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AMELIORATION OF GENOTOXICITY BY PAPAYA EXTRACT INDUCED BY ARSENIC CONTAMINATED DRINKING WATER

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ABSTRACT
Arsenic is an established genotoxic carcinogen in human. Arsenic trioxide (0.04mg/animal) when administered orally daily to albino swiss mice for 15 continuous days, increased the incidence of abnormalities to 30.6% in meiotic metaphase chromosome, 5.88% in the gross morphology of sperm head and decreased the sperm count per unit volume of caput epididymal suspension to 64.16 in comparison to the control. The concurrent treatment of papaya fruit extract and arsenic trioxide significantly decreased the abnormalities to19.6% in meiotic chromosome, 4.16% in the sperm head morphology and increased the mean count of sperm to 82.28. The increase in abnormality of meiotic chromosome as well as abnormality in gross morphology of the sperm head and decrease in sperm count, as observed upon arsenic trioxide treatment, were ameliorated by the aqueous papaya fruit extract. Therefore, it is suggested that the papaya fruit extract may reduce the risk of arsenic-induced genotoxicity.

INTRODUCTION

Human beings are much concerned about the direct effect of pollutants on their own health. Polluted water is a major cause of human disease and death. Arsenic is a major water pollutant and causes a disease called arsenicosis. High levels of arsenic in drinking water has been reported in many countries of the world. Arsenic exposure causes both chronic and acute toxicity and has been reported to be human carcinogen (Brown *et al*., 1989; Tsuda *et al*., 1995). Arsenic generates the reactive oxygen species and free radicals like hydrogen peroxide (Chen *et al*., 1998) or superoxide (Lynn *et al*., 2000). Increased free radicals favour oxidative stress which give rise to non-communicable diseases such as cardiovascular diseases, diabetes, cataract and cancer.

Plant and plant products are being used as a source of medicine since long. It has been estimated that 80% of the world population still use traditional medicine for their primary health care needs (Farnsworth, 1988). It has been well established that a plant based diet with high intake of fruits and vegetables and other nutrients rich plant food may reduce the risk of oxidative stress related diseases. Number of vegetables and plant parts has been reported to reduce the toxic effects of heavy metals. Aloe vera has got limited protective value against arsenic induced oxidative stress (Gupta and Flora, 2005a). The whole plant of *Centella asiatica* has been shown beneficial in improving alternations in arsenic induced oxidative stress besides it is also beneficial in depleting tissue arsenic concentration in limited way from the arsenic exposed animals (Saxena and Flora, 2006; Gupta and Flora, 2006a). Aqueous extract of amla have been found to show protective role against cytotoxic effect of lead and aluminium salt (Dhir *et al*., 1990) and arsenic (Biswas *et al*., 1999). Recently, coadministration of *Hippophae rhamnoides* with arsenic is more beneficial in reducing arsenic induced tissue oxidative stress than post arsenic exposure treatment (Gupta and Flora, 2005b, 2006b).Other important plant product as aqueous neem leaf extract (Kumari and Chourasia, 2007) and aqueous amla fruit extract (Bibha, 2009) have been reported to have beneficial effect on urea due to high content of antioxidant substance present. *Moringa oleifera* (particularly the seed) is another plant product which has recently reported to exhibit significant protection in arsenic burden from exposed animals (Gupta *et al*., 2005).

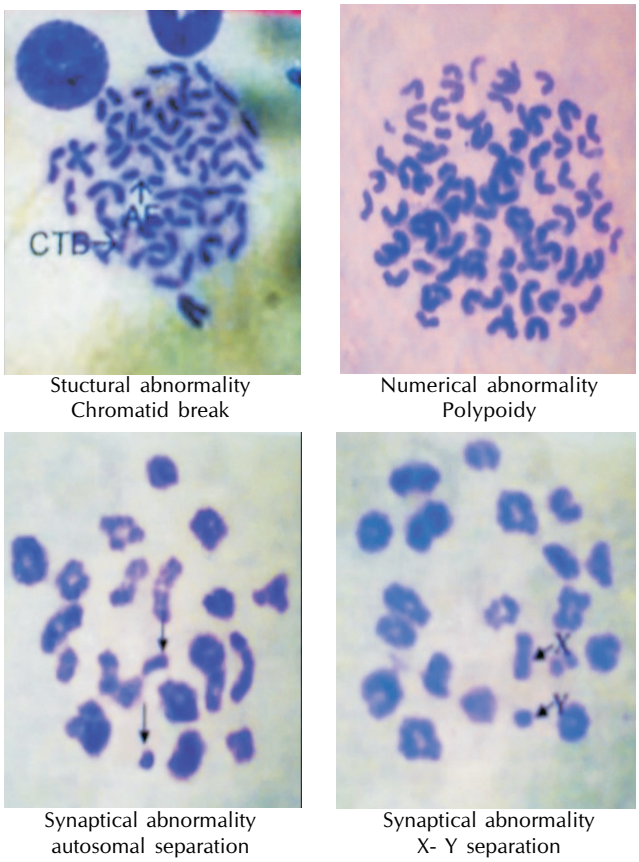
Among the fruits, papaya is one of the easily available, cheaper, grown extensively all over the region and have medicinal properties, use to treat several diseases and symptoms. Papaya fruit is one of the rich source of vitamin C and flavonoids like β-carotene, lutin, zeaxanthin and cryptoxanthins. These compounds are known to have antioxidant properties that play role in ageing and various disease process. Papaya can potentially lower the risk of many diseases, including heart diseases and colon cancer. Vitamins like ascorbic acid (Bose and Sinha, 1991; Sinha and Bose, 1992), tocopherol (Sinha and Prasad, 1989) and retinol (Sinha and Kumari, 1994; Kumari and Sinha, 1994) have been found to produce desired amelioration in counteracting mycotoxicosis in laboratory mammals because of their antioxidant nature. It has been also reported that ascorbic acid might play a therapeutic role against general arsenic toxicity (Singh and Rana, 2007; Sahu *et al*., 2006). Recently, papaya fruit could ameliorate arsenic induced genotoxicity in mitotic chromosome (Singh and

Kumari, 2012) of mice. Therefore, the present work was undertaken to ameliorate the arsenic contaminated drinking water induced genotoxicity in meiotically dividing primary spermatocyte, sperm head morphology and sperm count of mice by aqueous papaya fruit extract.

MATERIALS AND METHODS

Four to five week old male albino swiss mice (*Mus musculus*), 25gm, 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 standard laboratory condition ($25 \pm 5°C$, $RH = 50 \pm 10\%$). The animals were fed on food grains (maize and wheat) and tap water ad libitum. All animal treatments and protocols employed in this study received prior approval of the Institutional Head and Departmental Research Committee.

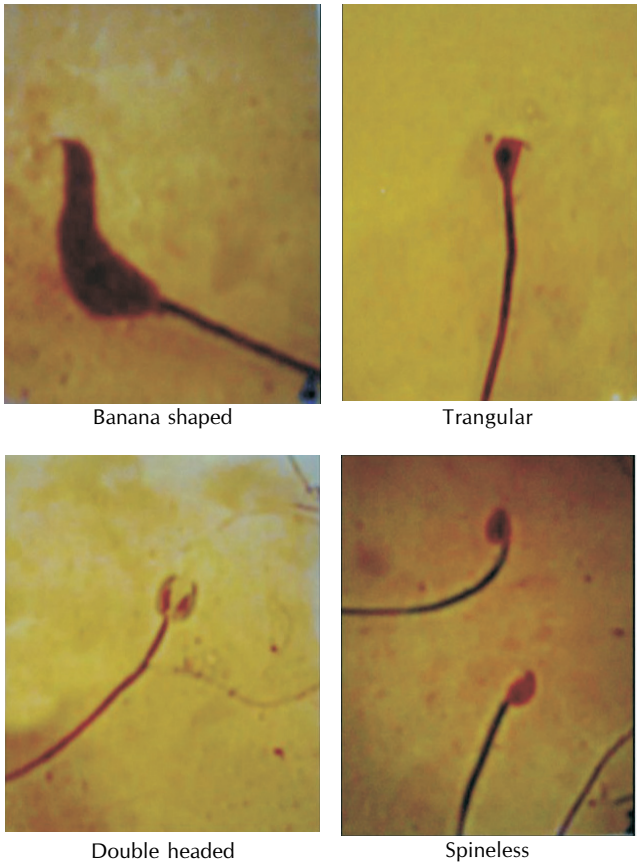
Male Albino Swiss mice were put into four groups and subjected to various treatments as shown in (Table 1). Arsenic trioxide was used as a water pollutant and ripe papaya (*Carica papaya*) fruit extract (aqueous) was used as an ameliorating agent. The animals in each group were subjected to treatment for 15 days. The summary of the experimental variant and treatment protocol are given in Table 1. Predetermined sub-lethal dose of arsenic trioxide (1.5 mg/kgb.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 papaya fruit extract (aqueous) was therapeutic at which dose genotoxicity of arsenic trioxide can be reduced.



Stuctural abnormality Chromatid break | Numerical abnormality Polypoidy

Synaptical abnormality autosomal separation | Synaptical abnormality X- Y separation

Plate 1: Abnormalities in meiotic metaphase chromosome of mice



Banana shaped | Trangular

Double headed | Spineless

Plate 2 : Various types of sperm head abnormalities

Meiotic chromosomes

The animals were sacrificed after treatment. The slides of meiotic chromosomes were prepared by following the technique suggested by (Das and Nayak, 1988). The chromosomal complex cell in Diplotene/Diakinesis/ metaphase-1 was screened for the structural, numerical and synaptical chromosomal abnormalities in each group (PLATE–I). Two repeats of experiments were performed. 300 well-spread metaphase plates from each group of animals were screened by random selection.

Sperm head morphology

The incidence of abnormalities in the gross morphology of sperm head was detected by using the methodology suggested by (Wyrobek *et al.*, 1984).Staining was done with 1% aqueous eosin-Y for 1h. Spermatozoa selected randomly for each

Table 1: Summary of the experimental protocol

Sl. No.	Experimental Variant	Symbol	Dose
1.	Control	C	No AT or P
2.	Arsenic Trioxide only	AT	0.04 mg/animal
3.	Papaya fruit extract only	P	100 mg/animal
4.	Arsenic Trioxide and Papaya fruit extract concurrently	AT + P	As 2 and 3

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

Experimental Variant	Structural abnormalities No. % ± S.E.		Numerical abnormalities No. % ± S.E.		Synaptical % abnormalities No. ± S.E.		Grand total abnormalities No.% ± S.E.	
Control	3	1 ± 0.15	7	2.33 ± 0.87	7	2.33 ± 0.87	17	5.6 ± 1.76
Papaya	2	0.66 ± 0.47	4	1.33 ± 0.66	5	1.66 ± 0.73	11	3.6 ± 1.07
Arsenic Trioxide	20	6.6 ± 1.43[ab]	41	13.6 ± 1.97[ab]	31	10.3 ± 1.75[ab]	92	30.6 ± 2.66[ab]
Arsenic Trioxide + Papaya	11	3.6 ± 1.07[ab]	29	9.6 ± 1.7[ab]	19	6.3 ± 1.40[ab]	59	19.6 ± 2.29[abc]

Table 3: Frequency of abnormal sperm head morphology in mice treated with arsenic trioxide and papaya

Exp. group	Total abnormal sperms	% ± S.E.
Control(6000)	190	3.16 ± 0.23
Papaya (6005)	140	2.33 ± 0.19[ab]
Arsenic Trioxide (6015)	348	5.88 ± 0.3[ac]
Arsenic Trioxide + Papaya (6015)	250	4.16 ± 0.26[abc]

Table 4: Mean sperm count of suspension in sample collected from caput epididymis of mice fed with arsenic trioxide and papaya

Experimental group	Mean ± S.E
Control	108.4 ± 1.25
Papaya	111.4 ± 1.04[b]
Arsenic Trioxide	64.16 ± 1.29[ac]
Arsenic Trioxide + Papaya	82.28 ± 0.86[abc]

mouse were examined microscopically (300-400) for the presence of any gross deviation from the normal head-morphology (PLATE–II).

Sperm count

The sperms were collected from the caput epididymis following the technique suggested by (Wyrobek *et al.*, 1984). The countings were made with the help of a haemocytometer in an RBC-counting chamber (Rastogi and Levin, 1987). The average of five different readings in each animal was used to calculate the final mean count in each group.

Difference in various assay parameters between pair of experimental groups were evaluated by the student's t-test, statistically significant at 5%.

RESULTS

Meiotic chromosomes

All the three types of abnormalities *i.e.* structural, numerical and synaptical chromosomal abnormalities were observed in each group. Arsenic trioxide supplementation increased the total incidence of abnormalities to 30.6% compared to 5.6% in control (Table 2). The percentage of abnormalities was 3.6% when papaya fruit was fed alone. However, when papaya fruit extract fed with arsenic trioxide (AT + P) significantly decreased the abnormality incidence to 19.6%.Thus, papaya was effective in reducing the abnormalities in meiotically dividing spermatocytes in mice.

a, b, c indicate significant difference at 5% with corresponding value in the control, papaya and arsenic trioxide variants, respectively.

Sperm - head morphology

The frequency of spermatozoa with abnormal head morphology was 3.16% in the control group of animals (Table 3), as high as 5.88% among the arsenic trioxide treated animals. A decrease in the frequency of abnormal sperm was noted, 2.33% on treatment with papaya fruit. Among the animals treated concurrently with arsenic trioxide and papaya (AT + P), the incidence of abnormal sperm was 4.16%, which was significantly less than arsenic trioxide treated mice.

a, b, c indicate significant difference at 5% with corresponding value in the control, papaya and arsenic trioxide variants respectively.

Sperm count

The mean count of sperm/ml in suspension was 108.4 in the control group. Arsenic trioxide significantly decreased this mean to 64.16 which is much less than the control. In the animals to which only papaya fruit was given, the mean count was 111.4. When the arsenic trioxide and papaya were fed concurrently (AT + P), the mean count was 82.28, which is a significant increase over the corresponding value in the arsenic trioxide fed animals.

a, b, c indicate significant difference at 5% with corresponding control, papaya and arsenic trioxide variants respectively.

DISCUSSION

The results show that arsenic trioxide is potent enough to damage meiotic chromosomes, as well as to induce changes in the gross morphology of the sperm, even the sperm count decreased. The present findings also show that concurrent treatment of papaya fruit extract and arsenic trioxide minimized the frequencies of these abnormalities as observed in case of mitotic chromosomal abnormalities (Singh and Kumari, 2012). The exact molecular mechanism of these genotoxic effects of arsenic is not known. However, it is believed that arsenic generates free radicals and these free radicals are suggested to damage macromolecules in cells. Trivalent arsenic toxicity could be carried out either directly by attacking sulfhydral groups or indirectly through generation of reactive oxygen species (Chen *et al.*, 1998). At the molecular level these radicals produce breaks in one or both strands of DNA molecules. Arsenic perturbes cells in numerous ways *i.e.* inducing chromosomal abberation, altering DNA repair or DNA methylation patterns and producing oxidative stress (Hamadeh *et al.*, 2002; Liu and Jan, 2000; Mass *et al.*, 2001).

The exact mechanism of papaya fruit extract is not well known. However the fruit extract could show ameliorating effect due to presence of vitamins that have antioxidant property. Antioxidants are substance when present in small quantities prevent the oxidation of cellular organelles by minimizing the damaging effect of reactive oxygen species or oxidative stress. These antioxidants can scavenge the chain propogating free radicals like peroxyl radicals and converting the reactive free

radicals to inactive products.

Many reports indicate that there is an inverse relationship between the dietary intake of antioxidant rich foods and incidence of human diseases (Sies, 1993; Halliwell, 1997). Papaya fruit, is one of the fruit with highest content of Vitamin C. Vitamin C is a potent antioxidant (Sato *et al*., 1990) which produces its antimutagenic effect both at extracellular and intracellular level (Ramel *et al*., 1986) through multiple inhibitory mechanism (De Flora and Ramel, 1988). Therefore, on the basis of these results it is suggested that human foods need to be supplemented with vitamin rich fruit to combat the arsenic contaminated water induced genotoxicity.

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