International Journal of Bioassays
ISSN: 2278-778X
Coden: IJBNHY
Open Access

Original Research Article

Amelioration Of Arsenic Induced Meiotic Chromosomal Abnormalities By *Psidium gyajava* (Guava) In *Mus musculus*

Andalib Iqbal* and Dharmshila Kumari
University Department of Zoology, T.M. Bhagalpur University, Bhagalpur-812007, Bihar, India

Received for publication: November 17, 2014; **Revised**: December 15, 2014; **Accepted**: February 21, 2015

Abstract: Arsenic trioxide (0.04 mg/animal/day) when administered orally to Albino Swiss mice (*Mus musculus*) for 15 days increased the frequency of abnormality 112 (37.33%±2.11) compare to 28 (9.33%±2.59) of control in meiotic chromosome. The abnormality was 22 (7.33%±2.39) in group fed only with guava fruit extract. However, when guava fruit extract and Arsenic trioxide fed concurrently the abnormality was 68 (22.66%±2.69) respectively. The result showed that arsenic trioxide is potent enough to damage meiotic chromosome and present finding also showed that concurrent treatment of guava fruit extract and arsenic trioxide minimize the frequency of meiotic chromosomal abnormalities. Therefore, it is suggested that the guava extract may reduce the risk of arsenic induced genotoxicity.

Key Words: Amelioration, Arsenic, Genotoxic, Guava, Meiotic chromosome, Oxidative stress, ROS, Water.

Introduction

The bioaccumulation of toxic heavy metals in the food chain can be highly dangerous to human health due to their persistent nature and potential toxicity. They can neither be created or destroyed nor can one heavy metal transformed into another i.e they are immutable. Arsenic contaminated drinking water threatens more than 150 million people all over the world. Arsenic is entering through the ground water by natural weathering processes and by the discharge of effluents of various industries. Drinking water is the main source of exposure of arsenic in human. It generates the reactive oxygen species and free radicals like hydrogen peroxide (Chen *et al.,* 1998) or superoxide (Lynn *et al.,* 2000) and has been found to be genotoxic in human cell (Gradacka *et al.,* 2001., Graham *et al.,* 2004). In addition, a few studies have shown that exposure to arsenic increases the frequency of micronuclei, chromosome aberrations, and sister chromatid exchanges both in humans and animals (Waclavicek *et al.,* 2001). Reactive oxygen species are an important factor in DNA damage, oxidative stress-induced damage and many other cellular processes (Fahmy *et al.,* 2009, Ye *et al.,* 2010). Members of Food and Nutrition Board of National Council (USA) recently defined a dietary antioxidant as a substance in food which significantly decrease the adverse effect of ROS, RNS or both in human. Various antioxidant have been shown to some protective role against arsenic toxicity (Gupta *et al*., 2005. Sahu *and* Paul., 2006). Epidemiological studies have indicated that frequent consumption of natural antioxidant is associated with a lower risk of various diseases and cancer (Renaud *et al.,* 2004). Fruits and vegetables contain high amount of antioxidant such as ascorbic acid, beta-carotene and folic acid which are useful to the body as a bioactive that is directly related to the prevention of lipid peroxidation and vascular diseases, (Frinazzi *et al.,* 2012). Ameliorating effect of papaya fruit has been found to some extent on arsenic induced meiotic chromosomal abnormalities in mice (Singh and Kumari, 2013). However, it could not negate completely. Guava fruit could also ameliorate the arsenic induced mitotic chromosomal abnormalities in mice (Iqbal *et al.,* 2014). Therefore, the present work was undertaken to study the ameliorating effect of another antioxidant rich fruit, guava on arsenic induced meiotic chromosomal abnormalities. Guava (*Psidium guajava*) is believed to overcome several health problems including a source of antioxidants (Sandra *et al.,* 2012) and free radical scavenges capacity (Chen *et al.,* 2007). It is one of the most common, easily available cheaper fruit which has long been used as a traditional medicine and also known as the poor's man apple.

***Corresponding Author:**
Andalib Iqbal,
University Department Of Zoology,
T.M. Bhagalpur University,
Bhagalpur-812007, Bihar, India.

Material And Method

Four to five week old male Albino Swiss mice (*Mus musculus*) were obtained from the laboratory inbred stock (seed colony supplied by Central Drug Research Institute, Lucknow) and maintained in the animal house of the department. They were housed individually in cage under the standard laboratory condition (25±5°C, RH=50±10%). The animals were fed on food grains (maize and wheat) and tap water ad libitum. All animal treatment and protocols employed in this study received prior approval of the Institutional Head and Departmental Research Committee.

Treatment

Male Albino Swiss mice were put into four groups and subjected to various treatments for 15 days as shown in Table-1. Arsenic Trioxide used as a water pollutants and ripe guava extract (aqueous) was used as an ameliorating agent. Predetermined sub lethal dose of Arsenic Trioxide (1.5mg/kg.b.wt/day) was selected (Yasmin *et al.,* 2011). Such treatment of Arsenic Trioxide have been given because at this dose mice can survive and above this dose mice would die. The dose of guava fruit extract was 60mg/animal in proportion to human recommended daily allowances (RDA) at which genotoxicity of Arsenic Trioxide can be reduced.

Slide Preparation

The animals of each group were sacrificed by cervical dislocation after the completion of treatments. The slides of meiotic chromosomes were prepared by following the technique suggested by Das and Nayak., 1988 ie Colchicine-Hypotonic-Acetoalcohal-Flame Drying-Giemsa Staining.

Screening of Slides

The chromosomal complex cell in Diplotene / Diakinesis / Metaphase I was screened under the microscope for screening the structural, numerical and synaptical chromosomal abnormalities in each group. 300 well spread metaphase-I plates from each group of animals were screened by random selection. Student t-test was applied for the data calculation.

Table 1: Summary of the treatment protocol

S.No	Experimental Variant	Symbol	Dose
1	Control	C	No AT or G
2	Arsenic Trioxide	AT	0.04mg/animal/day
3	Guava Fruit Extract	G	60mg/animal
4	Arsenic Trioxide and Guava Fruit Extract	AT+G	As 2 and 3

Result and Discussion

Structural, numerical and synaptical types of chromosomal abnormalities were observed in each group of mice. Among the four groups of mice the percentage of abnormality was 37.33% in Arsenic trioxide supplementation. Similar percentage of abnormality was observed in both the control (9.33%) and guava (7.33%) fed animals. However, guava fruit extract fed with Arsenic Trioxide (AT+G) significantly decreased the abnormality incidence to 22.66% from control group. Thus, the result showed the genotoxic effect of arsenic trioxide in meiotic cells like bone marrow cell (Saleha *et al.,* 2001., Singh and Kumari., 2012). The present finding also showed that concurrent treatment of guava and Arsenic trioxide had ameliorating property. The major mechanism behind this toxicity is oxidative stress that needs to a number of diseases in human. Oxidative stress and DNA damage induced by arsenic trioxide occur via production of superoxide and hydrogen peroxide radicals, specifically reactive oxygen species (Matsui *et al.,* 1999). Guava fruit is a rich source of Vit C and it acts as an antioxidant, scavenges the aqueous reactive oxygen species by very rapid electron transfer and inhibit lipid peroxidation (Barchowsky *et al.,* 1999). However, the mechanism of genotoxicity is not clearly understood, but may be due to the ability of arsenate to inhibit DNA and replicating or repair enzymes (Li *et al.,* 1989). Free radicals are produced in mice after acute exposure to inorganic arsenic (EPA., 2008). These free radicals are neutralized by an antioxidant defense system. Antioxidants act through different mechanism and different compartments but are mainly free radical scavengers). Therefore, from the above findings it is suggested that such type of cheap and antioxidant rich fruit in diet are beneficial for reducing the toxic effect of Arsenic contaminated drinking water.

Table 2: Incidence of chromosomal abnormalities in meiotic cells of mice treated with arsenic trioxide and guava fruit

Exp. Variant	Structural abnormality		Numerical abnormality		Synaptical abnormality		Grand Total	
	No	% ± S.E	No	% ± S.E	No	% ± S.E	No	% ± S.E
C	5	1.66 ± 1.25	12	4.0 ± 1.87	11	3.66 ± 1.80	28	9.33 ± 2.59
AT	27	9.0 ± 2.56	50	16.66 ±2.88[a]	35	11.66 ± 2.75	112	37.33 ± 2.11[a]
G	4	1.3 ± 1.13	10	3.33 ±1.73[b]	8	2.66 ± 1.56	22	7.33 ± 2.39[b]
AT+G	16	5.33 ± 2.11	31	10.33 ±2.67	21	7.0 ± 2.35	68	22.66 ± 2.69[abc]

a, b and c indicates the significant difference between C, AT and G.

References

1. Barchowsky A, Roussel RR, Klei LR, James PE, Ganju N, Smith KR and Dudek EJ. Low levels of arsenic trioxide stimulate proliferative signals in primary vascular cells without activating stress effect or pathway. In Toxicol. Appl. Pharmacol, 1999, 159, 65-75.
2. Chen YC, Lin JK. Involvement of reactive oxygen species and caspase 3 activation induced apoptosis, /Cell Physiol, 1998, 177, 324-33.
3. Chen HY and Yen GC, Antioxidant activity and free radical scavenging capacity of extracts from guava (Psidium guajava L.) leaves. Food Chemistry, 2007, 101, 2, 686-694.
4. Das RK and Nayak R, Spermatocyte chromosome alterations in mice inoculated with anti-rabies vaccine and tetanus toxoid separately and in combination. Mutation Research, 1998, 204, 277-282.
5. EPA, Health effects test guide line. OPPTS 870.5395 in vivo mammalian cytogenetics test: In Erythrocytes micronucleus Assays, 1996, 712-C-96-226.
6. Fahmy B, Cormier SA, Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells, Toxicol In Vitro, 2009, 23, 1365–1371.
7. Farinazzi-Machado FMV, Guiguer EL, Barbalho SM, Soares de Souza MSS, Santos Bueno PC, Mendes CG, Araújo AC, ART, Lara LimaLM, Marim NS, Brunnati ACS, Silva PMR and Sato IT, 2012. Effectsof Psidium guajava on the metabolic profile of Wistar

rats, J, Med. Plants Res, 2012, 618, 3450-3454. DOI, 10.5897/JMPR12.033.

8. Gupta R, Sharma M and Flora SJS, Therapeutic effect of Moringa olifera on arsenic induced toxicity in rats. Environ. Toxicol. Pharmacol, 2005, 20, 456-464.

9. Gradacka D. Palus J and Wasowicz W, Selected mechanism of genotoxic effects of inorganic arsenic compounds. In Int. J. Hyg. Environ. Health, 2001, 14, 4, 317-328.

10. Graham-Evans B, Cohly HHP, Yu H, Tchounwou PB. Arsenic-induced genotoxic and cytotoxic effects in human keratinocytes, melanocytes and dendritic cells, Int J Environ Res Public Health. 2004, 1, 83–89.

11. Iqbal A, Singh N and Kumari D. Ameliorating Effect of Guava fruit on drinking water pollutant induced genotoxicity. The Ecoscan an International Jouranl of Environmental science, special issue, 2014, Vol V, 129-134.

12. Li JH and Rossman TG. Inhibition of DNA ligase activity by arsenite: a possible mechanism of its co mutagenesis. Mol Toxicol, 1989, 2, 1–9.

13. Lynn S, Gurr JR. Lai HT and Jan KY, NADH oxidase activation involved in arsenite oxidative DNA damage in human vascular smooth muscle cells. Circ. Res, 2000, 86, 514-519.

14. Matsui M, Nishigori C, Toyokuni S, The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2′-deoxyguano sine in arsenic-related Bowen's disease. J Invest Dermatol, 1999, 113, 26–31.

15. Renaud SC, Guenguen RS, Chenker J, Hounted A, Alcohol and mortality in middle-aged men from Eastern France Epidemiology, 1998, 9, 184-88.

16. Saleha B, Danadevi BK, Jamil K, Ahuja YR, Visweraswara Rao K, Ishaq M, In vivo genotoxic effect of arsenic trioxide in mice using comet assay, In Toxicology, 2001, 162, 3, 171-177.

17. Sandra M, Barbalho SM, Farinazzi, Machado FMV, Goulart RA, Brunnati ACS, Ottoboni AMMB, Nicolau CCT, Psidium guajava (Guava) A plant of multipurpose medicinal application. Med Aroma Plants.2012, 1, 4, 1-6.DOL, 104172/2167-0412.1000104.

18. Sahu AP and Paul BN, Arsenic and human health effects: Prevention by dietary modification with functional food jiggery. 25th International Conference on crop. Hlth. Milan. Italy, 2006, 92.

19. Singh N and Kumari D, Genotoxicity of water pollntant and is amelioration. The Ecoscan. An International Jouranl of Environmental science, special issue 1, 2012, 209–212.

20. Singh N and Kumari D, Amelioration of genotoxicity by papaya extract induced by Arsenic contaminated drinking water. The bioscan, an international journal of environmental science, 2013 Vol 1, 2, 623-626.

21. Waclavicek M, Berer A, and Oehler L. Calcium ionophore: a single reagent for the differentiation of primary human acute myelogenous leukaemia cells towards dendritic cells. Br J Haematol, 2001, 114, 466–473.

22. Yasmin S, Das J, Stuti M, Rani M and D'Souza D, Sub chronic toxicity of arsenic trioxide on swiss albino mice. Int. J. Environmental Science, 2011, 1, 7, 1640-1674.

23. Ye JS, Li J, Yu YM, Wei Q, Deng WF, Yu LXL, Carnitine attenuates oxidant injury in HK-2 cells via ROS-mitochondria pathway. Regul Pept, 2010, 161, 58–66.

Cite this article as:
Andalib Iqbal and Dharmshila Kumari, Amelioration Of Arsenic Induced Meiotic Chromosomal Abnormalities By *Psidium Guajava* (Guava) In *Mus Musculus*, International Journal of Bioassays, 2015, 4 (03), 3738-3740.

Source of support: Nil
Conflict of interest: None Declared