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Attenuating Effect of N-Acetylcysteine on Apoptosis Associated with Imidacloprid Induced Testicular Damage in Male Albino Rats

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ABSTRACT

N-acetylcysteine (NAC), as a nutritional supplement, is a greatly applied antioxidant in vivo and in vitro. NAC is a precursor of L-cysteine that results in glutathione elevation biosynthesis. The therapeutic potential of N-acetylcysteine **(**NAC) has been investigated as a bioprotective agent against oxidative stress and ischemic injury. Also, it is used as a treatment for certain mental and physical illnesses. The aim of this study was to evaluate the protective and attenuating apoptosis effect of NAC on imidaclopride (IMI) induced testicular damage in rats. Forty male albino rats were classified randomly into four equal groups. Group1 (control). Group2 (IMI): rats received IMI orally day after day over a period of 8 weeks at a dose level of 21.2 mg/kg b.w (1/20 LD50). Group3 (NAC): rats received NAC (200 mg/kg body weight) orally for 8 weeks. Group 4 (NAC+IMI): rats received NAC (200 mg/kg body weight) orally for 4 days before and along with the administration of (IMI) over a period of 8 weeks. At the end of the experiment testes were isolated for the determination of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced Glutathione (GSH) and comet assay. Also, for histopathological examination.

The obtained results showed a significant increase in testicular tissue MDA, and DNA damage detected by comet assay in imidaclopride intoxicated rats. However, Testicular SOD activity, catalase, and GSH concentration were markedly decreased. Histopathological alteration caused by IMI toxicity were presented by slightly thickened tunica albuginea with less congestion of sub-capsular blood vessels, mild degeneration of the germinal epithelium of some seminiferous tubules with mild interstitial edema.

NAC protection to IMI induced testicular damage in rats caused a significant improvement of

all previous parameters and attenuate the histopathological changes. These results suggested that, NAC treatment exerts a protective effect on testicular damage by improving of oxidative stress markers and DNA damage detected by comet assay in rats through free radical scavenging and anti-inflammatory activities as well as regenerating endogenous antioxidant defense system mechanisms.
Keywords: Imidaclpride (IMI), Testicular damage, NAC, oxidative stress.

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1. INTRODUCTION

Antioxidants, which form a diverse group of compounds with different properties, operate by inhibiting oxidant formation, intercepting oxidants and repairing oxidant induced injuries (Ergüder et al., 2007).

N -acetylcysteine (NAC) is a metabolite of the sulphur-containing amino acid cysteine. It has the molecular formula $HSCH_2CH$ ($NHCOCH_3$) CO_2H. It can be administered orally or by intravenous infusion and can also be inhaled. Currently, it is used as an antioxidant and a mucolytic agent. The therapeutic potential of NAC has been examined and is currently being further investigated across a range of illnesses as an antidote for specific toxins, a bio-protective agent against oxidative stress and ischemic injury. It has been used for treatment of certain mental and physical diseases in a direct action or in a combination with some other medications. Also, it is a protected mucolytic drug that mellows tenacious mucous discharges (Stey et al. ,2000). In addition, it is sometimes used as a dietary supplement. NAC forms the dimers N –acetylcystine and N, N – diacetylcystine, it covalently bonds to plasma proteins and can be deactylated to form cysteine. The latter is the rate-limiting precursor for the endogenous antioxidant glutathione (Shahin et al., 2009). NAC is a powerful antioxidant acts directly as a scavenger of free radicals, especially oxygen radicals. It is also recommended as a potential treatment option for different disorders resulted from generation of free oxygen radicals **(**Deepmala et al., 2015).

Insecticides and pesticides act as reproductive toxicants in male rats and histologically induce severe focal necrosis of the germinal cells in seminiferous tubules associated with tubular atrophy (Narayana et al., 2006).

Testicular toxicity of insecticides is an important side effect on account of infertility. Extensive investigations were conducted to evaluate cellular and molecular aspects of gonadal damage in animals during spermatogenesis following chemical exposure to insecticides (Mandal and Das, 2012).

Imidaclopride is a chlorinated analog of nicotine, which belongs to the class of neonicotinoid insecticides. IMI has low vapor pressure and the technical product (94.0% IMI) has a moderate order of toxicity with respect to ingestion in the rat but appears to be less toxic when absorbed by the skin or inhaled. IMI may cause minimal redness to the eyes but is nonirritating to the skin (Mizzel and Sconyers, 1992).

Accordingly, the present study was carried out to investigate the protective effect of NAC on IMI subacute toxicity induces some oxidative stress, damage and histopathological changes in testes of male albino rats.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Forty white male albino rats of 8-10weeks old age and average body weight 150-200 g were used in this study. Rats were housed in separated polycarbonate cages (10 per cage) and they were kept on a well-balanced ration and fresh clean drinking water *ad-libitum*. Rats were kept at a constant environmental and nutritional condition throughout the whole period of experiment. All rats were left for 15 days for acclimatization before the beginning of the experiment.

2.2. Chemicals and antioxidant: -

Chemicals and the antioxidant used in the present study were:

2.2.1. *Imidaclopride*

Imidaclopride grade (96% active ingredient) were gifted from Pesticides Analysis Department, Central Agricultural Pesticides Lab., Dokki, Egypt.

-Imidaclopride solution preparation:

1000 mg of imidaclopride was mixed in 3 ml Dimethyl sulfoxide (DMSO) and complete dissolving was obtained. Then propylene glycol was added till 60 ml with vortex stirring to reach a final concentration of 21.2 mg Imidaclopride /ml of Solution.

2.2.2. *N-acetylcysteine (NAC):*

NAC was purchased from El Quahera for pharmaceutical & chemical industries, Cairo, Egypt. It was used as a protection to testicular disorders at a dose level of 200 mg/kg body weight and dissolved freshly in physiological saline (10 % NaCl) (Wang et al., 2007).

2.2.3. *Other chemicals used in this study* (Propylene glycol, DMSO and normal saline) were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

2.3. Experimental design: -

After acclimatization to the laboratory conditions, the animals were randomly classified into four groups (10 rats each) placed in individual cages and classified as follow:

Group 1 (control group): Rats received tap water, fed on basal ordinary diet during the entire experimental period of 8 weeks.

Group 2 (Imidaclopride treated group)**:** Rats received IMI orally by stomach tube day after day over a period of 8 weeks to induce testicular disorders in rats at a dose level of 21.2mg/kg b.w (1/20 LD50) which is 424 mg/kg body weight (United states EPA, 1995).

Group 3 (NAC treated group)**:** Rats received NAC orally once per day at a dose of 200 mg/kg body weight/day for 8 weeks.

Group 4 (NAC + IMI treated group): Rats treated with NAC orally by the stomach tube once per day at a dose of 200 mg/kg body weight/day 4 days before and along with the administration of IMI (day after day) over a period of 8 weeks.

2.4. Sampling:

2.4.1. Testes samples for biochemical analysis: -

At the end of the experiment the rats were sacrificed under light ether anesthesia. Both testes were isolated immediately, weighed and then one of them was cleaned by rinsing with cold saline and stored at -20 ºC for subsequent biochemical analyses. All testes samples were analyzed for the determination of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced Glutathione (GSH). Also, comet assay was detected.

2.4.1.1. Testicular tissue preparation for antioxidant determination: -

Briefly, testicular tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4 ºC then the resultant supernatant was used for the determination of the following parameters: MDA, SOD and CAT.

About 0.2 g of testicular tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed, incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clean supernatant was removed and used for determination of GSH concentration.

2.5. Methodology:

2.5.1. Antioxidants determination: -

Testicular tissues MDA concentrations were determined according to the method adapted by Mesbah et al., (2004), testicular superoxide dismutase (SOD) activity (Nishikimi et al., 1972**)** and testicular catalase activity (Aebi, 1984).

Determination of reduced glutathione (GSH) in testicular tissue by colorimetric method was carried out using kit (Bio Diagnostic. Co) with a catalog No.: GR 25 11.

2.5.2. Genotoxic analysis (Comet assay)

Comet assay (single cell gel electrophoresis, SCGE) was used to detect any prospective damage for DNA after various treatments. It detects DNA strand breaks and alkali labile sites by measuring the migration of DNA from immobilized nuclear DNA. In the current study, the comet assay was performed according to the protocol described by **(**Singh et al, 1988).

2.5.3. Histopathological examination:

The other testis was carefully examined by naked eyes for detection of any abnormalities and immediately fixed in 10% natural buffered formalin. Then after proper fixation, the samples were dehydrated in ascending grades of ethyl alcohol, then cleared in xylol, embedded in paraffin and finely blocking occurred. These samples were sectioned at 5 µm in thickness and stained with hematoxylin and eosin (H and E) for histopathological examination according to (Dray and Walling, 1976).

2.6. Statistical analysis:

The results were expressed as mean ± SE using SAS computerized program v. 9.2 (SAS, 2008) program to calculate the analysis of variance. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

3. RESULTS:

3.1. Antioxidants parameters:

The obtained results presented in table (1) indicated that, (G2) had a significant increase ($P\leq0.01$) in testicular tissue MDA activity when compared with control group (G1). As regard (G3) demonstrated no marked change when compared with control group (G1). In addition, (G4) protected rats revealed a significant decrease ($P\leq0.01$) in testicular tissue MDA activity when compared with (G2).

The mean level of SOD activity at G2 was insignificantly declined in testicular tissue when compared with control group (G1). While, NAC administration (G3) recorded no marked variation when compared with control group (G1). Incidentally, (G4) revealed an insignificant increase in testicular tissue SOD activity in comparison with (G2). The obtained data revealed that, (G2) showed a significant decrease ($P \leq 0.01$) in testicular tissue catalase activity when compared with control group (G1).

While, NAC administration (G3) recorded an insignificant elevation when compared with control group (G1). Catalase activity in (G4) testicular tissues revealed a significant increase ($P \leq 0.01$) when compared with (G2).

G2 revealed a highly significant ($P \leq 0.01$) decrease in testicular tissue GSH activity when compared with control group (G1). GSH level in testicular tissues recorded a significant ($P \leq 0.01$) decrease in G3 when compared with control group (G1). Moreover, (G4) revealed a significant increase in testicular tissue GSH activity when compared with (G2).

3.2. comet assay:

A comet assay which was performed to assess DNA damage in testicular tissues of imidacloprid-intoxicated rats and protected group with of N-acetylcysteine as compared to control. The results of comet assay were shown in Figure 1 and Table 2. Administration of imidacloprid (G2) led to significant ($P < 0.05$) increase in DNA damage that was indicated by increase in tail length, tail DNA% and tail moment as compared to control group. This increased DNA damage was reduced after protection with N-acetylcysteine (G4). On the other hand, no significant difference in DNA damage (tail length) was observed when we compare (G3) with control (G1). Histopathological examination:

Group 1 (Control rats)

Histopathological examination of the testes of the control group showed normal histological structure of seminiferous tubules and interstitial tissues as well as tunica albuginea (Fig. 2).

Group 2 (Rats treated with Imidaclopride 21.2 mg/kg b. w)

The microscopical examination of the testes obtained from rats treated with Imidaclopride for 8 weeks revealed that marked increase in the thickness of tunica albuginea with sub-capsular edema and severe congestion of sub capsular blood vessels (Fig. 3). Additionally, remarkable vasodilatation of testicular blood vessels in association with extensive inter-tubular edema was detected admixed with interstitial cells (leydig cells) in some cases. Fig.4&5

Group 3(N-Acetyicysteine treated group)

Testes obtained from rats treated with N-acetyicysteine showed normal histological structure of the germinal epithelium of seminiferous tubules and interstitial tissues were detected (Fig. 6).

Group 4 [Rats treated with N-Acetylcysteine (200 mg/kg b. w) plus Imidaclopride (21.2 mg/kg b. w)]:

The testes obtained from the animals of this group revealed mild histopathological changes when compared with the group treated with imidaclopride for the same period. Tunica albuginea of the testes of two animals appeared slightly thickened with congestion of sub-capsular blood vessels. Meanwhile, tunica albuginea of other animals in the same group appeared normal. Most of the seminiferous tubules were compact with each other in most animals (Fig.7)

Table (1): Effect of (NAC) administration on testicular oxidative stress markers [Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase ,(CAT) and Glutathione (GSH)] levels in IMI intoxicated male rats .

Parameter / Exp. group	MDA (nmol/gm)	SOD (u/g)	Catalase (u/g)	GSH (mmol/g)
Control (G1)	2.92 ± 0.146b	41.62 ± 2.023b	1.61 ± 0.068a	2.45 ± 0.024a
IMI (G2)	4.91 ± 0.518a	33.52 ± 4.656b	0.84 ± 0.167b	1.10 ± 0.019d
NAC (G3)	3.26 ± 0.300b	41.32 ± 1.791b	1.80 ± 0.072a	1.56 ± 0.082b
(NAC) +IMI (G4)	3.55 ± 0.362b	39.68±1.922b	1.84 ± 0.037a	1.47 ± 0.019bc

Data are presented as (Mean ± S.E). S. E = Standard error.Mean values with different superscript letters in the same column are significantly different at (P≤0.01). (n = 6) in each group.

Table (2): Comet assay parameters obtained by image analysis in cells of normal control group (G1) and treated groups (G2-4).

Parameter / Exp. group	Untailed cells (%)	Tail length (µm)	DNA in Tail (%)	Tail moment (U)
Control (G1)	98.5	1.38±0.12 ^{d}	1.54	2.12
IIM (G2)	71	9.42±0.68 ^{a}	8.11	76.40
NAC (G5)	97	1.54±0.11 ^{d}	1.68	2.59
(NAC) +IMI (G6)	88	4.51±0.21^{c}	3.56	16.06

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05). (n = 3) in each group.

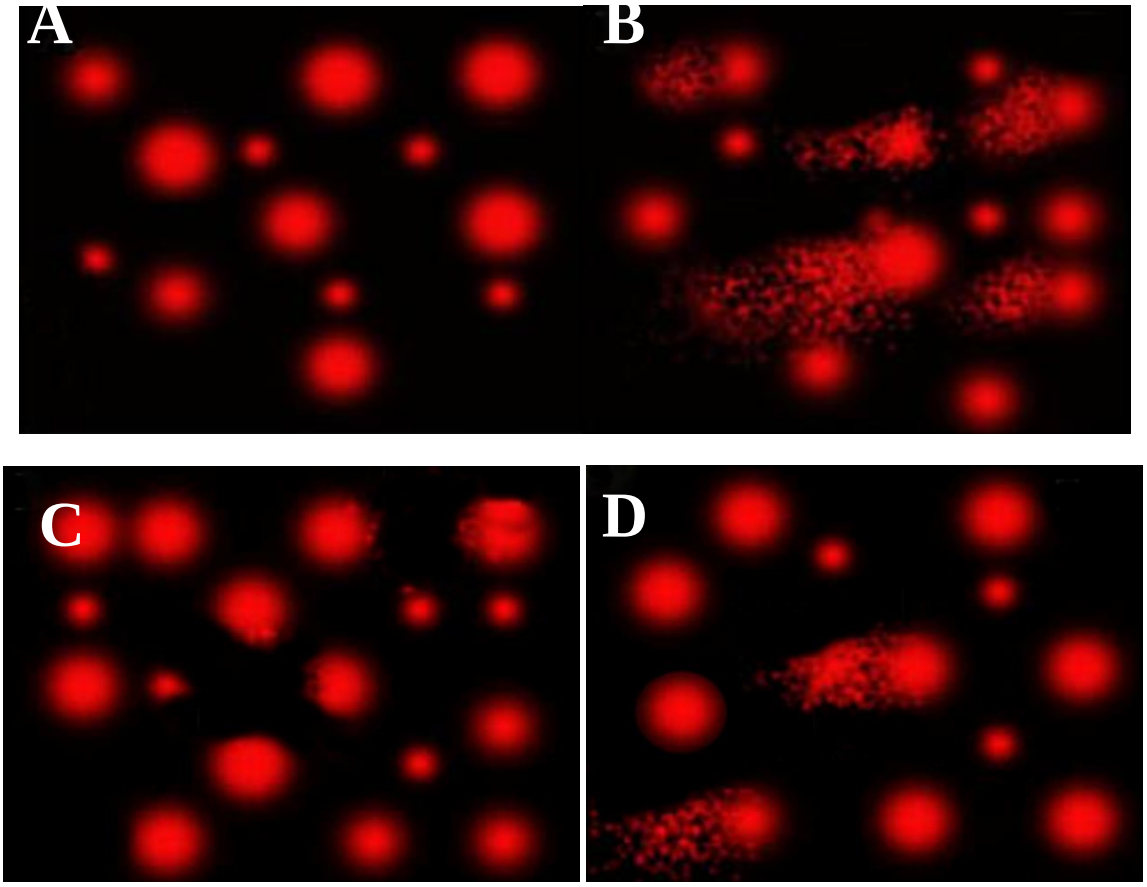

Figure (1): Photomicrographs representation of DNA damage in testicular tissues, using comet assay, in normal control group (A), Imidacloprid group (B), N-acetylcysteine group (C) and N-acetylcysteine + Imidacloprid (D).

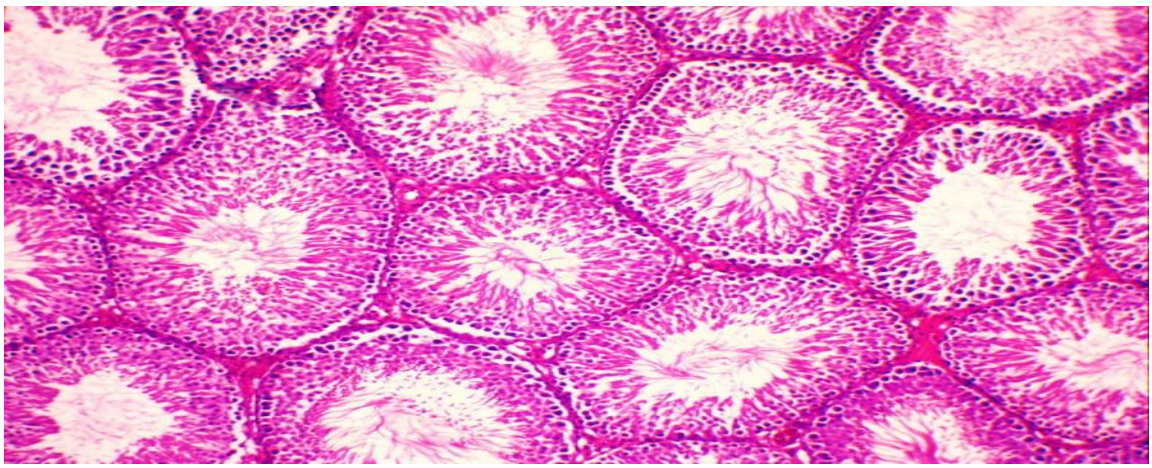

Fig. 2**.** Testes of control rat showing normal histological structures of seminiferous tubules and interstitial tissues. H&E stain x 100.

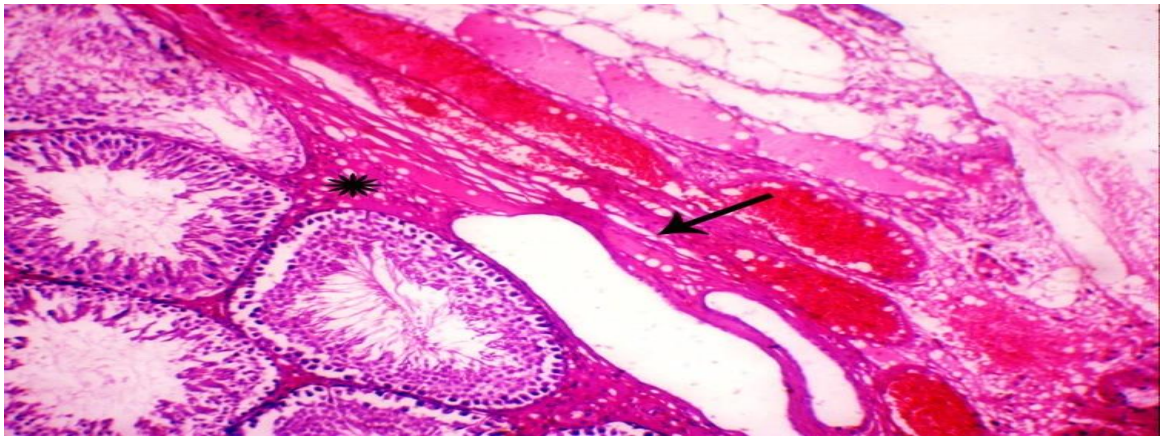

Fig. 3. Testes of rat**,** 8 weeks post administration of imidaclopride (21.2 mg/kg b.w), showing marked increase in the thickness of tunica albuginea (arrow) with sub-capsular edema (asterisk) and severe congestion of sub-capsular blood vessels. H&E stain x 200.

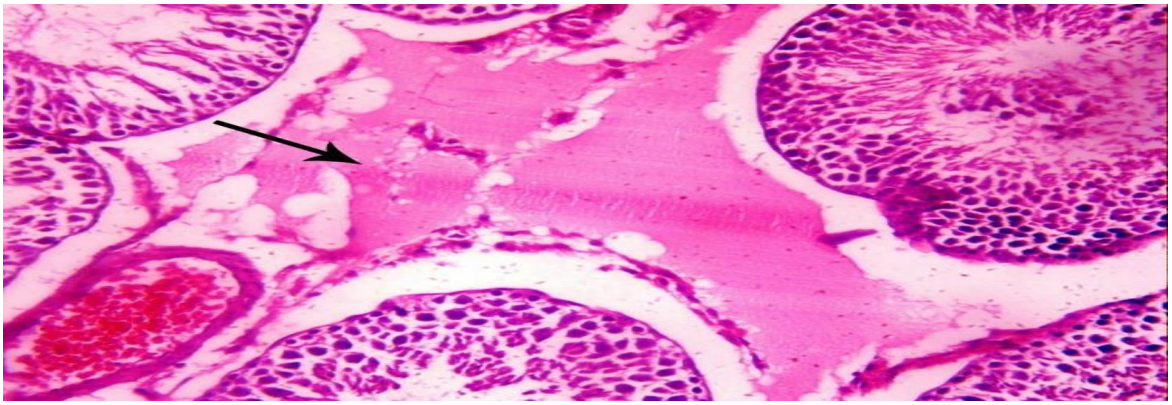

Fig. 4. Testes of rat**,** 8 weeks post administration of imidaclopride (21.2 mg/kg b.w), showing extensive inter-tubular edema (arrow).H&E stain x 200.

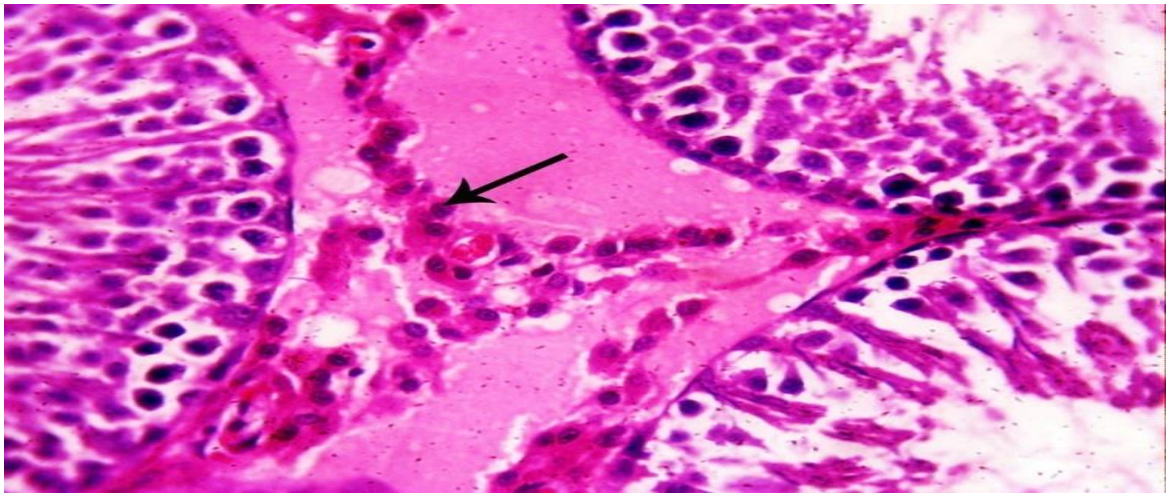

Fig. 5. Testes of rat**,** 8 weeks post administration of imidaclopride (21.2 mg/kg b.w), showing extensive inter-tubular edema admixed with interstitial cells (Leydig cells, arrow).H&E stain x 400.

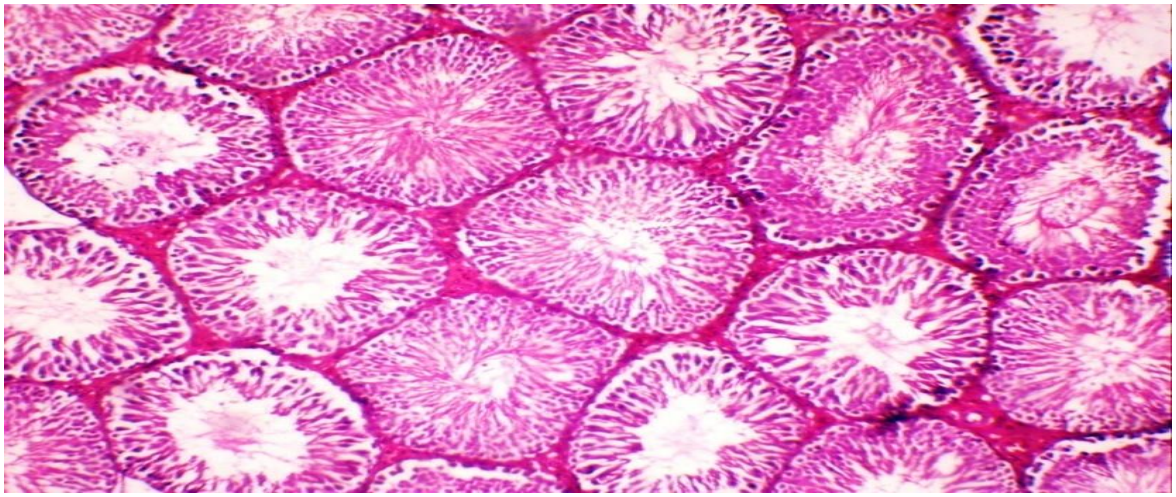

Fig. 6. Testes of rat**,**8 weeks post administration of N-acetylcysteine (200 mg/ kg b.w /day), showing Normal histological structure of the germinal epithelium of seminiferous tubules and interstitial tissues. H&E stain x 100.

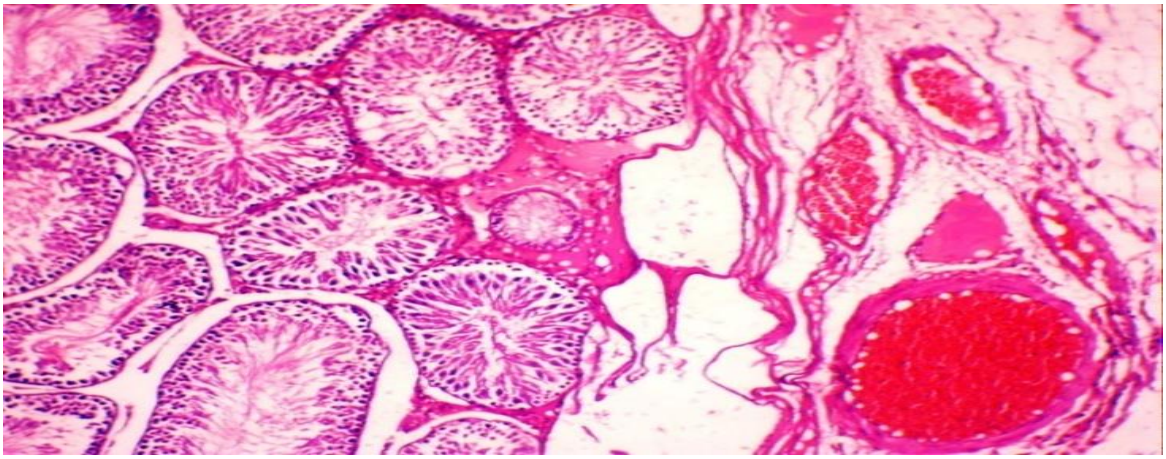

Fig. 7. Testes of rat**,** 8 weeks post administration of imidaclopride (21.2 mg/kg b.w) day after day) and daily oral administration with N-acetylcysteine (200 mg/ kg b.w /day) for 60 days, showing, slightly thickened Tunica albuginea with congestion of sub-capsular blood vessels. H&E stain x 200.

4. DISCUSSION:

It was observed that there is a positive association between pesticides exposures and alterations of male reproductive system functions (Yucra et al., 2006). Oxidative stress is a major causative factor responsible for the male reproductive failure (Kaur and Bansal, 2004). Reactive oxygen species (ROS) are

generated continuously in small amounts in normal cells since they are essential for many biological functions .If the metabolic production of ROS exceeds the capacity of the endogenous antioxidant defense systems, oxidative stress can occur (Spitz et al ., 2004) , ROS known to mediate many toxin-induced testicular injuries , its production causes DNA damage in the form of modification of all bases, and leads to production of base-free sites, DNA cross-links, and chromosomal re-arrangements (Gultekin et al ., 2000). Interaction of ROS with cellular membrane results in membrane lipid peroxidation which can be measured by using MDA, which is a marker of oxidative lipid damage and a major oxidative product of peroxidized polyunsaturated fatty acids (Zhang et al., 2004). Increased MDA suggests an increased production of free O2 radicals in rats (Mansour and Mossa, 2009). Enzymes such as SOD and CAT play an important role in the elimination of ROS produced during the biotransformation of xenobiotics and the induction of the SOD/CAT system may be the first defense mechanism against ROS (Lushchak, 2016).

The obtained results from table (1) showed that, IMI induced rats showed significant increase in testicular MDA and decrease in GSH, SOD and CAT levels when compared to normal control group. These results were nearly similar to Mohany et al. (2011) who demonstrated a significant increase in liver MDA activity in the IMI-treated male albino rats compared to the control group, with the highest levels recorded in the 1st and 3rd weeks. Similarly, decreased activity of SOD in rats was also reported with different pesticides like chlorpyrifos, , carbofuran and malathion, which shows decrease SOD activity in rats (Khan et al., 2005; Rai and Sharma, 2007; Mansour and Mossa, 2009).Moreover , our results go- hand- to hand with that of El-Kashoury and Tag El –Din (2010) who reported that oral administration of chlorpyrifos at dose of 21.4 mg/ kg b.w.for 28 days in male rats cause a significant increase in testicular MDA and decrease in GSH content incomparing to the control group.

These results are nearly similar to (Bal et al., 2012) who reported that the levels of testis MDA ($P < 0.05$) were significantly higher and those of GSH significantly ($P < 0.001$) lower in the rats treated with 8 mg/kg IMI than that in the controls.

In general, the inhibition of CAT activity has been related to the binding of toxicants to -SH groups of enzymes, increased H2O2 and/ or superoxide radical (Ruas et al., 2008). Thus, Navarro and Martinez (2014) suggested that the significant decrease in CAT activity in liver of P. lineatus might have resulted from its inactivation by the superoxide radical triggered by IMI exposure. The decrease activities of SOD, CAT and GSH content together with increase LPO may be attributed to induce free radicals in imidacloprid treated rats. Kapoor et al. (2010) had indicated that imidacloprid did not induce oxidative stress at 5 and 10 mg/kg b.w/day to female rats when exposed for 90 days. However, imidacloprid at 20 mg/kg b.w, / day had significantly induced oxidative stress to female rats. This may be due to dearrangement of cellular oxidative status as evidenced by

increased level of LPO (MDA), decreased activities of CAT, SOD, and reduced GSH level in vital tissues. Thus, on basis of parameters studied on that study, a dose 10 mg/kg body weight has been confirmed as No Observed Effect Level (NOEL) of imidacloprid in female rats.

GSH content significantly ($P<0.05$) decreased in imidacloprid-treated group (table, 1). These results are in agreement with Duzguner and Erdogan (2012) who suggested that imidaclopride caused oxidative stress and inflammation in central nervous system and liver in rats. The decreased brain GSH content observed in that study may reflect, at least partially, oxidation of GSH to glutathione disulfide (GSSG) due to the pesticide-induced generation of oxygen free radicals and their byproducts.

Pesticide also induces free radical generation that leads to DNA damage, protein degradation, LPO and finally culminating into damage to various vital tissues (Khan et al., 2005). These elevated free radicals and depressed antioxidant defense may lead to cell disruption, oxidative damage to cell membrane and hence increase susceptibility to LPO (Kapoor et al., 2009). Sperm DNA becomes susceptible to damage by three postulated routes: i. Improper packaging of DNA during spermiogenesis, ii. Oxidative stress and iii. Apoptosis (Tavalaee et al., 2009).

In present investigation, treatment of rats with imidacloprid increased DNA damage in rat's testis of G2 detected by comet assay (table 2). These results were nearly similar to wang et al. (2016) who detected that the sub-chronic exposure of imidacloprid caused DNA damage by increasing the percentage of DNA tail in comet assay after 7 days in earthworm Eisenia fetida.

Imidacloprid was found to induce DNA damage in a dose-related manner in earthworms as well as to increase the frequency of adducts in pesticide-treated calf thymus DNA, indicating agent-induced genotoxicity (Zang et al., 2000). Bal et al. (2012) observed that apoptosis and fragmentation of seminal DNA were higher in rats treated with imidacloprid by two doses 2 and 8 mg/kg/b.w. Excessive production of oxidants can result in oxidative damage, due to the oxidation of lipids, proteins and DNA in the cells (Ismail and Mohamed, 2012). Histological investigations revealed that there was a marked increase in the thickness of tunica albuginea with sub-capsular edema. This was supported by Najafi et al. (2010) who illustrated the increase of tunica albugina in thickness of mature wister rat's testis after day 30 of IMI exposure in the high dose (225 mg / kg b.w / group) and day 40 in the low dose (112 mg / kg b.w / group).

Protection with NAC to IMI intoxicated male rats (G4) caused a marked decrease in tecticular MDA (table, 1) and reduced DNA damage(table,2) when compared with IMI intoxicated rats (G2), while there was marked elevation in testicular tissue SOD, Catalase and GSH. These results confirmed by (Rao and Shaha, 2000) who recorded that NAC, which can serve as a precursor of GSH in the GSH biosynthetic pathway, increased germ cell and plasma GSH levels and was able to prevent several proapoptotic events including cytochrome c release, DNA fragmentation and ultimately cell death. GSH plays a very important role both during oxidative stress and during exposure to toxins**.** The mechanisms by which NAC reduces toxicity could be due to the capability of free thiol group of NACs to interact with electrophilic groups of ROSs generated in response to pesticides (Sen and

Packer, 2000). Our results were also supported by Cay et al. (2006) who suggested the protective effects of NAC in reducing cell death in testes against pesticide-mediated oxidative stress**.**

If a reduction in the intracellular levels of GSH was responsible for germ cell death, arguably, replenishing the levels of GSH by providing a substrate of the GSH biosynthetic pathway should result in the rescue of germ cells from undergoing apoptosis.

NAC may regulate expression of numerous genes by inhibiting activation of c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, redox sensitive activating protein, and nuclear factor B transcription factor. NAC directly modifies the activity of several enzymes (Zafarullah et al., 2003). NAC appears to restore GSH levels following experimental depletion but does not increase plasma GSH concentration under normal conditions (Burgunder et al.,1989). NAC protects against many aspects of radiation-induced oxidative damage by free radical scavenging activity rather than through its conversion to GSH (Neal et al., 2003). NAC could have exerted its effect by several mechanisms, such as scavenging ROS, regulating transcription of genes involved in the maintenance of cellular redox homeostasis, reducing antioxidative enzymes containing redox-sensitive cysteine residues, and/or enhancing intracellular biosynthesis of GSH. Treatment with N-acetylcysteine exhibited moderate improvement in the course of testicular degeneration produced by imidaclopride via improving the histopathological alterations in the seminiferous tubules, interstitial tissues and tunica albuginea, which reverted nearly to their normal histological structure this was supported by(Rao and Shaha, 2002) who reported Close-up of areas of seminiferous epithelium showing staining of different cell types in the seminiferous epithelium after NAC treatment by intraperitoneally injection at dose of 150 mg/kg of body weight for 3 days before challenging with MAA .

5. CONCLUSION:

The present study demonstrated that, administration of NAC relieved harmful effects caused by exposure to IMI induced testicular damage. IMI affected male reproductive organs mainly testis and these occurred through changes in several parameters. IMI induced testicular damage as it caused significant increase in testicular tissue MDA, a significant reduction in testicular tissue SOD, catalase, GSH, DNA damage and histopathological changes. NAC is able to ameliorate the toxic effect of IMI by restoring all the affected parameters to near its normal levels and alleviate the degenerative changes of the testicular tissues. So, these results confirmed the strong antioxidant, anti-inflammatory effects of NAC in testicular damage.

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