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Role of N-acetyl cysteine in mitigates testicular abnormalities in male rats induced by Atrazine

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Abstract: The effect of N-acetyl cysteine (NAC) against Atrazine (ATR) on epididymal sperm characteristic in experimentally orally treated rats with a herbicide Atrazine was evaluated. Adult male albino rats exposed to 200 mg/kg ATR, 200 mg/kg NAC or their combination day by day for 3 weeks.

In ATR treated group serum urea and uric acid are significantly exceeded compared to control. Epididymal sperm concentration, motility and vitality were declined, meanwhile the percentage of sperm abnormalities exceeded relative to control. Regarding oxidative stress markers, ATR caused increase in testicular contents of malondialdehyde (MDA) and nitric oxide (NO) in concomitant with decrease in activity of testicular antioxidants as SOD and CAT in addition to GSH content.

The present result show the positive correlation between chronic kidney disease and epididymal sperm abnormalities which may be related to disturbance in antioxidant system, NAC with its antioxidant capacity suppress the adverse effect of ATR.

keywords: Herbicides, Atrazine, NAC, Antioxidants

1.Introduction

Environmental and social factors may jeopardize the male reproductive system's health. Pesticides overexposure is thought to be one of the causes of male infertility by oxidative stress through producing reactive oxygen species (ROS) in different gonadal organs[1].

Oxidative stress is resulted from the redox imbalance because of overwhelming of antioxidant defense mechanism by ROS emerged through pesticides exposure. The excessive ROS may change the structure and function of cellular macromolecules that include proteins, lipids, and DNA, with production of pro-inflammatory cytokines and activation of stress-induced transcription factors[2].

Atrazine (ATR) is one of the most popular herbicides used worldwide from the group of triazines. It is resistant to biodegradation and easily found in ground, surface and rain water, so there is a concern on its effects on the gonads[3]. Male rats exposed to ATR show testicular atrophy, decrease reproductive organs weight and reduces sperm quantity and quality[4].

Uremic patients suffer from abnormalities in the hypothalamic pituitary testes axis (HPT)[5].

N-acetylcysteine (NAC) is the acetylated derivative of the amino acid L-cysteine, a thiol-based antioxidant with free thiol groups in its structure that easily penetrate cells due to their molecular structure[6].

N-acetylcysteine has a protective role in the quantity and quality of spermatozoa, as well as a reduction in spermatogenic cell death and the prevention of seminiferous tubule atrophy. It plays an important role in reducing testicular oxidation caused by environmental insults[7].

male rats.

The present work was designed to investigate the possible mitigative role of NAC on the ATR adverse testicular effect in male rats.

2. Materials and methods

The experimental protocol was carried out after approved by the local experimental animal ethics committee of Zoology Department, Faculty of Science, Mansoura University. Code number: Sci-Z-M-2021-30.

2.1 Chemicals:

Atrazine, the commercial product Atracom 80% WP was obtained from agent of Takamul National Agriculture Co (Riyadh, Saudi Arabia).

N-acetylcysteine 98% purity was purchased from agent of S D Fine-Chem Limited Company (SDFCL) (Mumbai, India). All other chemicals or kits used in the experiment were of analytical grade and high purity.

2.2 Animals:

Adult male albino rats weighed 155± 6 g were used. Rats were obtained from Egyptian Institute for Serological and Vaccine production, Helwan, Egypt; they were housed in the animal house of the Zoology Department, Faculty of Science, Mansoura University. Rats were placed in stainless steel cages containing wood-chip bedding, renewed daily. They were kept in a temperature-controlled environment 25º±2 C. All rats were acclimized a week before the commencement of the experiments. Rats were provided with normal chew diet and water *ad libitum* during the study.

2.3 Experimental design:

Twenty adult male albino rats were randomly divided into 4 groups each group has 5 animals as follows:

1. Control group: Rats kept on normal diet.

2. N-acetylcysteine group: Rats were orally administered 200 mg/kg freshly prepared NAC[8].

3. Atrazine group: Rats were orally administered 200 mg/kg freshly prepared ATR[9].

4. NAC + ATR group: Animals treated orally with both NAC and ATR as the 2nd and 3th group.

2.4 Samples collection

After 21 days, rats were fasted overnight, weighed and sacrificed under slight halothane anesthesia. Blood samples were collected into clean tubes without anticoagulant, left to colt and then centrifuged at 805 xg for 15 min. The clear non hemolyzed sera were removed and stored at-20^{o}C for further biochemical analysis. Testes and epididymes from each rat were removed & weighed. A known weight of testes samples were homogenized, stored at -20^{o}C. Spermatogenic samples were collected from epididymes.

2.5 Biochemical analysis

The content of testicular MDA and NO was estimated according to the method of [10] and [11] respectively. Testicular GSH content as[12], testicular activity of SOD and CAT determined by [13] and [14] respectively. Urea and uric acid were assessed using method of [15].

2.6 Spermatogenic parameters

The spermatozoa were counted using the Neubauer hemacytometer[16].The sperm motility and vitality were evaluated in duplicate, visually using the microscope [17].Sperms from each rat were examined for abnormalities in their different regions[18].

2.7 Statistical analysis

All statistical analyses were conducted using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, California, USA). Results were presented as mean ± standard error [$\overline{X}$±SE]. Differences were considered significant at $P \leq 0.05$[19].

3. Results

3.1 Some kidney functions markers

The data in table 1 indicated significant increase in serum urea and uric acid concentration in rats administered ATR compared with control group. Administration of NAC in concomitant with ATR showed a significant decrease in serum urea and uric acid concentration compared to ATR group.

3.2 Estimated spermatogenic parameters

Atrazine produced a significant decrease in the rat's spermatozoa concentration, motility and vitality and a significant increase in sperm abnormalities compared to control group as in table 2.

Moreover, rats treated with NAC + ATR showed significant increase in sperm concentration, motility and vitality and a

significant decrease in sperm abnormalities compared to ATR group

Table (1): Serum urea and uric acid concentration (mg/dl) of different animal groups.

Animal groups	C	NAC	ATR	NAC+ATR
Uric acid	4.38 ±0.26	3.86 ±0.51	10.38^{a}±0.78	4.84^{b} ± 0.53
Urea	26.00±4.71	23.00±3.51	68.40^{a} ±2.80	51.20^{ab}±3.85

Results are presented as means ± SE, n=5

a and b significant change compared to control or ATR groups respectively

Table (2): Sperm concentration ($x10^{6}$/g), Sperm abnormalities (%), Sperm motility (%) and Sperm vitality (%) of different animal groups.

Animal groups	C	NAC	ATR	NAC+ATR
Sperm concentration ($x10^{6}$/g)	944.9 ± 26.66	1006 ± 27.73	661^{a} ± 23.27	886.9^{ab}± 3.894
Sperm abnormalities (%)	17.90± 1.52	12.55± 1.03	76.98^{a}± 1.78	61.10^{ab}± 3.46
Sperm motility (%)	91.25±1.11	94.75±0.95	33.25^{a}±2.53	46.25^{ab}± 2.56
Sperm vitality (%)	91.99± 0.88	94.69± 0.92	40.16^{a}±2.42	62.50^{ab}± 1.42

Results are presented as means ± SE, n=5

a and b significant change compared to control or ATR groups respectively

3.3 Oxidative stress markers

The data presented in table 3 revealed that oral administration of ATR resulted in a significant increase in testicular content of MDA and NO compared to control group. Comparing the group treated with NAC+ ATR to that receive ATR, the data showed a significant decrease in MDA and NO content.

Table (3): Testicular malondialdehyde (MDA) content (nmol/g) and nitric oxide (NO) content (µmol/g) of different animal groups.

Animal groups	C	NAC	ATR	NAC+ATR
MDA(nmol/g)	666 ± 48.04	661.9 ± 46.5	1083^{a} ± 57.42	842.4^{b} ± 29.14
NO(µmol/g)	22.36 ± 3.20	21.94 ± 3.46	51.18^{a} ± 5.98	23.07^{b} ± 3.73

Results are presented as means ± SE, n=5

a and b significant change compared to control or ATR groups respectively

3.4 Non-enzymatic and enzymatic antioxidants

As shown in figure 1 and 2, rats administered ATR showed a significant decrease in testicular GSH content, CAT and SOD activity compared to that of control. Administration of NAC in concomitant with ATR showed a significant increase in testicular GSH content, CAT and SOD activity if compared with that receive ATR only.

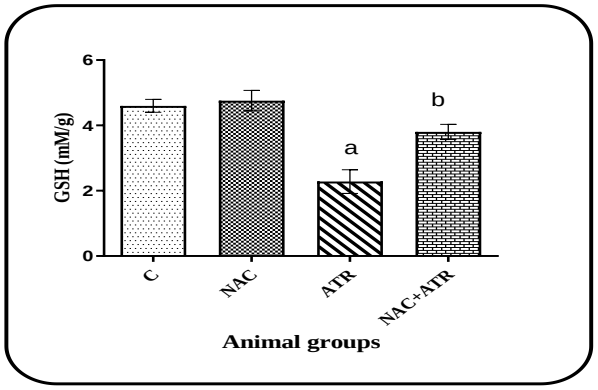

Fig (1): Testicular reduced glutathione (GSH) content (mM/g) in control and different treated animal groups

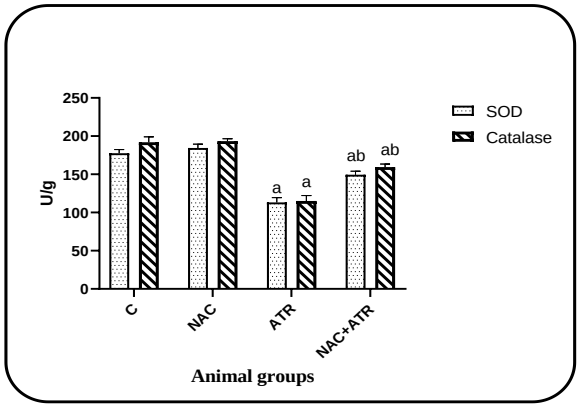

Fig (2): Testicular Superoxide dismutase (SOD) activity (U/g) and testicular catalase (CAT) activity (U/g) in control and different treated animal groups.

4. Discussion

Infertility is a global public health problem, involving developed and developing countries. Several factors can contribute in male infertility such as hormonal imbalances, anatomical causes, sexually transmitted diseases, genetic

factors as well as environment and lifestyles[20].

Pesticides are synthesized substances or biological agents used for attracting and destroying any pest. They are mainly applied in agriculture to protect crops from insects, weeds, and bacterial or fungal diseases during growth and to protect foods during storage from rats, mice, insects or diverse biological contaminants [21].

Pesticides, like herbicides results in production of ROS which in turn decrease the antioxidant levels and their defense against oxidative damage in the cellular system. Oxidative stress and ROS induce the long-term health effects such as carcinogenesis, neuro-degeneration, renal, endocrine and reproductive problems. When pesticides disturb the oxidative balance, they pave way for these diseases [22].

Atrazine is one of the most widely used herbicides in the world which may cause infertility[23].

N-acetylcysteine is an antioxidant and one of the endogenous precursors of GSH, can reduce the effects of oxidative stress and neutralizes the intracellular damages of free radicals. By increasing GSH, inhibiting inflammatory processes and preventing the expression of pre-apoptotic genes[24].

A significant increase in serum urea and uric acid in the ATR-treated group were recorded, which correspond to that of[25], these findings can be attributed to purine and pyrimidine degradation, as well as impaired kidney function, and/or a decrease in glomerular filtration rate in the kidney[26].

A significant decrease in serum urea and uric acid in NAC pre ATR treatment compared to the ATR group were noticed; these findings may be due to NAC's antioxidant power in protecting renal tissue from ATR damage[27].

Atrazine administration resulted in a significant decrease in sperm concentration, motility and vitality, as well as an increase in sperm abnormalities, these findings agree with[28].

The sperm characteristic in ATR treated group was significantly poorer than that of control rats. These data may be explained by the role of kidney dysfunctions which lead to hypogonadism caused by accumulation of uric acid and urea as well as other metabolites as reported by[29]. Moreover, it may be attributed to impairment of cystic fibrosis transmembrane regulator (CFTR) which is essential for sperm quality and associated with sperm concentration as well as their motility[30]. In addition to ATR's ability to induce ROS production leading to decrease activity of the membrane enzymes and disruption of the antioxidant defence system in the testes and prostate [31] and/or inhibiting cell division due to damage of DNA as noticed by[32].

The dangerous effect of ATR on DNA decreasing mitotic division activity must be taken into consideration to explain the decrease in sperm concentration[33].

The diffusion of H_2O_2 across the membrane in ATR treated group plays a role in abnormal sperm motility, as well as the decrease energy production by mitochondria[34]. The adenosine triphosphate (ATP) depletion as well as a decrease in axonemal protein phosphorylation may impair sperm motility[35].

The protective effect of NAC on spermatogenic parameters may be through its role in formation of acyl CoA hence produce energy for sperm respiration and motility[36].

N-acetylcysteine can also improve the process of spermatogenesis by increasing mitotic division of spermatogonia hence increasing spermatozoa concentration, vitality, motility, and normal morphology[37].

Exposure of male rats to ATR generates free radicals that react with membrane lipids and induce gonadal oxidative stress which indicated by a significant increase in testicular content of MDA in ATR treated rats is consistent with[38] this increase may be due to sensitivity of the spermatozoa plasma membrane that characteristics with high level of polyunsaturated fatty acids (PUFA) to oxidative stress that initiates lipid peroxidation, culminating in reduction of membrane fluidity and increase of cell permeability that alter spermatozoa cellular membranes[39].

Nitric oxide in the testes plays an important physiological role since it contributes to the regulation of steroidogenesis, vasodilatation and permeability of seminiferous tubules. In the

present study, the significant increase in testicular content of NO may be due to elevated nitric oxide synthetase activity (NOS) and increased inducible nitric oxide synthase gene expression (iNOS)[38]. The construct rats treated with NAC plus ATR exhibited a significant decrease in testicular contents of MDA and NO compared with the ATR group. This protective role of NAC may be attributed to its anti-lipid peroxidation property, ability to inhibit peroxynitrite formation and an increase in antioxidant capacity[40].

Superoxide dismutase is considered the first line of defense against free radicals in the cell by catalyzing the dismutation of superoxide anion radicals to hydrogen peroxide (H_2O_2), which is readily degraded by CAT. In the biological system, the antioxidant enzymes CAT protects SOD inactivation by H_2O_2, while the SOD reciprocally protects CAT against inhibition by superoxide anion. Thus, balance of this enzyme system may be essential to eliminate superoxide and peroxide radicals generated in the tissues[41].

A significant decrease in testicular GSH content, SOD and CAT activity of ATR-treated rats Fig 1 and 2 owing to ATR's ability to induce tissue oxidative damage[42] due to the primary antioxidant system's failure to act against free radicals[43].

Administration of NAC to rats prior to ATR may prevent the adverse role of ATR and exceed GSH content, SOD and CAT activity when compared to the ATR treated group by acting as a cysteine donor thereby, decrease generation of ROS post ATR treatment. Also, the mechanism by which NAC reduces ATR toxicity may be due to the ability of free thiol group of NAC to interact with electrophilic groups of ROS generated in response to ATR as mention by[44]. The protective role of NAC may be by regulating GSH and scavenging free ROS, restoring the balance between the pro-oxidant and antioxidant systems during oxidative stress and/or inhibiting lipid peroxidation, an explanation which run parallel with[45].

5. Conclusion

It seems that ATR reduces the antioxidant capacity in the body, increases lipid peroxidation, exceeds sperm abnormalities and decreases sperm concentration, motility and vitality that represents a high risk on the male fertility. However, NAC has partial protective role against the negative effect of ATR on reproductive organs through its antioxidant ability and anti-peroxidative property.

6. References

1 Asadi N., Bahmani M., Kheradmand A., Rafieian-Kopaei M. (2017).The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review, JCDR,**11(5)**: 1-5.

2 Ahmed Y. H., AbuBakr H. O., Ahmad I. M., Ahmed Z. S. O. (2022). Histopathological, Immunohistochemical, And Molecular Alterations In Brain Tissue And Submandibular Salivary Gland Of Atrazine-Induced Toxicity In Male Rats, ESPR,**29**(**20**): 30697-30711.

3 Abarikwu S., Oleribe A., Mgbudom-Okah C., Onuah C., Chikwendu C., Onyeike E. (2022).The protective effect of fluted pumpkin seeds against atrazine-induced testicular injury, Drug Chem Toxicol,**45**(**2**): 799-809.

4 Abarikwu S. O., Farombi E. O. (2016).Quercetin ameliorates atrazine-induced changes in the testicular function of rats, Toxicol Ind Health,**32**(**7**): 1278-1285.

5 Wang C. J., Cukor D., Johansen K. L. (2021).Sexual Dysfunction Among Patients With Chronic Kidney Disease, Seminars in Nephrology,**41**:534-549.

6 Mokhtari V., Afsharian P., Shahhoseini M., Kalantar S. M., Moini A. (2017).A review on various uses of N-acetyl cysteine, *Cell J*,**19**(**1**): 11-17.

7 Wang M., Su P. (2018).The role of the Fas/FasL signaling pathway in environmental toxicant-induced testicular cell apoptosis: An update, Syst Biol Reprod Med,**64**(**2**): 93-102.

8 Sethi R., Adameova A., Dhalla K. S., Khan M., Elimban V., Dhalla N. S. (2009).Modification of epinephrine-induced arrhythmias by N-acetyl-L-cysteine and vitamin E, JCPT,**14**(**2**): 134-142.

9 Lin J., Li H.-X., Xia J., Li X.-N., Jiang X.-Q., Zhu S.-Y., Ge J., Li J.-L. (2016).The chemopreventive potential of lycopene

against atrazine-induced cardiotoxicity: modulation of ionic homeostasis, Sci Rep,**6**(**1**): 1-12.
10 Ohkawa H., Ohishi N., Yagi K. (1979).Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem,**95**(**2**): 351-358.
11 Montgomery H., Dymock J. (1961).Nitric oxide assay, Analyst,86,p414.
12 Beutler E., Duron O., Kelly M. (1963).Determination of reduced glutathione in tissue homogenate, *Journal of Laboratory and Clinical Medicine*,**61**,p882.
13 Nishikimi M., Rao N. A., Yagi K. (1972).The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem Biophys Res Commun,**46(2)**: 849-854.
14 Fossati P., Prencipe L., Berti G. (1980).Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine, Clinical Chemistry,**26(2)**: 227-231.
15 Burtis C. A. (1999). Tietz textbook of clinical chemistry 3rd ed. In: Saunders, vol **37**. p 1915
16 Pant N., Srivastava S. (2003).Testicular and spermatotoxic effects of quinalphos in rats, *J Appl Toxicol*,**23(4)**: 271-274.
17 Sönmez M., Türk G., Yüce A. (2005).The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats, Theriogenology,**63**(**7**): 2063-2072.
18 Wyrobek A. (1978).The induction of sperm-shape abnormalities in mice and humans, Chem mutagens,**5**:257-285.
19 Armitage P., Berry G., Matthews J. (2008). Statistical methods in medical research,4th.,Place Published. John Wiley and Sons.
20. Babakhanzadeh E., Nazari M., Ghasemifar S., Khodadadian A. (2020).Some of the factors involved in male infertility: a prospective review, *Int J Gen Med*,**13**:29-41.
21 ajmohan K., Chandrasekaran R., Varjani S. (2020).A review on occurrence of pesticides in environment and current technologies for their remediation and management, Indian *journal of microbiology*,**60(2)**: 125-138.
22 Yadav I. C., Devi N. L. (2017).Pesticides classification and its impact on human and environment, Environ Sci Eng,**6**:140-158.
23 Szewczyk R., Kuśmierska A., Bernat P. (2018).Ametryn removal by Metarhizium brunneum: Biodegradation pathway proposal and metabolic background revealed, Chemosphere,**190**:174-183.
24 Zalewska A., Zięba S., Kostecka-Sochoń P., Kossakowska A., Żendzian-Piotrowska M., Matczuk J., Maciejczyk M. (2020).NAC supplementation of hyperglycemic rats prevents the development of insulin resistance and improves antioxidant status but only alleviates general and salivary gland oxidative stress, Oxid Med Cell Longev,(2020)
25 Kasteel E. E., Nijmeijer S. M., Darney K., Lautz L. S., Dorne J. L., Kramer N. I., Westerink R. H. (2020).Acetylcholinesterase inhibition in electric eel and human donor blood: an in vitro approach to investigate interspecies differences and human variability in toxicodynamics., Arch Toxicol,**94**(**12**): 4055-4065.
26 Walmsley R., White G. (1988).A guide to diagnostic clinical chemistry, *Journal of Clinical Pathology* **37 (8)**: 959–960.
27 Youness E., Agha F., El-Toukhy S., El-Naggar S., Selim A., Ibrahim A. (2016).The protective effect of orange juice on glyphosate toxicity in adult male mice, *Journal of Chemical and Pharmaceutical Research*,**8(3)**: 13-28.
28 Abarikwu S. O., Adesiyan A. C., Oyeloja T. O., Oyeyemi M. O., Farombi E. O. (2010).Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine, Archives of environmental contamination and toxicology,**58**(**3**): 874-882.
29 Xu H., Li H., Xu L., Zhang J., Chen W., Shi Q. (2012).The decline of fertility in male uremic patients is correlated with low expression of the cystic fibrosis

transmembrane conductance regulator protein (CFTR) in human sperm, Human reproduction,**27**(**2**): 340-348.

30 Li C., Jiang L., Chen W., Li K., Sheng H., Ni Y., Lu J., Xu W., Zhang S., Shi Q. (2010).CFTR is essential for sperm fertilizing capacity and is correlated with sperm quality in humans, Human reproduction,**25**(**2**): 317-327.

31 Fairuz A., Hashida N., Mahanem M. (2011).Effect of nicotine and goat milk co-administration on rat testis and sperm parameters, Austral *J Basic Appl Sci*,**5(12)**: 2738-2741.

32 Harper A. P., Finger B. J., Green M. P. (2020).Chronic atrazine exposure beginning prenatally impacts liver function and sperm concentration with multi-generational consequences in mice, Frontiers in Endocrinology,**11**:580124-580137.

33 Gao J.-G., He Y., Hua Y.-L., Chen S.-X., Jiang Y., Zheng J.-T., Wang P. (2020).Atrazine changes meiosis and reduces spermatogenesis in male mice, *Natl j androl*,**26(11)**: 963-968.

34 Hase Y., Tatsuno M., Nishi T., Kataoka K., Kabe Y., Yamaguchi Y., Ozawa N., Natori M., Handa H., Watanabe H. (2008).Atrazine binds to F1F0-ATP synthase and inhibits mitochondrial function in sperm, Biochem Biophys Res Commun,**366(1)**: 66-72.

35 Alahmar A. T. (2019).Role of oxidative stress in male infertility: an updated review, *J Hum Reprod Sci*,**12(1)**: 4-18.

36. Kanter M., Topcu-Tarladacalisir Y., Parlar S. (2010).Antiapoptotic effect of L-carnitine on testicular irradiation in rats, *J Mol Histol*,**41(2)**: 121-128.

37 Tsujiuchi T., Nakae D., Konishi Y. (2014).Multi-step lung carcinogenesis model induced by oral administration of N-nitrosobis (2-hydroxypropyl) amine in rats, Exp Toxicol Pathol,**66**(2-3): 81-88.

38 Owumi S., Akomolafe A., Imosemi I., Odunola O., Oyelere A. (2021).N-acetyl cysteine cotreatment abates perfluorooctanoic acid induced reproductive toxicity in male rats, Andrologia,**53(5)**: 14037-14055.

39 Akunna G. G., Saalu C. L., Ogunmodede O. S., Ogunlade B., Bello A. J. (2012).Aqueous extract of date fruit (Phoenix dactylifera) protects testis against atrazine-induced toxicity in rat, *World J Life Sci Med Res*,**2(2)**: 100-108.

40 Shadnia S., Dasgar M., Taghikhani S., Mohammadirad A., Khorasani R., Abdollahi M. (2007).Protective effects of α-tocopherol and N-acetyl-cysteine on diazinon-induced oxidative stress and acetylcholinesterase inhibition in rats, Toxicol Mech Methods,**17(2)**: 109-115.

41 Ighodaro O., Akinloye O. (2018).First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid, Alexandria *journal of medicine*,**54(4)**: 287-293.

42 Njoku R.-C. C., Abarikwu S. O., Uwakwe A. A., Mgbudom-Okah C. J., Ezirim C. Y. (2019).Dietary fluted pumpkin seeds induce reversible oligospermia and androgen insufficiency in adult rats, Syst Biol Reprod Med,**65(6)**: 437-450.

43 Abarikwu S., Duru Q., Chinonso O., Njoku R. (2016). Antioxidant enzymes activity, lipid peroxidation, oxidative damage in the testis and epididymis, and steroidogenesis in rats after co-exposure to atrazine and ethanol, Andrologia,**48**(**5**): 548-557.

44. Malmir M., Soleimani Mehranjani M., Naderi Noreini S., Faraji T. (2018).Protective antioxidant effects of N-acetylcysteine against impairment of spermatogenesis caused by paranonylphenol, Andrologia,**50(10)**: 13114-13124.

45 Hashim A. R., Bashir D. W., Yasin N. A., Rashad M. M., El-Gharbawy S. M. (2022).Ameliorative effect of N-acetylcysteine on the testicular tissue of adult male albino rats after glyphosate based herbicide exposure, *Journal of Biochemical and Molecular Toxicology*,**36(4)**: 22997-23008.