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The effect of the acetamiprid pesticide on rats: The role of curcumin to reduce these effects

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Abstract

Objective: The purpose of this study was to determine whether curcumin could protect male Wistar rats against acetamiprid-induced testicular damage, endocrine disruption, and oxidative stress. **Methods**: For three weeks, male Albino Wistar rats were divided into four experimental groups at random (n = 10 per group) and given the following oral treatment once a day: Group II (Curcumin) received 100 mg/kg BW of curcumin dissolved in maize oil; Group III (Acetamiprid) received 156 mg/kg BW of acetamiprid; and Group IV (Curcumin + Acetamiprid) received 100 mg/kg BW of curcumin followed by 156 mg/kg BW of acetamiprid. Biochemical indicators of oxidative stress, antioxidant enzyme activities, serum reproductive hormones, and epididymal sperm characteristics were all thoroughly assessed at the conclusion of the trial. **Results**: Acetamiprid exposure induced severe testicular oxidative damage and reproductive dysfunction, marked by elevated TBARS and H_2O_2 levels, depleted antioxidant enzymes (SOD, CAT, GPx, GR, GST), and hypothalamic-pituitary-testicular axis disruption that lowered testosterone while raising LH and FSH. Co-administration of curcumin significantly mitigated these toxic effects by scavenging free radicals, restoring endogenous antioxidant defenses, and normalizing reproductive hormone levels and sperm quality. **Conclusion**: Curcumin mitigates acetamiprid-induced testicular toxicity by leveraging its antioxidant capacity to scavenge free radicals, maintain membrane fluidity, and preserve cellular homeostasis.

Key words: Acetamiprid; Rats; Curcumin; Testicular toxicity.

Introduction

Acetamiprid, a selective agonist of nicotinic acetylcholine receptors, is among the most widely used neonicotinoids. Regarding acetamiprid's toxicity on the male reproductive system, not much is known (1). The liver, kidney, adrenal glands, and thyroid glands had the highest quantities of acetamiprid after oral administration. Some researchers showed that acetamiprid has detrimental effects on the immune, neurological, and respiratory systems in animal models (2). Furthermore, there have been reports of acute toxicity in humans after taking acetamiprid (3). Additionally, acetamiprid has been shown to impair a variety of species' reproductive systems (4). Male rats were used to demonstrate the reproductive toxicity of acetamiprid, which had a detrimental effect on testosterone levels, sex organs, and semen quality (5).

The hunt for naturally occurring bioactive substances derived from plants to combat systemic and organ-specific toxicities caused by pesticides

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has accelerated in recent years. Curcumin, a natural polyphenolic compound extracted from the rhizomes of Curcuma longa, has emerged as a highly promising therapeutic agent due to its exceptional pharmacological profile. Its strong intrinsic antioxidant, anti-inflammatory, and free radical scavenging properties across a range of physiological and chemical stress situations are well known (6).

Prior studies have demonstrated that curcumin administration effectively reduces testicular damage caused by pesticides and environmental toxins, mainly by inhibiting lipid peroxidation cascades and maintaining cell membrane fluidity (7). Furthermore, at a molecular level, Curcumin plays a critical role in preserving the structural and functional integrity of Leydig and Sertoli cells under chemical insults, thereby preventing apoptotic cell death in the spermatogenic epithelium (8).

Materials and methods

Curcumin (purity ≥ 95%) was purchased from Sigma-Aldrich (UK). It was eliminated in the vehicle (Corn oil) daily before oral administration. Sigma-Aldrich (UK) provided the Acetamiprid, an organic compound with the chemical formula $C_{10}H_{11}ClN_4$. amine with a purity of 99.6%.

Animals and experimental design

At the Faculty of Medicine at Alexandria University, male Albino Wistar rats weighing between 150 and 170g were used. The experimental methodology was authorized by the Regional Animal Welfare Committee, and animals were cared for in compliance with the guidelines for caring for lab animals outlined in the NIH guidance for the benefit of research animals.

The cages for the rats were made of wire with a stainless steel bottom, with a 12-hour light/dark cycle, a pellet diet, and unlimited access to water. Rats were randomly divided into four groups of ten after two weeks of acclimation. The following experimental methodology was followed for administering the test chemicals to the animals:

Group I (Control): Rats received daily oral administration of the vehicle (Corn oil) via oral gavage.

Group II (Curcumin): Rats received daily oral administration of Curcumin (100 mg/kg BW) dissolved in corn oil.

Group III (Acetamiprid): Rats received Acetamiprid orally (1/10 LD50; 156 mg/kg BW; dissolved in distilled water).

Group IV (Curcumin + Acetamiprid): Rats received Curcumin (100 mg/kg BW) followed by Acetamiprid (156 mg/kg BW) orally once a day for three weeks.

Based on reputable scholarly research, the chosen dosage of curcumin (100 mg/kg BW) and the use of maize oil as a hydrophobic vehicle were decided. This particular dosage of curcumin has been shown in earlier studies to successfully counteract acetamiprid-induced systemic oxidative stress and pathological changes in animals (9). Furthermore, Curcumin at 100 mg/kg has been proven to significantly alleviate chemical- and heavy metal-induced testicular toxicity, thereby restoring sperm count, motility, and normal morphology (10). This protective mechanism is strongly correlated with Curcumin's capacity to mitigate acute chemical insults to the male reproductive system (11) and suppress pesticide-induced nephro- and testicular damage through gastric gavage (12). Dynamically, Curcumin exerts its multi-pathway cytoprotective effects by upregulating endogenous antioxidant enzymes and suppressing inflammation in the male reproductive system under chemical stress (13).

Serum and Blood Sampling

Each rat's aorta was separately sampled for blood using non-heparinized glass tubes. Centrifugation was used to separate the serum for 15 minutes at 3000 rpm. Until analysis, the collected serum was

kept frozen at -18 °C for subsequent hormonal evaluations.

Tissue Specimens Preparation

Rats were sacrificed, and the testes were promptly removed, weighed, and cleaned with a cooled saline solution (0.9%). The testicular tissues were chopped and homogenized (10% w/v) in a Potter-Elvehjem type homogenizer using ice-cold sodium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl. The homogenates underwent centrifugation at 10,000×g for 20 minutes at 4°C. A variety of antioxidant enzyme activity, free radical concentrations, and other biochemical parameters were examined using the carefully collected supernatants.

Estimation of Testicular Hydrogen Peroxide (H_2O_2) Concentration

Following standard techniques, sample tissue (100 mg) was extracted with 5 ml of trichloroacetic acid (TCA; 0.1%, w/v) in an ice bath and centrifuged at 12,000×g for 15 minutes to estimate the concentration of hydrogen peroxide (H2O2) **(14).**

Assessment of Oxidative Stress Markers and Antioxidant Enzymes

The amount of thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation, was calculated using a standard curve created by repeated concentrations of tetramethoxypropane (TMP) (15).

Using commercially available colorimetric kits (Biodiagnostic, Egypt), the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT) in the tissue homogenate supernatants were measured spectrophotometrically in strict compliance with the manufacturer's operating instructions and specific standards. Following protein precipitation with metaphosphoric acid, reduced glutathione (GSH) was quantified by measuring the rate of DTNB oxidation to GSSG and TNB. The change in absorbance at 412 nm, reflecting TNB generation, directly correlated with sample GSH concentration. A GSH standard curve was utilized to determine the total glutathione content, and the final outcomes were represented as nmol/g tissue by dividing the sample's glutathione concentration by the weight of the tissue used. **(16).**

Determination of Reproductive Hormones

Hormonal profiles in rat serum were evaluated using Enzyme-Linked Immunosorbent Assay (ELISA) kits for the quantitative determination of Testosterone, Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH) (DRG International Co., USA). The assays were conducted strictly according to the standard protocols provided by the manufacturer **(17)**.

Semen Characteristics and Sperm Quality Evaluation

To assess sperm count, sperm motility, and sperm morphology, an epididymis was produced. According to the technique of **(18),** using an Olympus microscope and the Computer Assisted Semen Analysis (CASA System; Germany), was employed. Each rat's 200 spermatozoa were individually evaluated and classified as normal or abnormal using the stringent sperm morphological standards outlined in **(19).** According to the methodology, acrosome integrity was evaluated **(20).**

Statistical analysis

Data were fed to the computer and analyzed using the IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) The Shapiro-Wilk test was used to verify the normality of the distribution. Quantitative data were described using mean and standard error. The significance of the obtained results was judged at the 5% level. In addition, the test used is the F-test (ANOVA) for normally distributed quantitative variables, to compare more than two groups, and the Post Hoc test (Tukey) for pairwise comparisons.

Results and Discussion

The effects of acetamiprid, curcumin, and their combination administration on lipid peroxidation (TBARS), hydrogen peroxide (H2O2) concentrations, and reduced glutathione (GSH) levels in rat testes are shown by the biochemical parameters in Table 1.

Both TBARS (30.46 ± 0.613 nmol/g tissue) and H2O2 (14.74 ± 0.377 \mu/mol/g tissue) concentrations were significantly (P<0.05) higher in the testicular tissues exposed to acetamiprid alone than in the control group (20.70 ± 0.420 nmol/g tissue and 7.70±0.227 µmol/g tissue, respectively). Acetamiprid-treated rats showed a significant (P<0.05) reduction in the endogenous non-enzymatic antioxidant GSH (1.58 ± 0.049 mmol/mg protein) compared to control rats (2.79 ± 0.096 mmol/mg protein).

Surprisingly, co-administration of acetamiprid and curcumin considerably (P<0.05) reduced this oxidative damage. TBARS levels were considerably reduced to 22.23±0.712 nmol/g tissue in the combination group (curcumin + acetamiprid), and H2O2 concentrations were returned to levels close to control (8.06±0.258 µmol/g tissue). Additionally, compared to the group treated with acetamiprid alone, curcumin administration dramatically increased the concentration of GSH to 2.20 ± 0.059 mmol/mg protein, effectively reversing its depletion. Conversely, rats given curcumin alone showed a physiological improvement, with a notable increase in testicular GSH content (3.41 ± 0.079 mmol/mg protein) and a significant decrease in basal TBARS levels (17.47 ± 0.458 nmol/g tissue), confirming curcumin's strong intrinsic antioxidant and cytoprotective qualities under normal circumstances.

Acetamiprid's reproductive toxicity is primarily caused by the production of testicular oxidative stress. High levels of hydrogen peroxide and lipid peroxidation indicate an excessive production of reactive oxygen species (ROS) that exceeds the testicular tissue's normal buffering capability. Because acetamiprid is lipophilic, it easily passes across biological membranes and is metabolically activated, creating toxic intermediates that target macromolecules in cells. Due to its high concentration of polyunsaturated fatty acids, which act as direct substrates for the spread of free radicals, the mammalian testis is particularly susceptible to these oxidative assaults. Concurrently, a significant depletion of the cells' main non-enzymatic defense lines is reflected in the marked exhaustion of reduced glutathione (GSH).

Because GSH is a necessary co-substrate for peroxide-detoxifying enzymes and is dynamically utilized during the direct scavenging of free radicals, its depletion jeopardizes cellular survival and leaves the spermatogenic epithelium vulnerable. On the other hand, curcumin's strong cytoprotective and antigenotoxic effectiveness is demonstrated by its exceptional reduction of these oxidative markers. Curcumin is a strong chain-breaking antioxidant that efficiently donates electrons to neutralize free radicals and halt the lipid peroxidation cascade before it begins due to its unique chemical structure, which combines phenolic hydroxyl groups and a conjugated beta-diketone system. By promoting the production of endogenous glutathione, curcumin enhances the cellular adaptive response beyond direct scavenging and replenishes the non-enzymatic antioxidant pool. This dual approach maintains the membrane fluidity and metabolic equilibrium of the reproductive system by shielding the testicular architecture from pesticide-induced biochemistry. A harmful situation known as oxidative stress causes cell damage and eventual cell death as a result of the oxidation of essential cellular components like lipids, proteins, and DNA (21). Peroxidation of membrane lipids is one of the most harmful effects on cells since it can seriously compromise the structural and functional integrity of biological membranes, allowing potassium ions and other solutes to leak out and potentially killing cells (22).

Table (1): Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular TBARS, H_2O_2, and GSH levels in rats

Parameters	Groups			
	Control	Curcumin	Acetamiprid	Curcumin + Acetamiprid
TBARS (nmol/g tissue)	20.70±0.420^{c}	17.47±0.458^{d}	30.46±0.613^{a}	22.23±0.712^{b}
H_2O_2 (µmol/g tissue)	7.70±0.227^{c}	8.25±0.209^{d}	14.74±0.377^{a}	8.06±0.258^{b}
GSH (mmol/mg protein)	2.79±0.096^{b}	3.41±0.079^{a}	1.58±0.049^{d}	2.20±0.059^{c}

Each treatment group's n = 10 values are reported as means SE. ^{abcd} mean values in a row without a common superscript letter differed considerably (P<0.05).

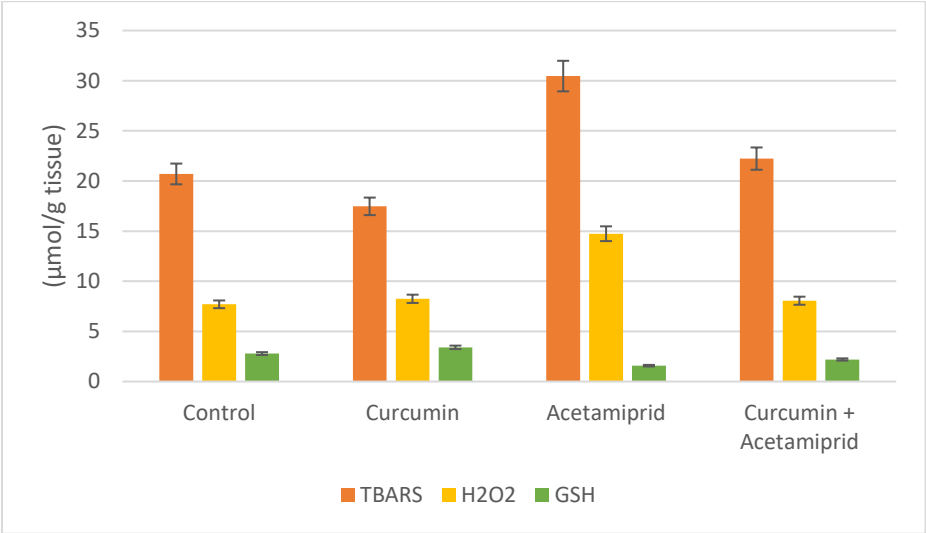

Figure (1): Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular TBARS, H_2O_2, and GSH levels in rats

The biochemical analysis in **Table 2** reveals that exposure to Acetamiprid leads to a significant reduction and down-regulation in the activities of all measured testicular antioxidant enzymes, including Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GP_x), Glutathione Reductase (GR), and Glutathione S-Transferase (GST).

On the other hand, co-administration of curcumin and acetamiprid has a noteworthy protective effect, leading to a remarkable restoration and up-regulation of these enzymatic defenses, returning their activities to normal. Additionally, baseline antioxidant enzyme activity is clearly elevated in rats given curcumin alone.

Under acetamiprid stress, antioxidant enzyme activity is significantly suppressed, indicating that the overproduction of free radicals has exhausted the enzymatic defense system. Inhibiting these enzymes biochemically causes hydrogen peroxide (H2O2) to build up, which promotes lipid peroxidation and modifies DNA, changing gene expression and resulting in cell death (23).

Superoxide anion (O2-) in aerobic organisms is converted by (SOD) to oxygen (O2) and hydrogen peroxide (H2O2), which is then reduced to water (H2O) by the H2O2-scavenging enzyme catalase (24,25). Curcumin is a powerful inducer of endogenous antioxidant systems, as evidenced by its ability to restore these enzyme activities. Curcumin successfully stops the oxidative cascade by sustaining the dynamic cycle of scavenging free radicals, protecting the testicular tissue's structural homeostasis against pesticide toxicity.

Table (2): Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular of activities of antioxidant enzymes in rat testes

Parameters	Groups			
	Control	Curcumin	Acetamiprid	Curcumin + Acetamiprid
SOD (U/mg protein)	69.27±1.91^{b}	85.01±2.12^{a}	36.67±1.23^{d}	58.19±1.68^{c}
CAT (U/mg protein)	8.11±0.291^{b}	9.60±0.267^{a}	5.68±0.143^{d}	5.52±0.166^{c}
GPx (U/mg protein)	7.96±0.265^{b}	9.36±0.311^{a}	3.34±0.078^{d}	7.14±0.147^{c}
GR (U/mg protein)	23.66±0.869^{b}	29.25±0.782^{a}	16.58±0.540^{d}	20.31±0.680^{c}
GST (µmol/hr/mg protein)	0.602±0.021^{b}	0.704±0.019^{a}	0.360±0.013^{d}	0.483±0.016^{c}

Each treatment group's n = 10 values are reported as means SE. ^{abcd} mean values in a row without a common superscript letter differed considerably (P<0.05).

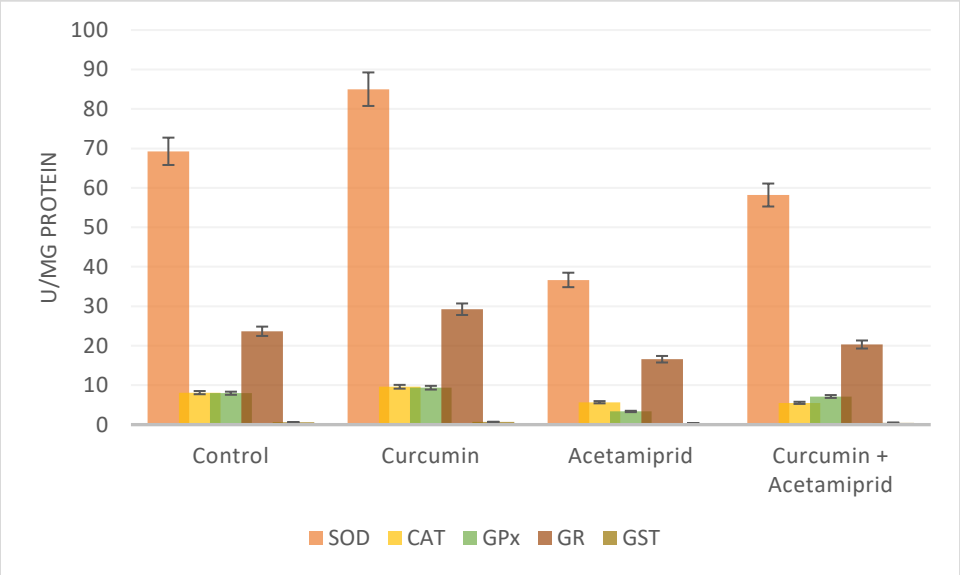

Figure (2) Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular activities of antioxidant enzymes in rat testes

According to Table 3 hormonal analysis, acetamiprid exposure significantly lowers testosterone concentrations. On the other hand, rat serum levels of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are significantly higher than those of the control group.

However, when curcumin and acetamiprid are administered together, there is a noticeable restorative effect that successfully returns these changed hormonal levels to baseline levels. These measured hormonal indicators do not significantly change in rats treated with curcumin alone.

Acetamiprid disrupts the hypothalamic-pituitary-testicular axis, as evidenced by the significant decrease in blood testosterone and the corresponding increase in LH and FSH. A structural or functional impairment of Leydig cells, the main locations of androgen synthesis in the testes, is confirmed by the drop in testosterone. When Leydig cell activity is compromised, the pituitary gland attempts to promote testicular output by increasing the secretion of LH and FSH under normal feedback processes.

Additionally, the pesticide's excessive creation of reactive oxygen species (ROS) damages Leydig cell

membranes and compromises the steroidogenic acute regulatory (StAR) protein, which is essential for the synthesis of testosterone.

Curcumin's ability to restore these hormone patterns highlights its potent protective function. Curcumin protects Leydig cells' structural integrity by scavenging free radicals and reducing oxidative stress in the testes' interstitial tissue. This preserves appropriate hormonal feedback control within the reproductive system and directly restores their steroidogenic potential.

Table (3): Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular hormone levels in rat serum

Parameters	Groups			
	Control	Curcumin	Acetamiprid	Curcumin + Acetamiprid
Testosterone(ng/ml)	19.11±0.539^{a}	18.61±0.808^{a}	5.60±0.242^{c}	12.11±0.654^{b}
FSH (ng/ml)	3.26±0.153^{c}	3.60±0.200^{c}	13.30±0.515^{a}	6.91±0.477^{b}
LH (ng/ml)	4.34±0.200^{c}	4.41±0.255^{c}	13.25±0.508^{a}	8.76±0.522^{b}

Each treatment group's n = 10 values are reported as means SE. ^{abcd} mean values in a row without a common superscript letter differed considerably (P<0.05).

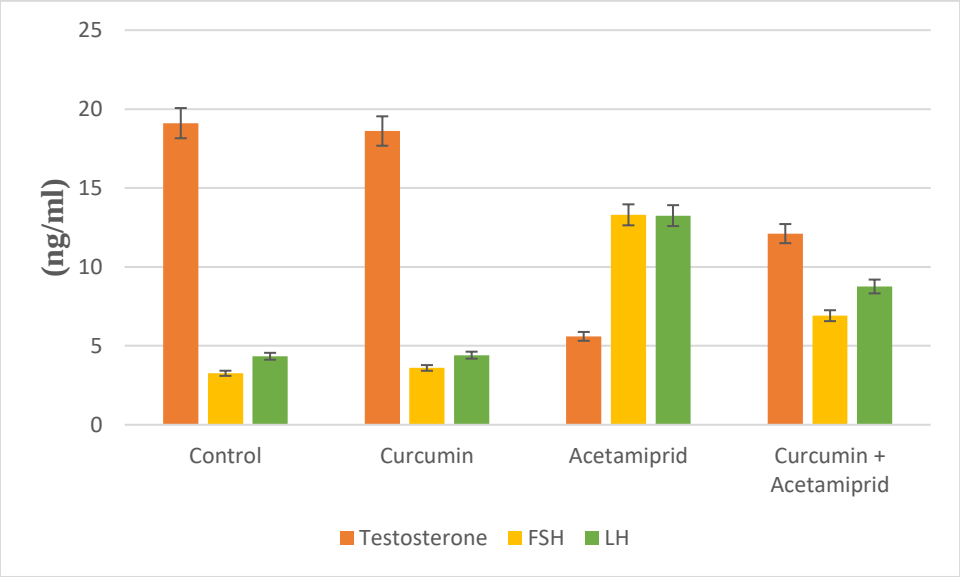

Figure (3): Ameliorative effect of Curcumin, Acetamiprid, and their combination on testicular hormones level in rat serum

Acetamiprid exposure significantly lowers both sperm count and motility, as shown by the spermatogenic evaluation in Table 4. On the other hand, when comparing the pesticide-treated rats to the control group, the percentage of aberrant sperm exhibits a significant rise and upregulation.

Conversely, co-administration of curcumin with acetamiprid shows a remarkable ameliorative effect, effectively reversing these parameters by considerably reducing sperm abnormalities toward normal levels and improving sperm count and motility. Rats given curcumin alone exhibit physiological improvements in the baseline parameters of their sperm.

Acetamiprid directly interferes with the process of spermatogenesis within the seminiferous tubules, as evidenced by the sharp decrease in sperm motility and count, as well as the increase in aberrant sperm morphology. Reactive oxygen species (ROS) overproduction, testosterone decline, and oxidative stress induction are all strongly associated with this decline. Spermatozoa are at risk from excessive ROS because of their high polyunsaturated fatty acid content. Acetamiprid reduces sperm motility, quantity, and viability while increasing the rate of acrosome deformity.

Antioxidant treatment may mitigate this impact. Curcumin's effectiveness as a potent antioxidant and cytoprotective agent is shown by its ability to preserve semen quality. Curcumin maintains sperm membrane fluidity and viability by efficiently scavenging free radicals and shielding lipid membranes from peroxidation. Curcumin also guarantees the continuation of normal spermatogenesis and shields developing germ cells from morphological abnormalities caused by pesticides by preserving ideal intratesticular testosterone levels.

Table (4): Ameliorative effect of Curcumin, Acetamiprid, and their combination on sperm quality

Parameters	Groups			
	Control	Curcumin	Acetamiprid	Curcumin + Acetamiprid
Sperm count (*10^6 cells)	80.71±2.28^{b}	87.86±1.92^{a}	18.00±1.20^{d}	44.00±1.70^{c}
Sperm abnormality (%)	7.14±0.857^{c}	7.43±1.21^{c}	45.71±5.14^{a}	34.43±2.35^{b}
Sperm motility (%)	77.14±3.47^{a}	83.43±3.03^{a}	19.71±0.747^{c}	44.57±2.94^{b}

Each treatment group's n = 10 values are reported as means SE. ^{abcd} mean values in a row without a common superscript letter differed considerably (P<0.05).

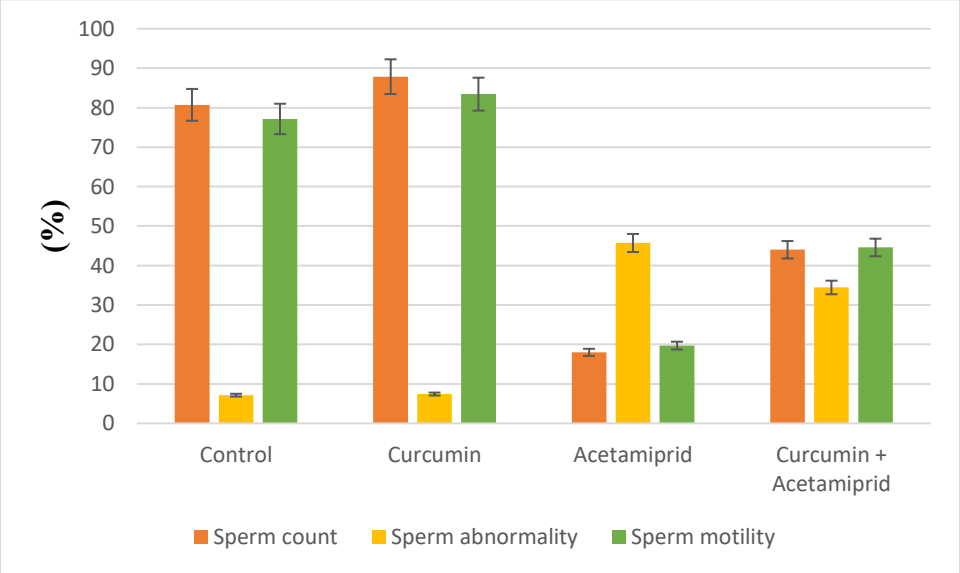

Figure (4): Ameliorative effect of Curcumin, Acetamiprid, and their combination on sperm quality

Conclusion

In summary, subchronic acetamiprid exposure in male Wistar rats induces severe testicular toxicity and reproductive failure. This impairment is primarily driven by oxidative stress, marked by extensive lipid peroxidation, GSH depletion, and the suppression of key antioxidant enzymes (SOD, CAT, GPx, GR, GST). These biochemical disruptions collapse the hypothalamic-pituitary-testicular axis, causing hormonal imbalances and severe semen quality deterioration (reduced count, motility, and abnormal morphology). Conversely, curcumin co-administration mitigates this toxicity. Driven by its potent antioxidant and radical-scavenging capacities, curcumin restores endogenous defenses, protects cellular membranes, and preserves Leydig cell and spermatogenic epithelial functions, thereby normalizing hormone profiles and sperm metrics. Consequently, curcumin represents a promising therapeutic agent against pesticide-induced male reproductive toxicity.

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Funding: **NIL**

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