Iraqi Journal of Biotechnology, May 2018, Vol. 17, No. 1, 67-77

Evaluation of antioxidants and reactive oxygen species (ROS) levels after combination exposure to chromium (III) and atrazine on liver in Wister Albion male rats

Sabah H. Enayah

Collage of Science/ Thi-Qar University / Human Toxicology Program, University of Iowa

Received: December 12, 2017 / **Accepted**: April 4, 2018

Abstract: Atrazine (ATZ)is remaining as one of the most widely broad spectrum pesticide used to control on annual grasses and broadleaf weeds. While Chromium Cr (III) it has been used currently as food supplemented. The current study was designed to determine impact of Cr at 7,30 and 300ppm on liver was in absence or in the present of (25 and 50mg/kg) of ATZ for 6 weeks in rats. Results indicated that deficiency or supplementation with Cr did not cause measurable toxicity and has no obvious effect on oxidative stress or anti-oxidant enzymes and lipid metabolism. Furthermore, the results showed that the mixture increased level of Aryl Hydrocarbon Receptor (AhR), Reactive Oxygen Species (ROS). Moreover, the mixture had effects on antioxidants such as super oxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH), activity where demonstrated more sever in liver tissue compared to Cr alone, especially as dose of ATZ increased. Moreover, as dose of Cr in the mixture was increased, the ROS, AhR, CAT, GSH activity was increased compared to low Cr values. However, SOD activity showed reduction in elevation of mixture concentrations. The results of this study suggest that there is synergic impact of co-exposure to ATZ and Cr on the parameters used as markers for liver toxicity in the current study. Further studies are warranted to investigate the interact of Cr and ATZ toxicity in more detail and to avoid having the Cr supplement where ATZ exposure is identified.

Keywords: Atrazine, Chromium, Reactive Oxygen Species(ROS), Cu/Zn Superoxide dismutase.

Corresponding author: should be addressed (Email: sabahhassainenayah-enayah@uiowa.edu , Sabah792007@yahoo.com).

Introduction:

Environmental and industrial persistent pollutants and pesticides, such as Atrazine ATZ which ongoing threating to human health with continuous exposure either through air or food(Jestadi et al. 2014). Atrazine (2-chloro-4-ethylamino-6-isopropylamine-1,2,5-triazine: ATZ) is a substance that can contaminate organisms at all tropic levels and their metabolites which would be cause of severe damage to these organisms (Nwani et al. 2010). It is well known for its persistence and powerful toxicity to hepatic cells and immunology system(Boffetta et al. 2013) ATZ is widely used in USA since 1960. Due to persistent of it under soil condition and its water solubility, it mostly contaminate many surface water or ground water where was found in 70% of all fresh water (McElroy et al. 2007) International Agency for Research on Cancer classified atrazine as possible carcinogenic to humans group 2B (Boffetta et al. 2013). Where several studies reported that ATZ exposure is associated with several health issues and cancers such as prostate cancer, dermatologic disease, reproductive system disorder and reparatory series diseases (Gely-Pernot et al. 2015) remarkable renal

dysfunction and renal cancer was noticed among animals exposed to ATZ (Lebov et al. 2015). Since ROS formation in hepatic cells meets all the required criteria to be considered as a serious situation of human health, it is essential to investigate the mechanism involved in the progression of their impact in present and absent of one of micronutrient such as chromium Cr.

Chromium (Cr) is essential micronutrient mineral which should be consumed through the diet and is involved in various biological activity (Rudolf and Cervinka 2009) It exists in trace amount in some of the plant products, where Cr is in the trivalent(III) and hexavalent (VI). Over decades, the anti- hyperglycemic activity of chromium (III) and its complex that known to regulates insulin in the body so is widely used as nutritional supplement. The action of Cr(III) and its complex is mediated via increasing adenosine 5'-momophosphate-activated protein kinase(AMPK)(Zhang et al. 2014). It has been reported that Cr (III) and its complex would be able when it is available in the supplement to regulate lipid metabolism (total cholesterol), normalize low and high density lipoprotein (HDL & LDL) as well as Cr (III)act to regulate triglyceride in diabetics (Suksomboon et al. 2014). Dietary supplements in functional food mostly has form of Cr (III). People with chromium (III) deficiency expressed inactive chromodulin which is important protein for enhance the signaling of insulin receptors and peripheral neuropathy with metabolic encephalopathy(Aupperle et al. 2001). Based on the author knowledge no study so far tried to explore the co-exposure to Cr and ATZ to elicited the influence of Cr would be exacerbate/mitigate on ATZ toxicity even at low doses.

Liver is one of the target organs of atrazine and Cr as reported in several research groups and big portion of ATZ would be ingested and bio accumulated in liver, thus, it is important to assess the changes in liver function and find out the rational relationship with chromium in different concentrations.

Therefore, the present study is designed to assess Cr in its capacity to abrogate ATZ morbidity and find out if moderate Cr shortage augment of ATZ impact. Moreover, the extent of Cr which has been able to change in hepatic micronutrients that well associated with ATZ toxicity. The role of Cr which would contributes in ATZ influence will also be learned.

Materials and methods:

Fifty-four of Wister Albion male rats at 4-5 weeks and around 100-200 g weight were used in the present study. All the animals housed in wire hanging cages in very controlled environment room. An experiment procedure is approved by the university ethics and complied with the laws and ethical recommendations currently in effect where the experiments performed. Room temperature 22 °C and 12-hour light dark cycle was maintained through all the experimental time. Animals then divided equally to three groups depending on fed diet. Varying text of chromium (III) (7, 30 and 300ppm) added to normal AIN-93G based diet following (Aupperle et al. 2001). The first group divided to three subgroups. 7ppm of chromium was chosen as lower level of Cr without resulting in deficiency to toxic level. 30ppm of chromium is adequate level as been set in normal AIN93G diet. 300ppm of

chromium level is supplemental level in rat diet. Animals were fed on their diets for three weeks. After 3 weeks feeding animals were administered through single IP injection to either normal saline or either 25mg/kg or 50mg/kg of body wet of atrazine. These doses were chosen based on studies investigated the effect of low doses of ATZ (Lim et al. 2009; Xia et al. 2017). While animals maintained on their respective diet, two weeks later animals were euthanized by carbon dioxide. Organs used in the current study were weighed and placed either in 10% formalin or flashed frozen in liquid nitrogen and kept in -80 °C for analysis. blood samples also were collected through cardiac puncture and collected in EDTA tubes.

For lipid extractions, pieces of liver weighing 0.2 g were ground with diatomaceous earth operating a mortar and pestle. Lipid extracted as described some were else (Bunaciu et al. 2007). The extraction done using with chloroform and methanol (2:1 v/v) utilizing an Enhanced Solvent Extractor (Dionex, Sunnvale, CA). samples were evaporated after concentrated to dry them in pre-weighed vials and sited in a desiccator. The vials weighed more than three times in several days, weight and values were normalized to tissue weight.

Two steps quantitative of Real Time-PCR were carried out to assess gene expression for selected proteins. RNeasy mini kit was utilized to isolate total RNA from hepatic tissue following kit instructions. cDNA reverse transcription kit. Cyp1A1 primer sequences used for the qPCR analysis of hepatic gene expression was (5'-ccatgaccaggaactatggg-3' 5'-tctggtgagcatccaggaca-3') and B-actin sequence

(5'-tagagccaccaatccacacag-3'

5'-cagccttccttcctgggtatg-3')

OxiSelect *in virto* ROS/RNS kit were purchased from Cell biolabs (San Diego USA) for ROS determination in hepatic cells. The assessment conducted following manufacturer's instructions. In brief, 10 and 20 mg of liver pieces homogenized in PBS and diluted to equal concertation. Fluorescence plat reader utilized to determine amount of ROS after DCFH2 and catalyst added to each well and incubated for 45 min. After incubation 480nm excitation and 530nm emission set up fluorescence densities were compared from each well to DCF standard curve. All the values were normalized to tissue weight.

Competitive inhibition assay was used to determine superoxide dismutase (SOD) activity as descried previously (Spitz and Oberley 1989; Spitz and Oberley 2001). In this assay superoxide inhibition mediated in reduction of xanthine to xanthine oxidase. CuZnSOD activity inhibition employing 5mM of sodium cyanide to conclude MnSOD activity. The activity of antioxidant is expressed as units in mg of protein following Lowry method where first time established in (Lowry et al. 1951)

Single cuvette and spectrophotometer employed to determine catalase activity CAT. 0.1 ml of H_2O_2 was added to 0.2ml of sample and the volume made up to 1ml using 5mM of phosphate buffer at pH of 7.4. Absorbance change at 240nm was recorded after the mixture was incubated for 2 min at room temperature, the rate of change indicated to the decomposition of H_2O_2 by CAT. Activities were calculated using the molar extinction(Simmonds et al. 1992).

Reduced glutathione (GSH) was measured in the liver homogenates

according to assay described previously by Sedlak and Lindsay (Sedlak and Lindsay 1968) Through this method aliquots (0.5mL) of liver homogenates tissue were deproteinized with TCA at 10%, supernatant was collected after centrifugation at 3000g for 10 min. 0.5 mL of the sample supernatant added to 4 mL phosphate buffer and 0.5 mL Ellman's reagent, 5,50-dithiobis 2-nitro benzoic acid was mixed and incubated in the dark at 25C for 5min. GSH standard used to compare it to yellow color that developed which was read immediately at 412nm using plate reader. The results in the current study were stated as microgram per milligram protein.

Glutathione peroxidase (GSH-Px) activity was estimated following method explained in detail elsewhere (Abarikwu et al. 2017) with slight modifications. The method briefly, mixture of 0.5 ml sample supernatant, sodium phosphate buffer (0.5mL), 0.1 ml sodium azide (10 mM), and 0.2 ml reduced glutathione (4 mM), finally, 0.1 mL mM H_2O_2 was mixt. The final total volume was brought up to 2.0 ml with duple distilled water. All the tubes were protected at 37 C for 3min and 0.5ml 10% TCA was used to terminated the reaction. The residual glutathione content also determined in the present study as following; after centrifugation, the supernatant was removed and added to 4.0ml of 0.3M disodium hydrogen phosphate solution then 1 ml of the Ellman's reagent were supplemented to the solution. A spectrophotometer was utilized to measure color development at 412 nm against a reagent blank containing only phosphate solution and Ellman's reagent on. The enzyme activity was stated here as units per milligram of protein.

Statistic:

All the experimental repeated three times. Two-Way ANOVA analysis carried out using Graphpad Prism 6 followed with Tukey multiple comparison. Significant differences were considered at $p \leq 0.05$. The statistical analyses were accomplished by GraphPad InStat, ver- sion7.01 (GraphPad, San Diego, CA). Moreover, a multiple linear regression (MLR) analysis with interactions conducted to evaluate overall variances and their directionality, i.e. increases or decreases. This was done using the proc reg function in SAS (v.9.3).

Results:

All the groups monitored for food intake through all the study period. Even though, no change in food consumption has been noticed, body weight increased with the time. Animals administrated to chromium showed significant elevation in weight comparing to sufficient at the end of the exposure time. ATZ exposure showed significant different in the weight comparing to the control of each group (figure 1).

Aryl Hydrocarbon Receptor (AhR) activation was evaluated to ensure canonically seen pathologies associated with ATZ exposure and their alteration with dietary of Cr. liver weight was significantly increased with ATZ exposure throughout Cr dietary groups (Figure 2A). Relatively great difference was observed among the diets where liver weight increased at 300ppm Cr comparing to 7 or 30ppm of Cr. Thymus weight was considerably decreased with ATZ exposure with no difference seen among the dietary Cr

levels (Figure 2B). The induction of CYP1A1 is likely the most well-known outcome of xenobiotic exposure and an efficient induction was seen with exposure (Figure 2C). Interestingly, the low dose of Cr supplemented, high ATZ exposure had a more robust induction of CYP450. This robust induction was not observed with Cr at 300ppm, high dose ATZ group where induction was more moderate. Extractable hepatic lipids, a measure of lipid accumulation, augmented with ATZ throughout the Cr dietary groups (Figure 2D).

Reactive Oxygen Species ROS has long been associated with herbicide exposure such as ATZ and was assessed here to investigate the role of Cr dietary in justifying or augmenting the oxidative stress. Hepatic ROS, as assessed by changes in DCFH2 oxidation, was amplified with ATZ exposure throughout the different Cr diets (Figure 3A). The basal dye oxidation levels in the complemented diet control treated animals was significantly elevated compared to the 30 ppm of Cr, like that seen with 7ppm. The greatest ROS was seen in the 300ppm of Cr and 50mg/kg of ATZ. Multiple linear regression (MLR) examination displayed that Cr in the diet positively affected the amount of dye oxidation. In addition, level of superoxide dismutase SOD activity was valued for the antioxidant ability of the hepatic tissue. All the SOD activities examined (CuZnSOD, MnSOD, and Total SOD), a decrease was obviously seen with treatment of ATZ, the promotion was most clearly seen with CuZnSOD (figure 3B) and total SOD activity (Figure 3D). Looking to the result would clearly noticed that CuZnSOD activity is altered somewhat by increasing Cr administration level; that is, inferior activity with the 7ppm and higher activity with the 300ppm diet. The variation of SOD activity also demonstrated in the total SOD level (figure 3D). CAT activity increased by ATZ exposure compared to the control. The activity of CAT was further increased with increasing level of Cr diet. ATZ co- administered with 300ppm Cr, CAT activity was even increased higher (figure 4A). Furthermore, GSH of hepatic cells increased at the end of the experiment whereas no significant change was observed with variety of Cr diets (figure 4B). Glutathione peroxidase GSH-Px significantly increased and continue to increase with raising of Cr diet. Significant different between 7ppm Cr administration and 300ppm (figure 4C).

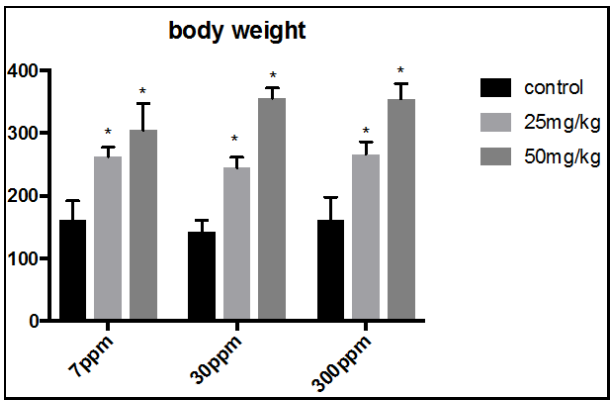

Figure (1): Impact of Cr or Cr and ATZ on the body weight. In the end of the experiment. * represent p≤0.05 compared to control (no ATZ) in the same Cr diet group.

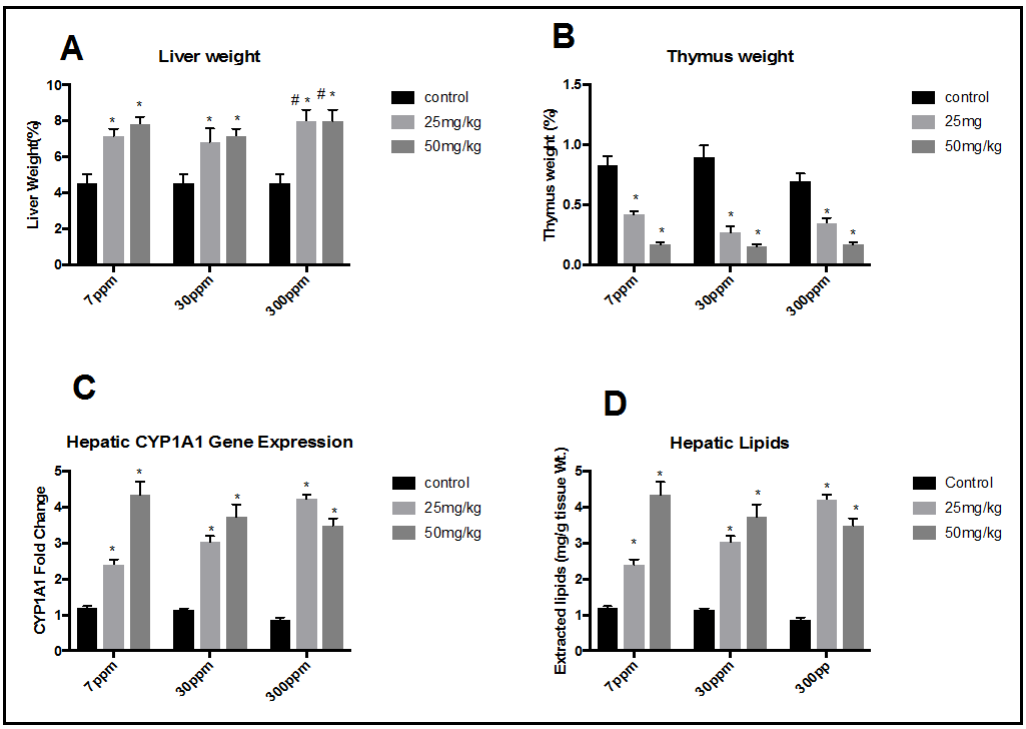

Figure (2): confirmation of hallmarks of ATZ induce hepatic toxicity. ATZ is known to activate NXR receptor so ordinary metrics were examed including liver (A) and thymus weight (B), CYP1A1 gene induction (C) and lipid accumulation in the liver cells (D). n=3 * represents p≤0.05 compared to control (no ATZ exposure) in same Cr diet; # represents p≤0.05 compared between 3 Cr levels of diet

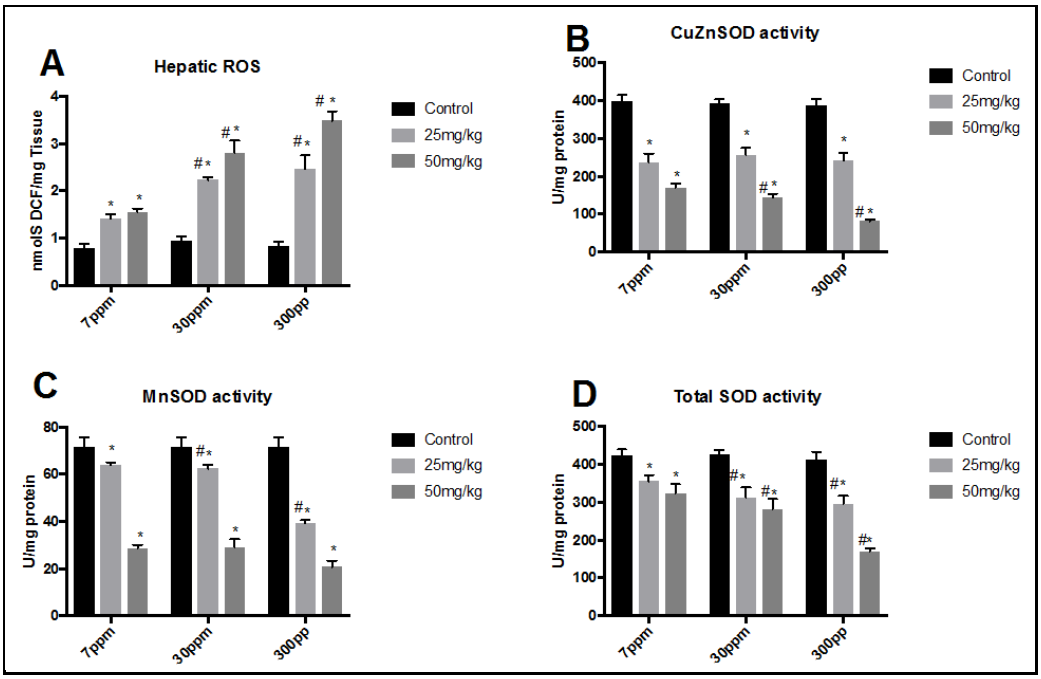

Figure (3): ROS induced by ATZ and three concentrations of Dietary. Total ROS in hepatic cells (A) along with CuZnSOD (B) mnSOD and total activity was measured to determine the change in ROS status caused by ATZ and Cr impact. n=3 *represents p≤0.05 compared to contral in same diet; # represents p≤0.05 compared to same treatment in extra Cr diet.

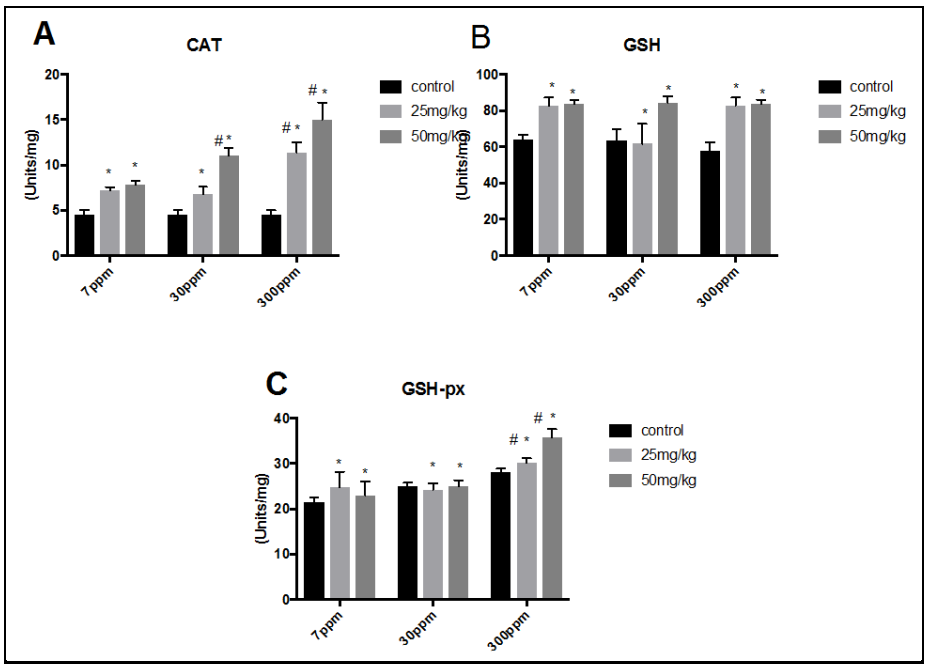

Figure (4): ATZ enhance antioxidants. Effects of different doses of ATZ on Catalase CAT (A), reduced glutathione GSH(B) and glutathione peroxidase GSH-px (C) activities in the liver cells n=3; * represents p≤0.05 compared in the same Cr treatment group; # represents P≤0.05 compared in the same Cr diet administration.

Discussion:

Toxic substance which effect on human health should be assessment to determine the sensitivity of toxicity and this is used to assets the degree of damage to target organs and physiological and behavioral obstruction (Nwani et al. 2010) In orally ingested atrazine the authors noticed that 15% of taken atrazine was detected in tissues such as liver and kidney(Ross et al. 2009). The rational explanation for the bioaccumulation of atrazine is probably due to the interact with phospholipid components of membranes which in turn would prevent atrazine excretion unless its undergoes a process that evaluate water solubility(Katagi 2010). In the current study ATZ and Cr effect on the body weight and cause the raising in most of the treatment groups. Previous studies noticed that, unlike the current studies, the body weights of ATZ exposed animals were generally decreased or unchanged. The different between our study and others is that the doses of ATZ used in these studies were 10 folds higher (Cantemir et al. 1997; Fukamachi et al. 2004). The main interpretation is the acute exposure to elevated level of atrazine is toxic and this would affect to prevent weight gain. In contrast, Low dose exposure for long time lead to mitochondrial damage which is similar to characteristic of insulin resistance that cause weight gain (Lim et al. 2009).

Clear marks of AhR activation in liver are established to include increase in cytochrome P450 expression, hepatic lipid accumulation as well as increase in liver and thymus weight(Lai et al. 2010). All these hallmarks were investigated in the current study. The

results were indicative of a positive AhR activation with ATZ exposure and Cr diet showed even more significant increase in the activation of AhR gradually with different Cr diet. Liver and thymus weight was dose dependent increased with both exposures. Furthermore, CYP450 induction synergicly increase with both administration. These finding is consistent with earlies study which indicated that ATZ can increase CYP expression by activating AhR (Xia et al. 2017). Alternately, pervious work showed that Cr do not effect on CYP450 activity alone (Elbekai and El-Kadi 2007). It has been observed in rats treated with atrazine that intracellular lipid content which support the interpretation for atrazine induce mitochondrial damage affects the insulin resistance and fat accumulation in metabolically tissue (EHIMIGBAI 2016).

Growing evidence reported that ATZ induce oxidative stress and cause hepatic damage and disrupt liver function by altering plasma ion levels at 250 and 500mg/kg and higher (Lin et al. 2016). Reactive Oxygen species ROS generated through ATZ ability to enhance oxidation production, suppressing antioxidant enzymes activity(Erinle et al. 2016; Liu et al. 2014). In the present study, ROS elucidated using dye oxidation; the degree of exacerbation/ mitigation by Cr diet also explored here. The study showed elevated level of ROS at 25 and 50mg/kg added to the Cr diet and this induction was higher at 300ppm of Cr supplement comparing to 7ppm. Interestingly, Cr alone showed no significant different in all the supplementation diet. This is contrary to what suggested previously where several studies reported increase level of ROS after Cr supplementations (Hassoun and Stohs 1995; Pandey et al. 2009; Wang et al. 2011). We think the does which had been used in our study was not high enough to produce any change alone. The most interesting results is that adding ATZ increased Cr ability to produce significant ROS. Superoxide dismutase activity SOD was decreased with combination of diet and ATZ exposure, consistent with what reported earlier (Erinle et al. 2016; Lim et al. 2009; Xia et al. 2017). Contention exists weather Cr dietary alone cannot alter the activity of Cu/znSOD or MnSOD (Doddigarla et al. 2016; Sinha et al. 2005) however other work showed Cr supplement do not alter SOD activity (Feng et al. 2015). Any decrease in SOD activity was depended on treatment of ATZ. Surprisingly, the SOD reduced at 300 ppm Cr and 50mg/kg exposure comparing to lower combination of Cr and ATZ. Decrease anti-oxidant enzyme as first defense would be due to impact of combination on gene expression or excessive intracellular superoxide induced which consumed much SOD and cause depletion of SOD activity(Zhao et al. 2014).

In birds, ATZ below 250 mg/kg activate Nrf2 pathway to protect against oxidative stress and enhancing antioxidants activity. Conversely, ATZ over 500mg/kg induced oxidative stress and apoptosis through suppressing antioxidants (Zhang et al. 2017). In agreement with our results where we noticed increasing in level of CAT as promoted after co-exposure to ATZ and Cr. Increase Nrf2 expression would induce the elevated in GSH and Gpx which consistent our work with ATZ exposure done earlier in several studies(Gandar et al. 2017; Zhang et al. 2017; Zhao et al. 2014).

Taking together the results, it can be assumed that Cr-ATZ co-exposure has potential to impair liver cells much more than which is observed with Cr treatment alone through the induction of hepatic oxidative damage. Consequently, the risk of ATZ induced liver damage may be highly influenced by Cr supplemented especially at high doses. This finding would approve a crucial insinuation for health especially for occupational exposure to ATZ and having Cr as food supplement. further studies are needed to better understand the effects of atrazine, including the chronic effect of low doses and targeting other organes.

Acknowledgments:

All the opinion provided by department faculty and lab members are appreciated. The author thanks Dr. Sattar for the statistics help. Author graciously acknowledges the support and training from university of Iowa, USA.

References:

1- Abarikwu, S.O.; Duru, Q.C.; Njoku, R.C.; Amadi, B.A.; Tamunoibuomie, A.; Keboh, E. (2017). Effects of co-exposure to atrazine and ethanol on the oxidative damage of kidney and liver in Wistar rats. Renal failure. 39(1):588-596.

2- Aupperle, H.; Schoon, H.A. and Frank, A. (2001). Experimental copper deficiency, chromium deficiency and additional molybdenum supplementation in goats--pathological findings. Acta veterinaria Scandinavica 42(3): 311-321.

3- Boffetta, P.; Adami, H.O.; Berry, S.C. and Mandel, J.S. (2013). Atrazine and cancer: a review of the epidemiologic evidence. European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP) 22(2):169-180.

4- Bunaciu, R.P.; Tharappel, J.C.; Lehmler, H.J.; *et al.* (2007). The effect of dietary glycine on the hepatic tumor promoting activity of polychlorinated biphenyls (PCBs) in rats. Toxicology 239(3):147-155.

5- Cantemir, C.; Cozmei, C.; Scutaru, B.; Nicoara, S. and Carasevici, E. (1997). p53 protein expression in peripheral lymphocytes from atrazine chronically intoxicated rats. Toxicology letters, 93(2-3):87-94 .

6- Doddigarla, Z.; Parwez, I. and Ahmad, J. (2016). Correlation of serum chromium, zinc, magnesium and SOD levels with HbA1c in type 2 diabetes: A cross sectional analysis. Diabetes & metabolic syndrome 10 (1 Suppl 1): S126-9.

7- Ehimigbai, R. (2016). Effect of Lead Nitrate on The Testis of Adult Wistar Rats At Different Dosage. International Journal of Forensic Medical Investigation (1): 5-7.

8- Elbekai, R.H. and El-Kadi, A.O. (2007). Transcriptional activation and posttranscriptional modification of Cyp1a1 by arsenite, cadmium, and chromium. Toxicology letters, 172(3): 106-119.

9- Erinle, K.O.; Jiang, Z.; Ma, B,; *et al.* (2016). Exogenous calcium induces tolerance to atrazine stress in Pennisetum seedlings and promotes photosynthetic activity, antioxidant enzymes and psbA gene transcripts. Ecotoxicology and environmental safety, 132: 403-412.

10- Feng, W.; Wu, H.; Li, Q.; *et al.* (2015). Evaluation of 90-day Repeated Dose Oral Toxicity, Glycometabolism, Learning and Memory Ability, and Related Enzyme of Chromium Malate Supplementation in Sprague-Dawley Rats. Biological trace element research, 168(1):181-195.

11- Fukamachi, K.; Han, B.S.; Kim, C.K.; *et al.* (2004). Possible enhancing effects of atrazine and nonylphenol on 7, 12-dimethylbenz [a] anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. Cancer science 95(5):404-410 .

12- Gandar, A.; Laffaille, P.; Canlet, C.; *et al.* (2017). Adaptive response under multiple stress exposure in fish: From the molecular to individual level. Chemosphere 188:60-72.

13- Gely-Pernot, A.; Hao, C.; Becker, E.; *et al.* (2015). The epigenetic processes of meiosis in male mice are broadly affected by the widely used herbicide atrazine. BMC Genomics 16.

14- Hassoun, E.A. and Stohs, S.J. (1995). Chromium-induced production of reactive

oxygen species, DNA single-strand breaks, nitric oxide production, and lactate dehydrogenase leakage in J774A.1 cell cultures. Journal of biochemical toxicology 10(6): 315-321.

15- Jestadi, D.B.; Phaniendra, A.; Babji, U.; Shanmuganathan, B. and Periyasamy, L. (2014). Effects of Atrazine on Reproductive Health of Nondiabetic and Diabetic Male Rats. International scholarly research notices 2014: 676013.

16- Katagi, T. (2010). Bioconcentration, bioaccumulation, and metabolism of pesticides in aquatic organisms. Reviews of environmental contamination and toxicology 204:1-132.

17- Lai, I.; Chai, Y.; Simmons, D.; *et al*. (2010). Acute Toxicity of 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126) in Male Sprague-Dawley Rats: Effects on Hepatic Oxidative Stress, Glutathione and Metals Status. Environment international 36(8):918-923.

18- Lebov, J.F.; Engel, L.S.; Richardson, D.; Hogan, S.L.; Sandler, D.P. and Hoppin, J.A. (2015). Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study. Environmental research, 143(Pt A):198-210.

19- Lim, S.; Ahn, S.Y.; Song, I.C.; *et al*. (2009). Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. PLoS One, 4(4):e5186.

20- Lin, J.; Zhao, H.S.; Xiang, L.R.; *et al*. (2016). Lycopene protects against atrazine-induced hepatic ionic homeostasis disturbance by modulating ion-transporting ATPases. The Journal of nutritional biochemistry, 27:249-256.

21- Liu, W.; Du, Y.; Liu, J.; *et al*. (2014). Effects of atrazine on the oxidative damage of kidney in Wister rats. International Journal of Clinical and Experimental Medicine, 7(10): 3235-3243.

22- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. The Journal of biological chemistry 193(1):265-275.

23- McElroy, J.A.; Gangnon, R.E.; Newcomb, P.A.; *et al*. (2007). Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in Wisconsin. Journal of exposure science & environmental epidemiology, 17(2):207-214.

24- Nwani, C.D.; Lakra, W.S.; Nagpure, N.S.; Kumar, R.; Kushwaha, B. and Srivastava, S.K. (2010). Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus (Bloch). International Journal of Environmental Research and Public Health, 7(8): 3298-3312.

25- Pandey, V.; Dixit, V. and Shyam, R. (2009). Chromium effect on ROS generation and detoxification in pea (Pisum sativum) leaf chloroplasts. Protoplasma, 236(1-4):85-95.

26- Ross, M.K.; Jones, T.L. and Filipov, N.M. (2009). Disposition of the herbicide 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine) and its major metabolites in mice: a liquid chromatography/mass spectrometry analysis of urine, plasma, and tissue levels. Drug metabolism and disposition: the biological fate of chemicals, 37(4): 776-786.

27- Rudolf, E. and Cervinka, M. (2009). Trivalent chromium activates Rac-1 and Src and induces switch in the cell death mode in human dermal fibroblasts. Toxicology letters, 188(3):236-242.

28- Sedlak, J. and Lindsay, R.H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry 25(Supplement C):192-205.

29- Simmonds, N.J.; Allen, R.E.; Stevens, T.R.J.; *et al*. (1992). Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. Gastroenterology 103(1): 186-196.

30- Sinha, S.; Saxena, R. and Singh, S. (2005). Chromium induced lipid peroxidation in the plants of Pistia stratiotes L.: role of antioxidants and antioxidant enzymes. Chemosphere 58(5): 595-604.

31- Spitz, D.R. and Oberley, L.W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. Analytical biochemistry, 179(1):8-18.

32- Spitz, D.R. and Oberley, L.W. (2001). Measurement of MnSOD and CuZnSOD activity in mammalian tissue homogenates. Current protocols in toxicology Chapter 7:Unit7.5.

33- Suksomboon, N.; Poolsup, N. and Yuwanakorn, A. (2014). Systematic review and meta-analysis of the efficacy and safety of chromium supplementation in diabetes.

Journal of clinical pharmacy and therapeutics, 39(3): 292-306.

34- Wang, X.; Son, Y.O.; Chang, Q.; *et al*. (2011). NADPH Oxidase Activation Is Required in Reactive Oxygen Species Generation and Cell Transformation Induced by Hexavalent Chromium. Toxicological Sciences 123(2): 399-410.

35- Xia, J.; Lin, J.; Li, X.N.; *et al*. (2017). Atrazine-induced environmental nephrosis was mitigated by lycopene via modulating nuclear xenobiotic receptors-mediated response. The Journal of nutritional biochemistry, 51:80-90.

36- Zhang, C.; Li, X.N.; Xiang, L.R.; Qin, L.; Lin, J. and Li, J.L. (2017). Atrazine triggers hepatic oxidative stress and apoptosis in quails (Coturnix C. coturnix) via blocking Nrf2-mediated defense response. Ecotoxicology and environmental safety, 137: 49-56.

37- Zhang, Q.; Xiao, X.; Li, M.; *et al*. (2014). miR-375 and miR-30d in the Effect of Chromium-Containing Chinese Medicine Moderating Glucose Metabolism. Journal of Diabetes Research, 2014: 6.

38- Zhao, F.; Li, K.; Zhao, L.; *et al*. (2014). Effect of Nrf2 on rat ovarian tissues against atrazine-induced anti-oxidative response. International journal of clinical and experimental pathology 7(6): 2780-2789.