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Research Article

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The Possible Protective Role of N-acetylcysteine against Testicular Toxicity Induced by Paracetamol Overdose in Adult Male Rats

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Abstract

Many substances, even medicines with proven therapeutic benefits, can harm cells by metabolically activating them into extremely reactive substances. Paracetamol is one of the most widely used over-the-counter analgesics. This study examines the harmful effects of paracetamol on the lipid peroxidation process in testes homogenates as well as enzymatic and non-enzymatic antioxidant activities. Also, examine the effects on male hormones and sperm count. The study also assesses if N-acetylcysteine protects against testicular damage induced by paracetamol excess. Forty mature male albino rats were created. Group 1 as a control, Group 2 paracetamol (650 mg/kg), Group 3 NAC (150 mg/kg), and Group 4 both paracetamol and NAC. Samples of blood and testicles were taken after 15 days to measure sperm and testicular biochemistry. Testicular tissues had considerably higher amounts of MDA and H2O2. SOD, GSH, and CAT levels significantly decreased. FSH and LH rise. On the other hand, testosterone levels decrease following paracetamol exposure. The administration of NAC generated changes in testosterone levels, FSH, LH, and antioxidant enzymes. The sperm morphology showed an increase in abnormalities but a significant decrease in motility and count. NAC effectively lowers the toxicity of paracetamol to the testicles while restoring biomarkers associated with normal testicular function.

Keywords: Sperms, Paracetamol, Testis, Albino Rats, N-Acetylcysteine.

INTRODUCTIO

Many substances, even medicines with proven therapeutic benefits, can harm cells by metabolically activating them into extremely reactive substances. Paracetmol is one of the most widely used over-the-counter analgesics. Called chemically N acetyl p aminophenol, paracetamol (PCM), also referred to as acetaminophen, is a moderate analgesic medication. It is frequently used to treat mild aches and pains, including headaches. It is also a key component of many cold and flu medicines. When combined with opioid analgesics, paracetamol is used to treat more severe pain, such as pain following surgery, and to give palliative care to patients with advanced cancer (Olaniyi and Agunbiade, 2018).

Paracetamol was introduced by Merring. (1893) as a potential analgesic drug. However, its effectiveness as a therapeutic drug was only realized in the 1960s. Its use has increased with time leading to its use as a combination in many other drugs. Even though paracetamol is used to

treat inflammatory pain, its limited antiinflammatory activity prevents it from being considered a nonsteroidal anti-inflammatory medicine (NSAID). (Jóźwiak-Bebenista and Nowak, 2014).

When paracetamol is taken therapeutically, the liver is mainly responsible for its metabolis this process produces metabolites that are easily eliminated through the kidney and do not cause any harm (El-Maddawy and El-Sayed, 2018). Hepatic cytochrome P450 isoenzymes bioactivate a part of paracetamol to form the hepatotoxic reactive metabolite N-acetyl-para-benzoquinone imine (NAPQI). (Koling *et al.,* 2007). The conjugation of hepatic glutathione (GSH), which is likewise safely eliminated by bile, quickly quenches NAPQI. However, due to insufficient glucuronidation and sulfation, high doses overwhelm the paracetamol detoxication pathways (Adil *et al*., 2016).

90% of the drug's administered dose (the therapeutic dose) is metabolized in the liver where it is conjugated in glucuronide and sulphate. The remaining drug is then hydroxylated to form N-Acetyl P Benzoquineime (NAPQI) (5-10%), a highly reactive oxidative product that conjugates with glutathione and GSH to form mercpturicacid, which is excreted in urine (Grahame-Smith and Aronson, 2002). Overdosing paracetamol alters reproductive parameters and produces harmful substances in the organs, such as hepatotoxicity, renal toxin, and testicular toxin (Radosavljevic *et al*., 2010). Elevted dosages of paracetamol seem to impact the masculine reproductive system, altering the quality of semen, namely the morphology of sperm and consequently their capacity to fertilize (Khayyat, 2021).

Early in the 1960s, N-acetylcysteine (NAC) was shown to have therapeutic value. Natural sources do not include this medication (Larsson *et al*., 2015). Furthermore, it is recognized as an antioxidant that directly benefits hepatic tissue by raising intracellular GSH (Ribeiro *et al*., 2011). It has an ideal thiol redox state, which is crucial for maximizing the cell's capacity to fend against inflammation and oxidative stress (OS). The amino acid N-acetyl cysteine has a thiol group. Because of its dual roles as a sulfhydryl (-SH) donor and a nucleophile, NAC has a protective effect against the toxicity of chemicals (Wang *et al*., 2013 and Mokhtari *et al*., 2017). When taken orally, NAC is absorbed in the stomach and intestines before traveling through the portal vein to the liver.NAC rapidly integrates peptides in the liver to produce a variety of metabolites and proteins (Lasram *et al*., 2015).

NAC exists in plasma in both reduced and different oxidized forms. Furthermore, it underwent oxidation to produce diacetylcystine, a disulfide. It has the potential to react with other low molecular mass thiols, such as glutathione and cysteine, to generate mixed disulfides. Furthermore, NAC may undergo oxidation through redox interactions with the plasma proteins' thiol groups. When given orally to rats, NAC is absorbed; just 3% of NAC is expelled in the feces. (Dodd *et al*., 2008).

De Andrade et al. (2015) state that cysteine is released and taken up by amino acid transporters into cells as a result of extracellular deacetylation of NAC. It is hypothesized that the production of GSH requires free cysteine. NAC prevents apoptosis and oxygen related genotoxicity in endothelial cells, which in turn increases intracellular glutathione levels and decreases mitochondrial membrane depolarization (Amin *et al* ., 2008; Elgindy *et al*., 2010).

Because it is a precursor to glutathione, one of the most significant naturally occurring antioxidants, NAC has antioxidant properties. N-acetylcysteine has a variety of pharmacological potentials for prophylaxis and therapy, including anti-inflammatory (Uraz *et al*., 2013) and antiox-

idant (Ahmed *et al*., 2011) effects. By scavenging free radicals and raising cellular GSH, they exercise their strong antioxidant properties and guard against lipid peroxidation (Dhouib *et al*., 2016). According to İçer *et al*. (2016), N-acetylcysteine has protective properties against hepatotoxicity induced by paracetamol.

According to El-Maddawy and El-Sayed (2018), it successfully maintained and restored liver, kidney, and testicular functions while preventing oxidative damage caused by paracetamol. According to Nencini *et al*. (2007), oxidative stress is caused by an imbalance between free radicals that can cause protein oxidation, DNA fragmentation, and lipid peroxidation, and reactive oxygen and nitrogen species (ROS and RNS) that are produced and scavenged. Protein structural and functional alterations, gene mutations, and a loss of membrane integrity are the outcomes of these damages (Reddy *et al*., 2009).

According to Sharma *et al*. (2011), oxidative stress plays a crucial role in several illnesses. Because the liver is the primary organ engaged in the body's detoxification of several medications and xenobiotics, it plays a critical role in the regulation of numerous physiological processes. Additionally, extra-hepatic organ damage such as brain impairment, kidney failure, and testicular failure can result from systemic oxidative stress that escalates with liver disease (Palma *et al*., 2014).

MATERIALS AND METHODS

Experimental animals

40 mature male albino rats weighing between 150 and 200 grams were acquired from the Animal House, Alexandria University, Egypt's Medical Technology Center and Research Institute. Before beginning the experiments, the animals were kept in plastic cages in an environmentally controlled room with a 12-hour light/dark cycle and a constant temperature of 2527°C. They were fed a standard rat diet consisting of 24% protein, 5% fat, 4% fiber, 55% carbohydrates, 0.6% calcium, 10% moisture, and 9% ash for ten days.

Chemicals

Sigma Chemical Co., St. Louis, MO, USA, provided the N-acetylcysteine (C5H9NO3S) 2-Acetamido-3-sulfanylpropanoic acid that was purchased. The supplier of paracetamol (C8H9NO2) was GlaxoSmithKline, Dungarvan Ltd. in Ireland. The highest purity and analytical grade were possessed by all other substances and solvents needed for the biochemical tests.

Experimental design

This study was carried out on 40 male rats, who were randomly divided into 4 equal groups (10 rats each) as follows: Group 1 (control group): Rats were administrated 1 ml distilled water by esophageal gastric syringe daily. Group 2 (Paracetamol group): Rats were administrated with 650mg/kg.b.w Paracetamol dissolved in 1ml distilled water by esophageal gastric syringe daily. Group 3 (N-acetylcysteine group): Rats were administrated 150mg/kg.b.w NAC dissolved in 1ml distilled water daily by esophageal gastric syringe. Group 4 (paracetamol + N-acetylcysteine): Rats were administrated (650mg/kg) Paracetamol daily after one hour followed by a dose of NAC (150mg/kg) by as in groups 2, 3. Two weeks passed during the experiment. The animals in the experiment were monitored for signs of death. The amounts of N-acetylcysteine and paracetamol were as per (Yousef *et al*., 2010).

Determination of antioxidant enzymes and oxidative stress in testes tissues

Testicular whole tissues were acquired through dissection, followed by a physiological saline wash and weighing. Next, a part of each rat's testicular tissue was kept in storage at 20°C. The piece to be

remembered was chopped and mixed thoroughly in 510 milliliters of cold buffer (potassium phosphate, 50 mM, pH 7.4, and ethylene diamine tetraacetic acid (EDTA). According to Goldberg and Spooner (1983), homogenates were centrifuged at 10,000×g for 20 minutes at 4°C. The clear supernatants were then utilized for MDA, glutathione peroxidase, superoxide dismutase, hydrogen peroxide, and catalase analyses.

Determination of serum testosterone level

The competitive inhibition enzyme immunoassay method is used in this assay. On a microplate, a monoclonal antibody that is specific to rat T has been pre-coated. Using the pre-coated rat T-specific antibody, a competitive inhibitory response is initiated between biotin-labeled rat T and unlabeled rat T (Calibrators or samples). The unbound conjugate is removed after incubation. Each microplate well is then filled with avidin conjugated to horseradish peroxidase (HRP), and the mixture is incubated. The concentration of T in the sample is inversely proportional to the amount of bound HRP conjugate. Following the addition of the substrate solution, the color created has an inverse relationship with the sample's T concentration.

Determination of serum Follicle stimulating level

Monoclonal anti-FSH antibody-coated wells are used to incubate biotin-conjugated anti-FSH and standard or sample in the Rat FSH ELISA Kit. Horseradish peroxidase (HRP) conjugated avidin is added and incubated for 30 minutes after washing and incubating for 15 to 18 hours. After washing, the HRP complex that was left in the wells reacted for 20 minutes with a chromogenic substrate (TMB). The reaction was then stopped by adding an acidic solution, and the absorbance of the yellow result was measured using spectrophotometry at 450 nm (the sub-wavelength is 620 nm). The absorbance and FSH concentration are almost directly correlated. Plotting absorbance versus standard FSH concentrations creates the standard curve. Using this standard curve, the FSH concentrations in unknown samples are ascertained.

Determination of serum luteinizing level

To detect LH in Shibayagi's Rat LH ELISA Kit, wells coated with monoclonal anti-LH antibody are treated with standards or samples. The biotin-labeled anti-LH antibodies is added and incubated for an additional hour to bind with captured LH after two hours of incubation and washing. Following washing, avidin labeled with horse radish peroxidase (HRP) is applied and incubated for half an hour. Following washing, the HRP complex that was left in the wells reacted for 20 minutes with a chromogenic substrate (TMB). The reaction was then stopped by adding an acidic solution, and the absorbance of the yellow result was measured at 450 nm using spectrophotometry. The absorbance and LH concentration are proportionate. Plotting absorbance versus standard LH values creates the standard curve. This standard curve is used to calculate the LH concentrations in unknown samples.

Quantitative and qualitative analysis of sperms

Collection of epididymis sperm and sperm function test: When the experiment concluded, all of the animals were given dimethyl ether without authorization, and their epididymis was removed right away. The caudal epididymis was utilized for sperm analysis. In short, Gray *et al*. (1989) described the process of collecting epididymis sperm by slicing the caudal epididymis. After cutting the epididymis with a sharp razor blade in 5 milliliters of physiological solution, it was incubated for 5 minutes at 35 degrees Celsius. Sperm obtained in the medium were used to measure sperm motility, count, and abnormalities after multiple washings. Sperm Vision TM CASA System (Eclipse E-200 Nikon Co., Japan) computer-aided semen analysis was used to measure these parameters. Krause (CASA). (1995).

Sperm motility, count and abnormalities

An Eclipse E-200 phase contrast microscope from Nikon Co., Japan, with a heat plate and Sperm Class Analyzer® software (SCA, full research version 5.1 from Microptic Co., Barcelona, Spain) comprised the CASA system. A video camera (Basler Vision, A312FC, Technologies' Co., Ahrensburg, Germany) with an X = 20 magnification was used to record the images at 50 frames per second. Within five minutes of the sperm suspension's separation from the epididymis, it was analyzed for this purpose. A 4 microliter sample of the sperm suspension was obtained and pipetted into a 10 Microliter makler counting chamber (Sefi-Medical Instruments, Germany). Before analysis, the loaded chamber was heated to 37°C on the microscope plate for three minutes. Next, a Nikon microscope was used to examine each sample. The following criteria were assessed for each rat: the proportion of motile and abnormalities, the sperm count expressed as (million/ml) under the last ten distinct and randomly selected fields.

Sperm morphology

Study of the morphology of sperm to assess the sperm morphological anomalies, smears were prepared using a portion of the sperm suspension. By Rezvanfar *et al*. (2008), a single drop of sperm suspension was added to an equivalent volume of 1% eosin-y 5% nigrosin, mixed, and smears were formed on clean glass slides and air-dried. The spermatozoa's abnormalities were assessed using a light microscope with an X= 400 magnification. Any deviation from the normal in the head, tail, or both morphology and structure was regarded as aberrant.

Statistical analysis

The mean ± SE is used to express the values. One-way analysis of variance was used in the statistical computation of the results using the statistical package for social sciences (SPSS software package, version 15). To compare groups, post hoc analysis of variance (ANOVA) testing was done. Regarding the LSD use. Significant differences were defined as $P<0.05$ (Howell, 1995).

RESULTS

Enzymatic and non-enzymatic antioxidants activities in the testis

Effects of paracetamol on both non-enzymatic and enzymatic antioxidants testis tissue levels of glutathione reduced (GSH), superoxide dismutase (SOD), and catalase (CAT) were displayed in Table 1 and Figures 1-3. There was a notable reduction in the activities of CAT, SOD, and GSH in the testes of rats administered PCM in comparison to the control group. A significant drop was obtained in CAT, SOD and GSH levels in rats treated with PCM. Treatment with NAC in combination with PCM significantly increased the activities of CAT, SOD and GSH levels ($P<0.05$) as compared with the PCM group.

Table: (1). Effect of N-acetylcysteine, paracetamol and their combination on enzymatic and non-enzymatic antioxidants in the testes of rats.

Parameters	Experimental groups			
	Control	PCM	PCM+ NAC	NAC
Catalase(CAT) (u/mg)	36.40±4.77	13.40±4.16[a]	26.80±6.65[ab]	37.60±5.90
Superoxide Dismutase (SOD) (u/mg)	64.40±6.58	22.40±7.50[a]	50.40±6.11[ab]	61.20±10.03
Glutathione reduced (GSH) (u/mg)	36.80±5.54	11.80±2.59[a]	33.40±5.22[b]	36.00±6.60

* Noteworthy at the 0.05 level. The values show the mean (± SE) of seven samples. (a) Means show a significant difference ($P<0.05$) from the control group. (b) Means show a significant difference ($P<0.05$) from the paracetamol group.

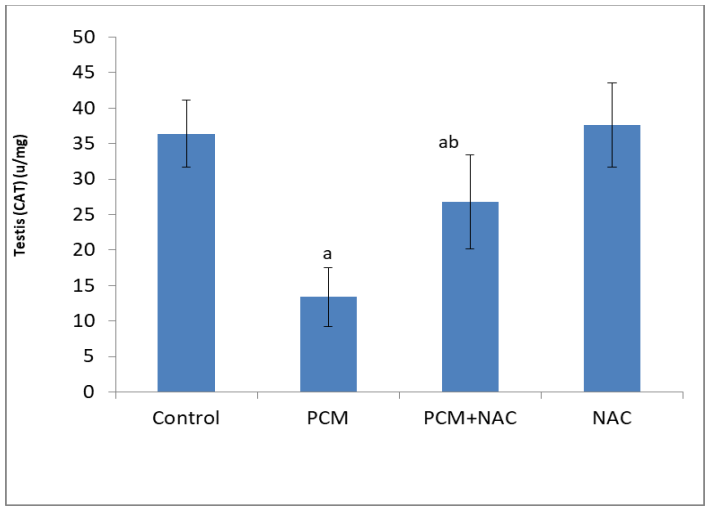

Figure: (1). Effect of NAC on testis homogenate's Catalase (CAT) level in rats given paracetamol in experimental groups.

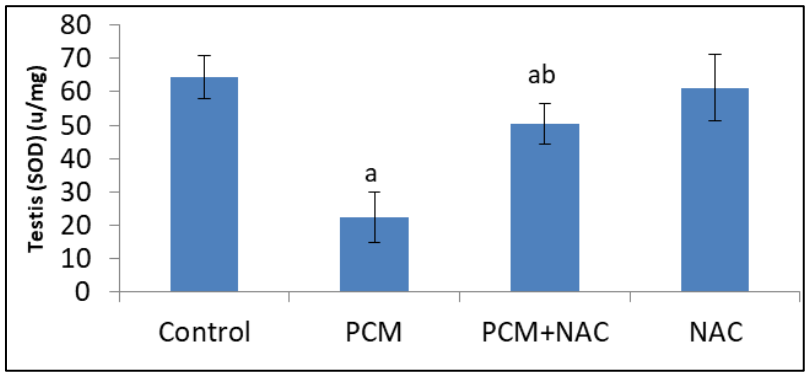

Figure: (2). Effect of NAC on testis homogenate glutathione reduced (GSH) levels in rats given paracetamol in experimental groups.

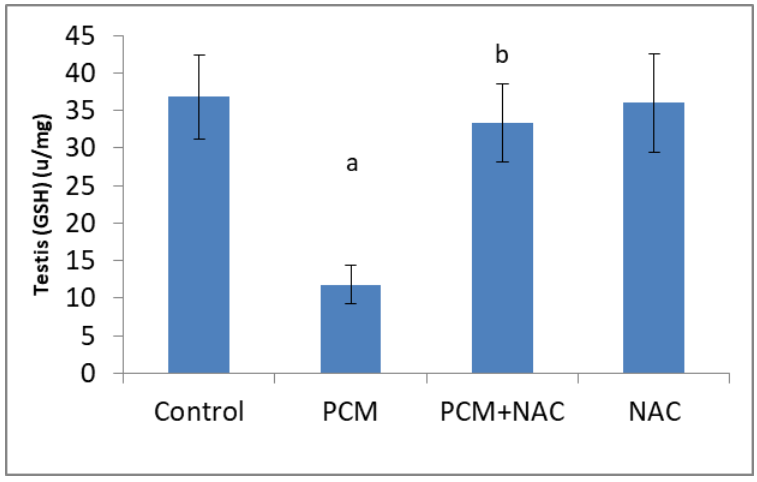

Figure: (3). Effect of NAC on testis homogenate levels of Superoxide Dismutase (SOD) in rats given paracetamol in experimental groups.

Measurement of hydrogen peroxide (H2O2) and malondialdhyde (MDA):

As a sign of free radical-mediated damage in testis tissue, the amount of hydrogen peroxide (H2O2) and lipid peroxidation end product (MDA) in the homogenate of the testes was measured. When compared to the control group, the PCM treated group showed a substantial rise in MDA and

H2O2. When compared to the group that received paracetamol, those who received NAC in addition to PCM demonstrated a partial recovery. (Figures 4,5), Tables 2.

Table: (2). Effect of N-acetylcysteine, paracetamol and their combination on lipid peroxidation tests in the testes of rats.

Parameters	Experimental groups			
	Control	PCM	PCM+ NAC	NAC
(MDA) testis tissue (nmol/g)	11.40±3.05	33.20±6.72[a]	21.00±3.16[ab]	9.20±3.11
H2O2(mM/g)	16.36±6.36	30.02±8.06[a]	20.20±4.76[b]	8.28±1.38

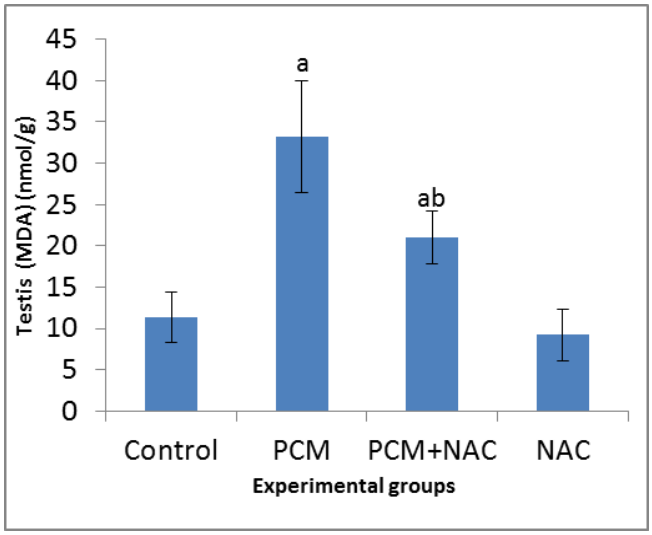

Figure: (4). Effect of NAC on testis homogenate levels of malondialdehyde (MDA) in rats given paracetamol in experimental groups

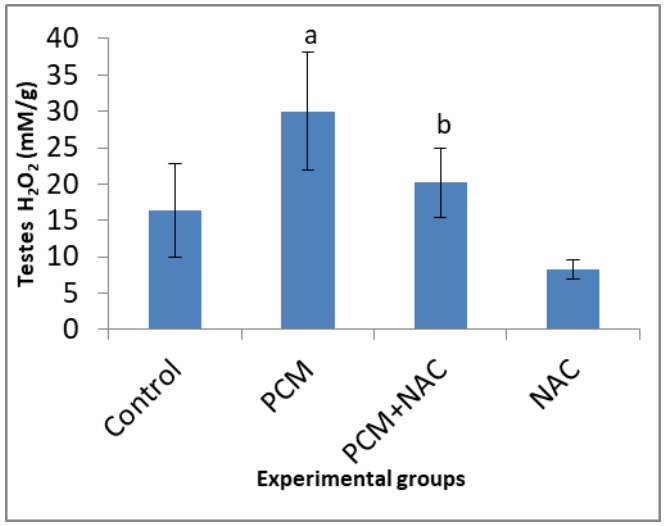

Figure: (5). Effect of NAC on Hydrogen peroxide (H2O2) level of testis homogenate in experimental groups of rats treated with paracetamol.

Determination of sex hormones

Rats receiving PCM showed a significant ($P < 0.05$) drop in testosterone levels when compared to the control group. The drop in testosterone levels was regulated by the combination of PCM and NAC therapy. Rats treated with APAP showed a significant ($P < 0.05$) rise in FSH levels when compared to the control group. When PCM and NAC were used together, the FSH level somewhat

recovered. The results of this investigation showed that the LH value increased significantly (P≤ 0.05) when treated with paracetamol in comparison to the control value. The LH value of rats given oral PCM+NAC therapy improved somewhat. Figures (6-8) and Table (3).

Table: (3). Effect of N-acetylcysteine, paracetamol and their combination on hormones tests in the testes of rats.

Parameters	Experimental groups			
	Control	PCM	PCM+ NAC	NAC
Testosterone(pg/ml)	4.86±0.76	1.32±0.52[a]	2.92±0.94[ab]	4.32±0.78
FSH(ng/ml)	0.37±0.07	0.78±0.10[a]	0.52±0.05[ab]	0.28±0.06
LH(ng/ml)	0.83±0.13	1.35±0.23[a]	0.90±0.13[b]	0.68±0.09

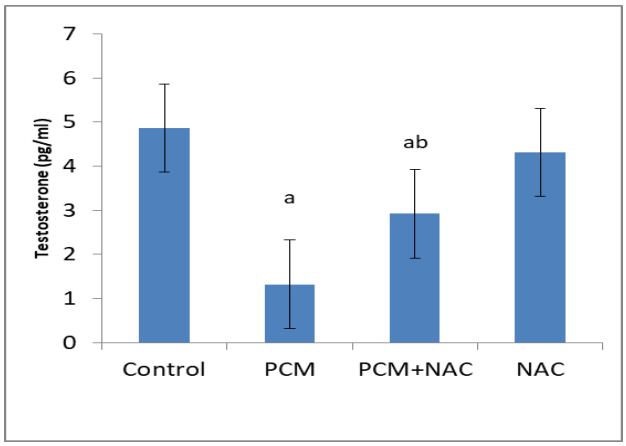

Figure: (6). Effect of NAC on testosterone hormone levels in experimental groups of rats treated with paracetamol.

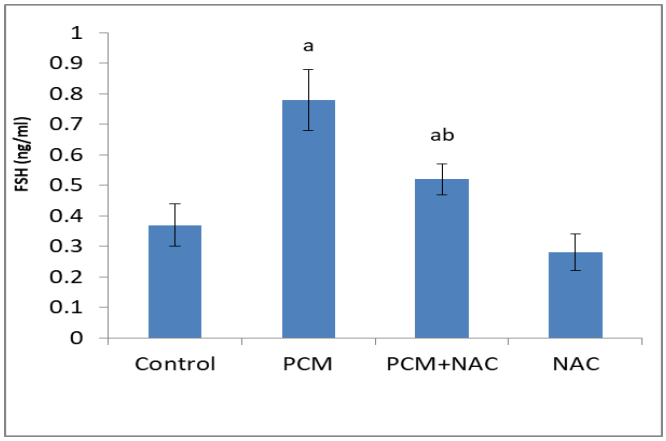

Figure: (7). Effect of NAC on the level of follicle-stimulating hormone (FSH) in rats given paracetamol in experimental groups.

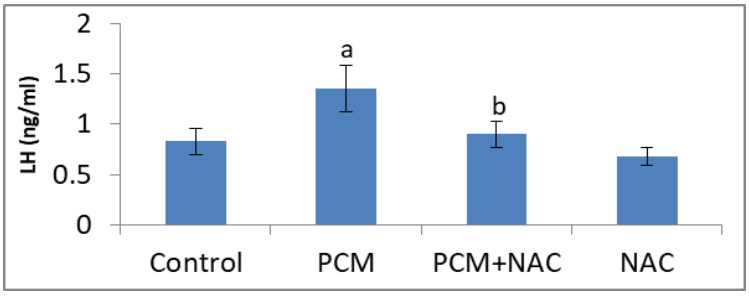

Figure: (8). Effect of NAC on levels of luteinizing hormone (LH) in rats given paracetamol in experimental groups.

Quantitative analysis and qualitative analysis of sperms

The sperm count, motility, and abnormalities treated with paracetamol, N-acetylcysteine, and their combination are displayed in Table (4) and Figures (9-12). Sperm count significantly decreased ($P < 0.05$) in male rats treated with PCM. When combined with PCM, NAC therapy significantly increased the number of sperm. When compared to the control group, the sperms' motility significantly decreased following the injection of paracetamol. Sperm motility was significantly reduced by administering NAC in addition to PCM. According to the current study, there was a significant increase ($P < 0.05$) in sperm abnormalities in the PCM-treated group as compared to the control group. The abnormality level was significantly reduced ($P < 0.05$) when (NAC) and (PCM) were administered together.

Table: (4). Effect of N-acetylcysteine paracetamol and their combination on sperm characteristics in the testes of rats.

Parameters	Experimental groups			
CASA	Control	PCM	PCM+NAC	NAC
Sperm Count (million/ml)	86.60±6.11	17.60±4.04[a]	61.40±7.77[ab]	83.60±16.1
Sperm Motility (%)	84.00±5.48	16.80±2.49[a]	52.40±14.60[ab]	82.40±8.44
Sperm Abnormality (%)	12.20±2.39	54.60±3.78[a]	34.40±7.02[ab]	10.80±5.40

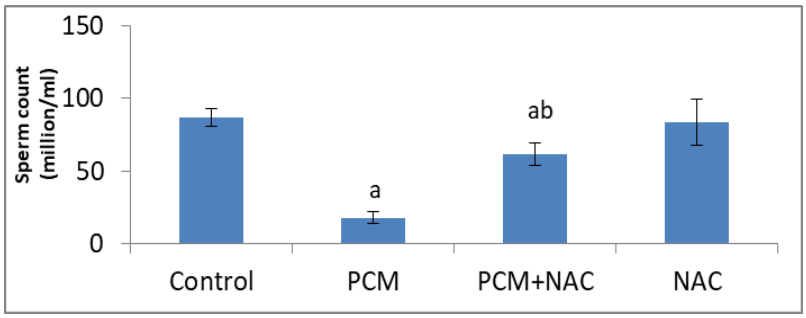

Figure: (9). Effect of NAC on sperm count in rats receiving paracetamol in an experimental setting.

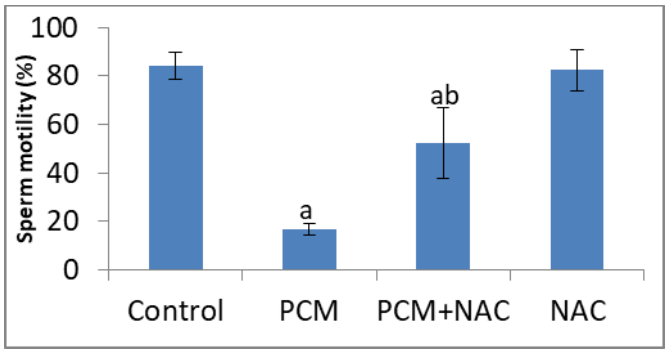

Figure: (10). Effect of NAC on motility of sperms in experimental groups of rats treated with paracetamol.

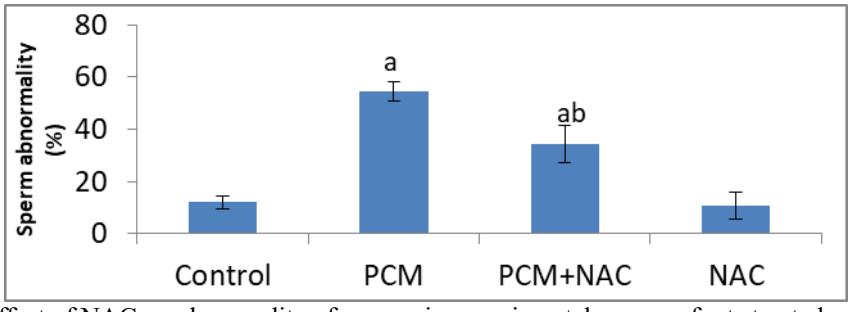

Figure: (11). Effect of NAC on abnormality of sperms in experimental groups of rats treated with paracetamol.

DISCUSSION

Important antioxidant markers are the CAT, GSH, and SOD. The body's antioxidant capacity shields the body against harm brought on by oxidative stress (Canayakin *et al*., 2016). Two crucial components of the toxicity process are the heightened production of reactive oxygen species and oxidative stress (Hinson *et al*., 2010). Lipid peroxidation (LPO) increases when oxidative stress increases due to the antioxidant enzymes' depletion, which scavenges harmful superoxide and hydrogen peroxide radicals (Kisaoglu *et al*., 2014).

Key antioxidant defense system enzymes, SOD and GSH, help detoxify reactive chemicals or repair the damage they cause to cells (Whidden *et al*., 2011). Lipid peroxidation is slowed down by natural antioxidants like SOD and CAT, according to Kisaoglu *et al*. (2014). While CAT directly neutralizes the increased H2O2 during oxidative stress, it also shields cells from the damaging effects of superoxide radicals. Where there is a large concentration of H2O2, catalase functions more efficiently.

The current study's findings showed that, in comparison to the control group, oral paracetamol administration was linked to a decrease in the activity of antioxidant enzymes (CAT, GSH, SOD) and an increase in MDA and H2O2 in testis tissues. However, when compared to the paracetamol group, these data showed a considerable improvement in the rats treated with PCM+NAC. These findings concurred with those of Mohammed & Sabry (2020); Kisaoglu *et al*. (2014), and Yousef *et al*. (2010). Testicular injury resulted from an increase in lipid peroxidation (Morsy *et al*., 2012; Lonare *et al*., 2016).

Yayla *et al.* (2014), indicated that an increase in antioxidant enzymes was accompanied by a significant reduction in the GSH levels. According to Karakus *et al*. (2013), oxidative stress and the loss of glutathione, coupled with an increase in the production of reactive oxygen species (ROS) and high doses of paracetamol, are crucial components of the toxicity process. Additionally, N-Acetyl P Benzo-quinine (NAPQI) causes the testis' intracellular GSH levels to decrease (EI-maddawy and EI-sayed, 2018).

Conversely, the testes are the site of the paracetamol toxicity mechanisms through metabolizing enzyme activity. According to Saito *et al.* (2010), the testes have lower relative levels of P450 against glutathione transferase and glutathione than the liver. Reactive metabolites produced in the liver are therefore unlikely to be transferred to the testicular cells. Ravinder Singh *et al*. (2011) discovered that glutathione was depleted in the testes as a result of paracetamol exposure, which is consistent with this theory. Furthermore, testicular toxicity was found to be generated by a mechanism other than the production of a reactive metabolite (Morakinyo *et al*., 2010). According to Heard (2008) and Olaleye and Rocha (2008), long-term or excessive usage of paracetamol can have negative consequences, such as altered testicular anatomy and decreased capacity for reproduction.

The testis antioxidant levels of CAT, GSH, and SOD were significantly elevated when NAC was administered, while testis MDA and H2O2 levels were significantly decreased. This indicated that the rats receiving PCM were better able to fend off the oxidative stress caused by paracetamol. These findings are in line with those of Morsy *et al*. (2012), Kisaoglu *et al*. (2014), and Lonare *et al*. (2016), who reported that because NAC possesses antioxidant and free radical scavenging properties, it can prevent testicular dysfunction and encourage the regeneration of injured cells.

Numerous investigations demonstrated that NAC might lower lipid peroxidation and restore a reduced level of antioxidant ability. In the current investigation, NAC stopped antioxidant enzyme depletion, including GSH. Additionally, this outcome agrees with Rushworth and Megson's work.

(2014). De Andrade *et al*. (2015) investigated that, the medication NAC's possible therapeutic applications as well as its capacity to fend off the toxicity brought on by a paracetamol overdose.

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are crucial for reproduction and sperm production by stimulating testosterone hormone release. Positive and negative regulatory mechanisms govern the activity of LH and FSH. Gonadotropin-releasing hormone (GnRH) is secreted by hypothalamic neurons. This hormone binds to gonadotroph receptors in the pituitary and stimulates gonadal secretion of LH and FSH. This, in turn, causes the testes gland to synthesize and release testosterone hormone, which in turn stimulates the gonadal secretion of the sex hormone testosterone (Tilbrook and Clarke, 2001).

In mammals and other vertebrates, the primary male sex hormone is testosterone, which is produced in the testes Leydig cells. According to Jensen *et al*. (2010), the primary functions of testosterone are to promote spermatogenesis and the secretion of the accessory sex glands. Numerous studies have demonstrated that testosterone withdrawal from the rat testes causes increased germ cell death, which in turn leads to diminished reproductive capabilities. Adult mammalian spermatogenesis is a testosterone-dependent process (El-Sharaky *et al*., 2010).

According to the current research, serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have significantly increased, but a drop in testosterone levels following the use of paracetamol. This is consistent with the findings of Mohammed & Sabry (2020) and Albert *et al*. (2013), who found that paracetamol exposure dramatically, reduced testosterone secretion.

According to Hassan (2013), male rabbits given large doses of paracetamol for an extended period experienced a significant drop in blood testosterone levels. The paracetamol toxicity mechanism, which is demonstrated by a significant elevation in the lipid peroxidation biomarker (MDA) and a reduction in the antioxidant molecules (CAT and GSH) in the testicles, is the cause of the decreased testosterone production caused by increased testicular oxidative stress. (Kheradpezhouh *et al*., 2010; Karthivashan *et al.,* 2016; Olaniyi and Agunbiade ., 2018).

Olaniyi and Agunbiade (2018) proposed a further explanation for the decrease in testosterone levels, stating that it can be brought on by an increase in gonadotropic hormones (FSH and LH) through a negative feedback process that affects the pituitary and hypothalamus.

According to research by Jensen *et al*. (2010); Kristensen *et al*. (2010, 2012), Snijder *et al*. (2012); Lind *et al*. (2013), and Mazaud-Guittot *et al*. (2013), paracetamol may be regarded as an endocrine disruptor that affects the development of the male reproductive system and the generation of testicular hormones.

According to Garu *et al*. (2011), LH and FSH activity depends on both the quantity of these hormones and the number of certain receptors in the testes. The manufacture and secretion of androgens, which are essential for male development and reproductive function, are carried out by the Leydig cells of the testes. Boekelheide (2005) stated that a decrease in the number of Leydig cells results in a fall in testosterone levels. Leydig and Sertoli cells, as well as the germ cells themselves, are the three primary target cells in the testes for toxicants that impair spermatogenesis.

In opposition to Olaniyi and Agunbiade (2018), there is another study found that the use of paracetamol increased levels of testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH). They found that testicular dysfunction was brought on by paracetamol's altered oxidative stress.

Follicle stimulating-hormone (FSH), luteinizing hormone (LH), and testosterone levels improved in the PCM+NAC-treated group of rats as compared to the PCM-only group, according to the current study. Because NAC possesses antioxidant and free radical scavenging potentials, similar results were observed by Morsy *et al*. (2012) and Lonare *et al*. (2016), confirming the ability of NAC to prevent testicular dysfunction and accelerate the regeneration of damaged cells. NAC also aids in increasing testosterone levels.

According to Zafarullah *et al*. (2003), NAC lowers lipid peroxidation in cell membranes and shields the cell from reactive oxygen species-induced oxidative stress. Additionally, as a defense against ROS-induced damage, cell growth and survival rates rose, which led to growth arrest and apoptosis.

It has been demonstrated by Kanter *et al*. (2010) and Del Vento *et al*. (2018) that supplementing the medium with N-acetylcysteine in vitro culture decreased the apoptosis of germ cells. According to El-Kirdasy *et al*. (2014), NAC has a crucial role in testicular protection as well as anti-apoptotic and anti-inflammatory effects on testicular function. Many different types of cells are protected by NAC.

According to the results of the current investigation, taking paracetamol damaged testicles. Toxicology from paracetamol has a negative impact on sperm count and motility. These findings concur with those of Oyedeji *et al*. (2013); Aksu *et al*. (2016), and Mohammed & Sabry (2020), who hypothesized that paracetamol would cross the blood-testis barrier and change the seminiferous tubule microenvironment as a result.

Olaniyi and Agunbiade (2018) claim that chemical agents' capacity to pass across the blood-testis barrier and produce a distinct microenvironment in the inner section of the seminiferous tubule wall is what caused the decrease in sperm motility. The effects of paracetamol on the testes and epididymis may be the cause of the effect (Oyedeji *et al*., 2013).

Additionally, the spermatozoa of rats given paracetamol showed a markedly higher incidence of anomalies related to sperm in the current investigation. Our findings are consistent with those of Morakinyo *et al*. (2010), who found that giving male rats paracetamol increased the occurrence of sperm. High doses of paracetamol have been linked to abnormalities in sperm.

According to studies by Ratnasooriya & Jayakody (2000) and Mohammed & Sabry (2020), paracetamol reduces sperm motility and quantity while also causing sperm cell death, which reduces testicular size and suggests the presence of mild testicular toxicity. Furthermore, excessive paracetamol dosages may result in lipid peroxidation, which may harm sperm fertilization potential by preventing glycolysis and reducing ATP supply, both of which aid in sperm motility. In a similar vein, Olaniyi and Agunbiade (2018) demonstrated that gonadotropic hormones (LH and FSH) and increased testicular oxidative stress caused sperm count, motility, and normal morphology to decline upon paracetamol (500 mg/kg b. w).

Numerous studies have shown that giving paracetamol can enhance oxidative stress by activating cytochrome P450, which can lead to an increase in reactive oxygen species (ROS). The body's built-in antioxidant resistance mechanisms are weakened by an excess of reactive oxygen species (ROS), which leads to oxidative stress and subsequent cellular damage. ROS takes two actions. It first weakens the sperm membrane and reduces its motility. Second, ROS has the ability to change sperm DNA, leading to a genetic abnormality (Wahyudi *et al*., 2015).

Rats given NAC+PCM in this study demonstrated a noteworthy improvement in sperm parameters (count, motility, and morphology) as compared to the group given paracetamol. Furthermore, NAC improved the characteristics of sperm. According to Samuni *et al*. (2013); Kumar *et al*. (2013) and

Takemura *et al*. (2014), these findings are consistent. It was believed that these advantages of NAC were related to the decrease in ROS, which enhanced sperm motility.

According to a study by Prasad *et al*. (2016), NAC improves sperm quality measures. Additionally, because of its antioxidant properties, it is useful against harmful substances that impair the quality of sperm. By enhancing the glutathione antioxidant mechanism, which is necessary for ideal sperm activities, NAC enhances male reproductive capabilities.

CONCLUSION

The results of this study showed that NAC, an antioxidant, improves most testis function biomarkers and causes improvement in sperm parameters and hormone levels in rats given paracetamol. Therefore, during oxidative stress, NAC can correct the imbalance between pro-oxidant and antioxidant systems. The high incidence of infertility in countries where paracetamol is consumed can be explained by the negative effects of excessive paracetamol usage on male fertility. To determine the proper dose of N-acetylcysteine in cases of paracetamol toxicity in humans, more research has to be done.

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