

AMELIORATING EFFECT OF AMLA ON DRINKING WATER FLUORIDE INDUCED GENOTOXICITY

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Abstract : Young weaning Swiss albino mice (*Mus musculus*) were orally administered by sodium fluoride (2 ppm/animal) daily 15 consecutive days, increased the frequency of abnormality 91 ((30.99±7.04) compare to 30 (10.0±30) of control in meiotic chromosome. The abnormality was 21 (7±0.217) in group fed only with amla fruit extract. However, when amla fruit extract and sodium fluoride fed concurrently the abnormality was 57 (19.0±2.26) respectively. The result showed the sodium fluoride is potent enough to damage meiotic chromosome and present finding also showed that concurrent treatment of amla fruit extract and sodium chromosomal abnormalities. Therefore, it is suggested that the amla fruit extract may reduce the risk of sodium fluoride induced genotoxicity.

Key Words : Amelioration, sodium fluoride, genotoxic, Amla, Meiotic chromosome, oxidative stress.

INTRODUCTION

One of the basic necessities of our life is water which is getting polluted day by day causes for pollution of water bodies may be due to anthropogenic or natural reasons. Many studies are related to water pollution, the effect of heavy metals is attracting widespread attention. Heavy metals are naturally found in earth's crust and is one of the causes of water pollution, exposure to heavy metals is a common phenomenon due to their environmental pervasiveness metal intoxication particularly neurotoxicity, genotoxicity or carcinogenicity is widely known. The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of reactive oxygen and nitrogen species. Despite many years of research, we are still far away from effective treatment against toxicity caused due to exposure to heavy metal. Supplementation of antioxidant rich fruit in diet proves to be a better treatment regimen. Epidemiological studies have indicated that frequent consumption of natural antioxidant is associated with a lower risk of various diseases and cancers. Antioxidants stabilize free radicals by completing the deficiency of electron possessed by free radicals that can cause oxidative stress. There are two types of antioxidants i.e. exogenous and endogenous. Exogenous outside the body entering through food or supplements. Fruits and vegetables are exogenous antioxidant rich diet. Among fruit Amla (*Phyllanthus emblica*) is one of the most effective edible fruit and has long used as a traditional medicine. It contains rich amount of vitamin C, Carotenoids and phenolic compounds as antioxidants.

Thus such type of cheap and antioxidant rich fruits in diet are beneficial for lower socio economic population to prevent the various disease caused by heavy metal exposure.

MATERIAL AND METHOD

Four to five weeks 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 orally with sodium fluoride 2 ppm/animal/daily. All animal treatment and protocols employed in this study received prior approval of the Institutional Head and Department 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. Sodium fluoride used as a water pollutants and ripe amla extract (aqueous) was used as an ameliorating agent. Predetermined sub lethal dose of sodium fluoride (2ppm/kg.b.wt/day) was selected. Such treatment of sodium fluoride have been given because at this dose mice can survive the dose of amla fruit extract was 40 mg/animal in proportion to human recommended daily allowances (RDA) at which genotoxicity of sodium fluoride can be reduced.

Slide preparation

The animals of each group were sacrificed by cervical dislocation after the completion of treatments. The slides of meiotic chromosome were prepared by following the technique suggested by Das and Nayak (1988) i.e. Colchicine – Hypotonic – Acetoalcohol – 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

Sr. No.	Experimental Variant	Symbol	Dose
01	Control	C	No (SF or A)
02	Sodium fluoride	SF	2 ppm (Kumari, 2011)
03	Amla	A	150 mg/kg (Ali et.al., 2013)
04	Sodium fluoride and Amla Concurrently	SF + A	As 2 & 3

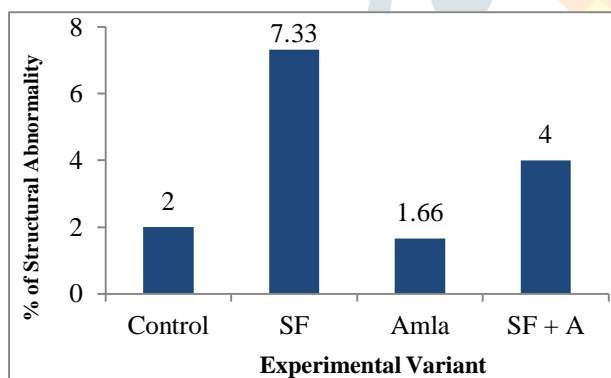
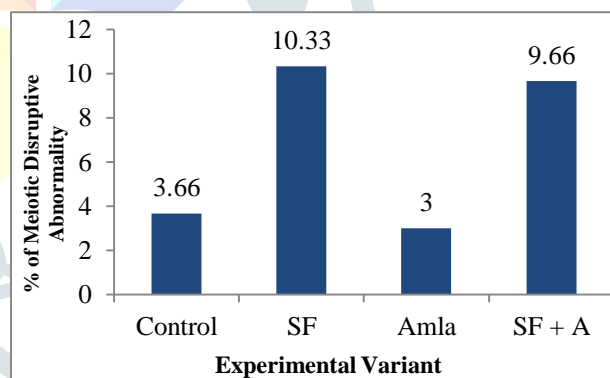
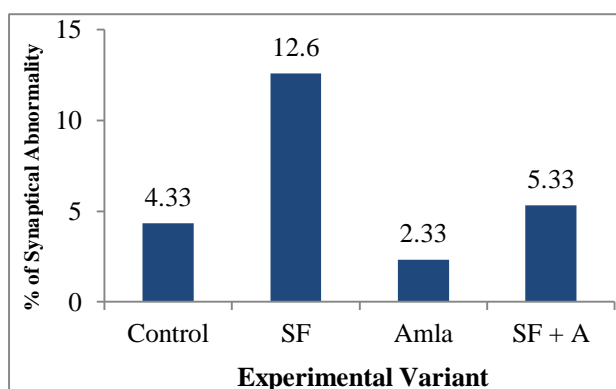
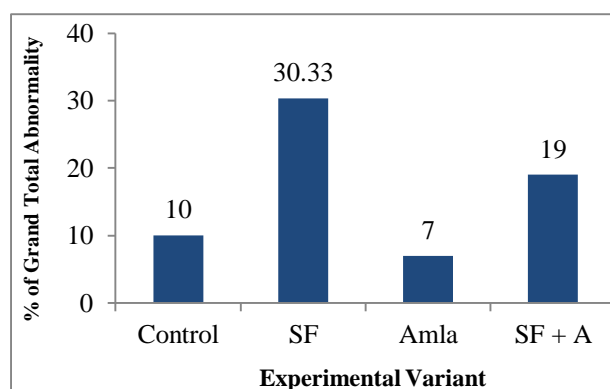
RESULT AND DISCUSSION

Structural, Numerical and Synaptical types of chromosomal abnormalities were observed in each group of mice. Among 4 groups of mice the percentage of abnormality was 30.33% in sodium fluoride supplementation. Similar percentage of abnormality was observed in both the control (10.0%) and amla (7.00%) fed animals. However, amla fruit extract fed with sodium fluoride (SF+A) significantly decrease the abnormality incidence to 2.0% from control group. Thus the result showed the genotoxic effect of sodium fluoride in meiotic cells like bone marrow cell (Saleha et.al., 2001; Singh and Kumari, 2012). The present finding also showed that concurrent treatment of amla and sodium fluoride had ameliorating properly. 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 sodium fluoride occur via production of superoxide and hydrogen peroxide radicals, 1999). Amla fruit is a rich source of vitamin C and its act as an antioxidant, Scavenges the aqueous reactive. Oxygen species by very rapid electron transfer and inhibit lipid, per oxidation (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). This free radicals are neutralized by an antioxidant defense system. Antioxidants act through different mechanism and different compartments but are mainly free radicals (Scavengers). Therefore, from the above findings it is suggested that such type of antioxidant rich fruit in diet and beneficial for reducing the toxic effect of sodium fluoride contaminated drinking water.

Table 2 : Incidence of chromosomal abnormalities in meiotic cell of mice treated with sodium fluoride and amla fruit extract (15 days).

Exp variant	Str. Abnormality		Meiotic Disruptive Abnormality		Synaptical Abnormality		Grand Total Abnormality	
	No.	% ± S.E.	No.	% ± S.E.	No.	% ± S.E.	No.	% ± S.E.
C	6	2.0 ± 0.653	11	3.66 ± 1.175	13	4.33 ± 1.380	30	10.0 ± 3.0
SF	22	7.33 ± 2.29	31	10.33 ± 1.73 ^a	38	12.6 ± 1.915 ^z	91	30.33 ± 7.04 ^a
A	5	1.66 ± 1.25	9	3.00 ± 0.97 ^b	7	2.33 ± 0.86 ^b	21	7.0 ± 0.217 ^b
SF + A	12	4.0 ± 1.87	29	9.66 ± 1.70	16	5.33 ± 2.11 ^{bc}	57	19.0 ± 2.26 ^c

a, b, c indicates the significant difference with corresponding value in sodium fluoride variant respectively at 5% significant level.

**Fig. (a)****Fig. (b)****Fig. (c)****Fig. (d)****Fig. (a, b, c, d) : Graphical representation of structural meiotic disruptive, synaptical and total abnormality in four different group of mice.**

CONCLUSION

Almost similar results were found in case of meiotic chromosomal abnormalities in SF treated animal and amla fruit extract 15 days could minimize the sodium fluoride induced genotoxicity.

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