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Comet Assay Application to Study the DNA Damage and Ameliorative Effect of Psidium guajava on Arsenic Induced Genotoxicity through Ground Water in Mus musculus

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Abstract
The study was performed to investigate the ameliorative effect of guava fruit extract against arsenic trioxide induced genotoxicity or DNA damage. Arsenic has been the subject of toxicological research, thepresent study aims to study DNA damage caused by arsenic trioxide (3mg/kg.b.wt/day/animal)and its amelioration through guava fruit extract (23.5g/kg.b.wt/day/animal) in Swiss albino mice in in vivo condition using alkaline single cell gel electrophoresis (SCGE) / Comet assay. Result showed a significant increase in both tail length and olive tail moment in AT treated groupincomparision to control and guava treated group When tail length and OTM observed in the group fed concurrently with arsenic trioxide and guava fruit extract were 17.10 ± 2.6 and 3.78 ± 1.2 respectively. AT+G treated mice significantly decreased the AT induced increased tail length and OTM.

Key word:*Single cell gel electrophoresis; Genotoxicity; Swiss albino mice; Arsenic trioxide; Guava; Olive tail moment; Tail length.*

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I. Introduction

Arsenic became a major cause of water bodies pollution now-a-days.The effect of arsenic as a heavy metal on organism is currently attracting widespread attention particularly in studies related to the pollution. The atmospheric deposition of heavy metal have dramatically altered the biological cycle [1].It basically results from natural sources like volcanic eruptions, weathering of rocks and anthropogenic sources. These activities are significantly increased in the past few decades as a result of burning of fossil fuels, industrial activities, automotive emissions, use of metal-enriched materials, mining, farm manures, wastewater irrigation, sewage sludge, pesticide usage, industrial and domestic wastes and many other factors. They can neither be created or destroyed nor can one heavy metal transformed into another ie they are immutable. Arsenic, being an environmental toxicant, leads to development of serious health hazards affecting human being via mainly drinking water, food, industrial processes and other sources. Drinking water is the primary and main route of exposure to arsenic. World Health Organisation (WHO) and US Environment Protection Agency (USEPA) has given a standard ie 10µg/l for arsenic in drinking water called as maximum contamination level (MCL). Arsenic is absorbed into the blood stream at the cellular level and is taken up by red blood cells, white blood cells, and other cells that reduce arsenate to arsenite[2,3]Arsenic affects nearly all organ system and also shows other health effect.The principal mechanism of genetic damage induced by arsenic is via oxidative mechanism. Inorganic i-As compounds are responsible for altered gene expression which is caused by the induction of oxidative stress [4,5]. Micronucleus and Comet Assay, are sensitive biomarkers of early biological effects of iAsexposure[6]. The most widely used method for assessment of DNA damage is the alkaline comet assay. The alkaline comet assay (pH>13) is a rapid and sensitive procedure for quantitating DNA lesions in mammalian cells [7]. It detects not only strand breaks but also alkalilabile sites, DNA crosslinking, and incomplete excision repair sites [8]. The *invivo* alkaline comet assay enables the detection of *in vivo* DNA damage in all targets of interest. The comet assay is now widely used in environmentalbiomonitoring and in assessments of genotoxicity[9].

The technology for arsenic removal for piped water supply is moderately costly and require technical expertise .Thus,it is difficult to afford arsenic free drinking water for low socio-economic status of large population of affected area. So the other option comes from nature *i.e.* natural antioxidant, which is mostly found in fruits and vegetables.The defensive effect of natural antioxidant in fruits and vegetables are related to

the three major groups i.e Vitamins, especially Vitamin C, Phenolics and carotenoids (β-Carotene)[10,11]. Antioxidants scavenge free radicals from the body, cells and prevent or reduce the damage caused by oxidation.Various antioxidant have been shown to some protective role against arsenic toxicity[12,13].Guava, as in many other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases.Therefore, the present work was undertaken to study the ameliorating effect of another vitamin C antioxidant rich fruit, guava (*Psidiumguajava*) on arsenic contaminated drinking water induced genotoxicity which would be consider a new data by a natural remedy.

II. Material And Method

Four to five week albino swissmice of both sexes obtained from an inbred laboratory stock were used as testanimal. They were fed upon grains,seeds,foods,pelletsetc. All animal treatment and protocols employed in their study received prior approval of the Institutional Ethical Committee and met the standardlaid down by the Govt.Of India.

*2.1:Treatment:*Mice are separated to four groups and subject to treatment for 15 continuous days. Arsenic trioxide used as water pollutant and guava fruit extract used as an ameliorating agent.
2.2:Slide preparation: Slides were prepared by smears of bone marrow with slight modification. The comet assay was carried out under alkaline condition basically described by Singh *et al*.,1988[14] with slight modification described by Buschini et al., 2015[15]. Briefly,0.8%LMPagarose was prepared in saline water and maintained at 39^0C to prevent solidification. Aftertreatment,thecellsweretrypsinizedand washedwithPBSand finally 1×10^5 cells weregentlymixedwith 250 µl of0.8%LMPagarose.Theresultingsuspensionwas layered on to thefrostedsideof full frostedslides.Theslideswereplaced on iceforapproximately5minute toallowtheagaroseto solidify.Subsequently,theywereimmersedinthelysissolutionfor 1hour to eliminateallnonnuclearcomponents.Theslideswerethenfurther immersed inalkalinebuffer(pH13)for20minute toallowDNAunwindingandconversionofalkalilabilesitestosingle-strand breaks.Next,electrophoresiswasconductedfor30minute at15V and 200mA,i.e.,attherateof0.6V/cm,usingacompactpower supply.Theslideswerethengentlywashedwith0.4MTris(pH 7.5)toremovethealkalianddetergents.Alltheslideswereplaced in ahumidchamberuntilstainingtopreventthegelfromdrying.
*2.3.Slide staining:*The cellswerestainedwithPropidium Iodide (40µg/ml).
*2.4: Slide screening:*Slides were observedundera fluorescence microscope(NikonEclipseTE2000S)andtheimages werecapturedusingadigitalcamera.About60–80 imagesper slide persamplewerecapturedfromdifferentimaging fields and analyzed.Theolivetailmoment(OTM)andtaillength(TL) parameters wereanalyzedusingCASPsoftware.

Table 1: Summary of the experimental group and treatment protocol

Experimental Group	*Symbol*	*Dose*
Control	C	No Dose
Arsenic Trioxide	AT	3mg/kg.b.wt/day
Guava	G	23.5g/kg.b.wt/day
Arsenic Trioxide and Guava	AT + G	As per above

III. Result And Discussion

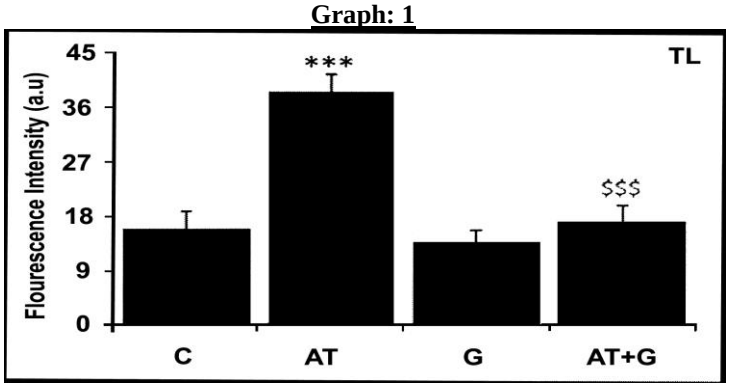
DNA damage was assessed by two comet parameters: tail length and olive tail moment. There is significant increase in both tail length and olive tail moment in AT treated group in comparision to control and guava treated group (table 2, graph 1 and 2). When tail length and OTM observed in the group fed concurrently with arsenic trioxide and guava fruit extract were 17.10 ± 2.6 and 3.78 ± 1.2 respectively. AT+G treated mice significantly decreased the AT induced increased tail length and OTM. Thus, guava fruit extract showed ameliorating effect against arsenic trioxide induced genotoxicity.

Table 2: Mean and standard error mean of comet tail moment and olive tail moment of cells from bone marrow of mice treated with Arsenic trioxide and Guava fruit extract.

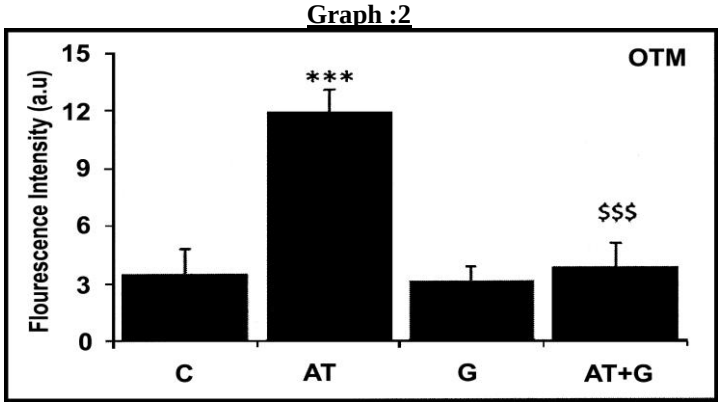
Treated Group	***TL***	***OTM***
	Mean±SEM	***Mean±SEM***
C	15.85 ± 2.8	3.50 ± 1.2
AT	38.73 ± 2.7[a,b]	11.86 ± 1.18[a,b]
G	13.78 ± 1.9	3.08 ± 0.7
AT+G	17.10 ± 2.6	3.78 ± 1.2

Notes: Values are expressed as mean±SEM and were analyzed by using one-way analysis of variance (ANOVA) for multiple comparisons. Newman-Keuls and Dunnets tests were used to examine the differences between samples. (a=p<0.001 Control vs. AT; b=p<0.001 AT vs AT+G).

Graph: 1



Graph :2



Graphical representation (1 &2) showing the significant increase in DNA damage after AT treatment. Co-treatment with guava attenuated the AT-induced DNA damage at low concentrations, data are expressed as mean ± Standard error meananalyzed by ANOVA post hoc Dunnetts and Newman–Keuls multiple comparison test. ***p<0.001, control *vs* AT treated. $$$p<0.001, control vs. AT+G.

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