[Now published in full after the initial publication as an Epub ahead of print on Mar 2, 2021 at www.fluorideresearch.online/epub/files/141.pdf]

LIVER AND KIDNEY FUNCTION IN RATS CO-TREATED WITH FLUORIDE AND ARSENIC FOR DIFFERENT TIME INTERVALS

Huma,[a] Yeshvandra Verma,[a] SVS Rana[a,*]

Meerut, India

ABSTRACT: The present study aimed to assess the combined effects of arsenic (As) and fluoride (F) on liver and kidney of rat after exposure to three different time periods i.e. 30, 60, and 90 days. For this, three months old female Wistar rats were administered through gavage sodium arsenate (4 mg/kg b.w.), sodium fluoride (4 mg/kg b.w.), and sodium arsenate and sodium fluoride (4 mg/kg b.w. each) together for three-time intervals i.e. 30, 60, and 90 days. Liver function in these rats was assessed through serum enzymes and bilirubin concentration. Kidney function was estimated through creatinine and uric acid determination in urine. Findings revealed, duration of exposure to As, F, and As+F influences the hepatic and renal toxic manifestations of these elements. Bioaccumulation of As and induction of metallochaprones seem to contribute to variations in their toxicity. Whereas, some results show antagonistic relationship between As and F, others exhibit synergism between the two. Majority of present observations favour a synergistic relationship between them. However, duration of exposure and bioconcentration appear to be important variables in their combined toxicity.

Keywords: Bioconcentration; Liver and kidney function; Sodium arsenate; Sodium fluoride.

INTRODUCTION

Arsenic (As) and fluoride (F), are both ubiquitous elements. A large section of human population over the globe consumes both As and F through drinking water. According to an estimate 300 million people around the world drink groundwater contaminated with As and F.[1,2] Toxicity profiles of arsenic[3] and fluoride[4] have been published by certain regulatory agencies. Endemicity of fluorosis and arseniasis has also been reviewed by different authors.[5,6] Epidemiological data link arsenic with skin, cardiovascular, cerebro-vascular, hepatic and renal diseases, and cancer in man.[7,8] Endemic arsenism is known to cause black foot disease in Taiwan.[9] Similarly, endemic fluorosis is known to occur in various countries including India.[10-13]

Liver and kidney have both been identified as their target organs. Hepatic and renal toxicity of inorganic as well as organic arsenic have been studied in the past by several workers.[14-16] However, only a few studies on their combined effects are available.[17] A few studies on the concurrent exposure to As and F in human population have also been made in Mexico and Argentina.[18,19] A few workers have studied their effects on cardiovascular systems, liver, and kidney of rats and cellular DNA damage in mice.[20] However, precise information on independent and antagonistic or synergistic relationships between these two elements remains so far inconclusive. Therefore, a study on their low dose, long term, and concurrent effects after different periods of exposure on liver and kidney of rat was proposed. Serum enzymes and bilirubin were selected as biomarkers of liver function. Two indicators of renal function i.e. creatinine and uric acid were also estimated. The results of this

[a]Department of Toxicology, Ch Charan Singh University, Meerut-250004, India;
*For correspondence: Dr SVS Rana, Professor Emeritus, Department of Toxicology, Ch Charan Singh University, Meerut 250004, India; Email: sureshvs_rana@yahoo.com; Mob: 91-9719113457.

study are expected to be helpful in delineating physiological relationship between these two elements.

MATERIALS AND METHODS

Reagents and chemicals: Sodium arsenate (As^{III}) and sodium fluoride (F) were procured from Sigma Chemical Co. St. Missouri (USA). Kits for the estimation of aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH), bilirubin (Bil), creatinine, and uric acid were purchased from Span Diagnostics (Surat, India). All other chemicals and reagents were used in this study were of highest purity.

Animals and their maintenance: Present study was performed on three months old female Wistar rats (140±20g), procured from the animal facility of All India Institute of Medical Science, New Delhi. After acclimatization for two weeks under standard laboratory conditions (room temp- 25±5 ºC, relative humidity- 60±10 %, and 12 hour light/dark cycle), the rats were separated into 12 groups, each containing five rats. They were housed individually, in polypropylene cages and fed on commercial food pellets and tap water *ad libitum*.

Experimental design and treatment protocol: The rats of groups A, B, and C were administered, through gavage, pre-determined sublethal dose of sodium arsenate (4 mg/kg body weight [b.w.]) on each alternate day for 30, 60, and 90 days, respectively. LD_{50} was determined by recommended method.[21] Similarly rats of groups D, E, and F were administered sublethal dose of sodium fluoride (4 mg/kg b.w.), on each alternate day for 30, 60, and 90 days, respectively after determining $LD_{50.}$ Rats of group G, H, and I were co-administered As and F both at a concentration of 4 mg/kg b.w. each, on every alternate day for 30, 60, and 90 days, respectively. Saline treated (4 mL/kg b.w.) rats of groups J, K, and L served as respective controls. Prior approval of the Institutional Ethical Committee was obtained to conduct these experiments.

Preparation of samples: On termination of respective treatments, rats were starved overnight and euthanized next morning by light ether anaesthesia. Before sacrifice, urine samples were collected through metabolic cages and stored at –80 ºC for further analyses. After sacrifice blood was collected directly by cardiac puncture and serum was separated through centrifugation.

Bioconcentration of arsenic (As) in urine: Bioconcentration of As in urine of rats was determined through inductive coupled plasma emission spectroscopy (ICPMS). Briefly, 1 mL of urine was added to 10 µL of concentrated nitric acid (A.R.) and diluted to 10 mL with ultra pure water. An aliquot of 5 mL was used for elemental analysis, employing ICPMS as suggested.[22]

Estimation of F in the urine: Concentration of in the urine samples was determined directly after dilution with equal volumes of TISAB (total ionic strength adjustment buffer) by a F specific electrode using an ion meter (Orion).[23]

Estimation of serum enzymes: Alanine amino transferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were analysed using a commercial kit applying the standard methods.[24,25]

Estimation of total bilirubin, creatinine, and uric acid: These were determined by recommended methods using their respective commercial kits as reported earlier [26,27]

[Now published in full after the initial publication as an Epub ahead of print on Mar 2, 2021 at www.fluorideresearch.online/epub/files/141.pdf]

RESULTS

As^{III} concentration in urine: Urinary excretion of As^{III} is a reliable indicator of its biotransformation and toxicity. Initially a treatment of 30 days increased its concentration followed by a non significant decrease after 60 days of exposure. However, it decreased significantly after 90 days of As^{III} treatment. In group co-treated with As + F, its concentration decreased after 60 days of treatment but increased after 90 days of treatment (Table 1).

Table 1. Arsenic and fluoride concentration in the urine samples of rats fed on As^{III} and As^{III} + F adjusted to normal specific gravity

Concentration	Exposure (days)	As^{III} / F	As^{III} + F	Control	f value
Arsenic(mg/L)	30	8.3 ±0.02*	5.3±0.02*	1.2 ± 0.03	0.112
	60	7.6±0.01^{NS}	3.0 ±0.05*	1.4 ± 0.02	0.484
	90	4.3±0.01*	1.4 ± 0.02^{NS}	1.2 ±0.03	0.204
Fluoride (mg/L)	30	2.8 ± 0.01*	2.1 ± 0.02*	1.6 ± 0.02	0.502
	60	3.0 ± 0.03*	3.5 ± 0.03^{NS}	1.0 ± 0.01	0.138
	90	2.0± 0.02^{NS}	3.0 ± 0.01*	0.8 ± 0.01	0.104

Result are expressed as mean ± SE (n=5); *P< 0.05 difference in comparison to controls; NS- Non significant; f, denotes the significance difference amongst groups.

of exposure. A surge in F concentration was recorded after 60 and 90 days of co-exposure to As^{III} + F (Table 1).

Aspartate amino transferase (AST) (EC 2.6.11): A comparison of enzyme values showed significant increase amongst the rats of all experimental groups. In As^{III} treated group, a progressive increase in enzyme activity in comparison to controls was registered after 30 days of treatments. Similar trend was observed amongst F treated rats. In As^{III} + F treated rats also; enzyme level increased significantly in comparison to control rats (Table 2).

Alanine amino transferase (ALT) (EC 2.6.1.2): Another enzyme biomarker, ALT also showed elevated values in activity after all the three treatments. Amongst As^{III} treated group, highest value of enzyme was recorded after 30 days of its treatment. However, these values declined after 60 and 90 days of exposure. Minimum value

[Now published in full after the initial publication as an Epub ahead of print on Mar 2, 2021 at www.fluorideresearch.online/epub/files/141.pdf]

was recorded after 90 days of exposure. Amongst F treated group, enzyme activity increased after 60 days of treatment but declined again after 90 days of exposure. In As^{III} + F treated group a progressive decline was recorded after 60 and 90 days of exposure (Table 2)

Table 2. Serum enzymes (ALT, AST, and LDH) and total bilirubin in the rats fed on As^{III}, F, and As^{III} + F

Parameter	Duration (days)	Arsenic	Fluoride	Arsenic +Fluoride	Control	f-value
ALT/(IU/L)	30	251.7 ±3.55*	200 ±5.08*	247.1 ±49.06*	43.0 ±1.0	28.37
	60	248.8 ±3.03NS	254.5 ±7.48*	242.0 ±5.08*	41.02 ±1.0	11.99
	90	200.5 ±41.63*	199.1 ±96.12*	217.1 ±21.14*	18.52 ±2.5	6.460
AST/(IU/L)	30	475 ±44.7*	244.6 ±48.06NS	386.4 ±41.2*	34.55 ±2.4	34.63
	60	318.6 ±81.6NS	232.6 ±34.33NS	313 ±78.0*	30.38 ±1.8	34.63
	90	305 ±86.99*	135.4 ±2.59*	360.3 ±58.31NS	28.36 ±10.2	6.078
LDH/(IU/L)	30	485 ±16.3*	344.9 ±16.1*	375.5 ±0.5NS	160.2 ±1.6	3.577
	60	413 ±15*	214 ±27.5*	405 ±1.3*	158.0 ±1.4	63.24
	90	347 ±49.5*	240 ±16.9NS	390 ±1.7NS	138.5 ±9.7	18.88
Total Bilirubin (mg/dL)	30	0.42 ±0.039*	0.16 ±0.054NS	0.51 ±0.09*	0.26 ±0.04	6.262
	60	0.43 ±0.023NS	0.24 ±0.01NS	0.35 ±0.04NS	0.22 ±0.03	5.557
	90	0.37 ±0.048*	0.30 ±0.09*	0.33 ±0.043NS	0.24 ±0.03	0.250

Result are expressed as mean ± SE (n=5); *P< 0.05 significance difference in comparison to controls; NS, Non significant; f, denotes the significance difference amongst the groups.

Lactate dehydrogenase (LDH) (EC 1.1.1.27): Lactate dehydrogenase activity is the expression of membrane damage. In present experiments, significant increase in its activity was observed in the rats of all the groups when compared to control rats. Higher enzymes activity was recorded in As^{III} and F treated group after all the three-time intervals. In As^{III} as well as F treated group, enzyme activity decreased with the increase in exposure period. However, in As^{III} + F treated group, enzyme values increased after 30 and 60 days of exposure but decreased after 90 days of treatment (Table 2)

Total bilirubin: Findings revealed, value of total bilirubin increased in As^{III} and As^{III} + F treated groups but decreased in the F treated rats, after 30 days of exposure. However, elevated values were recorded after 60 and 90 days of exposure. Amongst As^{III} treated rats a non-significant increase was observed after 60 days of treatment in comparison of 30 days of exposure. However, the values declined again after 90 days of exposure. Amongst As^{III} + F treated group, its values decreased with the increase in period of exposure (Table 2).

Creatinine: These results clearly indicate that kidney function is affected by As^{III} and F both. In all the groups treated for 30 days, significant increase in urinary, creatinine value was recorded in comparison to control rats. Amongst As^{III} treated rats, its values increased after 60 days of exposure. Similar trend was noticed in F and As^{III} + F treated groups. Maximum values for creatinine amongst rats of all the groups were recorded after 60 days of treatment (Table 3).

Table 3. Kidney function in the rats fed on As^{III}, F, and As^{III} + F

Parameter	Duration (days)	Arsenic	Fluoride	Arsenic +fluoride	Control	f-value
Creatinine (mg/dL)	30	1.9±0.05*	1.8±0.62^{NS}	0.9±0.07*	0.5±0.085	1.021
	60	3.1±0.29*	2.1±0.36*	1.5±0.36*	0.4±0.07	0.895
	90	2.0±0.23*	0.2±0.53*	1.2±0.31*	0.5±0.08	0.326
Uric acid (mg/dL)	30	2.7±0.15*	2.0±0.13^{NS}	2.2±0.18*	1.4±0.20	3.173
	60	2.2±0.13*	1.9±0.14*	2.8±0.36^{NS}	1.3±0.18	2.188
	90	2.5±0.29^{NS}	1.4±0.20*	2.6±0.02*	1.7±0.11	6.143

Result are expressed as mean ± SE (n=5); *P< 0.05 significance difference in comparison to controls; NS, Non significant; F, denotes the significance difference amongst group.

Uric acid: Both the elements individually as well as in combination enhanced uric acid concentration after 30 days of exposure. Amongst As^{III} treated group, minimum concentration was recorded after 60 days of exposure. In F treated rats, concentration decreased with the increase in exposure period. In As^{III} + F treated rats,

concentration increased after 60 days of treatment but decreased after 90 days exposure (Table 3).

DISCUSSION

A few reports on combined toxicity of As and F are available in literature.[18,19] While a few reports suggest antagonism, others support synergism between the two. Parameters of oxidative stress, antioxidant enzymes (SOD and GPx), and renal function favoured an antagonistic relationship.[28] Contrarily, combined effect of Cd+F exhibited synergism in rats.[29] Another study concluded that As^{III} and F exhibited synergistic effect on renal tissue even at WHO recommended water quality standards.[30] Present results showed that combined effects of As^{III}+F are influenced by their bioconcentration in liver and kidney as well as the rate of depurination. Long term exposure to F does not promote bioaccumulation of As^{III} in liver and kidney. As^{III} and F are known to accumulate in these soft tissues[31-35] and cause concentration/time dependent functional changes. Therefore, bioaccumulation factors responsible for retention of As^{III} in these organs and their modulation by F might determine consequent antagonism or synergism.

Serum enzymes are considered to be reliable markers of liver function. As^{III} and F both affected hepatic function. Nonetheless, no conclusive antagonistic or synergistic effect of As^{III} +F on ALT could be observed. Intriguingly, longer treatment of 90 days decreased enzyme activity in comparison to those treated for 30 and 60 days. It indicated an adaptive mechanism that needs to be explored further. Variations in the activity of AST were also observed in these three groups of rats. After an initial increase after 30 days, enzyme values decreased after 60 and 90 days of individual exposure to As^{III} and F. However, no decrease in enzyme activity in As^{III} + F treated rats even after 90 days of exposure was observed. These results suggested a synergistic effect on AST.

Effects of As^{III} and F on serum transaminases have also been studied earlier.[36,37] However, their combined effects on enzymes are not known. Longer treatments with As^{III} and F resulted into a decrease in LDH activity. In As^{III} + F treated group also, its activity increased after 60 days of treatment but non significant increase was recorded after 90 days of co-exposure.

LDH catalyses the conversion of lactate to pyruvate and back. Several xenobiotics are known to affect LDH activity in the liver and other organs.[38-40] A change in LDH activity reflects metabolic changes in affected organs.[41] As^{III} and F exposure also elevated LDH activity in a dose dependent manner.[42-43] Taken together, present results on LDH suggested that effects of As^{III} + F co-exposure on liver are influenced by period of exposure. Since liver parenchyma possess enormous functional reserve, longer treatments are expected to be less severe than shorter treatments.

During present experiments, As^{III} was found to cause hyperbilirubinimia, whereas no significant increase in total bilirubin was noted in the blood of F treated rats. Studies on metabolic profiles from the liver of As treated zebra fish[44] and toxicity studies on albino rats[45,46] have also reported elevated values for bilirubin after arsenic treatment. Present studies further add that exposure period does contribute in these effects amongst rats co-exposed to As^{III} + F.

As with liver, renal tissue is also known to accumulate As and F. Nephrotoxicity of As^{III} and F has been studied earlier also, employing two parameters of renal function i.e creatinine and uric acid.[47,48] Epidemiological results showed that urinary creatinine values increased in human population exposed to As.[49] A few reports have also described As and F mediated nephrotoxicity.[50,51] However, combined effects of As^{III} + F on renal function are not known. A report on Chinese population exposed to As^{III} + F has suggested their antagonistic effect on renal function.[28] Present observations on creatinine and uric acid both showed exposure period dependent effects. Values on creatinine indicated an antagonistic relationship between them, nevertheless, values on uric acid exhibited synergism between As^{III} and F.

CONCLUSIONS

Combined toxicity of As and F remains as an important ecotoxicological issue. Both elements are cumulative in liver and kidney. During combined exposure their absorption, bioaccumulation, binding with proteins, and rate of depurination differ from their individual toxicokinetic behaviours. Antagonism or synergism between the two appears to be an organ function dependent phenomenon. Further, during short exposures these elements in combination induce a greater toxicity in liver and kidney than with longer exposures. Therefore, duration of exposure may also be a factor in determining their combined toxicity.

ACKNOWLEDGEMENTS

The first author is thankful to and gratefully acknowledges the Director-General, Indian Council of Medical Research, New Delhi, India, and the Director of Central Instrument Facility, Indian Institute of Technology, Roorkee (India) for financial assistance (45/27/2018-BMS-PHA) and performing urine analysis, respectively.

REFERENCES

[1] Anderson DL. Chemical composition of the mantle. J Geoph Res 1983; 88:1341-52.
[2] Viswanathan G, Gopalakrishnan S, Siva IS. Assessment of water contribution on total fluoride intake of various age groups of people in fluoride endemic and non-endemic areas of Dindigul district, Tamil Nadu, South India. Water Res 2010;44:6186-200.
[3] ATSDR. Toxicological Profile for arsenic, Agency for Toxic Substances and Disease Registry. ATSDR/PB/2000/108021. (US Public Health Service, Atlanta, GA) 2000.
[4] Fawell J, Bailey K. Fluoride. In: Drinking-water, World Health Organization Emerging issues in Water and Infectious Disease Series. Geneva: World Health Organization 2006.
[5] McGill PE. Endemic fluorosis, Baillieres Clin Rheum 1995;9:75-81.
[6] Mandal BK, Suzuki KT. Arsenic round the world: a review. Talanta 2002;58:201-35.
[7] Chen CJ, Hsueh YM. Increased prevalence of hypertension and long-term arsenic exposure, Hypertension 1995;25:53-60.
[8] Kitchin KT. Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites. Toxico Appl Pharmaco 2001;17:249-61.
[9] Tseng CH, Huang YK. Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in black foot disease-hyperendemic villages in Taiwan. Toxico Appl Pharmaco 2005;206:299-308.
[10] Adler P, Armstrong, WD, Bell, ME, Bhussry, BR, Büttner W, Cremer, H-D, et al. Fluorides and human health. World Health Organization Monograph Series No. 59. Geneva: World Health Organization; 1970.

[11] Choubisa SL, Choubisa D. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ Sci Pollut Res 2016; 23(8):7244-54.

[12] Choubisa SL. A brief and critical review of endemic hydrofluorosis in Rajasthan, India. Fluoride 2018;51(1):13-33.

[13] Choubisa SL. A brief and critical review on hydrofluorosis in diverse species of domestic animals in India. Environ Geochem Health 2018;40(1):99-114.

[14] Allen T, Rana SVS. Effect of arsenic (A^{sIII}) on glutatnione-dependent enzymes in liver and kidney of fresh water fish *Channa punctatus*. Biol Trace Elem Res 2004; 100:39-48.

[15] Shi H, Shi X, Liu KJ. Mechanism of arsenic toxicity and carcinogenesis. Mol Cellul Biochem 2004;255:67-78.

[16] Yamanaka K, Kato K. The role of active arsenic species produced by metabolic reduction of dimethylarsinic acid in genotoxicity and tumorigenesis. Toxico Appl Pharmaco, 1999;198:385-93.

[17] Mondal P, Chattopadhyay A. Environmental exposure of arsenic and fluoride and their combined toxicity: A recent update. J Appl Toxicol 2009;40:552-66.

[18] Horta CG, Ballinas-Casarrubias L, Sanchez-Ramirez B, et al. A concurrent exposure to arsenic and fluoride from drinking water in Chihuahua, Mexico. Intl J Environ Res Public Health 2015;12:4587-601.

[19] Buchhamer EE, Blanes PS, Osicka RM, Gimenez MC. Environmental risk assessment of arsenic and fluoride in the Chaco Provice, Argentina: Research Advances. J Toxicol Environ Health A 2012;75:1437-450.

[20] Singh KP, Kukari R, Treas J, Dumond JW. Chronic exposure to arsenic causes increased cell survival, DNA damage and increased expression of mitochondrial transcription factor A (mtTFA) in human prostate epithelial cells. Chem Res Toxicol 2011;24:340-49.

[21] Horn HJ. Simplified LD_{50} (or ED_{50}) calculations. Biometrics 1956;12:312–22.

[22] Kawabata K, Inoue Y, Takahashi H, Endo G. Determination of arsenic species by inductively coupled plasma mass spectrometry with ion chromatography. Appl Organomet Chem 1994;12:312-22.

[23] Inkielewicz I, Krechniak J. Fluoride content in soft tissues and urine of rats exposed to sodium fluoride in drinking water. Fluoride 2003;36:263-66.

[24] Reitman S, Frankel SA. Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.

[25] King J. The dehydrogenase or oxidoreductase - lactate dehydrogenase, in: King J.(Ed.), Practical clinical enzymology, London: Van Nostrand Company Ltd; 1956.

[26] Burtis CA, Ashwood ER., editors. Textbook of clinical chemistry, 3rd ed, Philadelphia: WB Saunders; 1999. pp. 913-4.

[27] Henry RJ, Sobel C, Kima J. Modified carbonate-phosphotungstate method for the determination of uric acid and comparison with the spectrophotometric uricase method. Am J Clin Pathol 1957;28:152-60.

[28] Zeng Qi-bing, Xu Yu-yan, Yu Xian, Yang J, Hong F Zhang Ai-hua. The combined effects of fluorine and arsenic on renal function in a Chinese population. Toxicol Res 2014;3:359-66.

[29] Song J. Combined effects of fluoride and cadmium on liver and kidney function in male rats. Biol Trace Elem Res 2013;155:396-402.

[30] Wasana HMS, Perera GDRK. WHO water quality standards Vs Synergic effect(s) of fluoride, heavy metals and hardness in drinking water on kidney tissues. Sci Rep 2017; 14:42516

[31] Cui X, Okasayu R. Arsenic accumulation, elimination, and interaction with copper, zinc and manganese in liver and kidney of rats. Food Chem Toxicol 2008;46:3646-50.

[32] Kubota R, Kunito T, Tanabe S. Arsenic accumulation in the liver tissue of marine mammals. Environ Pollut 2001;115:303-12.

[33] Dubey NP, Jain SK, Maheshwari HS. Chronic study of arsenic trioxide-induced hepatotoxicity in relation to arsenic liver accumulation in rats. Toxicol Environ Chem 2014;96:491-9.

[34] Ashley JM. Fluoride exposure and kidney and liver function among adolescents in the United States: NHANES 2013–2016. Environ Intl 2019;105012.

[35] Perera T, Ranasinghe S, Alles N, Waduge R. Effect of fluoride on major organs with the different time of exposure in rats. Environ Health Prevent Med 2018;23:769-75.

[36] Singh N, Rana SVS. Arsenic toxicity in the pancreas of diabetic rats. J Toxicol Health 2014;105:487-93.

[37] Islam K, Haque A, Karim R, Fajol A, Hossain E, Salam KA, et al. Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. Environ Health 2011;10: doi:org. 10.1186/1476-069x-10-64.

[38] Nair SB, Jhala D, Chinoy NJ. Beneficial effects of certain antidotes in mitigating fluoride and/or arsenic induced in mitigating fluoride and/or arsenic induced hepatotoxicity in mice. Fluoride 2004;37(2):60-70.

[39] Keil E. Determination of enzyme activities in serum for the detection of xenobiotic effects on the liver. Exp Pathol 1990;39:157-64.

[40] Jaiswal SK, Siddiqui NJ, Sharma B. Studies on the ameliorative effect of curcumin on carbofuran induced perturbations in the activity of lactate dehydrogenase in Wistar rats. Saudi J Bio Sci 2016;25:1585-92.

[41] Omabe M, Omabe KH, Igwe, John OC, Uchenna SK, Elom S. Xenobiotics-induced liver damage is biochemically abrogated by treatment with lipophilic extracts of *Moringa oleifera in vivo***.** Bio Life Sci 2018;10: 313-25.

[42] El-Demberdesh FM. Oxidative stress and hepatotoxicity induced by synthetic pyrethroids-organophosphate insecticides mixture in rat. J Environ Sci Health Environ Carc Ecotox Rev 2011;29:145-58.

[43] Shalon MG, El-Hallous E. The role of ascorbic acid as a protective agent against sodium meta-arsenite food toxicity in rats. J Indian Soc Toxicol 2015;11:13-21.

[44] Li C, Li P, Ten YM. Metabolomic characterizations of liver injury caused by acute arsenic toxicity in zebra fish. PLoS One 2016;11:3 e0151225.

[45] Campbell C. Effects of arsenic trioxide poisoning on haematological parameters, liver marker enzymes and kidney of male albino rats. Pinna Bio Sci 2014;2:1-4.

[46] Singh S, Rana SVS. Amelioration of arsenic toxicity by L-Ascorbic acid in laboratory rat. J Env Bio 2007;28:377-84.

[47] Kinawy AA. Potential toxicity of aluminum and fluoride on some biochemical aspects of male rat's offspring. J Basic App Zool 2019; 80: doi.org/10.1186/s41936-019-0087-1.

[48] Peters BA, Hall MN, Liu X, Neugut YD, Pilsner JR, Levy D. Creatinine, arsenic metabolism, and renal function in an arsenic-exposed population in Bangladesh. PLoS One 2014;9:113760.

[49] Robles-Osorio ML, Sabath-Silva E. Arsenic-mediated nephrotoxicity. Renal Failure 2015; 37:542-47.

[50] Zheng L, Kuo C, Fadiwsk J, Agnew J, Weaver VM, Navas-Acien A. Arsenic and chronic kidney disease: a systemic review. Curr Environ Health Report 2014;1:192-207.

[51] Zhan X, Wang M, Xu Z, LiJ. Toxic effects of fluoride on kidney function and histological structure in young pigs. Fluoride 2006;39:22-6.