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# Combined effect of *Eichhornia crassipes* and *Emblica officinalis* on serum electrolytes and enzyme profile after arsenic intoxication in albino rats

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***Abstract:*** *The present investigation was carried out to evaluate the combined effect of Eichhornia crassipes and Emblica officinalis on serum electrolytes and enzyme profile after arsenic intoxication in albino rats. E. officinalis has been used as a valuable ingredient of various medicines in India. Recent investigations has given ample evidences of various uses of Eichhornia crassipes as hyper accumulator of various toxins. Three groups of ten adult male albino rats each were administered 0.102 mg/L arsenic present in drinking water (collected from Sikandara area of Agra) for 30, 45 and 60 days and another three groups of ten rats were treated first with arsenic water in the same way as mentioned above and then treated with combined E. crassipes (10gm/Kg body weight) and E. officinalis (500 mg/Kg body weight) fruit extract (mixed in ratio of 1:2) orally for 15, 30 and 45 days, respectively. Results showed a very highly significant ($p<0.001$) increase in arsenic in the serum, a very highly significant ($p<0.001$) decrease in calcium, magnesium, sodium, significant ($p< 0.05$) decrease in potassium, very highly significant ($p<0.001$) increase in serum AST, ALT and non-significant ($p>0.05$) increase in LDH after arsenic water intoxication; while a very highly significant ($p<0.001$) decrease in serum arsenic, a very highly significant ($p<0.001$) increase in calcium, potassium, a non-significant increase in magnesium, non-significant ($p>0.05$) decrease in sodium, a very highly significant ($p<0.001$) decrease in serum AST, ALT and non-significant ($p>0.05$) decrease in LDH after combined E. crassipes and E. officinalis treatment, respectively. E. crassipes reduces the serum arsenic burden through chelation bio-mechanism and provides nutrients; while E. officinalis acts as a strong antioxidant. We concluded that E. crassipes and E. officinalis have compensated the arsenic toxicity that affects the body metabolism.*

**Key Words**: Enzyme profile, Serum electrolytes, Albino rat, Arsenic water, *Eichhornia crassipes, Emblica officinalis.*

## Introduction

It has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential elements like As, Hg, Cd, and Pb. Arsenic is ubiquitously distributed in the environment in a number of organic and inorganic forms and thus exposure to this metalloid has become inevitable for both man and animals (Rmalli *et al.*, 2005). Arsenic is mainly transported in the environment by water. Arsenic has been growing rapidly during the last few years as a major pollutant of drinking water in several districts of West Bengal and many other states of India (Rehman *et al.*, 2002).

Common symptoms of acute arsenic poisoning are nausea, anorexia, vomiting, epigastric and abdominal pain, and diarrhea. Severe exposures can result in acute encephalopathy, congestive heart failure, stupor, convulsions, paralysis, coma, and death. General symptoms of chronic arsenic poisoning are weakness, general debility and lassitude, loss of appetite and energy, loss of hair, hoarseness of the voice, loss of weight, and mental abnormalities. Chronic exposure of humans to high concentrations of arsenic in drinking water is also associated with skin abnormalities.

particularly hyper pigmentation, hyperkeratosis, gangrene, peripheral vascular disease, hypertension, black-foot disease and a high risk of cancers (Chowdhury *et al.*, 1997).The current therapeutic approach to arsenic poisoning is to increase the excretion of arsenic by chelation. Co-administration of antioxidants such as vitamins C and E or N-acetyl cysteine and some essential metals such as zinc during chelation therapy has been found to be beneficial in increasing arsenic mobilization and assisting the recovery of altered biochemical variables (Modi *et al.*, 2005).

*Eichhornia crassipes a* water hyacinth is free floating aqueous weeds that grow in dense mats in tropical and subtropical freshwater. *E. crassipes* belonging to the family Pontedericeae. The mature plant consists of long, pendant roots, rhizomes, stolons, leaves, inflorescences and fruit clusters. The roots are very fibrous and provide the plant with all of their nutrients from the water. The plant is extremely tolerant towards heavy metals, such as Cd, Cr, Co, Ni, Pb and Hg etc. (Upadhyay and Tripathi, 2007). Water hyacinths have also been found to be hyperaccumulators of arsenic (Misbahuddin and Fariduddin, 2002). Water hyacinth is a rich source of amino acid, micronutrients and antioxidants, which could be a good solution for management of arsenicosis. It contains α-carotene, amino acid, melatonin, trace element, all of which have antioxidant property (Umar, 2007).

*Emblica officinalis* is a small to medium sized deciduous tree with globose, fleshy, pale yellow fruits of the family Euphorbiaceae. *Emblica officinalis* has many phytocemicals like phenolics, saponins, tannins, flavonoids, organic acids, sugars, free amino acids, minerals and vitamins (Rehman *et al.*, 2007). Pharmacological investigations on Amla have reported them to have antibacterial, antiviral, antimicrobial, antioxidative, anti-inflammatory and hypolipidemic properties (Saeed and Tariq, 2007). Amla is useful in cancer, age-related renal disease, diabetes and liver dysfunction (Qureshi *et al.*, 2009). The antioxidant activity of fruits of *E. officinalis* has been traced to its tannoid principles both in vitro and in vivo (Bhattacharya *et al.*, 2002). The present investigation deals with the combined effects of *E. crassipes* and *E. officinalis* on the serum electrolytes and enzyme profile in arsenic intoxicated albino rats.

## Materials and Methods

Ninety male albino rats (*Rattus norvegicus*) of wistar strain weighing 120 ± 25 gm and eight-weeks old were randomly divided into nine groups of ten rats each. Each group was kept in a separate polypropylene cage, and maintained in controlled temperature (25 ± 2°C), humidity (65 ± 10%) and proper circadian rhythm. The animals were acclimatized for 20 days before starting the experiment. During this period animals had free access to normal diet and the water given *ad libitum*.

The arsenic water was collected from Sikandara area in Agra region from as usual water sources like hand pumps in polypropylene bottles. The concentration of arsenic in drinking water was found to be 0.102 mg/L. The fruits of *Eichhornia crassipes* and *Emblica officinalis* were procured from local market and taxonomically identified by Department of Botany, School of Life Sciences, Khandari campus, Dr. B.R.A. University, Agra. The fruit pulp was macerated with the help of pistil and mortar. This macerated pulp was dissolved in 1 litre of distilled water, stirred intermittently and then left overnight. Finally, this solution was filtered by muslin cloth. The filtered extract was stored in refrigerator at a temperature of 2- 3 °C.

The selected dose of *Eichhornia crassipes* for entire research was 10 gm/kg body weight given to albino rats (Quayum, 2007). The therapeutic

dose of *Emblica officinalis* was selected at 500 mg/kg body weight (Thangaraj, *et al.*, 2007).The combined extract was formed by mixing the *Eichhornia crassipes* and *Emblica officinalis* (in ratio of 1:2) 15 min before the dosing.

The three groups of albino rats were treated as control for 30, 45 and 60 days. Another three groups were treated with arsenic water for 30, 45 and 60 days. Remaining three groups were treated first with arsenic water in the same way as described above and then treated with combined *E. crassipes* and *E. officinalis* fruit extract for 15, 30, and 45 days, respectively. The aqueous fruit extract was given orally to the rats by gavage tube. At the end of the respective treatment period, the rats were sacrificed under chloroform anesthesia by 24 hours after the last dosing.

The concentration of arsenic in water sample was measured by the method of Aggett and Aspell (1976). Serum arsenic was measured by the method of Campillo *et. al.* (2000), serum calcium by the method of Trinder (1960), serum magnesium by the method of Piper (1950) and serum sodium and potassium were estimated by method of Varley *et. al.* (1980). Serum AST and ALT were estimated by the method of Reitman and Frankel (1957) and serum LDH by the method of Oba and Uriteni (1982).

## Results and Discussion

The serum arsenic increases and is highly significant ($p<0.001$), serum calcium, magnesium, sodium decrease and are highly significant ($p<0.001$). Potassium decreases significantly ($p< 0.05$) after arsenic water ingestion for 30, 45 and 60 days; while serum arsenic decreases highly significantly ($p<0.001$), serum calcium, potassium increase highly significantly ($p<0.001$), serum magnesium increases non-significantly ($p>0.05$) and serum sodium decreases non-significantly ($p>0.05$) after combined *Eichhornia crassipes* and *Emblica officinalis* treatment for 15, 30 and 45 days with arsenic water ingestion treated groups, respectively (Table 1 and Fig.1-5). The serum AST and ALT increase and this is very highly significant ($p<0.001$) and LDH increases non-significantly ($p>0 05$) after arsenic water ingestion for 30, 45 and 60 days; while serum AST and ALT decrease. This is very highly significantly ($p<0.001$) while serum LDH decreases non-significantly ($p>0.05$) after combined *Eichhornia crassipes* and *Emblica officinalis* treatment for 15, 30 and 45 days with arsenic water ingestion treated groups, respectively (Table 2 and Fig. 6-8).

Metabolism of arsenic is associated with the conversion of the most potentially toxic forms of this element to the less toxic form, followed by accumulation in or excretion from the cell. However, this biomethylation process can easily become saturated and lead to the excess inorganic arsenic being deposited in the skin, hair and nails, where it binds tightly to keratin. It has long been known that arsenic is able to exert its toxic effects on the metabolic pathways by modulating the antioxidant defense system, interrupting the glycolytic pathway and citric acid cycle, and thus inhibits oxidative phosphorylation. Arsenic can alter activities of different enzymes such as transaminases, alkaline phosphatase, glyceralehyde-3-phosphate dehydrogenase, pyruvate decarboxylase, glutathione peroxidase etc. involved in normal functioning of different organs in human body (Schoolmeester and White, 1980).

It is established that the root of water hyacinth removes arsenic from arsenic contaminated water by absorption mechanism (Misbahuddin *et al.*, 2006). High protein content of water hyacinth may enhance the excretion of arsenic by increasing methylation. Water hyacinth is a rich source of amino acid, micronutrients and antioxidants, which could be a good solution for management of arsenicosis. *In vitro* and animal

**Table 1.** Biochemical changes in serum electrolytes in albino rats after arsenic water ingestion and combined *E.crassipes* and *E. officinalis* treatment.

| Parameters | Experimental Period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 days Control | 30 days A.W.I. | 15 days combined *E.crassipes* and *E.officinalis* treatment after 30 days A.W.I | 45 days Control | 45 days A.W.I. | 30 days combined *E.crassipes* and *E.officinalis* treatment after 45days A.W.I. | 60 days Control | 60 days A.W.I. | 45 days combined *E.crassipes* and *E.officinalis* treatment after 60 days A.W.I. |
| **Arsenic (µg/L)** | 3.79± 0.3544 | 13.95± 0.3377*** | 6.74± 0.1613*** | 3.55± 0.2852 | 15.83± 0.3062*** | 6.08± 0.1123*** | 3.45± 0.2963 | 17.37± 0.1398*** | 4.46± 0.2989*** |
| **Calcium (mg/dl)** | 9.9 ± 0.1914 | 8.016 ± 0.0022*** | 9.022 ± 0.0087*** | 10.0 ± 0.1914 | 7.57 ± 0.0249*** | 9.554 ± 0.0494*** | 10.558 ± 0.1898 | 6.962 ± 0.0195*** | 9.654 ± 0.0494*** |
| **Magnesium (mg/dl)** | 3.175 ± 0.0420 | 3.044 ± 0.0410* | 3.055 ± 0.0404 NS | 3.141 ± 0.0248 | 2.783 ± 0.0338*** | 2.792 ± 0.0340 NS | 3.14 ± 0.0244 | 2.684 ± 0.0339*** | 2.691 ± 0.0336 NS |
| **Sodium (mmol/L)** | 142.915 ± 0.4849 | 132.8 ± 0.4898*** | 131.8 ± 0.4898 NS | 143.187 ± 0.7697 | 126.00 ± 0.9189*** | 125.2± 1.0306 NS | 142.982 ± 0.8119 | 120.8 ± 0.3887 *** | 119.9 ± 0.4333 NS |
| **Potassium (mmol/L)** | 5.945 ± 0.1477 | 5.913 ± 0.1594 N.S | 5.991 ± 0.1476 N.S | 6.132 ± 0.2314 | 5.761± 0.2161 N.S | 6.309 ± 0.2359*** | 6.202 ± 0.1822 | 5.677 ± 0.1698* | 7.82 ± 0.0354*** |

A.W.I.- Arsenic Water Ingestion, NS- Non-significant, *-Significant ($p<0.05$), ***- highly significant ($p<0.001$)

**Table 2.** Biochemical changes in serum enzyme profile in albino rats after arsenic water ingestion and combined *E. crassipes* and *E.officinalis* treatment.

| Parameters | Experimental Period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **30 days Control** | **30 days A.W.I.** | **15 days combined *E.crassipes* and *E.officinalis* treatment after 30 days A.W.I** | **45 days Control** | **45 days A.W.I.** | **30 days combined *E.crassipes* and *E.officinalis* treatment after 45days A.W.I.** | **60 days Control** | **60 days A.W.I.** | **45 days combined *E.crassipes* and *E.officinalis* treatment after 60 days A.W.I.** |
| AST (SGOT) (U/L) | 64.7 ± 2.3335 | 82.9 ± 1.7156*** | 79.2 ± 1.6983*** | 65.9 ± 2.3211 | 102 ± 2.0110*** | 97.4 ± 1.7524*** | 66.5 ± 2.5177 | 136.5 ± 2.2570*** | 131.1 ± 2.3115*** |
| ALT (SGPT) (U/L) | 41.0 ± 5.0354 | 59.9 ± 5.6910* | 55.9 ± 5.6910*** | 43.6 ± 5.4041 | 67.4 ± 5.3462** | 62.3 ± 5.3459*** | 47.0 ± 4.7679 | 84.3 ± 4.6929*** | 80.6 ± 4.793[illegible] |
| LDH (U/L) | 446.2 ± 43.8251 | 456.2 ± 44.0340 N.S | 449.1 ± 44.2112 N.S | 445.1 ± 43.8011 | 464.4 ± 44.1155 N.S | 447.7 ± 43.7635 N.S | 444.0 ± 43.2093 | 465.3 ± 43.6188 N.S | 442.1 ± 43.1443 N.S |

A.W.I.- Arsenic Water Ingestion, NS- Non significant, *-Significant ($p<0.05$), **-Highly significant ($p<0.01$), ***- highly significant ($p<0.001$)

studies have indicated that amla have potent anti-oxidant effect against several test systems such as superoxide radicals, lipid peroxide formation induction by Fe+++/ADP ascorbate system, hydroxyl radical scavenging action and in systemic augmentation of antioxidant enzymes in the brain of laboratory animals (Mathur *et al.*, 1996).

In the present study, increased arsenic level in the serum is due to arsenic intoxication; while decreased arsenic level was seen in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of water hyacinth and Amla. Similarly, Quayum (2007) observed that an increased value of arsenic was observed in serum of rat due to accumulation of arsenic trioxide. Water hyacinth contains methionine. Nandi *et al.* (2005) showed the ameliorative potential of certain amino acids like cysteine, methionine and vitamins like ascorbic acid and thiamine on oxidative stress. The mechanism of water hyacinth may be chelation (Pal and Chatterjee, 2004). Similar findings have also been supported by Mittal and Flora (2006) in mice due to arsenic induced oxidative stress and reduced by supplementation of chelation therapy. Achliya *et al.* (2004), Rao *et al.* (2005) and Bhattacharya *et al.* (2002) in rat have noted the beneficial effect of amla on induced oxidative stress and Thangaraj *et al.* (2007) due to the antioxidant property of amla.

Decreased calcium level in the serum is due to arsenic intoxication; while increased calcium level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of water hyacinth and Amla. Similar observations have been supported by Nandi *et al.* (2008) and Flora *et al.* (2008) in rats due to arsenic toxicity and protection after supplementation of chelation therapy. Similarly, Biswas *et al.*, (2000) in goats and Islam *et al.* (2004) reported that in human arsenic toxicity led to change in the activities of antioxidant enzymes and protection after supplementation of nutritional antioxidants.

Decreased magnesium level in the serum is due to arsenic intoxication; while increased magnesium level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of water hyacinth and Amla. Similar observations have been supported by Mukherjee *et al.* (2006) in rats and Flora *et al.* (2008) in rats due to arsenic toxicity and protection after supplementation of chelation therapy. Similarly, Islam *et al.* (2004) in human reported that arsenic toxicity led to change in the activities of antioxidant enzymes. After supplementation of nutritional antioxidants there was protection against arsenic toxicity.

Decreased sodium level in the serum is due to arsenic intoxication; while decreased sodium level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of water hyacinth and Amla. Similar observations have been supported by Nandi *et al.* (2008) and Flora *et al.* (2008) in rats due to arsenic toxicity and protection after supplementation of chelation therapy. Similarly, Biswas *et al.* (2000) in goats and Islam *et al.*, (2004) in human reported that arsenic toxicity which led to change in the activities of antioxidant enzymes and protection after supplementation of nutritional antioxidants.

Decreased potassium level in the serum is due to arsenic intoxication; while increased potassium level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of water hyacinth and Amla. Similar observations have been supported by Nandi *et al.* (2008) and Flora *et al.*, (2008) in rats due to arsenic toxicity and

protection after supplementation of chelation therapy. Similarly, Biswas *et al.* (2000) in goats and Islam *et al.*, (2004) in human reported that arsenic toxicity led to change in the activities of antioxidant enzymes and protection after supplementation of nutritional antioxidants.

An increased AST level in the serum after arsenic water ingestion is due to arsenic toxicity; while decreased AST level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to chelating nature of water hyacinth and antioxidant characteristic of Amla. Similar findings have also been supported by Flora *et al.* (2008) and Mukherjee *et al.* (2006) in rats due to arsenic induced oxidative stress and reduced arsenic burden after supplementation of antioxidant treatment. Similarly, Wang *et al.* (2006) in pigs and Peraza *et al.* (2003) in human reported arsenic intoxication which led to change in the activities of antioxidant enzymes and protection after supplementation of nutritional antioxidants.

An increased ALT level in the serum is due to arsenic intoxication; while decreased ALT level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of *E. crassipes* and *E. officinalis*. Similar observations have been reported by Nandi *et al.* (2006) and Modi *et al.* (2007) in rats; while Rana *et al.* (2008) in cattle due to arsenic induced oxidative damage and protection after chelation therapy and antioxidant treatment with folic acid, vitamins, methionine and melatonin.

An increased LDH level in the serum is due to arsenic intoxication; while decreased LDH level in the serum after combined *Eichhornia crassipes* and *Emblica officinalis* treatment in same group of arsenic intoxication may be due to beneficial effect of *E. crassipes* and *E. officinalis*. Similar findings have been supported by Flora *et al.* (2008) and Pal and Chatterjee (2004) in rat due to arsenic induced oxidative stress and reduced arsenic burden after supplementation of antioxidant treatment with folic acid, vitamins, methionine and melatonin it was concluded that there is reduced oxidative stress exhibited by decreased serum AST, ALT and LDH level after combined treatment with E. crassipes and *E. officinalis* in arsenic intoxicated rats. There is also seen improvement in altered electrolytes levels. Thus, water hyacinth with Amla exerts beneficial effects on body metabolism and altered biochemical indices in arsenic toxicity. Application of this study will be useful in minimizing the side effects of arsenic intoxication in body in arsenic-rich areas.

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