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

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ABSTRACT: The present study was 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 (4mg/100g b.w.), sodium fluoride (4mg/100g b.w.), and sodium arsenate and sodium fluoride (4mg/100g 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 metallochaperones 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: Sodium arsenate; sodium fluoride; liver and kidney function; bioconcentration

INTRODUCTION

Arsenic (As) and fluoride (F), both are ubiquitous elements. A large section of human population over the globe consumes As and F both through drinking water. According to an estimate 300 million people around the world drink groundwater contaminated with As and F.¹² Toxicity profiles of arsenic³ and fluoride⁴ 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 both have been identified as their target organs. Hepatic and renal toxicity of inorganic as well as organic arsenic had 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 was also estimated. The results of this 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 of highest purity were used in this study.

**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 °C, relative humidity- 60±10 %, and 12hour 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 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 (4mg/100g body weight) on each alternate day for 30, 60, and 90 days, respectively. LD$_{50}$ was determined by commend method.$^{21}$ Similarly rats of groups D, E, and F were administered sublethal dose of sodium fluoride (4mg/100g body weight), 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/100 g body weight each, on every alternate day for 30, 60, and 90 days, respectively. Saline treated (4mL/100g body weight) rats of groups J, K, and L served as respective controls. Prior approval of the Institutional Ethical Committee was sought 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 °C for further analyses. After sacrifice blood was collected directly by cardiac puncture and serum was separated through centrifugation.
**Biconcentration of arsenic (As) in urine:** Bioconcentration of As in urine of rats was determined through inductive coupled plasma emission spectroscopy (ICPMS). Briefly, 1mL of urine was added 10µL 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 F 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

**RESULTS**

**AsIII concentration in urine:** Urinary excretion of AsIII 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 AsIII 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).

**F concentration in urine:** F excretion increased with the increase in exposure period of 60 days. However, a non-significant decrease was registered after 90 days of exposure. A surge in F concentration was recorded after 60 and 90 days of co-exposure to As+F (Table 1).

**Aspartate amino transferase (AST) (EC 2.6.1.1):** A comparison of enzyme values showed significant increase amongst the rats of all experimental groups. In AsIII 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 AsIII+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 AsIII 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 was recorded after 90 days of exposure. Amongst F treated group, enzyme actively increased after 60 days of treatment but declined again after 90 days of exposure. In As +F treated group a progressive decline was recorded after 60 and 90 days of exposure (Table 2).
**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 and F treated group after all the three-time intervals. In As\textsuperscript{III} as well as F treated group, enzyme activity decreased with the increase in exposure period. However, in As\textsuperscript{III}+F treated group, enzymes 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\textsuperscript{III} and As\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{III} treated rats, its values increased after 60 days of exposure. Similar trend was noticed in in F and As\textsuperscript{III} +F treated groups. Maximum values for creatinine amongst rats of all the groups were recorded after 60 days of treatment (Table 3).

**Uric acid:** Both the elements individually as well as in combination enhanced uric acid concentration after 30 days of exposure. Amongst As\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{28} Contrarily, combined effect of Cd+F exhibited synergism in rats.\textsuperscript{29} Another study concluded that As\textsuperscript{III} and F exhibited synergistic effect on renal tissue even at WHO recommended water quality standards.\textsuperscript{30} Present results showed that combined effects of As\textsuperscript{III}+F are influenced by their bioconcentration in liver and kidney as well as the rate of depurination. Long term exposure to F do not promote bioaccumulation of As\textsuperscript{III} in liver and kidney. As\textsuperscript{III} and F are known to accumulate in these soft tissues\textsuperscript{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 as 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 on As$^{III}$ and F on serum transaminases have also been studied earlier. However, their combined effects on enzymes are not known. Longer treatments to 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. A change in LDH activity reflects metabolic changes in affected organs. As$^{III}$ and F exposures also elevated LDH activity in a dose dependent manner. 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 hyperbilirubinemia, whereas no significant increase in total bilirubin was noted in the blood of F treated rats. Studies on metabolomic profiles from the liver of As treated zebra fish and toxicity studies on albino rats 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.

Alike 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. Epidemological results showed that urinary creatinine values increased in human population exposed to As. A few reports have also described As and F mediated nephrotoxicity. 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. 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.

**CONCLUSION**

Combined toxicity of As and F remains to be 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 than their individual toxicokinetic behaviour. Antagonism or synergisms between the two appears to be an organ function dependent phenomenon.Further, during short exposures these elements together induce greater toxicity in liver and kidney than longer exposures. Therefore, duration of exposure may also be a determining factor in their combined toxicity.

**ACKNOWLEDGEMENTS**

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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.

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

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 groups.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration (days)</th>
<th>Arsenic</th>
<th>Fluoride</th>
<th>Arsenic + Fluoride</th>
<th>Control</th>
<th>f-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT/(IU/L)</td>
<td>30</td>
<td>251.7±3.55*</td>
<td>200±5.08*</td>
<td>247.1±49.06*</td>
<td>43.0±1.0</td>
<td>28.37</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>248.8±3.03NS</td>
<td>254.5±7.48*</td>
<td>242.0±5.08*</td>
<td>41.02±1.0</td>
<td>11.99</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>200.5±41.63*</td>
<td>199.1±96.12*</td>
<td>217.1±21.14*</td>
<td>18.52±2.5</td>
<td>6.460</td>
</tr>
<tr>
<td>AST/(IU/L)</td>
<td>30</td>
<td>475±44.7*</td>
<td>244.6±48.06NS</td>
<td>386.4±41.2*</td>
<td>34.55±2.4</td>
<td>34.63</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>318.6±81.6NS</td>
<td>232.6±34.33NS</td>
<td>313±78.0*</td>
<td>30.38±1.8</td>
<td>34.63</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>305±86.99*</td>
<td>135.4±2.59*</td>
<td>360.3±58.31NS</td>
<td>28.36±10.2</td>
<td>6.078</td>
</tr>
<tr>
<td>LDH/(IU/L)</td>
<td>30</td>
<td>485±16.3*</td>
<td>344.9±16.1*</td>
<td>375.5±0.5NS</td>
<td>160.2±1.6</td>
<td>3.577</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>413±15*</td>
<td>214±27.5*</td>
<td>405±1.3*</td>
<td>158.0±1.4</td>
<td>63.24</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>347±49.5*</td>
<td>240±16.9NS</td>
<td>390±1.7NS</td>
<td>138±9.7</td>
<td>18.88</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>0.42±0.039*</td>
<td>0.16±0.054NS</td>
<td>0.51±0.09*</td>
<td>0.26±0.04</td>
<td>6.262</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>60</td>
<td>0.43±0.023NS</td>
<td>0.24±0.01NS</td>
<td>0.35±0.04NS</td>
<td>0.22±0.03</td>
<td>5.557</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.37±0.048*</td>
<td>0.30±0.09*</td>
<td>0.33±0.043NS</td>
<td>0.24 ±0.03</td>
<td>0.250</td>
</tr>
</tbody>
</table>

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

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

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.