DOSE-DEPENDENT TOXICITY OF ACETAMIPRID ON TESTES AND PROSTATE OF ADULT ALBINO RATS: ELUCIDATING TOXIC MECHANISMS

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ABSTRACT

Background: Acetamiprid (ACMP) is a neonicotinoid pesticide, broadly used as an alternate to organophosphates to control pests and its use is increasing day by day to increase crop yield. Traces of ACMP and its toxic metabolites have been detected in several foodstuffs, water and soil. **Aim of the work:** This experiment designed to elucidate the detrimental hazards of different doses of ACMP exposure on rat's testis, and prostate. **Material and Methods:** Twenty-eight adult male albino rats that were separated equally into 4 groups: control groups (negative and vehicle), ACMP groups (1/5 LD_{50} received 40 mg/kg/day; 1/20 LD_{50} received 10mg/kg/day). All treatments were given orally for 4 weeks. **Results:** ACMP-treatment (10 mg/kg/day) triggered a hormonal disturbance including elevated serum GnRH, and LH, and declined serum testosterone. Moreover, there were a significant deteriorated sperm characterization and triggered alteration of cellular redox homeostasis evidenced by increased malondialdehyde (MDA) levels with decreased total antioxidant capacity (TAC), also, elevation in pro-inflammatory (tumour necrosis factor alfa (TNF-α) allied with declining in anti-inflammatory (IL-10) in testicular and prostatic tissues. There were histopathological changes in the testicular and prostatic tissues. The previous abnormalities were more severe with a higher dose of ACMP (40 mg/kg/day) exposure. **Conclusion:** sub-acute ACMP exposure has detrimental effects on testis and prostate evident by functional and structural disturbance through triggering oxidative stress, and inflammatory process, which occurred in a dose-dependent manner. **Recommendation:** improve health awareness about ACMP proper use, and further studies to identify other mechanisms and how to improve their detrimental effects.

Keywords: *Acetamiprid, Prostate, Testis, TNF-α, Sperm.*

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INTRODUCTION

In the 1990s, a neonicotinoid group was produced, which considered as a new class of pesticides which act selectively as central agonist on insects' nicotinic acetylcholine receptor (nAChR) ***(Frederickson et al., 2016)***.

Neonicotinoids have fairly lengthy half-lives and low sorption in soil with high aquatic solubility, and. These features related to their persistence in the environment and a variety of metabolic and systemic diseases in the exposed workers ***(Bonmatin et al., 2021)***.

Acetamiprid (ACMP) is frequently used against a diversity of insects in agriculture and it is one of a neonicotinoid insecticide. In Egypt, ACMP widely used in controlling pests of major crops as to tomato plants, potatoes and rice. They tend to concentrate in plants and pollute water, this led to potential health hazards. ACMP half-life in soil is 31-450 days ***(Goulson, 2013 and Shalaby and Abdou, 2020)***.

Research was made on workers in agriculture including; farmers, insecticide sellers, and crop-dusting which were from the Dakahlia Governorate, Egypt, to blood screen for pesticide residues. The farmers, insecticide sellers, and spraying workers have a pesticide residue in their blood (48, 76, and 84% respectively). The blood of examined persons has eleven composites. 38.3% of the study cases have chlorpyrifos in their blood, 11.7% have ACMP and 10.7% have profenofos ***(Shalaby and Abdou, 2020)***.

Spermatogenesis involves four basic processes occurring in the testis: development of spermatogonium including stem cell and cell mitotic divisions, meiosis, spermiogenesis (development of spermatid including head and tail differentiation), and spermiation (mature sperm release into the seminiferous tubule lumen). The spermatozoa production and the testosterone secretion by the testis is both reliant on stimulation of the pituitary gonadotropins, (FSH and LH), that are released in response to hypothalamic GnRH. Initiation and maintenance of spermatogenesis depend on testosterone which is produced by the Leydig cell under LH stimulation ***(Mahmoud et al., 2019)***.

The formed sperms move into the epididymis which has an imperative role in providing the micro-environment for sperm storage. An important component of seminal plasma is prostatic fluid secreted by the rat prostate. This fluid is important for sperm motility and viability ***(Raymond et al. 2010)***.

Reproduction is one of the utmost sensitively to contaminants either environmental or occupational, principally pesticides. It has been stated that pesticides trigger free radicals' production that negatively affect reproductive system function; disruption of spermatogenesis; endocrinal disruption at any level of hormonal control ***(Mosbah et al., 2018)***.

Several researches concerning ACMP exposure have reported general mechanism of its toxicity could be generation of excess reactive oxygen free radicals in various tissues in rats (hepatotoxicity, neurotoxicity, nephrotoxicity, cytotoxicity and genotoxicity) ***(Rasgele et al., 2015; Annabi et al., 2019; Mehtap et al., 2020)***.

Exposure to ACMP is highly frequent as it is one of the most frequently used insecticides nowadays. Nevertheless, there is no gratified evidence on possible mechanism of the sub-acute ACMP toxicity (different doses) on male reproductive system including testis and prostate gland.

THE AIM OF THE WORK

This experiment designed to elucidate the detrimental hazards of different doses of ACMP exposure on rat's testis, and prostate.

MATERIAL AND METHODS

Acetamiprid: ACMP across Belgium 46393 was obtained from Sigma Aldrich Co. branch in Cairo, Egypt.

Distilled water (ACMP solvent), and sodium citrate solution (2.9-3%) and physiological saline solution (0.9%) (epididymal sperm analysis): they were gained from El- Nasr Co., Egypt.

Ethical Consideration:

This experiment was done in harmony with guidelines of the Institutional Animal Care and Use Committee, Zagazig University, Egypt *(Ethical approval number ZU-IACUC/3/F/90/2023).*

Methods:

According to ***Singh et al. (2012)*** study; considering LD_{50} value (198 mg/kg (approx.200 mg/kg) in mice), three different dose levels selected: $1/20^{th}$ (10 mg/kg), $1/10^{th}$ (20 mg/kg), $1/5^{th}$ (40 mg/kg) of the reported LD_{50} value were employed in their study, in the current study 1/20 of LD_{50} (lowest) and 1/5 of LD_{50} (highest) were selected.

According to mean±SD of normal sperm counts in control group (173.2±3.8) vs (178±3.5) in experimental group, so total sample size will be 28 adult male albino rats (each 150-200 gm) will be separated into 4 groups (n=7 in each group) power of test 80%, C.I 95%, using Epi program in community medicine department. The rats were gained from the Faculty of Medicine's animal house, Zagazig University. The rats were subjected to a week of passive prelamination for adaptation on the new environment before commencing experimentation to determine corporeal wellbeing and to eliminate a diseased one. Foodstuff was presented to all animals in equal quantities in each cage. Tap-water was accessible in isolated hygienic flasks.

All treatments were received orally for 4 weeks.

-Group I (negative control): no treatment was given (assessment of the basic parameters).

-Group II (vehicle control): 1 mL/day distilled water was given (used as ACMP solvent).

-Group III high dose ACMP (1/5 of LD_{50}): each rat gavaged orally with 40 mg/kg/day

ACMP (1/5 of LD_{50}) thawed in distilled water (4mL/kg body weight). LD_{50} of ACMP 200mg/kg in water ***(Williams, 2013)***.

-Group IV low dose ACMP (1/20 of LD_{50}): each rat gavaged orally with 10 mg/ kg/ day ACMP (1/20 of LD_{50}) thawed in distilled water (4mL/kg b.w) ***(Williams, 2013)***.

Twenty-four hours from the last dose of the treatment, at the experimental ending, the animals were anaesthetized with intra-peritoneal injection of 40 mg/ kg sodium thiopental ***(Mohamed et al., 2020)***. Then blood samples from each rat's retro-orbital plexus were collected according to ***Parasuraman et al. (2010)***, the serum was separated after spontaneous coagulation of the blood and centrifuged at 2500 xg for 15 minutes, and reserved at -80 °C for subsequent hormonal studies. The animals were then sacrificed and midline abdominal incisions were done to get the epididymis, prostates and testis. The epididymis was immersed at 37° C in phosphate buffered saline for the investigating sperm count, motility percent, and aberrant shape. One part of prostatic and testicular tissue was instantly fixed in formalin 10% for histological studies. The other parts of these tissues were transported on ice, and kept at -80 °C to obtain tissue homogenates for the analysis of oxidative stress markers and inflammatory and anti-inflammatory biomarkers.

I) Hormonal and bio-chemical parameters:

All parameters including hormonal and biochemical were assessed in Clinical Biochemistry Department, Faculty of Medicine, Zagazig University.

A) Hormonal studies:

Serum GnRH, LH and testosterone levels:

The serum GnRH, LH and testosterone were assessed by the competitive enzyme immunoassay technique using monoclonal antibodies specific for rat GnRH ***(Pappa et al., 1999)***, LH ***(Pappa et al.,1999)*** and testosterone ***(Zirkin and Chen, 2000)***. **Detection range**: (GnRH=12.35–1000 ng/L; LH= 0.3 mIU/ml–60 mIU/ml and testosterone= 0.13-25.6 ng/mL).

Sensitivity: less than 5.21 ng/L (the minimum detectable level of rat GnRH); less than 0.15 mIU/ml (minimum detectable level of rat LH), and 0.06 ng/mL (minimum detectable level of rat testosterone).

B) Biochemical studies:

1) Tissue inflammatory and anti-inflammatory biomarkers:

The proinflammatory TNF-α (Tumor necrosis factor alpha) and anti-inflammatory IL-10 (interleukin-10) in testicular and prostatic tissue were assayed colori-metrically according to ***DeCicco et al. (1998)*** and ***Pestka et al. (2004)***, correspondingly, using the commercial rat ELISA Kit (Ray Biotech, CUSABIO, United States) according to the manual instructions.

2) Tissue oxidative stress parameters:

The testicular and prostatic tissue of MDA (malondialdehyde) and TAC (total antioxidant capacity) were colorimetrically affording to ***Ohkawa et al. (1979)*** and ***Koracevic et al. (2001)***, correspondingly, using kits delivered from Bio-diagnostic Company; Dokki, Giza, Egypt.

II) Characterization of sperm:

The epididymis was divided into minor portions and located in 3 ml of warm PBS to create a suspension of sperm, then incubated for 10 minutes to permit the dispersion of sperm into the buffered solution. Evaluation of motility was done by counting both motile and non-motile one in the similar field. The suspension was filtered, and the number of spermatozoa (million/1 ml) was calculated by passing a predetermined volume of the suspension through Neubauer's counting chamber hemocytometer ***(Narayana et al., 2005)***. Under a light microscope, a piece of the filtrate was stained with 1% eosin to determine the total quantity of aberrant sperms ***(Wyrobek and Bruce, 1975)***. The percentages of aberrant shaped sperms were calculated. The normal values for sperm count, and percent of sperm motility, and percent of aberrant shaped sperms in rats were considered to be the control values.

III) Histological studies:

For hematoxylin and eosin (H&E) staining, the testicular and prostatic specimens were fixed in formalin 10% for 12 hours for paraffin blocks preparation according to ***Bancroft and Layton (2013)*** and examined by light microscope LEICA DM500 at the Medical Histology and Cell Biology

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Statistical Analysis:

Data were collected and managed using Statistical Package of Social Science (SPSS), software version 20 (2011; SPSS Inc). Statistical analysis of data was completed by Student T-test for two control groups comparison (the negative and the vehicle), One-way analysis of variance followed by post-hoc test (Least significance difference for multiple group comparison).

RESULTS

The results displayed no statistically significant discrepancy by using student T-test between control groups (the negative and the vehicle) concerning all the assessed parameters, so the group I (negative control) was used in the statistical comparison to other treated groups.

I) Hormonal studies:

Acetamiprid administration induced a significant upsurge in the serum levels of GnRH and LH and a significant diminution in the testosterone level when compared with negative control ($P<0.05$), and there was a significant variance in the serum hormonal levels of ACMP high dose group (40 mg/kg) when compared to ACMP low dose group (10 mg/kg) **(Table 1)**.

Biochemical studies:

1) Tissue inflammatory and anti-inflammatory biomarkers:

Acetamiprid administration induced a significant raise in TNF-α (proinflammatory cytokine), and a significant decrease in IL-10 (anti-inflammatory cytokine) in testicular and prostatic tissues in comparison with negative control ($P<0.05$), and there was a significant variance tissue TNF- α and IL-10 levels in ACMP high dose group (40 mg/kg) when compared to ACMP low dose group (10 mg/kg) **(Table 2).**

2) Tissue oxidative stress parameters:

Acetamiprid administration induced a significant upsurge in testicular and prostatic tissues prooxidant (MDA) and a significant lessening in testicular and prostatic tissues antioxidant (TAC) as compared to negative control ($P<0.05$), and there was a significant variance in the tissue MDA and TAC in ACMP high dose group (40 mg/kg) when compared to ACMP low dose group (10 mg/kg) **(Table 3)**.

II) Seminal analysis:

The current research displayed a significant declining in the sperm count, sperm motility percent and a significant rise in the aberrant shaped sperm percent in ACMP groups as compared to negative control ($P<0.05$). Similarly, there was a significant difference in the sperm count, motility and percent of aberrant shaped sperm in ACMP high dose group (40 mg/kg) when compared to ACMP low dose group (10 mg/kg) **(Table 4)**. Sperm aberrant were noticed in the either heads or tails; headless tails, curved one, and atypical shaped heads were found **(Figure 1)**.

III) Histological results:

Macroscopic examination of the testis and prostate of all the studied groups showed normal appearance with no significant size variations, no cysts or masses. Light microscopic findings of both negative and vehicle control groups were comparable with no observable differences in all examined tissues. Thereby, they were represented as the negative control group in the figures.

1) Testis:

Examination of control group sections revealed normal architecture of the testis. It was composed of abundant packed seminiferous tubules that were enclosed by connective tissue capsule. These tubules were lined by stratified germinal epithelium that had numerous cell types: spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Spermatozoa were found in the seminiferous tubules' lumen. Sertoli cells, supporting cells, had an imperative role in germ cells growth. Interstitium comprising interstitial cells and blood vessels was noticed in between seminiferous tubules **(Figure 2A, B)**.

Sections in the ACMP low dose (1/20 of LD_{50}) treated group revealed thickened connective tissue capsule, dilated congested capsular blood vessels and some disorganized seminiferous tubules. Some tubules' germinal epithelium either had lost their lining, or disorganized. Some tubules' Shrinkage with irregularity in basement membrane, widening among the tubules, and moderate diminution in the germinal epithelium lining height were

also seen. Dark pyknotic nuclei were noticed in Germinal epithelial cells**.** Acidophilic vacuolated hyaline material and cellular infiltration were also seen in- between seminiferous tubules **(Figure 2 C, D, E)**.

Examination of sections from the ACMP high dose (1/5 of LD_{50}) treated group revealed marked reduction in the germinal epithelium height in some seminiferous tubules, shrinkage of other tubules with irregularity in basement membrane, widening among the tubules were detected. In other sections, disorganized tubules were also seen, dark pyknotic nuclei were observed in most of germinal epithelial cells. Hyaline vacuolated material and cellular infiltration were showed in the interstitium **(Figure 2F, G)**.

2) Prostate gland:

Examination of control group sections revealed normal prostatic gland architecture. It was composed of variable sized acini containing prostatic secretions in their lumina and had columnar epithelial cells lining with basal nuclei and resting on a basement membrane. The acini were encircled by a fibromuscular stroma formed mostly of smooth muscle fibers and blood vessels (**Figure 3 A, B)**.

Conversely, examination of rat prostate sections from ACMP low dose (1/20 of LD_{50}) group revealed glandular epithelium with dark stained nuclei**.** Some prostatic acini have empty lumen and others had vacuolated acidophilic prostatic secretions**.** Thickening of the connective tissue stroma containing congested blood vessels**.** Some prostatic acini showed cell proliferation **(Figure 3 C, D)**.

The sections in ACMP high dose (1/5 of LD_{50}) treated group displayed marked deterioration in the structure of the gland; the prostatic acini had lining epithelium with dark stained nuclei, shedding of the epithelial lining in the lumen of the acini and thinning of the lining epithelium. Marked thickening of the connective tissue stroma containing congested blood vessels **(Figure 3 E, F, G)**.

Table (1): Serum level of male sex hormones of the studied groups.

Groups / Parameters	Negative control (I)	Vehicle control (II)	ACMP low dose (III)	ACMP high dose (IV)
GnRH (ng/L)	190.25± 4.78	189.92± 2.65	196.85± 6.86 [a,b]	205.98 ±5.40 [a,b]
LH (mIU/ mL)	11.057±0 .33	11.11±0 .48	11.68±0.24 [a,b]	12.90 ±0.58 [a,b]
Testosterone (ng/mL)	2.30±0 .19	2.21±0 .25	2.00±0.21 [a,b]	1.44± 0.23 [a,b]

Results values are expressed as mean ± standard deviation (SD) of n = 7 rats/group for 4 weeks
ACMP: Acetamiprid; GnRH: Gonadotropin Releasing Hormone; LH: Luteinizing Hormone.
[a] *Significant as comparing between the negative control and either low or high dose ACMP treated groups, P<0.05*
[b] *Significant as comparing between low and high dose ACMP groups, P<0.05.*

Table (2): Testicular and prostatic tissues inflammatory and anti-inflammatory biomarkers of the studied groups.

Groups / Parameters	Negative control (I)	Vehicle control (II)	ACMP low dose (III)	ACMP high dose (IV)
Testis TNF-α (Pg/mg tissue)	53.50±2.88	53.42±4.08	91.71± 8.65 [a,b]	501.57±45.82 [a,b]
Prostate TNF-α (Pg/ mg tissue)	52.07±4.21	55.91±3.01	92.14± 8.17 [a,b]	522.56±48.52 [a,b]
Testis IL-10 (Pg/mg tissue)	230.42±4.03	226.28±8.81	208.14± 6.61 [a,b]	115.57±9.86 [a,b]
Prostate IL-10 (Pg/mg tissue)	222.42±13.33	225.42±10.75	210.57±3.55 [a,b]	117.14±6.06 [a,b]

Results values are expressed as mean ± standard deviation (SD) of n = 7 rats/group for 4 weeks
ACMP: Acetamiprid; TNF-α: Tumor Necrosis Factor Alpha; IL-10: Interleukin 10.
[a] *Significant as comparing between the negative control and either low or high dose ACMP treated groups, P<0.05*
[b] *Significant as comparing between low and high dose ACMP groups, P<0.05.*

Table (3): Testicular and prostatic tissues oxidative stress biomarkers of the studied groups.

Groups / Parameters	Negative control (I)	Vehicle control (II)	ACMP low dose (III)	ACMP high dose (IV)
Testis MDA (ng/mg tissue)	6.62±0.33	6.71±0.33	7.31±0.34 [a,b]	11.32±0.39 [a,b]
Prostate MDA (ng/ mg tissue)	6.80±0.37	6.82±0.53	7.34±0.85 [a,b]	11.36±0.49 [a,b]
Testis TAC (nmol / mg tissue)	0.98±0.17	1.24±0.28	0 .81±0.06 [a,b]	0.41±0.03 [a,b]
Prostate TAC (nmol / mg tissue)	1.04±0.18	1.11±0.21	0 .92±0.09 [a,b]	0.36±0.07 [a,b]

Results values are expressed as mean ± standard deviation (SD) of n = 7 rats/group for 4 weeks
ACMP: Acetamiprid; MDA: malondialdehyde; TAC: total antioxidant capacity.
[a] Significant as comparing between the negative control and either low or high dose ACMP treated groups, P<0.05
[b] Significant as comparing between low and high dose ACMP groups, P<0.05.

Table (4): Sperm characterization (sperm count, motility, aberrant shape) of the studied groups.

Groups / Parameters	Negative control (I)	Vehicle control (II)	ACMP low dose (III)	ACMP high dose (IV)
Count (*10^6/ ml)	193.28± 10.11	188.00± 5.77	184.42± 4.54 [a,b]	116.142±5.61 [a,b]
Motility (%)	84.14± 3.07	87.57± 2.82	79.28±3.68 [a,b]	71.85±3.28 [a,b]
Total aberrant sperm shape %	3.70±0 .68	3.77±0 .74	23.28±4.20 [a,b]	38.14±3.03 [a,b]

Results values are expressed as mean ± standard deviation (SD) of n = 7 rats/group for 4 weeks
ACMP: Acetamiprid. [a] Significant as comparing between the negative control and either low or high dose ACMP treated groups, P<0.05. [b] Significant as comparing between low and high dose ACMP groups, P<0.05.

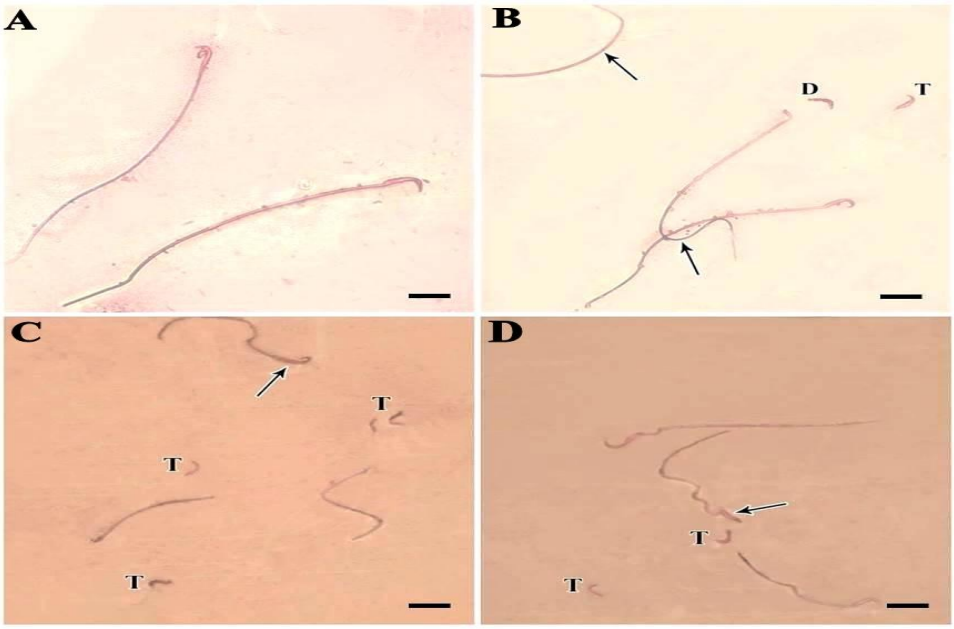

Figure (1): (A): A photomicrograph of normal sperms from control adult albino rat. (B): Sperms from acetamiprid 1/20 of LD_{50} (low dose) with deformed head (D), bent tail (arrow), and a tailless sperm (T). (C) & (D): Sperms of acetamiprid 1/5 of LD_{50} (high dose) showing abnormally deformed head (arrow) and tailless sperms (T). (A, B, C and D: Nigrosin and Eosin x 200 - Scale bar 30 µm)

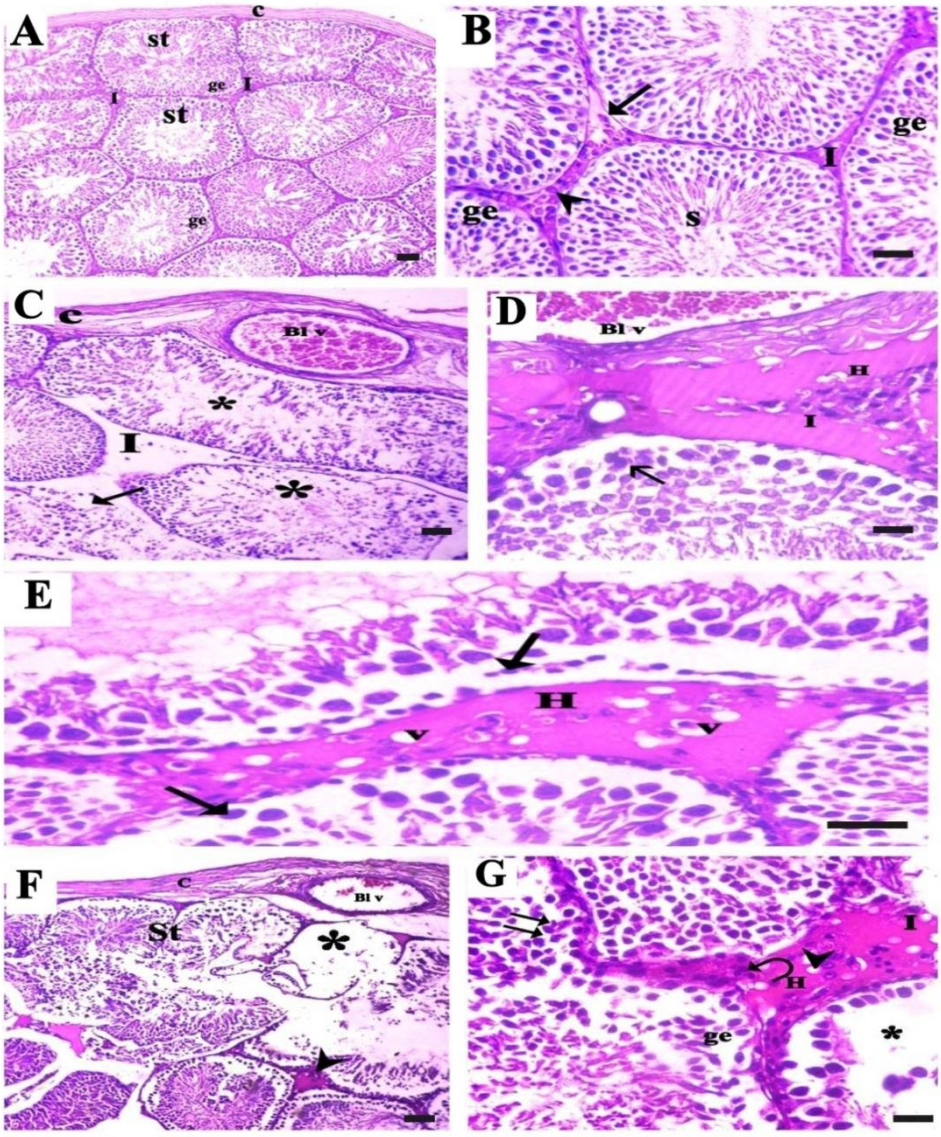

Figure (2): Photomicrographs of H&E-stained sections in rat testis of: (A) & (B) control group showing numerous packed seminiferous tubules (st) that are covered by connective tissue capsule (c). These tubules are lined by stratified germinal epithelium (ge) resting on basal lamina (arrow). Spermatozoa (S) are present in the lumen of these tubules. Interstitium (I) containing interstitial cells is present in between seminiferous tubules. (A: H&E X100 Scale bar 50 µm, - B: H&E X400 Scale bar 20 µm).

(C), (D) and (E): acetamiprid 1/20 of LD_{50} (low dose) group showing thickened connective tissue capsule (c), dilated congested capsular blood vessels (Bl v). Some seminiferous tubules (St) are lined by stratified germinal epithelium, other tubules (*) have lost most of their lining epithelium or disorganized epithelial lining (arrow). Wide interstitium (I) is also noticed. Higher magnification of the same group showing germinal epithelium (ge) with dark pyknotic nuclei (arrow). Acidophilic hyaline material (H) having vacuolations (v) is also present in the interstitium (I). (C: H&E X100 - Scale bar 50 µm, D and E: H&E X400 - Scale bar 20 µm).

(F) and (G): acetamiprid 1/5 of LD_{50} (high dose) group showing marked affection in the structure of seminiferous tubules (St); thickened connective tissue capsule (c), congested capsular blood vessels (Bl v), some tubules have marked decrease in germinal epithelial height (*), shrinkage of other tubules (arrow) with irregular basement membrane. Higher magnification of the same group showing disorganized tubules (St) with marked decrease in the height of the lining epithelium (*), most of germinal epithelium (ge) have dark pyknotic nuclei (double arrow). Interstitium (I) reveals acidophilic hyaline (H) vacuolated material (arrow head) and cellular infilteration (curved arrow). (F: H&E X100 - Scale bar 50 µm, G: H&E X400 Scale bar 20 µm)

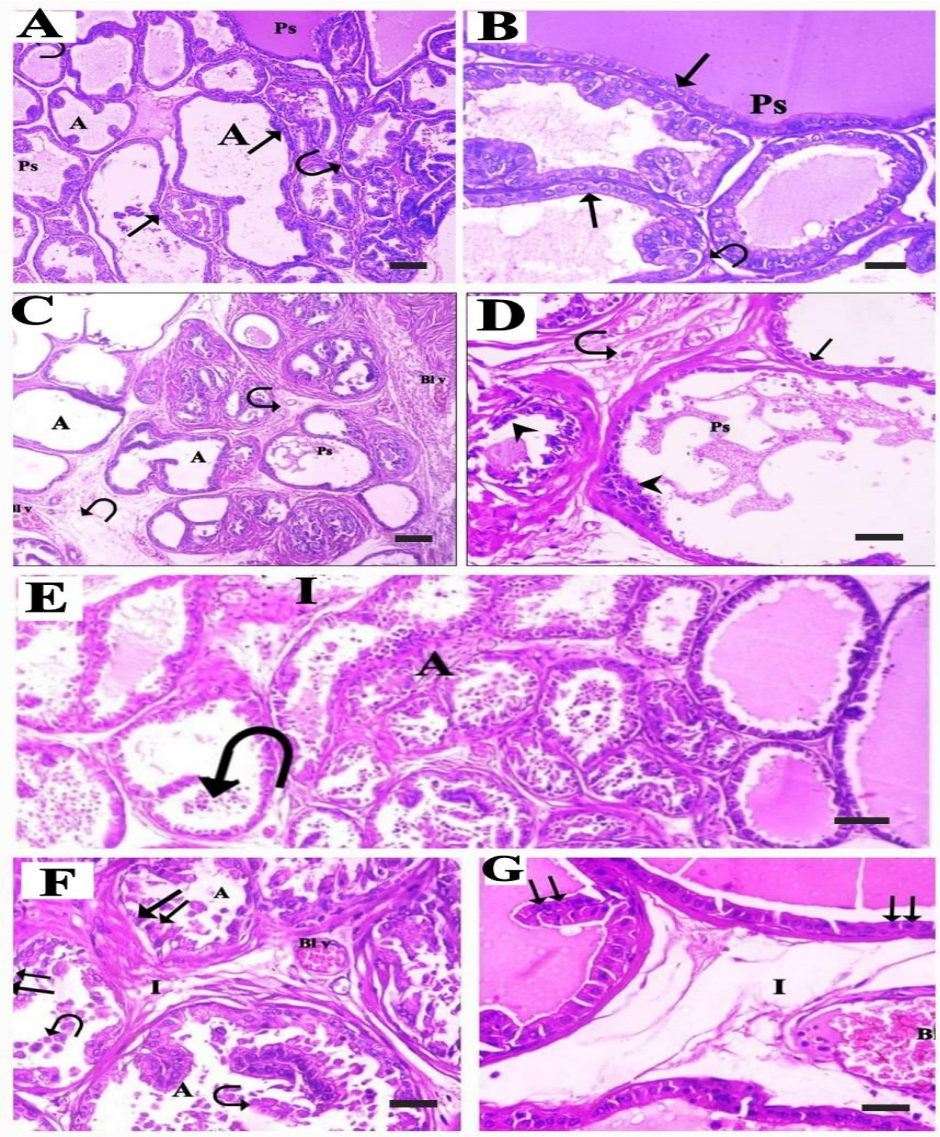

Figure (3): Photomicrographs of H&E-stained sections in rat prostate of: (A) & (B): control group showing variable sized acini (A) containing prostatic secretions (Ps) in their lumina and are lined by columnar epithelial cells (arrow). The acini are surrounded by a fibromuscular stroma (curved arrow). (A: H&E×100 Scale bar 50 µm, B: H&E×400 Scale bar 20 µm).

(C) & (D): acetamiprid 1/20 of LD_{50} (low dose) group showing variable sized acini (A), some prostatic acini (A) have empty lumen and others have vacuolated acidophilic prostatic secretions (Ps). Some acini show cell proliferation (arrow head). Glandular epithelium has dark stained nuclei (arrow). Thickening of the connective tissue stroma (curved arrow), that is present in between the acini containing congested blood vessels (Bl v). (C: H&E×100 Scale bar 50 µm, D: H&E×400 Scale bar 20 µm)

<u>(E), (F) &(G):</u> acetamiprid 1/5 of LD_{50} (high dose) group showing marked affected glands; the lining epithelium of the prostatic acini (A) have dark stained nuclei (double arrow), shedding of the epithelial lining in the lumen of the acini (curved arrow). Marked thickening of the connective tissue stroma (I) containing congested blood vessels (Bl v). (E: H&E×100 Scale bar 50 µm, F&G: H&E×400 Scale bar 20 µm)

DISCUSSION

Acetamiprid, a neonicotinoid, used to kill pests in various crops. It tends to environmental accumulation due to its persistence and widespread use ***(Han et al., 2018)***. So, this work aimed to study possible mechanisms the subacute ACMP reproductive toxicity particularly testis and prostate by using different doses in adult male albino rats. The results of the current research displayed a significant lessening in the serum testosterone levels and a significant surge in the serum GnRH and LH levels of ACMP groups in comparison with control one and there was a significant difference in the serum hormonal levels of the ACMP high dose group (40 mg/kg) in comparison with the ACMP low dose group (10 mg/kg).

Besides, the current research displayed a significant drop in the sperm count, sperm motility percent and a significant rise in the aberrant shaped sperm percent in ACMP groups in comparison with control one. Also, there a significant difference in the sperm count, sperm motility percent and abnormalities in the ACMP high dose group (40 mg/kg) in comparison with the ACMP low dose group (10 mg/kg).

The attributed reasons for the preceding changes are multi-factorial. ACMP has a significant disturbance in the reproductive hormones and a potential alteration of the spermatogenesis process. This indicated that ACMP has a repressive effect on reproduction.

These study results agreed with ***Zhang et al. (2011)*** who stated that ACMP (30mg/kg) significantly decline testosterone serum level, sperm count, motility percent in Kunming male mice. ACMP disrupt the spermatogenesis process as causing mitochondria and endoplasmic reticulum disorders of Leydig cells with resultant disruption in testosterone synthesis. They were explained that ACMP induce excessive ROS production which are toxic to sperms due to their high content of polyunsaturated fatty acid.

Besides, ***Awasthy (2013)*** reported that oral administration of ACMP with doses 26.25 and 52.5 mg/kg) for 8 weeks in adult rats lessened the sperm count and motility percent and augmented abnormalities in both head and tail. He also reported that the reduced number of spermatozoa is caused by interruption in the testicular enzymes' marker activities allied with the histo-pathological alterations.

Also, ***Kong et al. (2017)*** who stated that 30 mg/kg ACMP for 35 days revealed inhibition in synthesis of rat's testosterone through mitochondrial dysfunction and cytoplasmic ATP production in Leydig cells. This testosterone reduction led to a lessened sperm number and motility percent.

Moreover, ***Mosbah et al. (2018)*** stated that treatment with ACMP on Wistar rats with a dose (27mg/kg) for 45 days led to a reduced the testosterone level and a lessening the number of testicular spermatids, sperm count in epididymis, and motility percent and an upsurge in aberrant shaped sperm, this might be due to the depression of steroidogenic enzymes activities and/or a reduction in expression of the testicular steroidogenic acute regulatory protein, that aid in the cholesterol transmission in mitochondria and testosterone synthesis.

According to ***Arican et al. (2020)***, ACMP treatment at 12.5, 25, and 35 mg/kg for 12 weeks caused a drop in sperm count and testosterone levels that associated with increased GnRH, FSH, and LH levels and augmented aberrant shaped sperm and apoptosis in a dose-dependent manner. They also stated that ACMP may trigger free radicals' production in Leydig cells. Testosterone is the main circulating androgen, it also has anti-oxidant and anti-apoptotic activities on testis and shield sperm from DNA damage, so diminished levels lead to abnormal spermatogenesis.

Moreover, ***EL-Hak et al. (2022)*** reported that ACMP treatment with doses (20 and 10 mg/kg) significantly decreased LH level attributing these effects to oxidative stress and failure of antioxidant system. They also reported reduced testosterone levels in ACMP fed rats. They suggested that oxidative stress had a role, as disruption of the cell membrane lipid bilayer, resultant in diminished testosterone synthesis.

Acetamiprid induced reproductive function toxicity including hormonal and seminal

analysis disturbance were confirmed by histopathological finding in reproductive organ tissues including (testis and prostate) as follow:

In the current study, testis section from ACMP low dose group (10 mg/kg) revealed vacuole formation in some tubule's germinal epithelium and regular basement membrane was noticed. While, in the ACMP high dose group (40 mg/kg), there was a decreased spermatogenic germ cells number, damaged vacuolated spermatogenic cells and in a large number of seminiferous tubules lumen, immature cell rashes were noticed.

These results agreed with ***Zhang et al. (2011)*** who stated that treatment with 30 mg/kg ACMP for 35 days revealed vacuolization of the seminiferous tubules, and reduction of spermatids number and Leydig cells. Furthermore, some cells shed from the lumen of the seminiferous tubules, some primary spermatocytes showed vacuolization.

Also, ***Awasthy (2013)*** also reported oral administration of ACMP (26.25 and 52.5 mg/kg for 60 days) showed histo-pathological changes demonstrating seminiferous tubules degeneration in rat's testis, degenerative changes of Sertoli cells and disturbed spermatogenesis.

Similarly, ***Keshta et al. (2016)*** reported that rat's testis treated with 30 mg/kg ACMP for 35 days demonstrated abnormal seminiferous tubules with vacuolization, and there was shedding of spermatogenic cells, oedema, a drop in the sperm number.

Moreover, ***Kenfack et al. (2018)*** stated that the guinea pig's treatment with ACMP (26.67 mg/kg) revealed scarce undifferentiated germinal cells in the lumen. While, a quantity of undifferentiated cells was found in the lumen with uppermost doses (40 and 80 mg/kg) 90 days, also the interstitial tissue was markedly destroyed.

Also, ***Arican et al. (2020)*** reported that testis showed apoptotic cells in the tubules of entirely ACMP groups, frequently in spermatogonia and primary spermatocytes, the regular basement membranes of the seminiferous tubules were noticed in the both control and low-dose (12.5 mg/kg) groups. While, in the high-dose (35 mg/kg) one, the irregular basal membranes and disrupted structure in numerous seminiferous tubules were observed. Also, there were a reduction in spermatogenic germ cell number, vacuolization and luminal immature cell rashes in almost seminiferous tubules in high-dose group. They suggested that these testicular changes are due to oxidative stress and apoptosis induction.

According to study of ***Zayman et al. (2022)***, ACMP treated male mice with a dose 25ml/kg of ACMP for 21 days showed disorganization and degeneration in cell stratification in some seminiferous tubules' epithelium with large vacuoles, and Leydig cells showed hydropic degeneration. Likewise, there were reduction of spermatids number and interstitial areas showed of edematous acellular eosinophilic staining material accumulation. They supposed that this pathological damage caused by oxidative stress induction, sex hormones levels disturbance and mitochondria and endoplasmic reticulum dysfunction associated with upsurge in lysosomal accumulations in spermatogenic cellular line and Sertoli cells particularly in Leydig cells.

Also, ***EL-Hak et al. (2022)*** reported that the testis of rats given ACMP with doses (20 and 10 mg/kg) showed considerable degeneration of spermatogenic cells.

Additionally, in the present study, Haematoxylin and Eosin-stained section of prostate of the low dose of ACMP treated group (10 mg/kg) revealed glandular epithelium with dark stained nuclei. Some prostatic acini had empty lumen and others had vacuolated acidophilic prostatic secretions. Thickening of the connective tissue stroma containing congested blood vessels. Some prostatic acini showed cell proliferation.

While, sections of the ACMP high dose group (40 mg/kg) revealed marked deterioration in the histological structure of the gland; the prostatic acini had lining epithelium with dark stained nuclei, shedding of the epithelial lining in the lumen of the acini and thinning of the lining epithelium. Marked thickening of the connective tissue stroma containing congested blood vessels.

These results agreed with ***Awasthy (2013)***, who stated that treatment with ACMP (52.5

mg/kg) revealed severe thinning of glandular alveolar lining of rat prostate and affection of basement membrane with decrease amount of luminal secretion and low staining intensity, while, ACMP (26.25 mg/kg) treated rats reasonably revealed lesser thinning of epithelium with the existence of pinkish red homogenous mass.

Also, ***Gaber et al. (2024)*** reported that prostatic epithelium was hollow, and some areas were enlarged in rats treated with doses (94 and 281 mg/kg) of dinotefuran (a third-generation neonicotinoid).

Furthermore, in the present work, there was a significant surge in TNF- α and a significant diminution in IL-10 in testicular and prostatic tissue in ACMP treated groups as compared with negative control one. Likewise, there a significant difference in the tissue TNF- α and IL-10 in the ACMP high dose group in comparison with the ACMP low dose group.

Inflammatory cytokines are low molecular weight proteins that are released from specific cells of the immune system. They are signaling molecules that regulate inflammation, immunity, proliferation, differentiation, cell survival and death ***(Landskron et al., 2014)***. During inflammation, TNF-α is secreted by macrophages that result in necrosis or apoptosis after a varied range of signaling events within cells ***(Idriss and Naismith, 2000)***. While IL-10 serves as a potent anti-inflammatory cytokine that prevent inflammatory and auto-immune process ***(Iyer and Cheng, 2012)***.

These study results agreed with ***Erdemli et al. (2020)***, who stated that ACMP treated mice for 21 days with a dose 25 mg/kg showed significant increased renal tissue levels of TNF-α, IL-6, and IL-1β as compared with all other groups. Also, ***EL-Hak et al. (2022)*** reported that ACMP with doses (20 and 10 mg/kg) induced inflammatory response including migration and activation of inflammatory cells (resident and circulating), along with the cytokines release.

Moreover, ***Albrakati (2024)*** reported that ACMP treated Wistar rats with a dose (40 mg/kg) for 28 days revealed a significant upsurge in cerebral tissue level of TNF-α, NF-κB and IL-1β. He stated that ACMP upsurges pro-apoptotic proteins as caspase-3 and Bax with diminutions in the anti-apoptotic protein Bcl-2 that trigger oxidative stress and inflammatory process.

Additionally, In the present study, there was a significant escalation in testicular and prostatic MDA and a significant decline in testicular and prostatic TAC in ACMP groups in comparison with control one. Likewise, there a significant difference in the testicular and prostatic MDA and TAC in ACMP high dose group (40 mg/kg) when compared to ACMP low dose group (10 mg/kg).

The upsurge of free radicals can trigger the lipid peroxidation by oxidative breakdown of cell membrane polyunsaturated fatty acids. So, lipid peroxidation of sperm (lipid matrix in the spermatozoa membranes), result in axonemal damage, lessened sperm viability and amplified sperm abnormalities, and may be entirely hinders spermatogenesis in severe cases ***(Türk et al., 2007)***.

Malondialdehyde (MDA), sensitive indicator of lipid peroxidation, is chief product of peri-oxidized polyunsaturated fatty acids ***(Rahal et al., 2014)***. ***Pan et al. (2008)*** reported that it is a valuable measure of oxidative stress status. In addition, TAC considered the cumulative effects of all antioxidant's status (enzymatic or non-enzymatic) ***(Koracevic et al., 2001)***.

These study results were in agreement with ***Zhang et al. (2011)*** who reported that ACMP treatment causing upsurge in MDA level, representing lipid peroxidation induction. Excessive production of free radicals is toxic to sperms due to their high content of polyunsaturated fatty acid that result in loss of membrane integrity (structure and function). They also reported that ACMP lessen the tissue anti-oxidant activity (CAT, GSH-Px and SOD enzymes).

Also, ***Keshta et al. (2016)*** reported that ACMP and its toxic metabolites induced free radicals' production leading to damage of testicular and spermatogonia tissues associated with a lessening in sperm motility percent, even infertility. Also, ***Kong et al. (2017)*** reported that ACMP with a dose (30 mg/kg) led to an abnormal rise of ROS and damage of mitochondria in Leydig cells.

Moreover, ***Mosbah et al. (2018)*** reported that 27 mg/kg ACMP for 45 days led to oxidative

cell injury by excessive generation of free radicals associated with lessening in the antioxidant activities (SOD, CAT, and GSH-PX).

Besides, ***Anand et al. (2019)*** reported that 52.5 mg/kg ACMP for 28 days in rats led to inhibition of the antioxidant enzymes expression, resulting in oxidative stress and overwhelming of cellular antioxidant capacity.

Similarly, ***Arican et al. (2020)*** revealed that ACMP led to lipid peroxidation, GSH (glutathione) exhaustion, increase in total oxidant status, and reduction in total antioxidant status in testicular homogenates in a dose dependent way.

According to ***EL-Hak et al. (2022)***, ACMP treatment with doses (20 and 10 mg/kg) led to oxidative stress, rise in serum NO level and decrease in SOD and CAT levels suggesting that ACMP's toxic effects occurred by induction of oxidative stress.

CONCLUSION

In summary, subacute ACMP exposure has a toxic effect on the rat's testis and prostate which are characterized by sex hormones disturbance that leading to disturbance in spermatogenesis lead to abnormal semen analysis, as well as histopathological alterations of these organs. The mechanism of ACMP-induced testicular and prostatic insult occurs through the induction of oxidative stress and inflammation in the rat testicular and prostatic tissues. ACMP induced toxicity occurs in a dose-dependent manner.

RECOMMENDATIONS

From the previous study results, the following strategies are recommended: upgrading of health education programs to raise public awareness about protective measures that should be taken during application of ACMP; Using antioxidants supplementation may be useful in reducing of ACMP toxicity; Further researches are required to identify other mechanisms of ACMP-induced reproductive toxicity and elucidate the human risk, and to improve the global awareness about reproductive disrupting-chemicals.

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