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Short Communication

Red Onion Extract Attenuates Aluminum Induced Toxicity in Swiss Albino Mice

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CA, Asmatullah and SA conceived the idea, executed the research and wrote the manuscript. SH, MA and SA performed experimental work. CA, SH, MA, BZ performed enzyme assays, statistical analysis, manuscript editing.

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ABSTRACT

This study was planned to investigate the therapeutic potential of onion extract against aluminum (Al) induced toxicity in male albino mice, *Mus musculus*. Eighty, 8-9 week old mice were divided randomly into eight groups, each containing 10 mice and exposed to three sub-lethal concentrations (37.5µg/g; 18.7µg/g; 9.37µg/g B.W) of aluminum, with and without onion extract. Control and antidote groups were treated with distilled water and onion equous extract, respectively. Doses were administered orally by gavage for 30 consecutive days, once a day and mice were euthanized on the 31st day. Blood was collected in serum separating tubes through intracardiac puncture for biochemical analysis. Morphometric observations revealed significant decrease (P≤0.001) in body weight of mice in Al treated groups as compared to control but organs weight increased contrary to the body weight. Significant increase (P≤0.01-P≤0.001) was recorded in total alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) in liver and urea, and creatinine levels in kidneys, in Al exposed groups against control. In Al + antidote groups these deviations were less significant (P≤0.05). However, these morphometric and biochemical alterations improved to a great extent when onion extract was co-administered with Al. So it is concluded that Al caused morphological and biochemical disturbances in mice, whereas onion extract showed protective effects as well as regenerative potential against Al provoked toxicity in male mice.

Aluminum (Al) is the third most common element present in the earth's crust, almost 8% of total mineral component. It is found in the soil, clays, rocks and gems combined with oxygen, flourine, silicon and other elements. It is a constituent of cooking wares, drugs such as antacids, aspirins, vaccines, cosmetics, antiperspirants, deodorants, allergen injection and toothpastes (Abbasali *et al*., 2005). It even became the part of drinking water when used for purification purpose (Ochmanski and Barabasz, 2000; Turkez *et al*., 2010). Food and food additives contain small but inconsistent amounts of Aluminum so it gets easy access into the human body. As aluminum is broadly present in the environment and massively used in daily life, this ensures its recurrent exposure to human beings (Kumar and Gill, 2009).

Aluminum possesses substantial toxic potential for humans (Verstraeten *et al*., 2008). Studies showed neurotoxicity of Al in different regions of brain (Nehru and Bhalla, 2006). Al accumulation in liver causes hepatic damages at higher concentrations (Shati and Alamri, 2010). Al ions change structure and properties of cellular membranes, reduce the functional capabilities of many enzymes like alkaline phosphatase, acetylcholinesterase, and adenylcyclase (Qitu *et al*., 2002). Certain diseases enhance the absorption of Al through the gastrointestinal tract. For example, patients of chronic renal insufficiency or uremia have been reported to absorb Al more readily than normal individuals (Braunlich *et al*., 2006).

It is a well-known fact that diets rich in vegetables and fruits are preventative against a variety of diseases. Specially, antioxidants present in fruits and vegetables are considered to be the principal nutrients for their protective effects (Ross and Kasum, 2002). Onion (*Allium cepa*) is regularly used in everyday diet and likewise utilized as a folk remedy for its anti-septic properties and other valuable impacts. It has been scrutinized for its restorative abilities, as an antioxidant and anti-cancer agent by various researches (Augusti, 2009; Saleheen *et al*., 2004; Santas *et*

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al., 2008). It contains plenty of bioactive compounds such as amino acids, minerals, vitamins and sulphur (Roldán *et al*., 2008).

Literature showed that Al is present in environment ubiquitously and is a growing threat to humanity (Mitkus *et al*., 2011). Lot of studies have been done on neurotoxicity of Al but its potential biochemical toxicity on basic organs are not avaliable. Despite onion's antioxidant and anticancer properties, its remedial effect on Al caused damages have not been studied. The evaluation of probable toxicity of Al in mice and specific remedial effect of onion was therefore inevitable.

Materials and methods

Swiss Webster albino male mice (*Mus musculus*), 8-9 weeks old and approximately 28±2g weight, were raised in the animal house, Department of Zoology, University of the Punjab, Lahore. Mice were housed in ventilated steel cages (14x"10"x7") under well controlled environment, 12h light/dark cycle, temperature (27± 2°C) and with relative humidity 40-55%. Food and water were supplied *ad libitum*. All protocols and ethical procedures opted during this research were approved by local Ethical Committee of the Punjab University.

Aluminum chloride as source of aluminum (Al) was purchased from Sigma Chemical Co (Online). Keeping in view, LD_{50} of Al chloride in mice (0.77±0.12g/kg), three sub-lethal concentrations of Al were prepared in water, so that 0.1ml contained the required dose concentrations of 37.5µg/g, 18.7µg/g, 9.37µg/g body weight for mice. Onion extract was prepared by grinding 100g of onion in 60ml distilled water according to the reported procedure (Yoshinari *et al*., 2012). In short, fresh red onions purchased from local market of Lahore were peeled, cleaned and cut into pieces. Onion pieces were then minced using an electric blender and paste filtered using fine cloth to get liquid extract which was stored at 4°C.

In all eighty male albino mice were randomly divided into eight groups each of ten mice, which received following treatments for 30 days consecutively through oral gavage: (i) Control: received distilled water; (ii) D-I: received 9.37µg/g B.W of Al; (iii) D-II: received 18.7µg/g B.W of Al; (iv) D-III: received 37.5µg/g B.W of Al; (v) AD+D-I: received onion extract+9.37µg/g B.W of Al; (vi) AD+D-II: received onion extract+18.7µg/g B.W of Al; (vii) AD+D-III: received onion extract+37.5µg/g B.W of Al; (viii) AD: which received onion extract (1ml) only. The animals were euthanized by anesthetic inhalation Isoflurane for dissection on 31st day of dose administration.

Morphological and morphometric studies involved wet weight of testes, liver and kidneys measured by digital balance. Blood samples for biochemical assay were collected from intra-cardiac puncture into serum separating tubes and different enzymes were analyzed by methods described by Mastoi *et al*. (2010).

The arithmetic mean and standard error of means of all observations were calculated. The numerical data were processed statistically by using software SPSS version 16 (SPSS Inc., Chicago, IL, USA) by one-way ANOVA. Post Hoc Tukey test was applied on significant results for multiple comparisons.

Results and discussion

During current study, a significant ($P \leq 0.001$) decrease in mice body weight was observed in Al exposed groups as compared to control group. But average weight of mice in AD+Al groups was comparable with control and antidote groups (Table I). Our results were similar to those of Zhu *et al*. (2014) and Julka *et al*. (1996), who described that the oral administration of Al chloride had a detrimental effect on the body weights of rats.

Morphometric data revealed dose dependent increase ($P \leq 0.05$ to $P \leq 0.001$) in the mean weight of testes, kidneys and liver in Al exposed groups as compared to control. In the case of AD+Al group average weights of all tissues were comparable to control group except at higher concentrations of Al whereas, average liver weight increased noticeably ($P \leq 0.05$) in the mice exposed to onion extract only (Table I). These results are justified by the observations of Ighodaro *et al*. (2012) who reported marked degenerative changes and increased weight of testes and liver in Al exposed male rats. This was probably due to the functional compensation of the organs against oxidative stress caused by Al chloride.

Table I shows elevated levels ($p \leq 0.05$-$p \leq 0.001$) of liver function biomarkers ALT, ALP and AST in Al administered groups in dose dependent manner. These findings are supported by Chinoy and Memon (2001) who described that Al exposure promoted liver dysfunction. Groups in which onion extract was co-administered with Al, increase was less obvious except at higher concentrations of Al (AD+ D-III). Bilirubin level remained consistently less in Al group though a remarkable decrease was seen in AD+ Al group.

Table I also shows biochemical markers for kidney (urea and creatinine levels) which increased ($p \leq 0.05$-$p \leq 0.001$) notably in Al exposed groups though onion extract therapy increased urea and creatinine level inconsistently compared with control. These outcomes are similar to the study of Newairy *et al*. (2009). However, onion extract prior to Al significantly altered urea and creatinine level to someextent in normal range. These results are similar tothose of Prakash *et al*. (2007).

Table I. Effect of onion extract on body weight, weights of organs (liver, kidney and testis), liver function and renal function parameters of aluminum intoxicated male swiss albino mice.

Dose groups parameters	Control (n=10)	Al treated groups			Onion extract+ Al treatment			Onion extract treatment AD (n=10)
		D-I (n=10)	D-II (n=10)	D-III (n=10)	AD + D-I (n=10)	AD + D-II (n=10)	AD + DIII (n=10)	
Body weight (g)	30.0±0.92	27.2±0.50	26.4±0.28***	25.5±0.87***	28.95±1.3	29±0.35	29.3±1.35	28.5±1.18
Organ weight(mg)								
Liver	1208.8±33.77	1209±22.70	1482.6±23.77**	1802±29.50***	1351±15.85	1466.6±26.36*	1608±22.70***	1283.6±32.56*
Kidney	231.6±9.09	249±7.80*	259.4±4.01*	268±9.26**	223±5.61	251.6±5.80*	256.4±11*	215±18.56
Testis	53±2.03	61.6±5.19*	70.4±4.50**	72.7±3.55***	52.3±2.28	53.6±1.40	62.4±3.55**	50.5±2.28
Liver function test								
Bilirubin (mg/dl)	0.76±0.07	0.61±0.09	0.69±0.09	0.68±0.09	0.49±0.00	0.49±0.00***	0.51±0.21***	0.39±0.00**
ALT (U/L)	19.3±0.08	23.04±0.25*	21.2±0.43*	27±0.27***	18.94±0.16	23.66±0.52*	24.84±0.37*	39.38±0.41
AST (U/L)	18.1±0.58	26.6±0.21**	25.8±0.37**	29.8±0.09***	20.8±0.24*	14.6±0.18	22.8±0.10*	41.46±0.34**
ALP (U/L)	150.3±0.56	201.5±0.22***	188±0.41***	215.6±0.31***	205.5±0.44***	199.3±0.31***	213.6±0.43***	175.7±0.39*
Renal function test								
Urea (mg/dl)	31.8±3.73	28.46±0.24	37.76±0.10*	42.84±0.09**	30.9±0.11*	27.76±0.11	29.80±0.09*	28.1±3.25
Creatinine (mg/dl)	0.48±0.09	0.8±0.05***	0.78±0.01***	1.18±0.00***	0.81±0.01***	0.68±0.00*	0.63±0.03*	0.58±0.00

Control, untreated; D-I, D-II, D-III, Al exposed groups; AD+D-I, AD+D-II, AD+D-III, Al + onion extract treated; AD, onion extract treated; n, number of animals. Data presented as Mean± SEM, Oneway ANOVA, * P≤0.05, **P≤0.01, ***P≤0.001. ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase.

Despite these findings and clear evidences on hepatic, renal, and testicular toxicity of Al in mammalian model, this study had some limitations. First, very low concentration of Al is expected to be absorbed using oral administration and unfortunately we were not able to detect the levels of Al accumulation within the tested tissues by HPLC.

Conclusion

So by these observations, it is concluded that Al toxicity is proved by various parameters (Morphology, morphometry, biochemistry). Side by side, therapeutic effect of onion extract is justified, when given with Al, it showed positive remedial potential. Perhaps here onion extract acts as metal chelating agent and binds with Al. Contrary to these findings, another noticeable thing was hepatotoxicity and serum level of liver specific enzymes was also increased significantly in only onion extract exposed mice, probably due to its sulphur content.

Statement of conflict of interest

The authors have declared no conflict of interest.

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