Potential toxicity of aluminum and fluoride on some biochemical aspects of male rat’s offspring

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Abstract

Background: This work was designed to evaluate the potential hazards of sodium fluoride (NaF) and aluminum chloride (AlCl$_3$) given separately or in conjugation throughout the prenatal and up to weaning time or till the postnatal 70th day. The levels of the following parameters were then assessed; vitamin C (ascorbic acid), glutathione (GSH) and oxidized glutathione (GSSH), malondialdehyde (MDA), total protein, albumin, total calcium, ionized calcium, creatinine, urea, uric acid, and bilirubin. In addition, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. In this study, female pregnant rats were allocated into four groups. The first received the drinking deionized water and served as a normal group, the second was given a daily dose of NaF (0.15 g/L) dissolved in deionized water from day 6 of gestation till the end of the weaning period, and the third was given a daily dose of AlCl$_3$ (500 mg/L) for the same period of time. The fourth group was given drinking water containing combined doses of NaF + AlCl$_3$ for a similar length of time. Each group was further divided into two subgroups; the first continued to be treated with the same pollutants in drinking deionized water at the same dose level until the age of 70 days, whereas the second group was supplied with pure deionized water free from the intoxicating substances for the same period of time.

Results: The results revealed that either NaF or AlCl$_3$ given separately or in conjunction with each other abated the quenching effects of the antioxidant system and induced oxidative stress and several perturbations in the aforementioned parameters. The metals caused significant increase in the levels of urea, uric acid, creatinine, and activities of ALT and AST activities, whereas the levels of total and ionized calcium in serum and the concentration of vitamin C, GSH, and GSH/GSSG ratio in hepatic tissues were significantly decreased and the levels of MDA were markedly increased in the liver as a response to the studied metals.

Conclusion: Based on the present results, the exposure to sodium fluoride or aluminum chloride induced profound perturbation in the liver and kidney functions.

Keywords: Fluoride, Aluminum, Vitamin C, Reduced glutathione, Oxidized glutathione, Lipid peroxidation, Liver and kidney functions.

Background

Chronic exposure to fluoride and aluminum or aluminum/fluoride leads to accumulation of both elements in the lung, brain, gastric endocrine glands, kidney, and liver. This is concomitant with metabolic alternation and tissues damage; fluoride is an essential trace element, present in air, water, and food that may produce chronic alterations in human health when they are exposed to it (Kinawy & Al-Eidan, 2018; Kinawy & Ezzat, 2013; Tan, Liu, Zhang, Liu, Lu, Yu, Tu, & Cui, 2011). On the other hand, either aluminum or sodium fluoride (NaF) toxicity causes severe imbalances in the oxidant–antioxidant system and as consequences generate the reactive oxygen species (ROS) that contain unpaired electrons; these are highly reactive and can cause damage to the nucleic acid, lipids, proteins, and carbohydrates (Bényettou, Kharoubi, Hallal, Bényettou, Tair, Belmokhtar, Aoues, & Ozaslan M, 2017; Kinawy & Ezzat, 2013). Oxidative stress is developed when there is an
increase in the production of free-radical derivatives from oxygen and may serve as common mediators of neurotoxicity in response to fluoride and aluminum toxicity (Kaur, Bijarnia, & Nehru, 2009). Previous literature hypothesize that sodium fluoride is capable to penetrate the soft tissues such as liver by simple diffusion and induce several perturbations in the metabolic homeostasis and liver detoxification capacity, and subsequently initiate reactive oxygen species and generation oxidative stress in the liver and kidneys tissues (Chattopadhyay, Podder, Agarwal, & Bhattacharya, 2011; Dabrowaska, Letko, & Balunowska, 2006; Zhang, Zhou, Wang, Wang, Song, Liu, & Xi, 2014). In addition, elevation of the liver enzymes may lead to cellular degeneration of hepatic cells. The liver is considered the main organ which is responsible for metabolism and detoxication. In addition, NaF induce several perturbations in antioxidants defense system with higher rate of lipid peroxidation membrane damage (Anuradha, Kanno, & Hirano, 2001; Chen, Proestou, Bourbeau, & Wang, 2000; He & Chen, 2006; Nabavi, Habtemariam, Jafari, Sureda, & Nabavi, 2012; Trivedi, Verma, Sangai, & ChinoY, 2011), that leads to membrane dysfunction and as a consequence increases its permeability to calcium ions influx and mitochondrial injuries. On the other hand, the in-utero exposure to aluminum and fluoride has deleterious irreversible effects of some biochemical and physiological aspects. In this regard, the problem of exposure of the prenatal and early postnatal to aluminum and fluoride are of particular interest because it may have devastating, and probably irreversible impacts on several vital processes. This is likely because the metabolic pathways, biotransformation, elimination mechanisms, gut, liver, neuro-hormonal balances and renal functions, neural development, and particularly brain barrier formation are not fully developed in early stages (Bishop, Morley, Chir, Day, & Lucas, 1997; Kinawy & Al-Eidan, 2018; Kinawy & Ezzat, 2008, 2013; Weaver, Laker, & Nelson, 1984). In addition to the high affinity of fluoride for aluminum, this complex may occur spontaneously in body fluids. Furthermore, central pathophysiological mechanism may be responsible for much of the toxicity of aluminum and aluminium-fluoride compounds on the soft tissues (Blaylock, 2012). Vitamin C (ascorbic acid) is a cofactor for several enzymes; in addition, it prevents free radicals and acts as antagonistic to the toxic, mutagenic, and carcinogenic effects of environmental pollutants by stimulating liver detoxifying enzymes (Jan et al., 2015; Kojo, 2000). Consequently, this study was carried out to investigate the impact of aluminum chloride and sodium fluoride given separately or in combination throughout the prenatal and weaning periods or up to day 70 of postnatal stages.

Material and methods

Animals

This study was carried out using adult female waster rats (180–220 g). The animals were obtained from the animal house of the Institute of Ophthalmic Disease Research. All animals were maintained under a controlled environment at 20 °C with 12 h dark/light cycle and 50–70% humidity. Pregnant rats and the neonates were fed milk and bread and provided with water ad libitum.

Breeding program

Each female was mated with a proven male of the same strain in the separate cage following the traditional commercial system. Vaginal smears were taken to detect the presence of true estrus and sperm to confirm pregnancy according to the protocol described by Ozbilgin, Boz, Inan, and Vatansever (2012).

Experimental design

This experiment was conducted to investigate the effects of the hazards of sodium fluoride and aluminum chloride given separately or in combination through the prenatal life and up after weaning or up to 70 days of postnatal stages. Eighty pregnant female rats were randomly divided into 4 equal groups. The first group represent the control group that received the pure drinking deionized, the second, ingested NaF 0.15 g/L (He & Chen, 2006) dissolved in pure deionized water, the third exposed to 0.5 g/L aluminum chloride (AlCl3) (Fulton & Jeffery, 1990) dissolved in pure deionized water, and the fourth administered NaF plus AlCl3 dissolved in pure deionized water; after parturition, the mothers continued to have the same polluted substances and the pups were exposed to polluted substance through breastfeeding and left for natural maternal care during the first period of their development. Then after just the weaning stage, only the male rats were used and each group was divided into two subgroups; the first one supplied with pure ionized water and the other subgroups continued to have a daily the same dose level of polluting substances (NaF 0.15 g/L, 0.5 g/L AlCl3 separately and NaF + AlCl3 dissolved in drinking deionized water respectively) until day 70 of postnatal periods. Thus, the experimental groups were:

- Normal group (NPP-G): normal (N) offspring given deionized H2O throughout the pre- (P) and postnatal (P) periods until 70 days old.
- FPP-G: offspring exposed to sodium fluoride (F) throughout pre- (P) and postnatal periods until 70 days old.
- FP-G: offspring exposed to NaF (F) throughout the prenatal (P) period only.
APP-G: offspring exposed to AlCl₃ (A) throughout pre- (P) and postnatal (P) periods until 70 days old.
AP-G: offspring exposed to AlCl₃ (A) throughout the prenatal (P) period only.
FAPP: offspring exposed to NaF + AlCl₃ (FA) throughout pre- (P) and postnatal (P) periods until 70 days old.
FAP: offspring exposed to NaF + AlCl₃ (FA) throughout the prenatal (P) period only.

The rats were slightly anesthetized with pentobarbital (30 mg/kg, i.p. injection), and blood was collected from the abdominal dorsal aorta. Blood was collected and serum was separated for the biochemical and physiological analyses. Determination of serum levels of total protein, urea, and albumin was performed by using a commercial diamond diagnostics kit and determined by the colorimetric method according to the method of Young (2001). Serum creatinine, alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST), and calcium were determined by bio-systems kits according to the method of Young (2001); and free calcium was calculated according to the following equation:

\[
\text{Ionized calcium} = \frac{6 \times \text{total calcium} - K/3}{K + 6}
\]

where:

\[K = (0.19 \times \text{T.protein}) + \text{albumin}\]

Serum concentration of urea was determined by the method of Patton and Crouch (1977). Serum bilirubin was determined by Sigma-Aldrich (Merck) by colorimetric methods by Bulmer, Verkade, and Wagner (2012).

HPLC determination of the malondialdehyde (MDA) as lipid peroxidation marker and ascorbic acid concentrations: weighing and homogenization of the tissue: 0.1 g of liver tissues was freezing in liquid hydrogen, was homogenized in 1 ml 0.1% trichloroacetic acid for pre-oxidation of protein and then was homogenized with 10% perchloric acids and centrifuged at 5000 rpm for 5 min then the supernatant was injected to HPLC determination up to just after the weaning stage. Blood was collected and the abdominal dorsal aorta. Blood was collected and serum was separated for the biochemical and physiological analyses. Determination of serum levels of total protein, urea, and albumin was performed by using a commercial diamond diagnostics kit and determined by the colorimetric method according to the method of Young (2001). Serum creatinine, alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST), and calcium were determined by bio-systems kits according to the method of Young (2001); and free calcium was calculated according to the following equation:

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Measurement of oxidized and reduced glutathione of liver was done by weighing and homogenization of 0.1 g of iced KCl followed immediately by deproteinization and acidification with sulfosalicylic acid. A liver tissue was eluted by using 0.0025 M sodium phosphate buffer at pH 3.50 (with 0.005 M tetrabutylammonium phosphate (Waters, Milford, MA) and 13% methanol). The homogenate was spun at 5000 rpm for 10 min and the supernatant was used for glutathione (GSH) and oxidized glutathione (GSSH) determination immediately extracted from the trace elements and lipids by the use of solid phase 30-cm × 3.9-mm C-18 mu Bondapak column and detected with an ultraviolet (UV) detector at 190 nm. Flow rate: 1 ml/min (Jayatilleke & Shaw, 1993).

Statistical analysis
Kolmogorov-Smirnov test indicated that the data were normally distributed, and therefore the parametric statistical analyses were applied. One-way analysis of variance (ANOVA) was applied to analyze the effect of treatments on the studied parameters of male rat offspring, then followed by Duncan’s test of homogeneity (post-ANOVA hoc) to compare between all variables at significant level α = 0.05 (P < 0.05). Statistical analyses were done by the aid of the IBM Statistical Package for the Social Sciences (SPSS) version 24.

Results
The group that was given NaF and AlCl₃ separately or in combination throughout the prenatal and weaning period followed by the same treatment up to day 70 and had a significant decrease in the levels of the protein in comparison with the normal group. On the other hand, the groups exposed to the pollutants from day 6 of gestation up to just after the weaning stage did not reveal any significant change in comparison with the normal group that was lower than each one group administered the polluted substances. The group that was given the combination of NaF + AlCl₃ exhibited lower level than the group administered the polluted substances during the prenatal and weaning stages. Albumin was significantly reduced in the group exposed to the combination of NaF + AlCl₃ than the control and aluminum groups. The level of uric acid was significantly increased in the groups given NaF, AlCl₃ alone, or in combination until the age of 70 days when compared with the control values, and these groups approached each other, and higher than the group exposed to NaF + H₂O, AlCl₃ + H₂O, and NaF + AlCl₃ + H₂O. Urea level was elevated in all treated groups in comparison with the normal group. The group administered NaF, AlCl₃ separately, or in combination until the age of 70 days was significantly higher than the groups given the polluted substances for a short time from the prenatal to weaning stages (Table 1).
Table 2 shows that the levels of ALT had a significant increase in all treated groups as compared with the control values. On the other hand, both groups exposed to NaF + AlCl₃ and NaF + AlCl₃ + H₂O exhibited a higher level than that in the other groups. The level of creatinine values in the group exposed to NaF individually or in combination with aluminum were significantly higher than in the corresponding control group; moreover, the group exposed to the aluminum and sodium fluoride exceeded significantly the other each one group; further, the group exposed to NaF + H₂O did not reveal significant changes when compared with the normal.

The offspring in all groups exposed to NaF, AlCl₃ alone, or in combination throughout the prenatal and weaning periods followed by the same treatment up to day 70 had a significant decrease in the level of the vitamin C when compared with the corresponding normal, NaF + H₂O, AlCl₃ + H₂O, and NaF + AlCl₃ + H₂O. However, the groups administered NaF + H₂O, AlCl₃ + H₂O, and NaF + AlCl₃ + H₂O did not reveal significant changes when compared with the normal. Lipid peroxide was higher in all tested groups than the normal group, except AlCl₃ + H₂O group which had no significant change in comparison with the normal values, however, is lower than all treated groups. Moreover, the combination of AlCl₃ and NaF exhibited lower levels than all groups exposed through the prenatal and weaning stages. Reduced glutathione was reduced in both groups exposed to NaF or NaF + AlCl₃ in comparison with the normal.

Each group ingested AlCl₃ alone did not reveal any significant change from the normal values. Most of studied parameters in groups given NaF + AlCl₃ were significantly higher than all groups exposed to AlCl₃ alone. The level of oxidized glutathione was significantly increased in all groups that administered polluted substances than the normal group. Moreover, the group that was given the combination of NaF + AlCl₃ exhibited a higher value than NaF, NaF + H₂O, AlCl₃ + H₂O, and NaF + AlCl₃ + H₂O exposed groups, but approached to

Table 1

| Experimental groups | Total proteins (g/dl) | Albumin (g/dl) | Uric acid (mg/dl) | Urea (mg/dl) |
|---------------------|---------------------|----------------|------------------|-------------|
| N-G                 | 5.35 ± 0.1          | 3.36 ± 0.05    | 2.62 ± 0.08      | 9.36 ± 0.13 |
| FPP-G               | 4.9 ± 0.12          | 3.17 ± 0.1     | 3.11 ± 0.07      | 11.16 ± 0.35|
| FP-G                | 5.4 ± 0.03          | 3.2 ± 0.065    | 2.34 ± 0.08      | 10.05 ± 0.24|
| APP-G               | 4.9 ± 0.09          | 3.25 ± 0.05    | 3.27 ± 0.07      | 11.99 ± 0.09|
| AP-G                | 5.25 ± 0.07         | 3.2 ± 0.03     | 2.45 ± 0.06      | 9.89 ± 0.12|
| FAPP-G              | 4.7 ± 0.08          | 3.02 ± 0.05    | 3.37 ± 0.21      | 12.3 ± 0.19 |
| FAP-G               | 5.6 ± 0.12          | 3.2 ± 0.09     | 2.65 ± 0.06      | 9.92 ± 0.08 |

Values are expressed as a mean of eight offspring ± SEM
N: normal offspring given deionized H₂O throughout the pre- and postnatal periods until 70 days old
FPP: offspring exposed to NaF throughout pre- and postnatal periods until 70 days old
FP: offspring exposed to AlCl₃ throughout the prenatal and up to the end of weaning stages
APP: offspring exposed to AlCl₃ throughout pre- and postnatal periods until 70 days old
AP: offspring exposed to AlCl₃ throughout the prenatal and up to the end of weaning stages
FAPP: offspring exposed to NaF + AlCl₃ throughout pre- and postnatal periods until 70 days old
FAP: offspring exposed to NaF + AlCl₃ throughout the prenatal and up to the end of weaning stages
a,b,c,d,e,f: Significant difference in comparison with the corresponding N, FPP, FP, AP, APP, and FAPP groups at α = 0.05 (P < 0.05)

Table 2

| Experimental group | ALT U/L | AST U/L | Creatinine mg/dl | Bilirubin mg/dl |
|--------------------|---------|---------|-----------------|-----------------|
| N-G                | 32.87 ± 2.3 | 147 ± 2.5 | 0.64 ± 0.015    | 1.145 ± 0.004   |
| FPP-G              | 41.62 ± 1.83 | 180 ± 2.07 | 0.76 ± 0.05     | 1.162 ± 0.0025  |
| FP-G               | 37.87 ± 1.89 | 146 ± 1.7  | 0.68 ± 0.005    | 1.155 ± 0.004   |
| APP-G              | 39.87 ± 0.69 | 173 ± 2.85 | 0.65 ± 0.013    | 1.151 ± 0.023   |
| AP-G               | 38.19 ± 0.6 | 150 ± 2.48 | 0.566 ± 0.01    | 1.159 ± 0.008   |
| FAPP-G             | 48.45 ± 2.20 | 180 ± 3.78 | 0.92 ± 0.06     | 1.16 ± 0.023    |
| FAP-G              | 47.25 ± 2.5  | 154 ± 2.5  | 0.75 ± 0.02     | 1.157 ± 0.055   |

Caption description and details as in Table 1
A long-term exposed group. The group exposed to AlCl₃ alone exceeded the group given NaF alone, AlCl₃ + H₂O, and NaF + AlCl₃ + H₂O-treated groups. The GSH/GSSH ratio was significantly increased in tested groups as compared to the control group; on the other hand, the group that was given the combination of NaF + AlCl₃ had a significantly higher level than the other treated groups. The level of bilirubin was not significantly different when the comparison was made with the other treated group (Table 4).

Table 3: The levels of vitamin C, malondialdehyde (MDA), glutathione (GSH), and glutathione disulphide (GSSG) and the ratio of GSH to GSSG in the liver of offspring rats exposed to NaF or AlCl₃ alone and in combination through the pre- or and postnatal stages until 70 days

| Experimental groups | Vitamin C (μmol/g) | MDA (nmol/g) | GSH (μmol/g) | GSSG (μmol/g) | GSH/GSSG |
|---------------------|--------------------|--------------|--------------|--------------|----------|
| N-G                 | 10.64 ± 0.065      | 57.37 ± 1.8  | 89.2 ± 0.23  | 60.17 ± 1.6  | 0.149 ± 0.006 |
| FPP-G               | 9.34 ± 0.18        | 72.87 ± 2.5a | 75.1 ± 0.24a | 65.7 ± 0.75a | 0.114 ± 0.004a |
| FP-G                | 10.36 ± 0.063b     | 67.85 ± 1.3a | 79.6 ± 0.215a | 64.92 ± 1.12a | 0.123 ± 0.0035a |
| APP-G               | 9.40 ± 0.33c       | 72.62 ± 2.3a | 85.8 ± 0.24b | 71.63 ± 1.42b | 0.12 ± 0.0053a |
| AP-G                | 10.52 ± 0.34d      | 61.66 ± 1.8b | 88.3 ± 0.40c | 66.94 ± 0.578ad | 0.132 ± 0.006ab |
| FAPP-G              | 9.35 ± 0.34c       | 77.24 ± 1.73c | 72.9 ± 0.27c | 73.67 ± 1.135c | 0.099 ± 0.0041abcde |
| FAP-G               | 10.41 ± 0.24d      | 69.34 ± 1.1c | 79.8 ± 0.23d | 66.64 ± 0.481c | 0.125 ± 0.0058cd |

Caption description and details as in Table 1

Discussion
The present results revealed that the offspring that received sodium fluoride, aluminum chloride, individually or in combination with each other had significantly elevated lipid peroxides (MDA) levels, which is accompanied by decrease in the ratio of GSH/ GSSH which acts as an indicator of redox potential and antioxidant capacity; furthermore, vitamin C and both calcium ion and total calcium, as well as total protein were reduced in the groups that ingested the polluted substance throughout the prenatal and postnatal up to the day 70. In addition, albumin was reduced in the group administered with sodium fluoride plus aluminum. Kidney function is altered and induces an elevation in the creatinine, urea, and uric acid. On the other hand, kidney and liver are highly sensitive to fluoride and aluminum. So, there is an interrelationship between liver function, kidney function, and ingestion of sodium fluoride or aluminum chloride separately or in combination with each other. A lot of theories have been forwarded to explain the mechanism of aluminum or sodium fluoride intoxication including the free radicals and oxidative stress with disturbances in the antioxidant defense system and as consequences disturb cellular nucleotide and induce metabolic disorder (Mailloux, Lemire, & Apana, 2011; Pawłowska-Goral, Kurzeja, & Stec, 2013; Viezeliene, Jansen, Rodovicius, Kasauskas, & Ivanov, 2011). Yildirim et al. (2017, 2018) showed that the chronic exposure to NaF in the drinking water caused a significant increase in the activities of AST, ALT, and the levels of creatinine and MDA, whereas the levels of GSH in the liver was significantly decreased, and these finding are consistent with our data. They attributed the hepatic and renal functions disturbance to the oxidative stress of these tissues to the response the liberated of the reactive oxygen species (ROS) that caused the degeneration and necrosis of the hepatocytes caused as a result of exposure to NaF. Histologically, they found that NaF induced oxidative stress to the cardiac muscle, damage of the hepatic, renal, and the cardiac muscle inflammation that causes myocarditis, hyaline degeneration, and leading to Zenker's necrosis. In addition, they attributed the observed hydropic degeneration and necrosis in hepatocytes in the acinar
region, along with hyperemia in the livers to the chronic fluorosis rats.

It was also reported that Al or sodium fluoride exposure significantly enhanced liver and kidney damage with concomitant alterations in the enzymatic antioxidant defense status, thus having a serious bearing on the functions and structures of soft tissues (Atmaca, Atmaca, Kanici, & Antepioğlu, 2014; El-Demerdash, 2004; Hamza, El-Shenawy, & Ismail, 2015). In addition to the high affinity of fluoride for aluminum, this complex may occur spontaneously in body fluids and magnified the hazards effect. The present results are in agreement with the antecedent studies indicating that aluminum or fluoride caused an elevation in brain MDA accompanied with a decrease in GSH content and SOD activity, in this regard, oxidative damage, reduces the levels of non-enzymatic antioxidant glutathione and disturbs calcium homeostasis (Benyettou et al., 2017; Blaylock, 2012; Kinawy & Ezza, 2013; Walton, 2012). On the other hand, both ascorbic acid and GSH are among the most active reducing substances in living tissues that attenuate the reactive oxygen species. It is relevant to mention that the increased enzyme levels ALT and AST activity are considered as an indicator of liver damage and degeneration of the hepatic cells and thus alterations in liver function (Bouaziz et al., 2006; Bouaziz, Amaraa, Essefia, Croute, & Zeghal, 2010; Eraslan, Kanbur, & Silici, 2007; Hassan & Yousef, 2009; Kanbur, Eraslan, Silici, & Karabacak, 2009; Lu et al., 2017; Xiong et al., 2007; Zhou et al., 2015). With respect to the total protein level, either fluoride or aluminum concentration can lower the serum total protein level; on the other hand, Al might have interfered with the process of translation at a post-transcriptional level to inhibit the production of protein end products (Chatterjee, Mahapatra, & Sarkar, 2016; Chinoy & Memon, 2001; El-Demerdash, 2004; Michael, Barot, & Chinoy, 1996; Sallam, Nasser, Yousef, El-morsy, & Mahmoud, 2005; Yousef, 2005). Moreover, total bilirubin was unaffected in all experimental groups. These findings are basically in agreement with the results obtained from similar investigations by Liang et al. (1999). In addition, plasma creatinine concentration is a more sensitive indicator than urea in the first phases of kidney disease; more seriously, the higher level of urea and creatinine in blood might be due to an inability of kidney to excrete the toxic metabolic product (Azab, Albasha, Jibireal, & Adwas, 2018). In addition, fluoride and aluminum possess a higher capacity to cross cell membranes and easily, inter-visceral organs especially brain, liver, and kidney, as consequences, are burdened with the high levels of these elements (Abdel Aziz & Masad, 2013; Wasana, Perera, Gunawardena, & Bandara, 2015).

Conclusions
In conclusion, the present study explicitly indicates that exposure to sodium fluoride and aluminum chloride during the early stages of life results in several imbalances and disturbances in liver and kidney functions and as a consequence, serious alterations in metabolic homeostasis.

Abbreviations
AlCl₃: Aluminum chloride; ALT: Alanine aminotransferase; Ascorbic acid: Vitamin C; AST: Aspartate aminotransferase; GSH: Reduced glutathione; GSSH: Oxidized glutathione; MDA: Malondialdehyde; NaF: Sodium fluoride; UV: Ultraviolet.

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Authors’ contributions
Single author. The author read and approved the final manuscript.

Ethics approval
Animals were maintained at the College of Science, Taif University animal house, where handling and use of animals were strictly in agreement with the regulations and guidelines set by living Creatures/University of Taif.

Consent for publication
Not applicable.

Competing interests
The author declares that she has no competing interests.

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