Evaluation of Haematological and Hepatorenal Functions of Selected Plant Extract on Aluminium Treated Mice

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Abstract

In the recent times, there has been growing interest in exploring the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects. In today's life the exposure of aluminium (Al) is inevitable as it is released primarily from natural process and anthropogenic sources. Humans are exposed to Al by packaging, medicines, water and air. Al generates ROS which causes oxidative deterioration of cellular lipids, protein, DNA and interferes with number of body functions such as liver, and kidney. Al can be neurotoxic. The present study has implicated the importance of herbal preparations in protecting living organism against the toxic effect of aluminium exposure. Swiss albino mice weighing (25±5g) were taken and randomly divided into various groups of five animals each. Group I served as normal control, Group II was toxicant treated group. Group III, IV, V, VI and VII were treatment groups and were administered *Allium sativum*, Triphala, *Terestris tribulus, Boerrhavia diffusa* and *Aloe vera per se* respectively. Hb% decreased remarkably due to Al thereby indicating altered metabolism. Al showed significant rise in serum transaminases, creatinine, blood urea and lipid peroxidation in brain. Screening studies with herbal preparations showed that there was a reversal of the biochemical parameters thereby recouping the variables towards normal levels. Marked recoupment was observed with *Allium sativum per se* and in combination with Triphala thereby generating marked therapeutic efficacy.

Keywords- Aluminium Chloride, *Allium sativum*, Triphala, Haematology, *Terretris tribulus*, Transaminases.

Introduction

Aluminium is a ubiquitous element with a moderate toxic effect on living organism comprising about 8% of the earth's crust. It is a non essential metal to which humans are frequently exposed. Food ingredients, antacids, buffered vaccines, allergens, injections, food preparations all contain considerable amount of aluminium. The small amount (<1%) is systematically absorbed and excreted principally in the urine and to lesser extent in the faeces. Aluminium has been reported to be neurotoxic, when injected directly into the brain of animals. ATSDR reported that aluminium is distributed mainly in bone, liver, testis, kidneys and brain (Fahaid, 2009). Patients on dialysis or on long-term treatment with total parenteral nutrition (Klein, 1993) have been shown to accumulate this metal in different organs. Different forms of Al are environmental xenobiotics that induce free radical mediated cytotoxicity and reproductive toxicity. Al causes oxidative stress within brain tissue and has direct effect on haematopoiesis. Excess Al has been shown to induce microcytic anemia (Swartz *et al*., 1987).

In the recent past, there has been growing interest in exploring the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects (Naik *et al*., 2003 and Chopra *et al*., 1956). Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring the traditional system of medicine in India. Many plant extracts have been used as a source of medicinal agents to cure urinary tract and helmintic infections gastrointestinal disorders, respiratory, parasitic, protozoan and inflammatory processes (Leite *et al*., 2006 and Biradar *et al*., 2008).

Medicinal plants have been used by all civilizations as a source of medicines since ancient times (Paliwal *et al*., 2011). Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs, and the bioprospecting of new plant-derived drugs (Sharma *et al*., 2010). Plants are the essential and integral part in treatment strategy against metal toxicity as they form secondary metabolites such as proteins, flavanoids, alkaloids, steroids and phenolics which in turn are used to restore health and heal many diseases. There is an inverse relation between the dietary intake of antioxidant rich foods and the incidence of human diseases. Among myriad of plants and herbal formulations an attempt has been made to screen few herbal preparations against aluminium intoxication.

Triphala is a traditional ayurvedic herbal formulation consisting equal parts of three medicinal plant fruits namely *Terminalia chebula, Terminalia bellerica, Emblica officinalis*. Triphala has been used extensively as a drug against number of diseases. *E.officinalis has* been reported as a rich source of vitamin C, which plays an important role in scavenging free radicals. Garlic (*Allium sativum L.*) is one of the earliest known medicinal plants (Metwally, 2009) which possess many healthful properties that are related to its bioactive compounds. It contains vitamins, minerals, Selenium and Germanium as trace elements (Lewis *et al*., 2003). Garlic was studied in different forms of extracts: aqueous and alcoholic as well as dried powders (Gruenwald, 2004 and Gorinstein, 2006). Garlic exhibits a wide range of properties including immunomodulatory, hepatoprotective, antimutagenic and anticarcinogenic effects (Shin, 2004 and Ameen *et al*., 2003). Aloe Vera (*Aloe barbadensis*) commonly known as Aloe Vera is widely distributed in Asia, Africa and other tropical areas (Yagi *et al*., 2002). It is used in the traditional medicine of many cultures and said to be beneficial in the treatment of disorders such as arthritis, gout, dermatitis etc and wounds such as peptic ulcer and burns (Grindlay and Reynolds, 1986). The fresh gel, juice and formulated products have long been used for medical and cosmetic purposes and general health (Chithra *et al*., 1998 and Reynolds, 1999). Use of Aloe gel preparation has increased dramatically in the last decade. The mucilaginous gel from paranchymatous cells in the leaf pulp of aloe vera is incorporated in ointments, creams, lotions and other preparations essentially for topical use. (Anshoo *et al*., 2005). In spite of its wide use in folk remedies any influence on various heavy metals/ metalloid induced altered biochemical and physiological processes have not yet been described in detail (Gupta *et al*., 2005). *Tribulus terrestris*, also called "puncture vine" is a plant long used around the world for the treatment of various ailments. Tribulus terrestris has also been known as hypoglycemic drug. *Boerrhavia diffusa*, also called punarnavasava contains alkaloids and flavonoids. The root is mainly used to treat gonorrhea, inflammation, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumors, and cancers (Rajpoot *et al*., 2011). It is also administered orally as a blood purifier and to relieve muscular pain (CSIR 1988).

The present study has implicated the importance of plants in protecting living organism against the toxic effect of aluminium exposure.

Materials and Methods

Chemicals

Aluminium Chloride anhydrous sublimed for synthesis was purchased from Merck specialities Pvt Ltd.

Determination of $AlCl_3$ daily dose

In our study, mice received a daily i.p. of 4.2 mg $AlCl_3$ / kg of body weight (Horie et al., 1989).

Treatments

Garlic buds (about 250 gms) were dried under shade. It was grinded with mortar and pestle to make fine powder dried in direct sun light and was stored at -200C until use. 10% aqueous garlic extract was administered orally for five days.

Triphala was commercially procured from Dindayal Aushadhalaya. 10% aqueous garlic extract was administered orally for five days.

Boerhavia diffusa and Terestris tribulus were commercially procured from Dindayal Aushadhalaya. This was suspended in distilled water to give 10% aqueous extract.

Aloe vera gel was prepared according to Flora *et al.*, 2005 with slight modifications from fresh leaves, ground in a blender and centrifuged at 10000 g to remove the fibers. The supernatant was lyophilized and stored at room temperature. This was suspended in distilled water to give 10% aqueous extract.

Experimental design

The animal care and handling was done according to the guidelines CPCSEA. Swiss albino mice (25 ± 10g) were used for the study. The mice were housed in standard polypropylene cages (05 mice /cage) in an environmentally controlled well ventilated room with a constant temperature of 25±2°C and a 12 hr light/dark cycle. The mice were fed a "standard pellet diet (Pranav agro Industries Delhi)" and water *ad libitum*. Five groups of 5 mice each were used for the experiments. The groups were treated as follows: Group I served as normal control, received a daily dose of 1 ml/Kg b.w. normal saline (orally); Group II was experimental control group, received $AlCl_3$ at a dose 4.2 mg/ kg body weight (ip) daily for 21 days; Group III, IV,V ,VI,VII were administered $AlCl_3$ as in group II followed by administration of *Allium sativum* , Triphala, *Terestris tribulus, Boerhavia diffusa* and *Aloe vera per se* orally. All the animals were sacrificed 5days after the last treatment.

Autopsy of animal and Collection of blood serum

Blood was collected directly from retro-orbital sinus and serum was separated then Animals were sacrificed by cervical dislocation .The tissues viz; liver, kidney and brain were quickly excised, washed in ice cold, normal saline and blotted individually freed from extraneous material on ash-free filter paper. The tissues were then homogenized separately in hypotonic solution, using a Potter-Elvenjham homogenizer at 600-1000 rpm in ice cold conditions.

Preparation of Tissue homogenate

The crude tissue homogenate was then centrifuged at 10,000 rpm for 15 min (0-4°C). The supernatant was collected and stored at 20°C until used for estimating hematological, serum and Tissue Biochemical Parameters, and Lipid Peroxidation. Blood urea, Serum creatinine determined by commercially prepared kit method, Transaminases activity were determined with Reitman and Frankel, 1957 and content of the compounds reacting with thiobarbituric acid (TBARS) in tissues according to Sharma and Krishnamurthy, 1968.

Results

Effects of administration of Aluminium chloride and different plant extracts orally with Aluminium chloride on body weight along with selected biochemical parameters in mice tissue and blood serum is presented in Table 1-4.

Table 1: Body weight of mice treated with Aluminium chloride ($AlCl_3$) and extracts of various plants.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Body Weight (g)	32 ± 2.3	27 ± 2.3	42 ± 2.3	40 ± 2.3	38 ± 2.1	40 ± 2.2	44 ± 0.4
Liver weight (g)	2 ± 0.16	1.5 ± 0.14	2.1 ± 0.16	2.2 ± 0.25	2.0 ± 3	2.2 ± 0.28	2.16 ± 1.9
Kidney weight (g)	0.68 ± 0.07	0.38 ± 0.03	0.68 ± 0.03	0.53 ± 0.04	0.62 ± 0.03	0.8 ± 0.08	0.7 ± 0.05
Brain weight (g)	0.48 ± 0.05	0.37 ± 0.03	0.5 ± 0.05	0.49 ± 0.04	0.45 ± 0.05	0.58 ± 0.06	0.8 ± 0.07

Table 2: Serum biochemistry& changes in the activities of serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (AST) of mice treated with aluminium chloride ($AlCl_3$) and extracts of various plants.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Hb (g%)	12.4 ± 0.7	8 ± 0.7	12.5 ± 0.9	12.6 ± 0.8	11.6 ± 2	12.1 ± 0.8	12 ± 0.18
Serum AST(IU/L)	59.1 ± 13	97.2 ± 10	58.5 ± 11	56.2 ± 9	56 ± 11	54.5 ± 0.5	62.8 ± 0.59
Serum ALT (IU/L)	45.2 ± 16	52.1 ± 12	39.4 ± 3	40.5 ± 3	39.8 ± 3	41.6 ± 0.35	48.6 ± 0.38
Blood Urea (mg/dl)	32.4 ± 3	41.3 ± 5	32.1 ± 2	29.0 ± 3	28.7 ± 2	35.5 ± 0.29	34 ± 0.28
Serum creatinine	1.18 ± 0.1	1.78 ± 0.2	1.2 ± 0.5	1.72 ± 0.7	1.4 ± 0.9	1.62 ± 0.7	1.42 ± 0.8

Table 3-4: Protein & TBARS estimation of mice treated with aluminium chloride ($AlCl_3$) and extracts of various plants.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Serum(g/100ml)	38.8 ± 14	60 ± 9	49.8 ± 14	38.4 ± 17	47.2 ± 15	42.8 ± 12	38.6 ± 16
Liver (mg/100g)	27.2 ± 22	20.7 ± 28	28.6 ± 26	28.5 ± 19	26.3 ± 22	25.1 ± 21	25.8 ± 22
Kidney(mg/100g)	14 ± 23	10 ± 9	17 ± 22	15 ± 19	12 ± 16	11 ± 15	13 ± 15
Brain(mg/100g)	27 ± 22	23.7 ± 23	30.7 ± 22	29.2 ± 22	26.7 ± 24	22 ± 21	22.6 ± 22

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Liver LPO (n mole of MDA/ mg protein)	0.29 ± 0.02	1.2 ± 0.01	0.49 ± 0.03	0.44 ± 0.03	0.45 ± 0.03	0.5 ± 0.028	0.6 ± 0.05
Kidney LPO (n mole of MDA/ mg protein)	0.34 ± 0.02	1.2 ± 0.1	0.49 ± 0.04	0.44 ± 0.03	0.39 ± 0.02	0.43 ± 0.03	0.51 ± 0.02
Brain LPO (n mole of MDA/ mg protein)	0.4 ± 0.05	1.5 ± 0.08	0.9 ± 0.0.05	01 ± 0.0.3	0.8 ± 0.02	0.6 ± 0.04	0.5 ± 0.04

Body weight decreased remarkably on the contrary there was decline in the Hb content after Al exposure which recouped considerably with aqueous extract of garlic. Elevation in total serum protein was observed after the exposure of toxicant and it was recovered after the administration of garlic and total protein content in tissue was decline and it was recouped after triphala therapy. Rise in serum transaminases on aluminium intoxication followed by recoupment with garlic was obseres (Table 1,2). Rise in total serum protein was observed after the exposure of toxicant and it was recovered after the administration of garlic and total protein content in tissue was decline and it was recouped after triphala therapy (Table 3). In present study elevation in LPO level was observed in kidney followed by brain and liver after the toxicant exposure. Marked recovery was observed in groups administered with garlic and triphala. There was rise in urea and creatinine level after the toxicant and it is recouped after the administration of garlic and triphala treatment (Table 4).

Discussion

The present investigation deals with the exposure of aluminium following phytotherapy in mice model. Different plant extracts were used *per se* to aluminium intoxicated mice to screen the effectiveness in recouping the toxicity induced by aluminium.

Measurement of damage caused by metal can be assessed biochemically by observing alterations in the enzyme activity which then indicate the degree of injury and its response to corrective treatment.

Body weight decreased remarkably by intraperitoneal administration of aluminium chloride solution to mice for 21 days. This is because toxicant may decrease feed intake by modulation of appetite. The decrease in body weight was also supported by Sallam *et al*., 2005. Where, mean value indicated that treatment with aluminium chloride caused significant ($P< 0.05$) decrease in the live body.

Haemoglobin is a parameter which is a marker of the overall status of the body. There was decline in the Hb content after Al exposure which recouped considerably with aqueous extract of garlic. This study was supported by Altmann, 1988, he found decline in haemoglobin. This may be because the modest aluminium accumulation found in most dialysis patients has a pronounced inhibitory effect on haemoglobin synthesis.

In this study rise in total serum protein was observed after the exposure of toxicant and it was recovered after the administration of garlic and total protein content in tissue was decline and it was recouped after triphala therapy. Helal *et al*., 2006, found the decline in total protein level in $NaNO_2$ exposure which was recouped after the administration of aqueous extract of garlic. A decline in the protein content due to toxicant was observed in the present investigation in vital organ which may be due to decreased ATP production.

Transaminases i.e. AST and ALT are excellent markers of tissue damage. Rise in serum transaminases has also been reported with other metal exposure which substantiates our results (Nivaskar *et al*., 2006). The stabilization of transaminases with administration of garlic is a clear indication of the improvement in the functional status of hepatic and renal cells. Aluminium causes necrosis to the liver and kidney, AST significantly increases in such cases. ALT level indicates the existence of liver diseases, as this enzyme is present in large quantities in the liver. Level increases in serum when cellular degeneration or destruction occurs in this organ. Therefore, the increase of these enzymes in plasma is indicative of liver damage and thus altering the liver function (Oluwole, 2001).

In present study elevation in LPO level was observed in kidney followed by brain and liver after the toxicant exposure marked recovery was observed in groups administered with garlic and triphala, this study was also supported by Dhanalakshmi *et al*., 2000. Aluminium has the ability to generate ROS (Reactive Oxygen Species) which causes peroxidation of membrane lipids. Lipid peroxidation (LPO) is a multiphasic process which involves initiation, propagation and termination leading to the generation of peroxides and hydroperoxides, and many cytotoxic products such as aldehydes, peptones and alcohols as exemplified by malondialdehyde (MDA). Increased LPO and their products contribute to neuronal loss in conductions associated with oxidative stress induced by highly toxic metals like arsenic, lead mercury etc. The process of lipid peroxidation may reflure the cell membrane and release destructive lysosomal enzymes. Al intoxication lead to severe increase in the level of lipid peroxidation LPO damage in the liver cells. The increased TBARS as seen in the present study is due to tissue injury and failure of antioxidant defense mechanism thus increased accumulation of lipid peroxidation products which may be a consequence of progressive degradation of necrotic tissue.

In our study there was rise in urea and creatinine level after the toxicant exposure because of kidney dysfunction and it is recouped after the administration of garlic and triphala treatment. Jaijoy *et al*., 2010 found decline in urea and creatinine after the exposure of triphala. Marked increase of serum urea and creatinine in animals receiving aluminium chloride is of interest. The increase of serum urea and creatinine concentration can be a consequence of critical accumulation of this metal in kidney and following renal failure development as aluminium is excreted mainly by kidney (Kowalczyk *et al*., 2004).

Conclusion

Potential activity of garlic and triphala dry powder recouped aluminium induced alterations considerably, indicating their effectiveness as a therapeutic agent against aluminium induced toxicity. Further investigations are required to prove the efficacy of these agents against aluminium intoxication.

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