

## Evaluation of the Effects of *Citrus aurantifolia* (Lime) Juice in Lead-induced Hematological and Testicular Toxicity in Rats

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### ABSTRACT

**Background:** *Citrus aurantifolia* (Lime) is a citrus fruit and an excellent source of vitamin C and flavonoids which have unique antioxidant activities. This study evaluates the protective effects of *Citrus aurantifolia* (Lime) juice on lead-induced toxicity on hematological and reproductive functions and some organ weights in the male rat. **Materials and Methods:** The animals were given Pb nitrate (4, 8 or 12 mg kg<sup>-1</sup>), or 0.1 mL Lime juice + 8 mg kg<sup>-1</sup> Pb nitrate, or vehicle daily for 28 days by oral gavage. **Results:** Lead treatment caused lymphocytosis with accompanying neutropenia and anemia. Also, lead significantly increased sperm motility, counts and viability but did alter percentage of abnormal sperm cells (morphology). Testicular weight, as well as liver and kidney weights were equally significantly reduced by lead treatment. Pretreatment with lime, 1 h prior to lead (8 mg kg<sup>-1</sup>) administration prevented hematological effects of lead. The Citrus juice also inhibited lead-induced reductions in liver and kidney weights but not on testicular weight. Lime pretreatment also inhibited lead-induced reduction in sperm count but did not affect sperm motility and viability. **Conclusion:** *Citrus aurantifolia* juice prevents lead-mediated bone marrow toxicity and may also prevent alteration of liver and kidney functions, which may be attributed to the antioxidants (flavonoids and vitamin C) in the plant. On the contrary, *Citrus aurantifolia* juice does not protect lead-induced testicular toxicity but may rather have potential for promoting testicular dysfunction, which mechanism remains unclear.

**Key words:** *Citrus aurantifolia*, flavonoids, lymphocytosis, testis

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### INTRODUCTION

Lead is one of the oldest environmental pollutant and toxicant. Lead poisoning, which can be traced to prehistoric time, is a major problem in most urban cities due to its wide application in industrial activities (Bilandzic *et al.*, 2009). Used widely in the manufacture of batteries and ammunition occupational exposure to the metal is high among workers in such industries (ATSDR, 2007). Lead is also used in the manufacture of toys, paints, X-ray apparatus (as protective shield), eye cosmetics, gasoline, electric cables, water pipes and tanks (Stokinger, 1981; Fischbein *et al.*, 1992; Florea and Busselberg, 2006). With the wide application of lead in paints and toys, children are especially at risks of lead, even at relatively low concentrations.

The major routes of lead exposure include inhalation, gastrointestinal tract and the skin. The inhalational and skin exposures are more significant for occupationally exposed groups, while the latter contributes a greater proportion of those for the general

population (Florea and Busselberg, 2006). Because of effective control measures in reducing lead use (like the stoppage of lead in gasoline and reduction of the amount of lead in paints) by different regulatory bodies such as the Environmental Protection Agency (EPA), Consumer Product Safety Commission (CPSC), Centers for Disease Control (CDC) etc; acute lead toxicity is greatly reduced. Chronic toxicity on the other hand, is much more common and occurs at blood lead levels of 40-60 µg dL<sup>-1</sup>, which in severe cases, is characterized by persistent vomiting, anemia, encephalopathy, lethargy, delirium, convulsions and coma (CDCP/NCEH, 2005).

Several systems in the body could become targets in lead intoxication. The main targets include the hematopoietic (Anetor *et al.*, 2002; Flora *et al.*, 2006), nervous (White *et al.*, 2007) and cardiovascular systems (Navas-Acien *et al.*, 2007). Lead also affects renal (Ekong *et al.*, 2006) and reproductive functions (ATSDR, 2007; Makhoulouf *et al.*, 2008; Zhu *et al.*, 2010). Existing data suggest that lead exposure results in oxidative stress and this has been reported to be the major mechanism of lead toxicity in most biological systems (ATSDR, 2005; Flora *et al.*, 2012).

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*Citrus aurantifolia* (Lime) is a citrus fruit and an excellent source of vitamin C and flavonoids which have unique antioxidant activities (Patil *et al.*, 2009; Shrestha *et al.*, 2012; Karoui and Marzouk, 2013). *Citrus aurantifolia*, which belongs to the Rutaceae family, is believed to reduce oxidative stress by inhibiting cellular lipid peroxidation and increasing cellular antioxidant systems (Shrestha *et al.*, 2012; Karoui and Marzouk, 2013). Gharagozloo and Ghaderi, 2001; Patil *et al.* (2009) have also reported that *Citrus aurantifolia* (Lime) protect against proliferation of cancer cells but there is limited data on its protective effects in other metal induced toxicities. Since *Citrus aurantifolia* juice contains potent antioxidants and such antioxidants have been demonstrated to prevent metal-induced toxicities (Gurer *et al.*, 1998; Obianime *et al.*, 2010; Sajitha *et al.*, 2010; Ayinde *et al.*, 2012), it is logical to hypothesize that *Citrus aurantifolia* juice will protect lead-induced organ toxicities in the rat. The present study aims to evaluate the protective effects of *Citrus aurantifolia* (Lime) juice on lead-induced toxicity on hematological and reproductive functions and some organs in male rats.

## MATERIALS AND METHODS

Lead nitrate (Burgoyne Burgoynes and Co, India) was obtained from the Department of Pharmacology, University of Port Harcourt.

**Plant material:** Fresh fruits of *Citrus aurantifolia* used in this research were obtained from an orchard located at the University of Port Harcourt, Nigeria. The fruits were harvested from three trees and juice was expressed out, filtered with muslin and used fresh for the experiments.

**Animals:** Adult male Wistar albino rats, weighing between 180-230 g were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt and used for the study. The animals were maintained in a ventilated room at a room temperature of  $28.0 \pm 2^\circ\text{C}$  and natural lighting condition. The animals were fed with standard rodents chow and water was given ad libitum. The animals were handled in accordance with the Guide for Care and Use of Laboratory Animals (CCAC, 2009) and the experimental protocol was approved by the Committee for Ethics in Animal Experimentation of the University of Port Harcourt.

**Experimental design:** The animals were randomly distributed into five groups ( $n = 6$ ) and administered with different concentrations of Pb as nitrate daily for 28 days by oral gavage. Group I was given vehicle (distilled water) and was used as the control. Group II was given  $4 \text{ mg kg}^{-1}$  ( $\approx 27 \text{ ppm}$ ) Pb. Group III received  $8 \text{ mg kg}^{-1}$  ( $\approx 54 \text{ ppm}$ ) Pb. Group IV received  $12 \text{ mg kg}^{-1}$

( $\approx 80 \text{ ppm}$ ) Pb. Group V was given *Citrus aurantifolia* juice (0.1 mL) for 1 h before administering  $8 \text{ mg kg}^{-1}$  ( $\approx 54 \text{ ppm}$ ) Pb. At the end of the drug administrations, the rats were sacrificed under deep diethylether anesthesia. Blood was collected into EDTA bottles and hematological parameters were measured. The testes were excised out, weighed and sperm was collected from the epididymis to analyze sperm count, motility, morphology and viability. Animal livers and kidneys were also removed and weighed.

**Hematological analysis:** Whole blood collected into EDTA bottle was assayed for Packed Cell Volume (PCV), White Blood Cell (WBC) counts and WBC differentials lymphocyte and neutrophil counts using an auto analyzer.

**Sperm analysis:** The method of Amelar *et al.* (1973) was used in collecting sperm cells from the epididymis. Briefly, the testis was excised and the caudal epididymis was carefully isolated and placed in a Petri dish containing 3 mL of  $\text{NaHCO}_3$  buffered Tyrodes's Lactate solution. Several (1 mm) incisions were made on it and sperm was gently drawn into a plastic transfer pipette and transferred into 5 mL test tubes and vigorously shaken for homogeneity and dispersal of sperm cells. Sperm was then analyzed to determine sperm motility, sperm count, percentage of abnormal sperm cells (sperm morphology) and percentage of viable sperm cells (sperm viability) following standard procedures (WHO, 1999).

**Statistical analysis:** Data were expressed as Mean  $\pm$  SEM. Statistical differences between the groups were evaluated by one way ANOVA, followed by Dunnett's comparison test to compare between treated and control groups. Differences yielding  $p < 0.05$  were considered statistically significant. Statistical analyses of data were performed using GraphPad Prism 5 software.

## RESULTS

**Hematological indices:** Hematocrit or Packed Cell Volume (PCV) values obtained in Groups III and IV were significantly lower than control rats (Table 1). White Blood Cell (WBC) counts of only lead treated rats were higher compared to control but only that of group IV was significant,  $p < 0.05$  (Table 1). Similarly, lymphocyte counts of only lead treated rats were higher compared to control but only that of group IV was significant,  $p < 0.05$  (Table 1). Neutrophil counts of only lead treated rats were decreased but group IV value was also significant,  $p < 0.05$  when compared to the control (Table 1). The blood counts of WBC and its differentials in *Citrus aurantifolia* juice pretreated rats (Group V) were not all significantly different from controls (Table 1).

Table 1: Blood levels of some hematological indices in Wistar rats following 28 days oral administration of lead nitrate and *Citrus aurantifolia* (Lime) juice pretreatment

Group	PCV(%)	WBC count ( $\times 10^6$ mL <sup>-1</sup> )	Neutrophil count (%)	Lymphocyte count (%)
Control (I)	42.00 $\pm$ 3.54	3.58 $\pm$ 0.28	56.25 $\pm$ 5.27	43.25 $\pm$ 5.59
4 mg kg <sup>-1</sup> (II)	38.75 $\pm$ 0.95	8.20 $\pm$ 1.38	48.00 $\pm$ 4.83	52.00 $\pm$ 4.83
8 mg kg <sup>-1</sup> Pb (III)	30.00 $\pm$ 4.10*	11.53 $\pm$ 2.76	40.00 $\pm$ 4.56	59.75 $\pm$ 4.31
12 mg kg <sup>-1</sup> Pb (IV)	26.50 $\pm$ 2.53*	12.95 $\pm$ 2.54*	32.25 $\pm$ 6.92*	67.75 $\pm$ 7.92*
Lime+ 8 mg kg <sup>-1</sup> (V)	40.50 $\pm$ 2.06	4.10 $\pm$ 0.28	51.25 $\pm$ 8.98	48.25 $\pm$ 8.78

Data represented as Mean $\pm$ SEM, n = 6, \*Significant at p<0.05

Table 2: Sperm parameters in Wistar rats following 28 days oral administration of lead nitrate and *Citrus aurantifolia* (Lime) juice pretreatment

Group	Sperm count ( $\times 10^{12}$ )	Sperm motility (%)	Sperm viability (%)	Abnormal sperm cells, morphology (%)
Control (I)	65.25 $\pm$ 6.76	55.00 $\pm$ 2.04	70.05 $\pm$ 9.72	10.00 $\pm$ 2.04
4 mg kg <sup>-1</sup> (II)	60.00 $\pm$ 4.36	50.00 $\pm$ 4.08	58.65 $\pm$ 5.20	13.75 $\pm$ 3.75
8 mg kg <sup>-1</sup> Pb (III)	55.00 $\pm$ 1.41	30.00 $\pm$ 4.08 <sup>b</sup>	43.75 $\pm$ 4.39*	13.75 $\pm$ 3.75
12 mg kg <sup>-1</sup> Pb (IV)	47.50 $\pm$ 0.50*	23.75 $\pm$ 2.39 <sup>f</sup>	39.45 $\pm$ 0.48 <sup>g</sup>	8.75 $\pm$ 1.25
Lime+ 8 mg kg <sup>-1</sup> (V)	52.75 $\pm$ 2.78	21.25 $\pm$ 5.54 <sup>f</sup>	36.78 $\pm$ 2.32 <sup>g</sup>	10.00 $\pm$ 2.04

Data represented as Mean $\pm$ SEM, n = 6, \*Significant at p<0.05, <sup>b</sup>Significant at p<0.01, <sup>f</sup>Significant at p<0.001

**Sperm parameters:** Mean epididymal sperm count, motility and viability obtained in Pb-treated rats (Groups II, III and IV) were dose-dependently decreased. However, only the sperm motility and viability values in Groups III and IV (8, 12 mg kg<sup>-1</sup> Pb-treated) rats were significant (Group III, p<0.01, p<0.05; Group IV, p<0.001, p<0.01), compared to Group I (control) rats, (Table 2). These Pb-induced levels corresponded to 45.5 and 56.8% decreases, respectively in sperm motility and 37.3 and 43.7% decreases, respectively in sperm viability. Also, only the sperm count in Group IV (12 mg kg<sup>-1</sup> Pb-treated) rats was significant, p<0.05 (Table 2), which was equivalent to 22.6% decrease, compared to the control. Percentages of abnormal sperm cells (sperm morphology) in Pb-treated rats (Groups II, III and IV) were not significantly different from control rats (Table 2). Furthermore, while sperm count obtained in *Citrus aurantifolia* pretreated rats (Group V) was not significantly altered, sperm motility and viability were decreased, p<0.001, p<0.01 when compared to control rats (Table 2). The respective level of reductions in motility and viability were 61.4 and 47.5%. There was no significant difference in the percentage of abnormal sperm cells (sperm morphology) between (Group V) and control rats (Table 2).

**Organ weights:** Weights of testis, liver and kidney in Pb-treated rats (Groups II, III and IV) were significantly

Table 3: Weights of some organs in Wistar rats following 28 days oral administration of lead nitrate and *Citrus aurantifolia* (Lime) juice pretreatment

Group	Weight (g)		
	Testis	Liver	Kidney
Control (I)	1.64 $\pm$ 0.24	8.24 $\pm$ 0.35	1.16 $\pm$ 0.06
4 mg kg <sup>-1</sup> (II)	1.07 $\pm$ 0.06*	4.81 $\pm$ 0.69 <sup>f</sup>	0.84 $\pm$ 0.07*
8 mg kg <sup>-1</sup> Pb (III)	0.72 $\pm$ 0.09 <sup>g</sup>	4.43 $\pm$ 0.43 <sup>f</sup>	0.78 $\pm$ 0.09*
12 mg kg <sup>-1</sup> Pb (IV)	0.62 $\pm$ 0.04 <sup>f</sup>	3.18 $\pm$ 0.16 <sup>f</sup>	0.71 $\pm$ 0.03 <sup>g</sup>
Lime+ 8 mg kg <sup>-1</sup> (V)	1.06 $\pm$ 0.11*	7.10 $\pm$ 0.26	0.89 $\pm$ 0.06

Data represented as Mean $\pm$ SEM, n = 6, \*Significant at p<0.05, <sup>g</sup>Significant at p<0.01, <sup>f</sup>Significant at p<0.001

decreased in dose-dependent manners, compared to Group I (control) rats (Table 3). Weight of testis of *Citrus aurantifolia*-pretreated rats (Group V) were also significantly lower than control rats but there were no changes in liver and kidney weights between *Citrus aurantifolia*-pretreated (Group V) and control rats (Table 3).

## DISCUSSION

Lead is a widespread environmental pollutant associated with toxicity of many body functions, including the hematopoietic and reproductive systems in humans and experimental animals (Chandra *et al.*, 1981; Anetor *et al.*, 2002; Bellinger, 2005; Ekong *et al.*, 2006; ATSDR, 2007). Low Packed Cell Volume (PCV) or hematocrit, which results in anemia, is a common sign of lead toxicity (Jacob *et al.*, 2000; Anetor *et al.*, 2002). This supports the observation of the reduction of PCV by lead in this study. Chronic exposure of high levels of lead (2,000 ppm) has been reported to induce significant immunosuppression in rats (Ercal *et al.*, 2000). The results of this study however, indicate that low levels of lead exposure would cause elevation in white blood cell (WBC) levels with proliferation of lymphocytes and reduction of neutrophil levels, indicative of bone marrow toxicity. This observation suggests that responses of immunological cells to lead may depend on its concentration. The lead-induced lymphocytosis with accompanying neutropenia and anemia has a high implication for the development of lymphoproliferative neoplasms (Dhodapkar *et al.*, 1994; George, 2012).

Testicular function is assessed in part by analysis of spermatogenic indices including sperm count, motility, viability and morphology (Zinaman *et al.*, 2000; Eliasson, 2003). Measurements of these parameters in the spermatozoa give an indication of the quality and functionality of the sperm. As normal sperm motility and count are vital for male fecundity (Zinaman *et al.*, 2000), reductions in the number and motility of sperms by lead in this study indicates that the metal could alter normal testicular function. Additionally, the percentages of viable sperms (i.e., spermatozoa with intact cell membrane) and

morphologically abnormal sperms are critical indicators of testicular function (Menkveld *et al.*, 2011). Lead-induced decrease in viability and increase in morphologically abnormal sperm cells observed in this study is strongly suggestive of testicular dysfunction. Organ weights are indices often employed in toxicological evaluations (Michael *et al.*, 2007). Reduction in testis and other organ weights in lead treated rats in this study demonstrate lead toxicity. This observation also suggests that lead may cause atrophy of the testis, liver and kidney and ultimately affect their physiological functions.

Earlier studies have reported that *Citrus aurantifolia* extracts protect against lead induced bone marrow toxicity (Gharagozloo and Ghaderi, 2001; Hosseinimehr *et al.*, 2003; Patil *et al.*, 2009). However, lead is deleterious to several biological systems and the protective potential of *Citrus aurantifolia* on other organ functions, including reproductive function has not been studied. It is important to note that the fruit juice of *Citrus aurantifolia* is more commonly used than most of the other parts of the plant. It also has a higher vitamin C and flavonoid contents than other parts of the plant (Karoui and Marzouk, 2013). Unfortunately, *Citrus aurantifolia* fruit juice has not been assessed for the prevention or amelioration of heavy metal induced toxicity, even though similar antioxidant compounds have been shown to exhibit beneficial properties in this regard (Gurer *et al.*, 1998; Obianime *et al.*, 2010; Sajitha *et al.*, 2010; Ayinde *et al.*, 2012). In the present study, *Citrus aurantifolia* fruit juice prevented the hematological effects of lead (anemia, lymphocytosis and neutropenia), just as it inhibited the lead-induced reduction of sperm count. However, the low percentages of sperm motility and viability observed in *Citrus aurantifolia* juice pretreated rats indicate that the effects of lead on these sperm indices were unchallenged by *Citrus aurantifolia* juice. Additionally, no effect observation on liver and kidney weights in *Citrus aurantifolia* juice pretreated rats clearly shows that lead-induced atrophy of these organs was inhibited. Interestingly, *Citrus aurantifolia* juice failed to prevent testicular atrophy caused by lead, similar to its effects on sperm motility and viability. When the level of alterations in these parameters were analyzed between only lead treated and *Citrus aurantifolia* juice pretreated rats, it was observed that sperm motility was reduced by 56.8 and 61.4%, respectively; sperm viability was reduced by 43.7 and 47.5%, respectively and weight of testis was reduced by 62.2 and 35.4%, respectively.

*Citrus aurantifolia* contains antioxidants that may be responsible for the amelioration of lead-mediated toxicity of bone marrow, liver and kidney. However, the findings of the present study provide evidence that *Citrus aurantifolia* juice may not protect lead-induced

testicular damage. With higher levels of alterations in some of the spermatogenic indices by *Citrus aurantifolia* juice pretreatment, compared to only lead treatment, *Citrus aurantifolia* juice may independently cause adverse effects on testicular function and indeed have antifertility potential in the male. Besides antioxidants, *Citrus aurantifolia* juice also contains high amounts of organic acids like citric acid, coumaric and ferulic acids (Patil *et al.*, 2009; Shrestha *et al.*, 2012). As testicular milieu is highly sensitive to most chemicals, the testicular effects of *Citrus aurantifolia* juice may be attributed partly to the acid constituents of the plant.

## CONCLUSION

The results of this study show that *Citrus aurantifolia* juice may protect lead mediated bone marrow toxicity and alteration of liver and kidney functions. On the contrary, *Citrus aurantifolia* juice does not protect lead-induced testicular toxicity but may rather have potential for promoting testicular dysfunction.

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## REFERENCES

- Amelar, R.D., L. Dubin and C. Schoenfeld, 1973. Semen analysis: An office technique. *Urology*, 2: 606-611.
- Anetor, J.I., O.O. Babalola, F.A.A. Adeniyi and T.S Akingbola, 2002. Observations on the hemopoietic system in tropical lead poisoning. *Nig. J. Physiol. Sci.*, 17: 9-15.
- ATSDR, 2005. Toxicological Profile for Lead. U. S Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2007. Toxicological Profile for Lead. Department of Health and Human Services, ATSDR, Atlanta, USA.
- Ayinde, O.C., S. Ogunnowo and R.A. Ogedegbe, 2012. Influence of vitamin C and vitamin E on testicular zinc content and testicular toxicity in lead exposed albino rats. *BMC Pharmacol. Toxicol.*, Vol. 13. 10.1186/2050-6511-13-17
- Bellinger, D.C., 2005. Teratogen update: Lead and pregnancy birth defects. *Res. A. Clin. Mol. Teratol.*, 73: 409-420.
- Bilandzic, N., M. Sedak, D. Vratarić, T. Perić and B. Simić, 2009. Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. *Sci. Total Environ.*, 407: 4243-4247.

- CCAC, 2009. The Care and Use of Farm Animals in Research, Teaching and Testing. CCAC., Ottawa, ON., pp: 12-15.
- CDCP/NCEH, 2005. Third National Report on Human Exposure to Environmental Chemicals. CDC, National Center for Environmental Health, Atlanta, GA., ISBN: 0309071402, Pages: 12.
- Chandra, A.V., M.M. Ali, D.K. Saxena and R.C. Murthy, 1981. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. *Arch. Toxicol.*, 49: 49-56.
- Dhodapkar, M.V., C.Y. Li, J.A. Lust, A. Tefferi and R.L. Philyky, 1994. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: A T-cell clonopathy of undetermined significance? *Blood*, 84: 1620-1627.
- Ekong, E.B., B.G. Jaar and V.M. Weaver, 2006. Lead-related nephrotoxicity: A review of the epidemiologic evidence. *Kidney Int.*, 70: 2074-2084.
- Eliasson, R., 2003. Basic Semen Analysis. In: *Current Topics in Andrology*, Matson, P. (Ed.). Ladybrook Publishing, Perth, WA, pp: 35-89.
- Ercal, N., R. Neal, P. Treeratphan, P.M. Lutz, T.C. Hammond, P.A. Dennery and D.R. Spitz, 2000. A role for oxidative stress in suppressing serum immunoglobulin levels in lead exposed Fisher 344 rats. *Arch. Environ. Contam. Toxicol.*, 39: 251-256.
- Fischbein, A., J. Wallace, S. Sassa, A. Kappas, G. Butts, A. Rohl and B. Kaul, 1992. Lead poisoning from art restoration and pottery work: Unusual exposure source and household risk. *J. Environ. Pathol. Toxicol. Oncol.*, 11: 7-11.
- Flora, G., D. Gupta and A. Tiwari, 2012. Toxicity of lead: A review with recent updates. *Interdiscip. Toxicol.*, 5: 47-58.
- Flora, S.J.S., G. Flora and G. Saxena, 2006. Environmental Occurrence, Health Effects and Management of Lead Poisoning. In: *Lead Chemistry, Analytical Aspects, Environmental Impacts and Health Effects*, Cascas, S.B. and J. Sordo (Eds.). Elsevier Publication, Netherlands, pp: 158-228.
- Florea, A.M. and D. Busselberg, 2006. Occurrence, use and potential toxic effects of metals and metal compounds. *Biometals*, 19: 419-427.
- George, T.I., 2012. Malignant or benign leukocytosis. *Hematol. Am. Soc. Hematol. Educ. Program.*, 2012: 475-484.
- Gharagozloo, M. and A. Ghaderi, 2001. Immunomodulatory effect of concentrated lime juice extract on activated human mononuclear cells. *J. Ethnopharmacol.*, 77: 85-90.
- Gurer, H., H. Ozgunes, R. Neal, D.R. Spitz and N. Ercal, 1998. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. *Toxicology*, 128: 181-189.
- Hosseini-mehr, S.J., H. Tavakoli, G. Pourheidari, A. Sobhani and A. Shafiee, 2003. Radioprotective Effects of Citrus Extract Against Gamma-Irradiation in Mouse Bone Marrow Cells. *J. Radiat. Res.*, 44: 237-241.
- Jacob, B., B. Ritz, J. Heinrich, B. Hoelscher and H.E. Wichmann, 2000. The effect of low-level blood lead on hematologic parameters in children. *Environ. Res.*, 92: 150-159.
- Karoui, I.J. and B. Marzouk, 2013. Characterization of bioactive compounds in tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. *BioMed. Res. Int.*, 10.1155/2013/345415
- Makhlouf, M.M.M., H.M.S. Eldien, D.A.M. Zagloul, E.A.B. Dief and N.G. Abd ElHaliem, 2008. The effect of lead acetate on testicular structure and protective effect of vitamin e in adult albino rat. *Egypt. J. Histol.*, 31: 406-418.
- Menkveld, R., C.A.G. Holleboom and J.P.T. Rhemrev, 2011. Measurement and significance of sperm morphology. *Asian J. Androl.*, 13: 59-68.
- Michael, B., B. Yano, R.S. Sellers, R. Perry and D. Morton *et al.*, 2007. Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. *Toxicol. Pathol.*, 35: 742-750.
- Navas-Acien, A., E. Guallar, E.K. Silbergeld and S.J. Rothenberg, 2007. Lead exposure and cardiovascular disease: A systematic review. *Environ. Health Persp.*, 115: 472-482.
- Obianime, A.W., N.J. Ahiwe and J.S. Aprioku, 2010. Effects of vitamins C and E pretreatments on cadmium-induced serum levels of some biochemical and hormonal parameters in the female guinea pig. *Afr. J. Biotechnol.*, 9: 6582-6587.
- Patil, J.R., K.N. Chidambara Murthy, G.K. Jayaprakasha, M.B. Chetti and B.S. Patil, 2009. Bioactive compounds from Mexican lime (*Citrus aurantifolia*) juice induce apoptosis in human pancreatic cells. *J. Agric. Food Chem.*, 57: 10933-10942.
- Sajitha, G.R., R. Jose, A. Andrews, K.G. Ajantha, P. Augustine and K.T. Augusti, 2010. Garlic oil and vitamin E prevent the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats. *Indian J. Clin. Biochem.*, 25: 280-288.
- Shrestha, R.L., D.D. Dhakal, D.M. Gautum, K.P. Paudyal and S. Shrestha, 2012. Variation of physiochemical components of acid lime (*Citrus aurantifolia* swingle) fruits at different sides of the tree in Nepal. *Am. J. Plant Sci.*, 3: 1688-1692.

- Stokinger, H.E., 1981. The Metals. In: Patty's Industrial Hygiene and Toxicology, Clayton, G.D. and F.E. Clayton (Eds.). 3rd Edn., John Wiley and Sons, New York, pp: 1687-724.
- White, L.D., D.A. Cory-Slechta, M.E. Gilbert, E. Tiffany-Castiglioni and N.H. Zawia *et al.*, 2007. New and evolving concepts in the neurotoxicology of lead. *Toxicol. Applied Pharmacol.*, 225: 1-27.
- WHO, 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th Edn., Cambridge University Press, New York, USA., ISBN-13: 9780521645997, Pages: 128.
- Zhu, M., E.F. Fitzgerald, K.H. Gelberg, S. Lin and C.M. Druschel, 2010. Maternal low-level lead exposure and fetal growth. *Environ. Health Perspect.*, 118: 1471-1475.
- Zinaman, M.J., C.C. Brown, S.G. Selevan and E.D. Clegg, 2000. Semen quality and human fertility: A prospective study with healthy couples. *J. Androl.*, 21: 145-153.