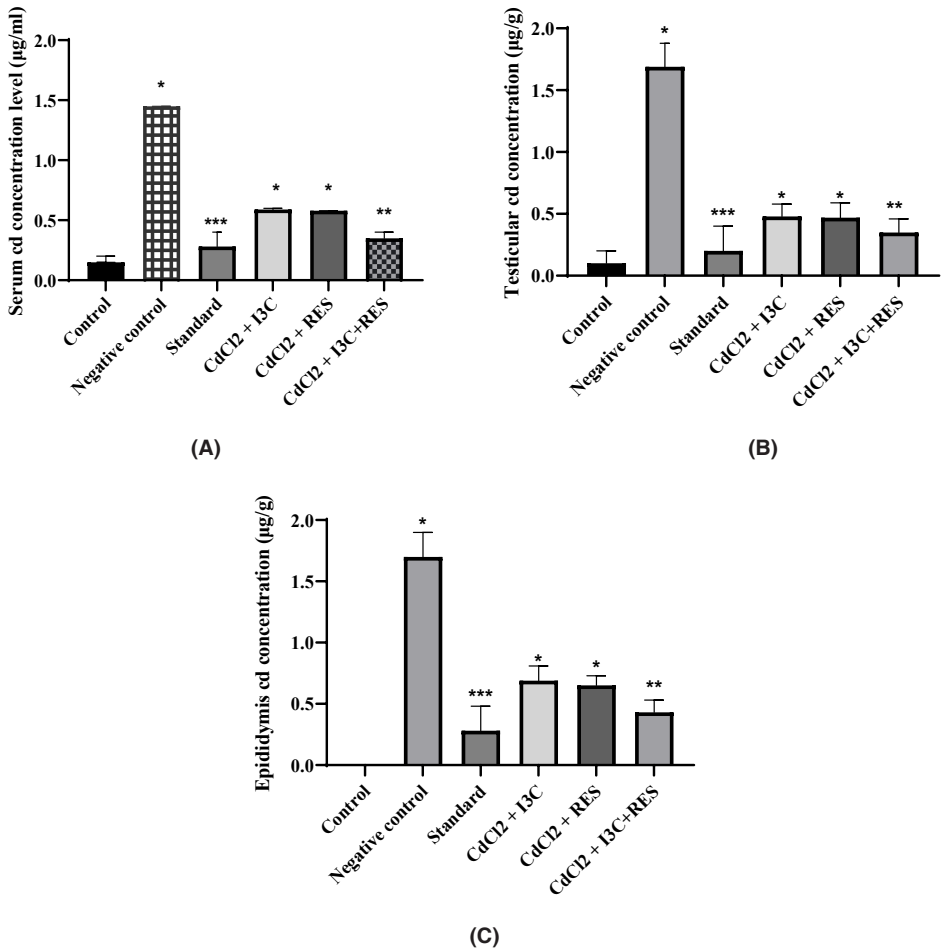


Figure 1 (A, B, C): Rats' serum, testis and epididymis Cd concentrations after 3 weeks of different treatments are shown in (A), (B) and (C), respectively. Values are mean±SEM, $n = 6$, ***p<0.001, **p<0.01, *p<0.05 vs negative control group *p<0.001.

Table 3: Effect of $CdCl_2$, Indole-3-carbinol, resveratrol on semen analysis.

Factors	Control	Negative control	Standard	$CdCl_2$+I3C	$CdCl_2$+RES	$CdCl_2$+I3C+RES
Semen pH	6.2±0.1	5.8±0.1	6.3±0.1	6.2±0.1	6.3±0.1	6.2±0.1
Epididymal sperm number (million/ml)	79.7±2.1	29.5±1.8*	83.5±0.1***	51.8±1.2*	30.4±1.6*	80.2±1.9**
Sperm viability (%)	78.2±1.8	33.8±1.1*	88.2±1.9***	55.5±1.5*	31.5±1.8*	80.9±1.8**
Sperm motility (%)	85.5±1.5	40.6±1.7*	87.2±1.7***	48.9±2.1*	39.8±2.1*	81.8±2.1**
Abnormal morphology (%)	7.0±0.4	35.0±1.8*	5.2±1.5***	8.1±1.2*	7.1±2.1*	6.5±1.6**

ESN stands for epididymal sperm number and values are mean SEM, n=6. ***p<0.001, **p<0.01 and *p<0.05. vs negative control group *p<0.001.

substantially higher serum LH concentrations (***p< 0.001) Figure 2B. Also, in comparison to the negative control, serum LH levels rose considerably (*p<0.05) in the $CdCl_2$+I3C and $CdCl_2$+RES groups.

When compared to the negative control group (*p<0.001), the standard (***p<0.001) and $CdCl_2$+I3C+RES groups had considerably higher serum testosterone concentrations (*p<0.05) the $CdCl_2$ group Figure 2C. When compared to the negative control, the blood testosterone levels in the $CdCl_2$+I3C and $CdCl_2$+RES groups increased significantly (*p<0.05) Figure 2C.

Testicular histopathology

The testis' histology was investigated Figure 3A-F. In comparison to the negative control (*p<0.001) which showed maturation arrest, there was a substantial (***p<0.001), (*p<0.05) improvement shown in the standard group, $CdCl_2$+I3C and $CdCl_2$ +RES groups. The space within the seminiferous tubule was reduced in the combination treatment with $CdCl_2$+I3C+ RES, which led to an increase in spermatogenesis and showed significant difference (**p<0.01) when compared to the negative control group.

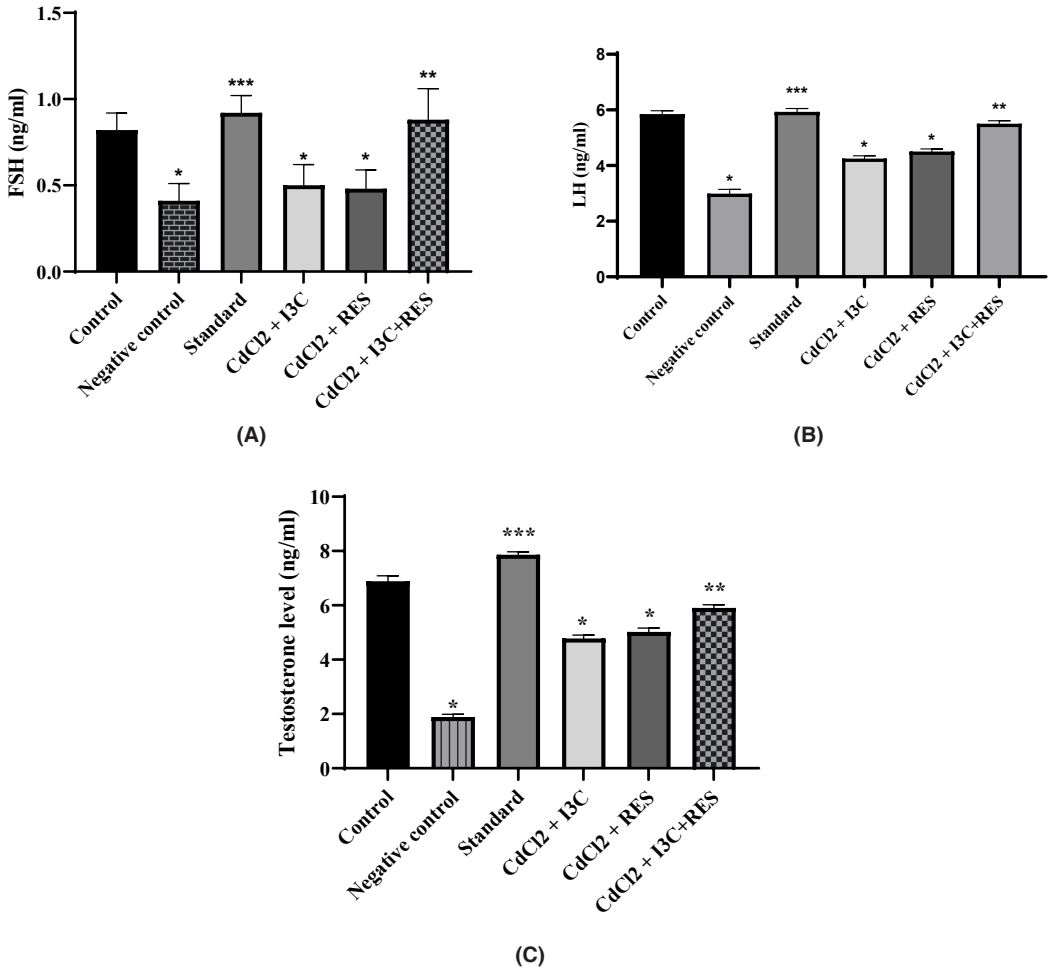


Figure 2 (A, B, C): All data were shown as mean SEM ($n = 6$); significant comparisons to the *$p<0.001$ negative control were determined using ***$p<0.001$, **$p<0.01$ and *$p<0.05$.

Photomicrographs of the Control group show healthy interstitial tissues and seminiferous tubules with spermatocytes and spermatogonia at 10x magnification. The Cadmium-induced group exhibits deteriorated interstitial tissue and impaired germ cell development at 10x magnification. The Standard-treated group displays normal interstitial tissues, seminiferous tubules and healthy germ cells (spermatogonia, spermatocytes, spermatids and spermatozoa), Sertoli, Leydig cells and flagella at 10x magnification. The I3C-treated group shows healthy germ cells, including spermatogonia, spermatocytes, spermatids, spermatozoa, Sertoli and Leydig cells at 10x magnification. The RES-treated group reveals increased germ cell presence, including spermatogonia, spermatocytes, spermatozoa, spermatids, Sertoli and Leydig cells at 10x magnification. The I3C and RES-treated group shows a significant increase in germ cells (spermatocytes, spermatogonia, spermatozoa, spermatids), along with Sertoli and Leydig cells, at magnifications of less than 10x.

SUMMARY

Cadmium is a common heavy metal that is harmful to the environment and is found in practically all parts of the environment.[46] It is employed extensively in a variety of applications. According to a growing number of studies, exposure to cadmium in animal's results in significant testicular injury and consequent infertility.[47] The main sources of cadmium exposure for people include food, water, tobacco smoke and industrial or agricultural goods.[48] As a result, several studies have employed various techniques in recent years to lessen the damaging effects of cadmium on the testicles. Some items with anti-inflammatory and antioxidant characteristics have been utilised to lessen the testicular toxic levels of Cd because oxidative stress and inflammation play a significant role in cadmium-mediated testicular damage.[49–54] The purpose of the present research is was to determine how I3C and resveratrol affected male wistar rats' cadmium-induced toxicity via

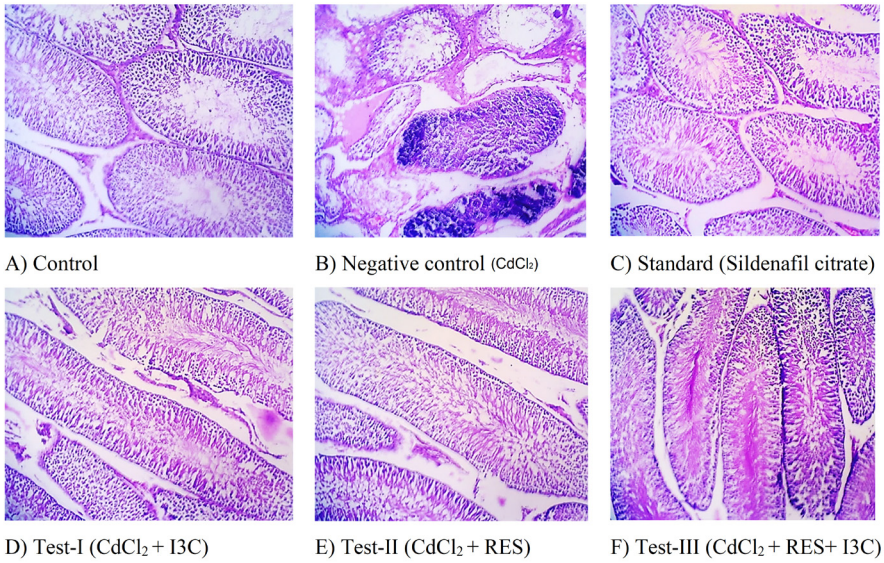


Figure 3 (A-F): Male wistar rat testicular histology. Hematoxylin and eosin staining was applied to sections.

suppressing the aromatase enzyme in both *in vivo* and *in silico*. The findings demonstrated that sperm motility, sperm viability, sperm count and serum testosterone were all considerably reduced in rats exposed to Cd. Additionally, a histological analysis of the testis anatomy revealed abnormalities in the seminiferous tubule structure and a reduction in the number of Leydig cells in the cd-treated groups. Numerous investigations have demonstrated that cadmium has harmful effects on hormone functioning, inflammatory response activation and oxidative stress.[55] The effects of $CdCl_2$-induced alterations in histology, hormonal levels, sperm characteristics and serum testosterone levels were also reduced by the administration of sildenafil, I3C and resveratrol. In accordance with those results, sildenafil, Indole-3-carbinol and resveratrol treatment avoided the severe changes in the testes caused by $CdCl_2$ poisoning. As a result of maintaining hormone levels and restoring sexual activity, normal histological structure and promoting testosterone production, therapy with Indole-3-carbinol and resveratrol can alleviate Cd-intoxication-induced testicular injuries in rats.

DISCUSSION

The current study demonstrates the therapeutic potential of Indole-3-carbinol and resveratrol as effective natural aromatase inhibitors to mitigate cadmium-induced male infertility. Cadmium, a toxic environmental pollutant, is well-documented for its detrimental effects on male reproductive health, primarily through oxidative stress and hormonal disruption. Previous research on aphrodisiacs like *Tribulus terrestris* and *Panax ginseng* has highlighted their ability to enhance sexual performance and sperm quality by modulating testosterone levels and combating oxidative stress.[56] Consistent with these findings, our study showed that I3C and RES improved sperm motility, viability and overall sexual behaviour in cadmium-exposed rats, suggesting similar protective mechanisms. The role of aromatase inhibitors in male infertility management is well established, with both synthetic (e.g., anastrozole) and natural inhibitors being explored for their efficacy.[57] Our *In silico* analysis confirmed strong binding affinities of I3C and RES to the aromatase enzyme, supporting their action as natural inhibitors. This aligns with prior computational studies that validated the potential of flavonoids and lignans as effective aromatase inhibitors.[58] The *In-vivo* results further confirms this, as treatment with I3C and RES not only restored hormonal balance but also improved testicular histology, indicating enhanced spermatogenesis and increased Sertoli and Leydig cell counts, akin to the effects reported for *Eurycoma longifolia* and *Ashwagandha* in similar experimental methodology.[59]

Histological examination provided additional evidence of the protective effects of I3C and RES, demonstrating improved testicular architecture and increased spermatogenic activity compared to the cadmium-only group. This suggests that these compounds can mitigate cadmium-induced testicular damage and support normal reproductive function, paralleling previous findings on resveratrol's antioxidant capabilities in protecting against testicular oxidative damage.[60] The synergistic effect observed with the combination of I3C and RES further underscores the potential of using natural aromatase inhibitors in addressing male infertility associated with environmental toxin exposure. Given the promising

outcomes, further research is warranted to explore the applicability of these compounds in human infertile populations and establish standardized dosing regimens for therapeutic use.

CONCLUSION

The docking simulations, in conclusion, showed possible interactions between the aromatase inhibitor and the ligands I3C, resveratrol and sildenafil citrate, suggesting that these compounds have the capacity to control aromatase activity. I3C and resveratrol's aphrodisiac properties were demonstrated by the mating behaviour study's increased sexual behaviour parameters. The study also emphasised the possible defence mechanisms of I3C and resveratrol against Cd-induced toxicity as well as the effects of these compounds on hormone levels and testicular histology. These results provide credence to the idea that aromatase inhibitors, such as I3C and resveratrol, may be investigated as aphrodisiac drugs, albeit further study is required to confirm these effects in actual clinical settings.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ABBREVIATIONS

CID: Compound Identifier; **Cd:** Cadmium; **AAS:** Atomic Absorption Spectrophotometry; **$CdCl_2$:** Cadmium Chloride; **DMSO:** Dimethyl Sulfoxide; **DNA:** Deoxyribonucleic Acid; **ESN:** Epididymal sperm number; **FSH:** Follicle-Stimulating Hormone; **H and E:** Hematoxylin and Eosin; **LH:** Luteinizing Hormone; **I3C:** Indole-3-carbinol; **RES:** Resveratrol; **SEM:** Standard Error of Mean; **SPZ:** Spermatozoa; **ST:** Seminiferous Tubules; **IT:** Interstitial tissues; **ST** seminiferous tubules; **SP:** Spermatogonia; **SPI:** Spermatocytes; **SPt:** Spermatids; **SPZ:** spermatozoa; **STc:** Sertoli; **LeyC:** Leydig cells; **Flg:** Flagellum; sperm cells; **DM:** Disrupted maturation; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institute Animal Ethics Committee; **MF:** Mount Frequency; **IF:** Intromission Frequency; **ML:** Mount Latency; **IL:** Intromission Latency; **EL:** Ejaculation Latency; **PDB:** Protein data bank.

SUMMARY

The study investigated the effects of Indole-3-carbinoland resveratrol on male Wistar rats exposed to cadmium-induced infertility. The findings highlighted their potential as aromatase inhibitors, capable of reducing cadmium toxicity, improving sperm quality and restoring hormonal balance. The *In vivo* and *In silico* results demonstrated improved sexual performance and protective effects against testicular damage. These natural compounds offer promising therapeutic avenues for treating male infertility caused by environmental toxins, though further research is needed to validate their efficacy in human subjects.

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