University of Thi-Qar Journal Of Science (UTsci)
Website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq
Volume 6, Number 4, June 2018

The protective role of N-Acetyl Cysteine and vitamin C against atrazine-Induced Dopaminergic Neurodegeneration in N27 Cells

Sabah Hassain Enayah Ahmed k. Al Atya Afrah A. Maktoof

Biology department- Collage of science- The university of Thi-Qar

Abstract:

Background: Atrazine (ATZ)has been associated with its capability to interact and damage DNA in neuronal cells. However, the molecular mechanisms of ATZ are not fully understood. In vitro and In vivo studies indicated that atrazine induces cytotoxicity and oxidative stress in human and animals. This study sought to determine the role of two antioxidants against the toxic impact of atrazine. **Main Methods:** N27 cell line pretreated to N-acetyl Cysteine NAC or Ascorbic acid AA alone or in combination before 24h atrazine exposure. Multiple intercellular antioxidant parameters measured such as (GSH, GSSG, MDA). Genotoxicity represented by single gel electrophoreses assay (comet assay). **Results:** the outcomes of this work demonstrated that higher protection against atrazine exposure at combination of NAC and AA comparing to the single exposure to each. The antioxidant acted in synergistic way to rescue cells from reactive oxygen species generated from atrazine exposure. DNA breaking or damage also prepared in better way in combination exposure comparing to the single exposure. **Conclusion:** the evidence of atrazine increase the oxidative damage have been approved by enhancing defense system to increase cell availability and DNA repair. While antioxidant (NAC&AA) prevent oxidative damage that causes by atrazine via increase cell viabilities and DNA fix.

Keywords: Atrazine, Ascorbic acid, N- Acetyl Cysteine, antioxidants, N27 cells.

Introduction:

The main contribution of environmental pesticides that influence on etiology neurons dysfunction, is now being broadly recognized(1). Atrazine ATZ is widely used as agriculture pesticides and now it is documented as one of the most risk neurodegenerative causes(2). Recently, atrazine is continually persisting in environment of many contraries (3). Chronic exposure to atrazine cause raise and accumulation level in several species including fish, frogs and rodents (4-6)

In vitro studies on variety of cell lines, exposed to ATZ, showed decrease in cell growing(7, 8). Furthermore, human's and animal's studies confirmed the connotations between ATZ exposure and cancer incidence. Apoptosis and oxidative stress rises were inveterate in toxicity of ATZ in most of these studies(9, 10).

N- acetylcysteine (NAC) is serve as a potent Reactive Oxygen Species (ROS) inhibitor and been known widely to counter the adverse effects that arising from oxidative stress (11). It is working to increase intracellular GSG and can easily act as precursor to GSH biosynthesis through been resource for sulfide group. In addition, it is stimulator of the cytosolic enzymes at the cellular level which included and work in glutathione regeneration(12). 4- hydroxynonenal 4-HNE would induce neuronal death can be inhabited and protected by NAC (13). The effectiveness of NAC in protecting cell has been understood with context of mechanism including oxidative stress(14)

Ascorbic Acid AA is the reduced form of vitamin C which is essential for metabolite for a variety of organisms(15). It is found in multiple fruits and vegetables and can be synthesized from glucose in liver of some mammalian to allow the conservation of physiological levels(16). In human, the lack in enzymes included in AA synthesis making the exogenous sources are the only sources(17). The exogenous nutritional source led to classify it as vitamin and it has been reported that its deficiency caused scurvy disease(18). It is playing biological role by antioxidant action with variety of cellular function. Its activity

inside the cells are included; detoxification activities, contribute in some enzyme action as an enzyme co-factor. Moreover, it is categorized as one of the first line antioxidant defense compound and it can protect lipid membrane and save DNA and proteins from oxidative damage(19, 20).

Even though, the mechanism of pretreatment in NAC and AA protecting cells against oxidative stress produced by atrazine is still not clearly elucidated. In the current study, we systematically tested 27 different grouping of NAC/AA to find out the optimized concentrations which can produce maximum protection to N27 cells suffering from atrazine influence. We then clarified the role of single exposure and compared to co-exposure to both antioxidants. The identification of their roles included the antioxidant and DNA single or double broken as consequence of atrazine damage.

Materials and Methods:

All the chemicals purchased from Sigma Chemical Company (St. Louis, MO). unless mentioned. The immortalized rat mesencephalic dopaminergic cells (N27 cells) were obtained as gift and cultured in RPMI 1640 medium that supplemented with 10% fetal bovine serum (FBS), 2 mM L- glutamine, 50 units of penicillin, and 50 μg/mL streptomycin. The previous media referred as completed media. To prompt cell differentiation, cells were incubated with 3mM of DB-cMAP in complete media for 48h. The perfect condition for these cells is that cells were grown in 5% CO2 at 37◦C and growth factor added, until they were 60–70% confluent. After culturing and cells reached the wanted confluent, cells were plated in 6-well plates at a density of 25,000 cells/well. All the exposures were initiated for the differentiated N27 cells.

Atrazine, 2-chloro-4-(ethylamine)-6-(isopropylamine)-s-triazine ATZ (purity: 98.9%) were purchased from Chem Service, Inc. (West Chester, PA, USA). ATZ were freshly prepared in a 75-mM stock in 0.5% of ethanol. The cells were pre-incubated with 0.1 to 0.9mM of ascorbic acid which prepared daily or/ and with NAC at final concentration of 1-9mM for 2h

All the pretreatments and atrazine treatments were made in a complete RPMI medium. All the experiments conducted in triplicate

The experiment designed as following

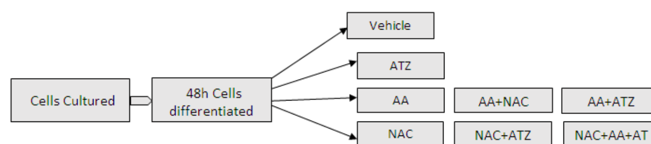

Figure 1: Experimental design. Atrazine (ATZ), N-acetyl-L-cysteine (NAC), Ascorbic Acid (AA)

Cell viability:

Cell feasibility was assessed by trypan blue staining and MTT assay. Cells were detached with 0.05% trypsin. Cells centrifuged and cell pellets were re-suspended in 100µl Hank's buffer. Mixture of 5 µl cells and 5µl trypan blue solution (Invitrogen) were used to count the cells. Cell viability was determined by counting the viable cells and dead cells by phase contrast microscopy. For the MTT assay, methylthiazoletetrazolium (MTT, Sigma) was added to culture medium (final concentration at 0.4 mg/mL) and incubated for two hours. Culture medium was removed and precipitates were dissolved in 0.04 M HCl in isopropanol. Cell viability was measured by a plate reader at OD_{590}.

GSH Analysis:

HPLC were used to measure the reduced GSH levels. The HPLC system (Shimadzu) contained a model LC-10A pump, an injector with a Rheodyne insertion controller with a 20 lL stuffing loop, and a Model Rf-535 fluorescence spectrophotometer that functioning at an excitation wave length of 330 nm and a 375 nm of emission wave length. The HPLC column was 4.6 mm and was filled with 5 lm atoms of C18 stuffed material. Chromatopac Model CR601 integrator (Shimadzu) was utilized to quantify the peaks from the HPLC system. The mobile phase was performed with 70% acetonitrile and 30% water adjusted to a 2.5 of pH with adding 1 mL/L of both acetic and o-phosphoric acids. The products were calculated from the column at a flow rate of 1 mL in minute.

Before we started cells were homogenized in grade water (HPLC water). 20mL of the diluted cell homogenate was mixed with 230 ml of HPLC water and then added to 750ml of NPM. Product solutions were kept for 5 at room temperature. 5 ml of 2 N HCl was added to stop the reaction. Finally, all samples were filtered through a 0.2 mm filter and injected into the HPLC system (21).

GSSG Analysis:

HPLC also used to detect level of oxidized glutathione (GSSG). 40 ml straight cell homogenate added to 44 ml of HPLC grade water, and mixed with 16 ml of 2- vinyl pyridine. The mixture was incubated at dark for 1h in room temperature for block any preexisting GSH. 1h later, mixture of 95 ml of a 2 mg/mL solution of NADPH and 5ml of 2 units/mL glutathione reductase solution were added to the original solution. 150ml of aliquot from pervious mixture was mixed with equal amount of HPLC water and then750 ml of NPM also added. 5min later the reaction was stopped by adding 5 ml of HCl. To purified the samples, all samples were filtered before HPLC injection (21).

MDA Analysis:

For MDA measurement reverse –phase of HPLC system was used as previously described (Draper et al. 1993) with some modification. Briefly, 350 ml of straight cell homogenate added to 100 ml butylated hydroxytoluene, and 550 ml of 10% trichloro acetic acid. The mixture was merged and boiled for 30 minutes. Then cooled on ice and centrifuged for 10 min. 500ml of the supernatant was removed and added to 500ml of thiobarbituric acid. The samples were boiled again for 30 minutes, and then cooled on ice. 500 ml of result solution was removed and mixed with 1.0 ml of n-butanol. The mixture was then vortexed, and centrifuged for 5 min to facilitate a phase separation. The top layer was then filtered and injected into a reverse-phase HPLC system. The mobile phase comprised of 0.6% tetrahydrofuran 69.4% sodium phosphate, 30% acetonitrile. The excitation wavelength was 515nm; the emission wave length was 550 nm.

Single cell preparation and comet assay:

several techniques can be used to assess DNA fragmentation such as micronucleus and chromosomal aberration assays(22). single-cell gel electrophoresis or comet assay, A suitable approach for evaluating DNA damage or broken which is reported as simple, sensitive, and fast technique for counting and analyzing DNA impairment in separate cells(23). After all the treatments, the cell suspended in phosphate buffered saline(PBs). Then, thry homogenized in Nisei Ace Homogenizer. Afterward material was centrifuged at 5009g for 3 min and suspension generated is used. The alkaline single cell gel electrophoresis of N27 cells was completed as following. In brief, normal melting point agarose (NMPA) (1.0%) was prepared in (PBs), agarose melted using micro-waved. Thin layer of NMPA on frosted slides were prepared. Diluted sample with 1% low melting point agarose (LMPA) was loaded and evenly spread. The slides were saved on ice for 15 min to let the gel to harden. The third layer of 0.5% of agarose was added onto each slide and retained to be on ice for 10 min. The slides were kept in lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris) with 10% DMSO and 1% Triton X-100. After that, all the slides placed in electrophoresis buffer for 30min. the cells then trsported utilizing a horizontal electrophoresis platform in fresh, chilled electrophoresis buffer for 15 min at 28 V and 300AM. Tris–HCl buffer (pH 7.5) used for neutralized and stained with ethidium bromide and examed with fluorescent microscope and DNA damage qualify(24).

Protein Assay:

The protein level was determined following the Bradford method with Coomassie Blue (Bio-Rad) (25).

Statistical Analysis:

One-way -ANOVA with Bonferroni post-test multiple comparisons post-test was performed for analysis using GraphPad Prism version 7.0 software (GraphPad Software, Inc., San Diego CA). p values < 0.05 were reflected statistically significant.

Results:

Expose N27cells line to different concentration of ATZ for 24h showed cytotoxicity dependent of does manner and the reduction in cell viability start to be significant at 200 µM up to 300 µM figure (2A). In this study, we pre-treat cells with NAC or AA to protect cell against atrazine induced cell death. The outcome of this study showed that NAC reduce the toxicity of atrazine but is not efficient enough to produce fall recovery of atrazine action figure (2B). As shown in figure (2C), pre-incubation with AA alone enhance the protection alongside the atrazine exposure but still not efficient enough to remove the toxic impact of Atrazine toxicity.

MDA measurement results explained that atrazine would significantly increase MDA level. Single exposure to NAC or AA exhibited significant different comparing to the control (without any treatment) and revealed significant different comparing to 300 µM atrazine exposure. Furthermore, NAC/AA in

combination indicated return MDA level close to the control figure (3A).

Atrazine exposure depleted GSH from the cell. Treatment with NAC as GSH precursor displayed significant different with control as well as with atrazine alone. In other word, single exposure worked to bring up the GSH level to the normal but it was not meaningful enough to be impersonator to control level. AA exposer also served as NAC single but was not as what has demonstrated with co-exposure to NAC and AA figure (3B).

Moreover, the induction of GSSG after 24h exposure to atrazine reduced at NAC or AA single, but the reduction was significant to the control and atrazine exposure. Pre-treatment for 2h to NAC and AA was adequate to resolve the influence of atrazine exposure figure (3C).

Comet assay results demonstrated that amount of DNA in the tail increased comparing to the head after atrazine exposure. DNA amount decreased after pre-treatment to NAC or AA separately. The combination was satisfactory to repair the DNA table (1) and figure (4).

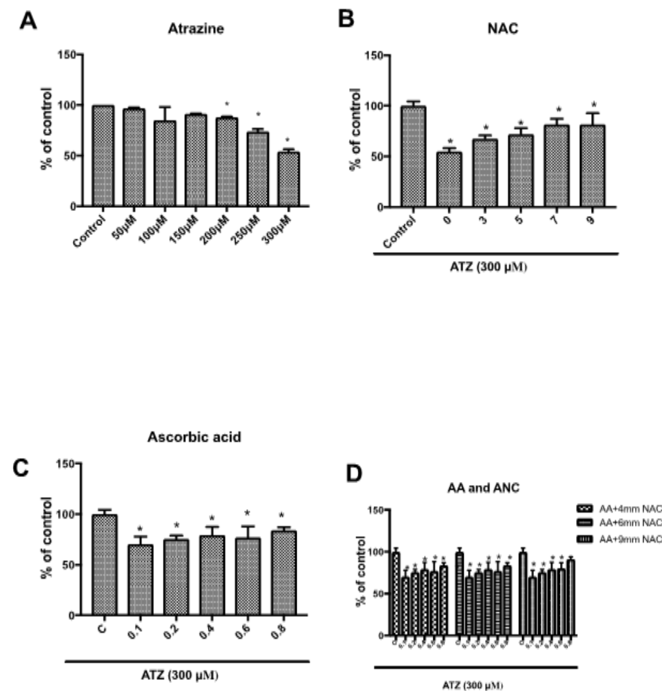

Figure 2: N27 cells viability, (A) cells exposed to (50-300 µM) for 2h. (B) cells pretreated for 2h to NAC then to atrazine for 24h. (C) cells pretreated with AA alone for 2h before atrazine exposure. (C) pretreatment of N27 cells to AA and NAC at different concentration to figure out best combination impact with atrazine exposure for 24h.

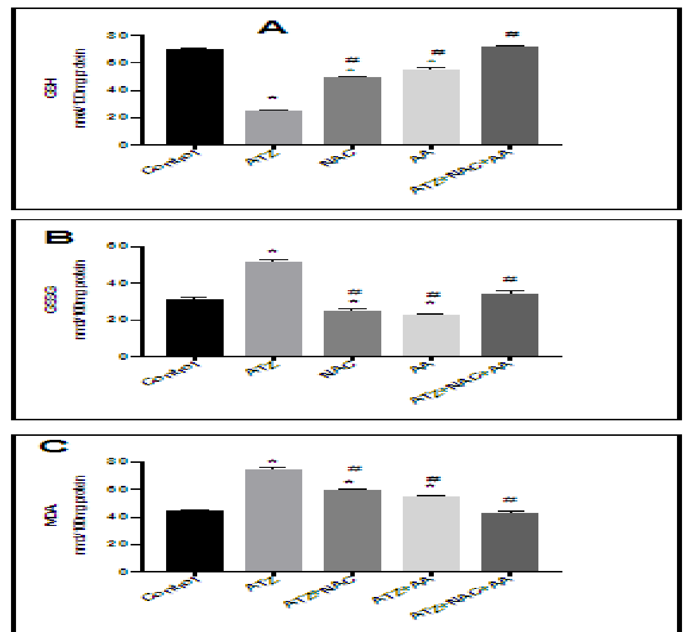

Figure 3: Intercellular of GSH, GSSG and MDA in N27 cells with and without pretreatment with NAC or AA for 2h. (A) GSH. (B) GSSG. (C) MDA. * significant different compared to the normal control cells; # significant different compared to atrazine treatment at 300 µM for 24h, P<0.05.

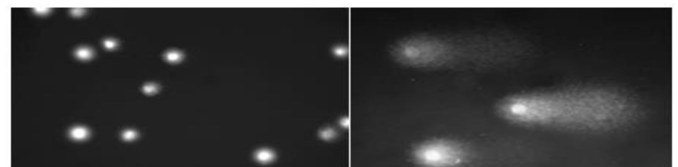

Figure 4: Comet assay pictures showing the control cells with intact DNA and exposed cells with tail where broken DNA migrated

Table 1: Comet assay parameters after single and double exposure. * significant different compared to the normal control cells; # significant different compared to atrazine treatment at 300 µM for 24h, P<0.05.

	Head DNA (%) Mean±SD	Tail DNA (%) Mean±SD	Head diameter Mean±SD	Tail length Mean±SD	Tail moment Mean±SD	Tail mean intensity Mean±SD	Tail intensity Mean±SD
Control	92.32±0.34	6.8±0.23	49.32±0.51	3.9±1.12	0.2±0.03	978±210	2685± 82
Atrazine	40.88±9.23*	59.78±7.72*	20.43±4.32*	35.34±4.56*	2.90±0.02*	1678±542*	7852±67*
NAC	98.32±0.94#	1.8±0.83#	44.36±0.51#	2.9±1.82#	0.01±0.05#	988±210#	435± 82#
AA	97.65±0.62#	2.05±0.53#	23.82±0.51#	1.49±1.82#	0.3±0.15#	778±290#	4357± 54#
Atrazine +NAC	68.48±7.35*#	45.43±9.44*#	12.78±7.51*#	19.91±5.16*#	3.54±0.30*#	1438±542*#	7843±67*#
Atrazine +AA	77.48±6.43*#	30.43±9.44*#	15.78±7.51*#	15.28±3.95*#	3.54±0.30*#	1438±542*#	7652±67#
Atrazine +NAC+AA	88.36±4.97#	10.43±9.44#	10.78±7.51#	5.89±2.56#	3.54±0.30#	1438±542#	6453±67#

Discussion:

Our results elucidate that pretreatment with thiol antioxidant NAC and vitamin C in combination would potentially protect against ATZ neurotoxicity and exerted the main action of reactive oxygen species effect better than individual antioxidant exposure. As well as we showed that the protection with combination of antioxidants may be able to inhibit the impact of ATZ capacity to break down DNA comparing to single exposure. In the current study, we treated N27 cells with various concentrations of Atrazine for 24h. Because ATZ can provoked alternation in intracellular ATP to be less efficient, it will cause toxicity to dopaminergic neurons(26). The toxicity started to be significant comparing to the control at 200 μM was below the atrazine stated to cause neuron toxicity, which typically reported at 300 μM in previous studies and in our study as well (27, 28). Atrazine would also influence on the cell morphology and this would have led to energy perturbation and increasing in ADP: ATP ratio. Augmentation level of ADP and the alteration in level of ATP can be correlated with increase cell death in neuronal cells (29). Evaluation the protection effects of NAC action as antioxidant, cells were pre-incubated for 2h with NAC. Several studies have shown that NAC and AA can suppress atrazine induced in brain (30, 31) One mechanism by which NAC may prevent the impact of toxicant via its antioxidant effects such reactive oxygen species or enhancing GSH level (30). It has been approved that both AA and NAC are analogue and precursor of glutathione and they are able to possess a broad array of biological properties which are fundamentals in the pathophysiological protective under certain circumstance, they are acting as pro-oxidant more than anti-oxidant (31). Previous study demonstrated that the pretreatment in NAC and AA would promote cell proliferation through their action to suppress cyclin dependent kinase inhibitor in stem cells (28).

Atrazine suppressed GSH in N27cells which is reduced by atrazine. Generally attenuated level of GSH will reduce the protection of neuron from reactive oxygen species ROS that generated from reactive species such as hydrogen peroxide and superoxide(14) There for, this study was designed to investigate weather atrazine alters GSH level and role of double antioxidants effect . GSH depleted with 24h exposer to atrazine at 300 μM. Next, we assessed the role of NAC and AA as GSH precursor that save cells from atrazine influence. While the treatment with each of NAC or AA alone mad some alteration in the GSH level, the combination showed better results, which was close to the control. NAC could enhance GSH inside the cells. This result will confirm role of oxidative stress as an early event of atrazine exposure because neurotoxicity would be attenuated by increasing some of antioxidants as Trolox and GSH(32). Furthermore, loss of protein sulfhydryl, including transporter proteins, might be associated with GSH depletion after (33).

Environmental adulteration may be cause lipid peroxidation and DNA break directly through their parental or metabolites or secondarily by ROS generation (34). The significant induction of GSH can protect DNA from damage. Therefore, besides measuring GSH as antioxidant biomarkers, we evaluated DNA damage with pre-treatment to single and double of antioxidant following by atrazine to measure their role in protections. Oxidative stress induction comping atrazine exposure, initiation ROS in cells trigger DNA damage due to obstruct DNA repair enzymes(35, 36). Our results, using comet assay to evaluate the toxicity outcome, showed increase DNA damage with atrazine exposure but the antioxidant improved this impact. The present study confirms the genotoxicity of herbicide increased and the antioxidant would reduce this disruption, this observation is supported by other studies (37)

Even though, there is different in the structure feature and intrinsic characteristic of GSSH and MD, they are significantly influenced by atrazine exposure. Atrazine caused GSSH and MDA increased and this induction could be also related to the rising formation of ROS as an optical defense mechanism(38). NAC and AA are also reported to scavenge free radicals by aggregate level of GSSH and MDA(39). The second experiment was to test weather NAC or /and AA could protect N27 cells from Atrazine oxidation effects. In this experiment, we treated N27cells with different concentrations of NAC alone or AA alone and the combination of both for 2h followed by exposure to atrazine for 24h showed that N27 cells were significantly saved by combination of NAC and AA in does dependent manner.

In summary, pretreatment with mixture of antioxidant would protect neuronal cells from oxidative damage produced by atrazine through oxidant reduction and DNA protection. The author strongly recommended to consider NAC and AA in the nutrition or treatment to workers or farmers who expose to atrazine.

Acknowledgment:

The author thankful for all the researcher in the university who help him to conduct the current work. The author thankful to the head of department and his lab team.

Conflicts of Interest:

The author declares no conflict of interest.

Reference:

Abarikwu, S. O., Farombi, E. O., Kashyap, M. P., & Pant, A. B. (2011). Kolaviron protects apoptotic cell death in PC12 cells exposed to atrazine. *Free Radic Res, 45*(9), 1061-1073. doi:10.3109/10715762.2011.593177.

Abarikwu, S. O., Farombi, E. O., & Pant, A. B. (2011). Biflavanone-kolaviron protects human dopaminergic SH-SY5Y cells against atrazine induced toxic insult. *Toxicol In Vitro, 25*(4), 848-858. doi:10.1016/j.tiv.2011.02.005.

Agarwal, R., & Shukla, G. S. (1999). Potential role of cerebral glutathione in the maintenance of blood-brain barrier integrity in rat. *Neurochem Res, 24*(12), 1507-1514.

Arakawa, M., & Ito, Y. (2007). N-acetylcysteine and neurodegenerative diseases: basic and clinical pharmacology. *Cerebellum, 6*(4), 308-314. doi:10.1080/14734220601142878.

Arakawa, M., Ushimaru, N., Osada, N., Oda, T., Ishige, K., & Ito, Y. (2006). N-acetylcysteine selectively protects cerebellar granule cells from 4-hydroxynonenal-induced cell death. *Neurosci Res, 55*(3), 255-263. doi:10.1016/j. neures.2006.03.008.

Bakke, B., De Roos, A. J., Barr, D. B., Stewart, P. A., Blair, A., Freeman, L. B., . . . Vermeulen, R. (2009). Exposure to atrazine and selected non-persistent pesticides among corn farmers during a growing season. *J Expo Sci Environ Epidemiol, 19*(6), 544-554. doi:10.1038/jes. 2008.53.

Bohn, T., Cocco, E., Gourdol, L., Guignard, C., & Hoffmann, L. (2011). Determination of atrazine and degradation products in Luxembourgish drinking water: origin and fate of potential endocrine-disrupting pesticides. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 28*(8), 1041-1054. doi:10.1080/ 19440049.2011.580012.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem, 72*(1), 248-254. doi:https://doi.org/10.1016/0003-2697(76)90527-3

Campos-Pereira, F. D., Oliveira, C. A., Pigoso, A. A., Silva-Zacarin, E. C., Barbieri, R., Spatti, E. F., . . . Severi-Aguiar, G. D. (2012). Early cytotoxic and genotoxic effects of atrazine on Wistar rat liver: a morphological, immunohistochemical, biochemical, and molecular study. *Ecotoxicol Environ Saf, 78*, 170-177. doi:10.1016/j.ecoenv .2011.11.020.

Cavas, T. (2011). In vivo genotoxicity evaluation of atrazine and atrazine-based herbicide on fish Carassius auratus using the micronucleus test and the comet assay. *Food Chem Toxicol, 49*(6), 1431-1435. doi:10.1016/j.fct. 2011. 03.038.

Chandramani Shivalingappa, P., Jin, H., Anantharam, V., Kanthasamy, A., & Kanthasamy, A. (2012). N-Acetyl Cysteine Protects against Methamphetamine-Induced Dopaminergic Neurodegeneration via Modulation of Redox Status and Autophagy in Dopaminergic Cells. *Parkinsons Dis, 2012*, 424285. doi:10.1155 /2012/424285.

Dhawan, A., Bajpayee, M., & Parmar, D. (2009). Comet assay: a reliable tool for the assessment of DNA damage in different models. *Cell Biol Toxicol, 25*(1), 5-32. doi:10.1007/s10565-008-9072-z.

Di Monte, D. A. (2003). The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? *Lancet Neurol, 2*(9), 531-538.

Du, J., Cullen, J. J., & Buettner, G. R. (2012). Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochim Biophys Acta, 1826*(2), 443-457. doi:10.1016/j.bbcan.2012.06.003.

Foradori, C. D., Zimmerman, A. D., Hinds, L. R., Zuloaga, K. L., Breckenridge, C. B., & Handa, R. J. (2013). Atrazine inhibits pulsatile gonadotropin-releasing hormone (GnRH) release without altering GnRH messenger RNA or protein levels in the female rat. *Biol Reprod, 88*(1), 9. doi:10.1095/biolreprod.112.102277.

Fukami, G., Hashimoto, K., Koike, K., Okamura, N., Shimizu, E., & Iyo, M. (2004). Effect of

antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. *Brain Res, 1016*(1), 90-95. doi:10.1016/j. brainres. 2004 .04.072.

Harrison, F. E., & May, J. M. (2009). Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med, 46*(6), 719-730. doi:10.1016/j.freeradbiomed.2008.12.018

Kammann, U., Bunke, M., Steinhart, H., & Theobald, N. (2001). A permanent fish cell line (EPC) for genotoxicity testing of marine sediments with the comet assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 498*(1), 67-77. doi: https://doi.org/10.1016/S1383-5718(01)00268-6

Lachapelle, M. Y., & Drouin, G. (2011). Inactivation dates of the human and guinea pig vitamin C genes. *Genetica, 139*(2), 199-207. doi:10.1007/s10709-010-9537-x.

Li, C. J., Sun, L. Y., & Pang, C. Y. (2015). Synergistic protection of N-acetylcysteine and ascorbic acid 2-phosphate on human mesenchymal stem cells against mitoptosis, necroptosis and apoptosis. *Sci Rep, 5*, 9819. doi:10. 1038/srep 09819.

Los, M., Mozoluk, M., Ferrari, D., Stepczynska, A., Stroh, C., Renz, A., . . . Schulze-Osthoff, K. (2002). Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. *Mol Biol Cell, 13*(3), 978-988. doi:10.1091/mbc.01-05-0272.

Lynnyk, A., Lunova, M., Jirsa, M., Egorova, D., Kulikov, A., Kubinova, S., . . . Dejneka, A. (2018). Manipulating the mitochondria activity in human hepatic cell line Huh7 by low-power laser irradiation. *Biomed Opt Express, 9*(3), 1283-1300. doi:10.1364/boe.9.001283.

Maksimchik, Y. Z., Lapshina, E. A., Sudnikovich, E. Y., Zabrodskaya, S. V., & Zavodnik, I. B. (2008). Protective effects of N-acetyl-L-cysteine against acute carbon tetrachloride hepatotoxicity in rats. *Cell Biochem Funct, 26*(1), 11-18. doi:10.1002/cbf.1382.

Manske, M. K., Beltz, L. A., & Dhanwada, K. R. (2004). Low-level atrazine exposure decreases cell proliferation in human fibroblasts. *Arch Environ Contam Toxicol, 46*(4), 438-444.

May, J. M. (2012). Vitamin C transport and its role in the central nervous system. *Subcell Biochem, 56*, 85-103. doi:10.1007/978-94-007-2199-9_6.

Millea, P. J. (2009). N-acetylcysteine: multiple clinical applications. *Am Fam Physician, 80*(3), 265-269.

Narayan, S., Liew, Z., Bronstein, J. M., & Ritz, B. (2017). Occupational pesticide use and Parkinson's disease in the Parkinson Environment Gene (PEG) study. *Environ Int, 107*, 266-273. doi:10.1016/j. envint.2017. 04.010.

Nishikimi, M., Kawai, T., & Yagi, K. (1992). Guinea pigs possess a highly mutated gene for L-gulono-gamma-lactone oxidase, the key enzyme for L-ascorbic acid biosynthesis missing in this species. *J Biol Chem, 267*(30), 21967-21972.

Oliveira, M., Maria, V. L., Ahmad, I., Serafim, A., Bebianno, M. J., Pacheco, M., & Santos, M. A. (2009). Contamination assessment of a coastal lagoon (Ria de Aveiro, Portugal) using defence and damage biochemical indicators in gill of Liza aurata--an integrated biomarker approach. *Environ Pollut, 157*(3), 959-967. doi:10.1016/j. envpol.2008.10.019.

Powell, E. R., Faldladdin, N., Rand, A. D., Pelzer, D., Schrunk, E. M., & Dhanwada, K. R. (2011). Atrazine exposure leads to altered growth of HepG2 cells. *Toxicol In Vitro, 25*(3), 644-651. doi:10.1016/j.tiv.2011.01.001

Ramirez, S. H., Potula, R., Fan, S., Eidem, T., Papugani, A., Reichenbach, N., . . . Persidsky, Y. (2009). Methamphetamine disrupts blood-brain barrier function by induction of oxidative stress in brain endothelial cells. *J Cereb Blood Flow Metab, 29*(12), 1933-1945. doi:10. 1038/jcbfm.2009.112.

Raza, H., & John, A. (2006). 4-hydroxynonenal induces mitochondrial oxidative stress, apoptosis and expression of glutathione S-transferase A4-4 and cytochrome P450 2E1 in PC12 cells. *Toxicol Appl Pharmacol, 216*(2), 309-318. doi:10.1016/j.taap.2006.06.001.

Rodriguez, K. A., Wywial, E., Perez, V. I., Lambert, A. J., Edrey, Y. H., Lewis, K. N., . . . Buffenstein, R. (2011). Walking the oxidative stress tightrope: a perspective from the naked mole-

rat, the longest-living rodent. *Curr Pharm Des, 17*(22), 2290-2307.

Singh, M., Sandhir, R., & Kiran, R. (2008). Atrazine-induced alterations in rat erythrocyte membranes: ameliorating effect of vitamin E. *J Biochem Mol Toxicol, 22*(5), 363-369. doi:10.1002/jbt.20249.

Stang, A., & Witte, I. (2009). Performance of the comet assay in a high-throughput version. *Mutat Res, 675*(1-2), 5-10. doi:10.1016/j. mrgentox.2009. 01.007.

Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., . . . Sasaki, Y. F. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen, 35*(3), 206-221.

Winters, R. A., Zukowski, J., Ercal, N., Matthews, R. H., & Spitz, D. R. (1995). Analysis of glutathione, glutathione disulfide, cysteine, homocysteine, and other biological thiols by high-performance liquid chromatography following derivatization by n-(1-pyrenyl) maleimide. *Anal Biochem, 227*(1), 14-21. doi:10.1006/abio.1995.1246.

Zhang, X., Banerjee, A., Banks, W. A., & Ercal, N. (2009). N-Acetylcysteine amide protects against methamphetamine-induced oxidative stress and neurotoxicity in immortalized human brain endothelial cells. *Brain Res, 1275*, 87-95. doi:10.1016/j.brainres.2009.04.008.

Zhu, L., Dong, X., Xie, H., Wang, J., Wang, J., Su, J., & Yu, C. (2011). DNA damage and effects on glutathione-S-transferase activity induced by atrazine exposure in zebrafish (Danio rerio). *Environ Toxicol, 26*(5), 480-488. doi:10. 1002/tox.20575.