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The attenuating role of N-acetylcysteine in the haematological and antioxidant disruption induced by Atrazine in male rats

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Abstract: The protective role of NAC against haematological abnormalities and disruption of antioxidant system in male rats given ATR was evaluated. Adult male rats orally receive ATR 200 mg/kg b.w. for 3 weeks, NAC 200 mg/kg b.w. or rats received NAC+ATR by the same dose for the same period. Rats given ATR showed significant decrease in RBCs count, Ht %, platelets count and neutrophile % in concomitant with increase in total WBCs count and lymphocyte %. Regarding the oxidative stress markers, there are increase in cardiac MDA, H_2O_2 as well as NO content, meanwhile cardiac GSH content and the activity of SOD and CAT declined. NAC significantly reconsider the haematological abnormalities and oxidant / antioxidant balance caused by ATR. It is concluded that, ATR induced cardiac damage via the enhancing of NO production and disrupt the hematopoietic functions, in addition to depletion of oxidant / antioxidant balance.

keywords: NAC-ATR-Haematopoiesis-Antioxidants-ROS

1.Introduction

Atrazine (ATR) is an extensively used herbicide of the triazine class to control broadleaf and grassy weeds on some crops such as corn, sorghum and sugarcane. ATR remains in surface water and groundwater for a long time and is difficult to degrade, thus it causes serious pollution to the ecological environment [1]. In experimental rats, ATR causes oxidative stress and alteration in cardiac function. ATR is linked to heart disease, carditis, and an increase in cardiovascular mortality [2].

Diet particularly those with antioxidant properties found in botanical products such as polyphenols, organosulfur compounds, and flavonoids, may reduce the incidence of Cardiovascular disease (CVD) [3]. N-acetylcysteine (NAC), a safe natural compound found in several vegetables, including Garlic and Onion, in addition it is a well-tolerated thiol reducing agent [4]. It is essential for the production of glutathione, which plays an important role in intracellular defense against oxidative stress, and it is involved in the detoxification of numerous molecules [5].

NAC has antioxidant, anti-angiogenic, and anti-cancer properties [6]. It also serves as a cysteine donor and keeps glutathione content intracellularly stable [7]. Furthermore, NAC can protect against oxidative stress and DNA damage [8] also it has anti-inflammatory properties and protects the heart by lowering the release of inflammatory mediators such as Nuclear Factor Kappa B (NF-κB) in the myocardium [9]. NAC has a cardioprotective effect on myocardial infarction by reducing oxidative damage [10].

Nitric oxide exerts different effect on heart activity in addition to the pathogenesis of CVD [11]. The exceed NO formation cause many myocardial diseases [12].

The aim of this study was to investigate the association between ATR-impaired blood components, reduced antioxidants and the chemoprotective potential of NAC on these abnormalities.

2. Materials and methods

2.1. Chemicals

ATR the commercial product Atracom 80%

WP was obtained from agent of Takamul National Agriculture Co (Riyadh, Saudi Arabia). NAC 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

Twenty adult male albino rats were used. Rats were obtained from Egyptian Institute for Serological and Vaccine production, Helwan, Egypt; they were housed in the animal house of 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 25 ± 2°C. Rats were provided with standard chew diet and water *ad libitum* during the whole period of study.

2.3. Experimental design

The experimental protocol was carried out in accordance with the guide of the National Research Council for the Care and Use of Laboratory Animals and was approved by the local experimental animal ethics committee of the Department of Zoology, Faculty of Science, Mansoura University. Code number: Sci-Z-M-2021-28.

Adult male albino rats weighed 155 ± 6 g divided into 4 groups, each group has 5 animals as follow: Control group: did not receive any treatment, NAC group: administered orally NAC (200 mg/kg b.w day by day) for 21 days [13], ATR group: orally ATR administration (200 mg/kg b.w day by day) for 21 days [14], NAC + ATR group: animals treated orally with NAC + ATR by the same route and dose of the 2nd and 3rd groups.

2.4. Blood and tissue sampling

At the end of the experimental period (21 day), rats were sacrificed under slight halothane anesthesia, blood samples were collected on EDTA containing tube for CBC enumeration. The hearts were excised, washed, and weighed. Known weight of the heart samples were homogenized in saline solution. The samples were kept at -20°C till used for further estimations.

2.5. Estimated parameters

2.5.1. Hematological study. The count of RBCs, WBCs, differential leukocyte counts, Ht %, Hb concentration and platelets count were conducted using haematological analyzer (Sysnex Ts-21) Japan.

2.5.2. Biochemical analysis. cardiac MDA, H_2O_2, NO, GSH contents, cardiac SOD and CAT activities were estimated using specific kits supplied by Biodiagnostic Company, Egypt.

2.6. Statistical analysis

All results were analyze using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, California, USA). Results were presented as mean ± standard error [$\overline{X} \pm SE$]. Statistical comparisons were made by one way analysis of variance (ANOVA) followed by Neuman-Keuls post-hoc test [15]. Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Blood tested parameters

Table (1) showed that, ATR administered rats displayed a significant decrease in (RBCs count, Ht %, platelets count and neutrophil %) accompanied by a non-significant decrease in Hb concentration, while there was a significant increase in (WBCs count and lymphocytes%) compared to control group. NAC treatment before ATR administration significantly prevented the alterations in estimated hematological parameter.

3.2. Oxidative stress markers

Data presented in Fig. (1) revealed that oral, administration of ATR resulted in a significant increase in cardiac content of MDA (+57.89%), H_2O_2 (+312.23%) and NO (+112.55%). NAC pre-ATR treatment to some extent prevented alterations in these parameters compared to ATR group.

3.3. Non-enzymatic and Enzymatic antioxidants

Data presented in Fig. (2) showed that oral, administration of ATR resulted in a significant decrease in the cardiac content of GSH and the activity of cardiac SOD and CAT. Rats administered NAC pre-ATR treatment, showed partially prevention of these estimated parameters that occur following only ATR

treatment.

4. Discussion

The actual prevention and management of haematopoietic toxicity continues to be an essential health issue. This study demonstrates the role of NAC against ATR-induced oxidative stress, to reduce the risk of haematopoietic toxicity.

In ATR administered group there are alterations in haematological parameters, these data run parallel with [16]. These results may be explained by the inability of injured renal parenchyma to produce erythropoietin [17], in addition to DNA breakdown [18], thus decrease the bone marrow ability to divide for blood corpuscles synthesis [19]. Furthermore, exceed oxidative stress and increased ROS production due to lipid peroxidation may contribute to cell membrane degeneration. ATR may also cause an increase in the rates of biosynthesis of thromboxane A2 in blood platelets, which directly aids in their accumulation and adhesion to one another thus decrease blood platelets numbers [20].

A significant increase in WBCs counts accompanied by increase in lymphocyte % were noticed in ATR treated group, these increase may indicate activation of the animal's defense mechanisms and immune system as a result of inflammation caused by ATR toxicity [21].

The dosage of NAC partially prevents the adverse effect of ATR on estimated haematological parameters, this effect may be attributed to NAC's role in scavenging free radicals and eliminating their cellular toxic effects, as recorded by [22], which keep the maintenance of selective permeability of RBCs and platelet membranes and also the preservation of their nucleic and cytoplasmic components. Furthermore, the powerful antioxidant NAC may have stimulated a remarkable increase in the number of RBCs in the blood through stimulate liver, kidney, and bone marrow healthy, as well as their Hb concentration and Ht%, by supporting the enzymatic and non-enzymatic antioxidant systems as well as protein synthesis from liver. Total WBCs values were significantly lower after NAC pre-ATR treatment compared to ATR received group; these findings were consistent with those of [23] and may be due to their preventive inflammation effect.

Depletion of reduced GSH, SOD and CAT induced by ATR may be due to over production of ROS resulted from ATR administration that forming singlet oxygen, hydroperoxides, and hydrogen peroxides that promoted lipid peroxidation [24].

ATR administration significantly increases cardiac MDA, H_2O_2 and NO contents, these concur well with [25], these toxic manifestation may be attributed to the hypersensitivity of polyunsaturated fatty acid (PUFA) of the heart membrane to peroxidation by ATR, an explanation which in agreement with [26]. The free radical production by ATR, which can damage cells via lipid peroxidation must be taken into consideration [27].

Excessive free radical production by ATR reduces the enzyme activity of tricarboxylic acid cycle (TCA) that are present in mitochondrial membrane to generate energy by oxidizing Co-A. ROS inhibition of these enzymes may affect mitochondrial substrate oxidation, resulting in reduced substrate oxidation, slower rate of transfer of reducing equivalents to molecular oxygen, and cellular energy depletion, this may explain the increase in oxidative stress and increase in ROS such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) [28].

NO is a key signaling molecule that regulates processes of the cardiovascular system. 3 nitric oxide synthase (NOS) isoforms, iNOS, eNOS and nNOS, which differ in distribution and function, catalyze a process that results in the formation of NO [29]. The increase in cardiac NO content may be due to increase the production of cardiac myocyte iNOS mRNA which cause an increase in the NOS activity and cardiac NO content that lead to haematopoietic toxicity [21].

The obtained exceed in MDA and depletion of GSH in ATR treated group may be through the formation of secondary toxic oxidizing species as peroxynitrite (ONOO) after the interaction between NO and other ROS leading to enhancement of lipid peroxidation [30].

The present study showed significant increase in cardiac H_2O_2 content that may be due to decline in CAT activity, lead to H_2O_2

accumulation and disrupt the heart structure and functions, in addition to vascular remodeling and endothelial dysfunction as reported by [31]

N-acetylcysteine pre-ATR treatment significantly decreases the content of cardiac MDA, H_2O_2 and NO relative to ATR given group. The protective effect of NAC on ATR induced haematopoietic toxicity run parallel with previous study of [9]. NAC's hydrogen atom on the thiol group is capable to neutralizing free radicals, making it a useful free radical scavenger in the body [32], this explanation is consistent with [33] and may be due to its actions in increasing oxygen free radical scavenging activity and decreasing damage caused by lipid peroxidative. The obtained decrease in H_2O_2 in this group may be due to the increase in CAT activity [34].

Oral administration of ATR resulted in a significant decrease in the cardiac content of GSH and activity of cardiac SOD and CAT these results are in consistent with the finding of [35]. The possible explanation of these results could be related to the proposed role of GSH in the active excretion of ATR by binding to the thiol group of GSH and then being excreted [36]. A decrease in GSH content, a principal antioxidant, could lead to oxidative stress. Moreover, increased ROS consequently depletes SOD activity that plays a substantial role in oxygen dismutation and conversion of highly reactive O_2^- into H_2O_2 thus inducting OH^- production and oxidative stress in ATR-intoxicated rats [37]. The reduction of CAT activity may be due to the exhaustion of antioxidant capacity through the ATR toxicity which in concomitant with [38]

Rats administered NAC showed an increase in cardiac GSH content, SOD and CAT activities, which may be due to NAC efficiently transported into the cell and converted to cysteine for GSH synthesis, scavenging free radicals, improving intracellular detoxification and reducing ROS liberation [39].

5. conclusion

It seems that ATR disrupts the haematological parameters, reduces the body antioxidant capacity and increases lipid peroxidation that represents a high risk for CVD. NAC through its antioxidant ability and anti-peroxidation property can protect the myocardial membrane integrity reducing, the risk factor for CVD.

Table (1): Hematologic parameters in the different groups

GroupsParameters	Control	NAC	ATR	NAC +ATR
RBCs ($\times10^6$/mm^3)	7.25±0.16	7.35±0.04	6.74±0.06[a]	7.16±0.12[b]
Hb (g/dl)	14.20±0.19	14.30±0.06	13.67±0.07	14.11±0.19
Ht %	39.96±0.22	40.38±0.37	37.76±0.11[a]	39.46±0.07[b]
WBCs ($\times10^3$/mm^3)	13.78±0.422	13.38±0.8514	20.66±0.7011[a]	17.3±0.9191[ab]
Neutrophil %	55.40±0.75	55.00±0.31	50.24±0.79[a]	54.24±0.67[b]
Lymphocyte %	36.00±1.10	36.06±0.53	42.82±0.57[a]	36.56±0.81[b]
Monocyte %	6.20±0.37	6.24±0.19	5.54±0.21	6.18±0.27
Platelets ($\times10^3$/mm^3)	777.2±34.43	701.6±27.30	572.4±38.17[a]	730.8±39.65[b]

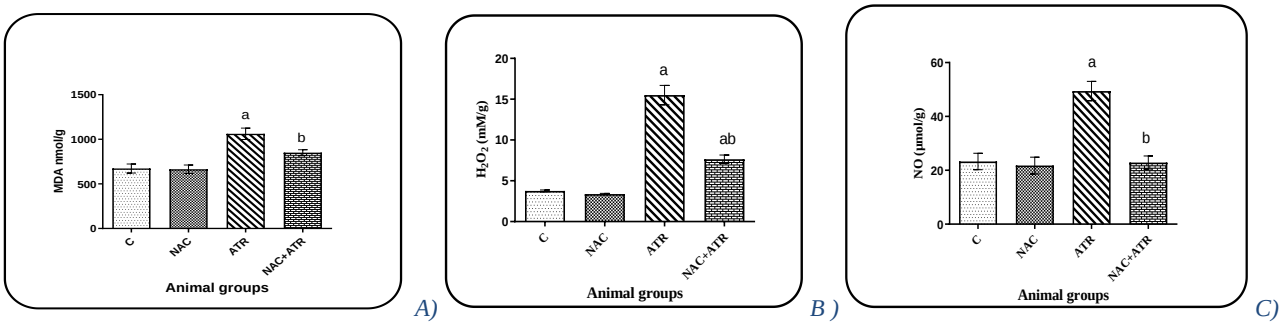


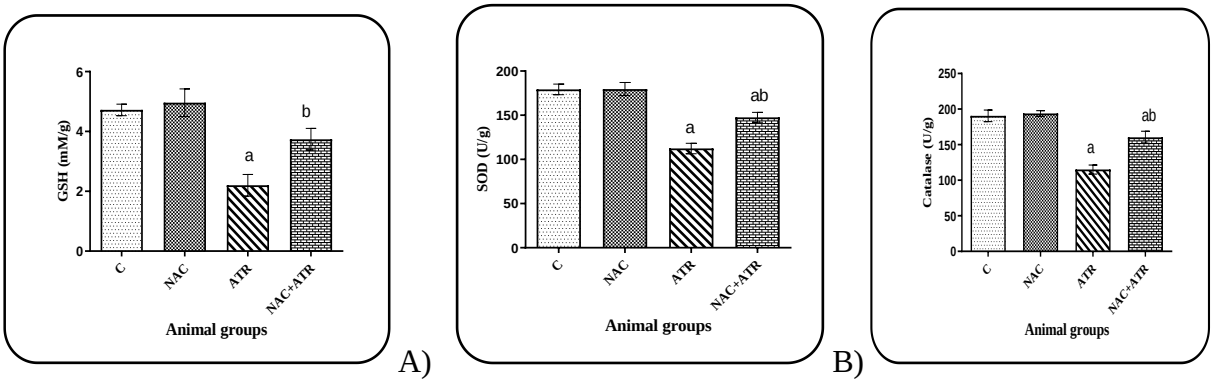
Fig. (1): Effect of administration of N-acetylcysteine (NAC) and atrazine (ATR) on cardiac content of A: MDA, B: H_2O_2. C: NO in different experimental groups.

Data are presented as $\bar{X}$ ±SE, n=5.

Values are expressed as $\bar{X}$±SE, n= 5

a: Indicated the significant change as compared to control group.

b: Indicated the significant change as compared to ATR group



A) B) C)

Fig. (2): Effect of administration of N-acetylcysteine (NAC) and atrazine (ATR) on cardiac content of A: GSH, cardiac activity of B: SOD and C: CAT and the activity of serum in different experimental groups.

Data are presented as $\bar{X} \pm$SE, n=5.

a: Indicated the significant change as compared to control group.
b: Indicated the significant change as compared to ATR group.

5. References

1 Zhu S., Zhang T., Wang Y., Zhou X., Wang S.and Wang Z. (2021). Meta-analysis and experimental validation identified atrazine as a toxicant in the male reproductive system, Environmental Science and Pollution Research, **28** (**28**)**,** 37482-37497.

2 Fijorek K., Püsküllüoğlu M., Tomaszewska D., Tomaszewski R., Glinka A.and Polak S. (2014). Serum potassium, sodium and calcium levels in healthy individuals—literature review and data analysis, Folia Med. Crac., **54** (**1**)**,** 53-70.

3 Basha R.H.and Priscilla D.H. (2013). An in vivo and in vitro study on the protective effects of N-acetylcysteine on mitochondrial dysfunction in isoproterenol treated myocardial infarcted rats, Exp. Toxicol. Pathol., **65** (**1-2**)**,** 7-14.

4 Hsu C.-c., Huang C.-n., Hung Y.-c.and Yin M.-c. (2004). Five cysteine-containing compounds have antioxidative activity in Balb/cA mice, *J Nutr*, **134 (1),** 149-152.

5 Samuni Y., Goldstein S., Dean O.M.and Berk M. (2013). The chemistry and biological activities of N-acetylcysteine, Biochim Biophys Acta Gen Subj., **1830** (**8**)**,** 4117-4129.

6 Wang X., Martindale J.L.and Holbrook N.J. (2000). Requirement for ERK activation in cisplatin-induced apoptosis, *J. Biol. Chem.* , **275 (50),** 39435-39443.

7 Oh S.-H.and Lim S.-C. (2006). A rapid and transient ROS generation by cadmium triggers apoptosis via caspase-dependent pathway in HepG2 cells and this is inhibited through N-acetylcysteine-mediated catalase upregulation, Toxicol. Appl. Pharmacol., **212** (**3**)**,** 212-223.

8 Hamdi H., Rjiba-Touati K., Ayed-Boussema I., M'nassri A., Chaabani H., Rich S.and Abid-Essefi S. (2022). Epoxiconazole caused oxidative stress related DNA damage and apoptosis in PC12 rat Pheochromocytoma, NeuroToxicology, **89,** 184-190.

9 Wu X.Y., Luo A.Y., Zhou Y.R.and Ren J.H. (2014). N-acetylcysteine reduces oxidative stress, nuclear factor-κB activity and cardiomyocyte apoptosis in heart failure, Mol. Med. Rep., **10** (**2**)**,** 615-624.

10 San-Martín-Martínez D., Serrano-Lemus D., Cornejo V., Gajardo A.I.and Rodrigo R. (2022). Pharmacological Basis for Abrogating Myocardial Reperfusion Injury Through a Multi-Target Combined Antioxidant Therapy, Clinical pharmacokinetics**,** 1-16.

11 Mohamad A. (2001). Glutathione peroxidase and nitric oxide in patients with chronic liver diseases, Egyptian *Journal of Schistosomiasis and Infectious and Endemic Diseases*, **23,** 27-46.

12 Weinstein D.M., Mihm M.J.and Bauer J.A. (2000). Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice, *Journal of Pharmacology and Experimental Therapeutics*, **294 (1),** 396-401.

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

14 Lin J., Li H.-X., Xia J., Li X.-N., Jiang X.-Q., Zhu S.-Y., Ge J.and Li J.-L. (2016). The chemopreventive potential of lycopene against atrazine-induced cardiotoxicity: modulation of ionic homeostasis, Sci. Rep., **6 (1),** 1-12.

15 Armitage P., Berry G.and Matthews J.N.S. (2008). Statistical methods in medical research, 4th ed, John Wiley & Sons.

16 Ezenwaji N.E., Nelo W.C.and Oluah N.S. (2012). Effect of oral administration of sublethal concentration of atrazine on the haematological profile of albino rat, Anim. Res. Int., **9** (**2**)**,** 1572-1578.

17 Yousef M.I., Omar S.A., El-Guendi M.I.and Abdelmegid L.A. (2010). Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat, Food Chem. Toxicol. , **48** (**11**)**,** 3246-3261.

18 AL-Shimmari N.R.and Jawad R.A. (2020). Study the Protective Role of Arabic Gum Extract on Some Physiological and Histological Criteria for Liver of Male Rabbit Treated with Atrazine, Prof. RK Sharma, **14** (**2**)**,** 485.

19 Koury M.J.and Bondurant M.C. (1990). Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells, Sci., **248** (4953)**,** 378-381.

20 Abdul-Zahra M.T.and Jwad S.M. (2020). The Effect of Alcoholic Extract of Pomegranate Peel on the level of Erythropoietin and some of the Blood Properties in Albino Male Rats Treated with Erythromycin, *Int. J. Psychosoc. Rehabilitation*, **24 (04),** 6350-6361.

21 Li X.-N., Lin J., Xia J., Qin L., Zhu S.-Y.and Li J.-L. (2017). Lycopene mitigates atrazine-induced cardiac inflammation via blocking the NF-κB pathway and NO production, *J. Funct. Foods*, **29,** 208-216.

22 El-Maddawy Z.K.and El-Sayed Y.S. (2018). Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats, ESPR, **25** (**4**)**,** 3468-3479.

23 De la Fuente M.and Hernanz A. (2012). The antioxidant N-acetylcysteine in vitro improves several functions of peritoneal leucocytes from old mice approaching their values to those of adult animals, *J Appl Biomed*, **10 (2),** 79-90.

24 Lin J., Xia J., Zhao H.-S., Hou R., Talukder M., Yu L., Guo J.-Y.and Li J.-L. (2018). Lycopene triggers nrf2–AMPK cross talk to alleviate atrazine-induced nephrotoxicity in mice, *J. Agric. Food Chem.*, **66 (46),** 12385-12394.

25 Mansour H.H.and Hasan H.F. (2015). Protective effect of N-acetylcysteine on cyclophosphamide-induced cardiotoxicity in rats, Environ. Toxicol. Pharmacol., **40** (**2**)**,** 417-422.

26 Hussein A., Ahmed A.A., Shouman S.A.and Sharawy S. (2012). Ameliorating effect of DL-α-lipoic acid against cisplatin-induced nephrotoxicity and cardiotoxicity in experimental animals, Drug Discov Ther, **6** (**3**)**,** 147-156.

27 Arıca V., Demir I., Tutanc M., Basarslan F., Arıca S., Karcıoglu M., Öztürk H.and Nacar A. (2013). N-Acetylcysteine prevents doxorubucine-induced cardiotoxicity in rats, Hum Exp Toxicol, **32** (**6**)**,** 655-661.

28 Katare P.B., Nizami H.L.and Banerjee S.K. (2019). Mitochondrial Dysfunction and Oxidative Stress: Focusing on Cardiac Hypertrophy and Heart Failure. Oxidative Stress in Heart Diseases, Springer.

29 Barouch L.A., Harrison R.W., Skaf M.W., Rosas G.O., Cappola T.P., Kobeissi Z.A., Hobai I.A., Lemmon C.A., Burnett A.L.and O'Rourke B. (2002). Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms, Nature, **416** (**6878**)**,** 337-339.

30 Elbaky N.A.A., El-Orabi N.F., Fadda L.M., Abd-Elkader O.H.and Ali H.M. (2018). Role of N-acetylcysteine and coenzyme Q10 in the amelioration of

myocardial energy expenditure and oxidative stress, induced by carbon tetrachloride intoxication in rats, Dose-Response, **16** (**3**)**,** 1559325818790158.

31 Ajibade T.O., Oyagbemi A.A., Omobowale T.O., Asenuga E.R., Afolabi J.M.and Adedapo A.A. (2016). Mitigation of diazinon-induced cardiovascular and renal dysfunction by gallic acid, Interdiscip. Toxicol., **9 (2),** 66-77.

32 Thanoon S., Taqa G.and A Alkataan M. (2022). The Protective Effect of N-acetyl Cysteine on Mitochondrial Copy Number of Salivary Glands after Induction of Oxidative Stress in Albino Rats, Egyptian *Journal of Veterinary Sciences*, **53 (3),** 391-402.

33 Dong S., Tong X., Liu H.and Gao Q. (2012). Protective effects of pomegranate polyphenols on cardiac function in rats with myocardial ischemia/reperfusion injury, Nan Fang yi ke da xue xue bao= *Journal of Southern Medical University*, **32 (7),** 924-927.

34 Turkmen R., Birdane Y.O., Demirel H.H., Yavuz H., Kabu M.and Ince S. (2019). Antioxidant and cytoprotective effects of N-acetylcysteine against subchronic oral glyphosate-based herbicide-induced oxidative stress in rats, Environmental Science and Pollution Research, **26** (11)**,** 11427-11437.

35 El-Kholy W.M., Hemieda F.and El-Zarif N. (2015). Protective effect of aqueous extract of Anacyclus pyrethrum root on atrazine-induced male reproductive disorders in rats, IJAR, **3 (10),** 1701-1715.

36 Bhatti J., Sidhu I.and Bhatti G. (2011). Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes, Mol. Cell. Biochem., **353** (**1**)**,** 139-149.

37 Helen O.T., Emmanuel U., Asuquo A.E., Utibe J.D., Omolabake O.O.and Violet A.-N. (2022). Determination of Atrazine/Streptozotocin-Induced Oxidative Stress and Mitigating Effect of Virgin Coconut Oil in Male Wistar Rats, Current Aspects in Pharmaceutical Research and Development Vol. 7**,** 65-82.

38 Khalaf H.A.and El-Mansy A.A.E.-R. (2019). The possible alleviating effect of saffron on chlorpyrifos experimentally induced cardiotoxicity: Histological, immunohistochemical and biochemical study, Acta Histochem., **121 (4),** 472-483.

39 van der Pol A., van Gilst W.H., Voors A.A.and van der Meer P. (2019). Treating oxidative stress in heart failure: past, present and future, European *Journal of Heart Failure*, **21 (4),** 425-435.