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Modulating effect of *Phyllanthus* fruit extract against lead genotoxicity in germ cells of mice

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Abstract: *The objective of the present study was to evaluate the protective effect of Phyllanthus emblica against clastogenicity induced by lead nitrate on the incidence of sperm head abnormalities in the germ cells of mice. At higher concentration of lead, a significant increase in the percentage of sperm head abnormalities was noted but when animals primed with Phyllanthus fruit extract (PFE), a reduction in the frequency of sperm head abnormalities was observed. It can be suggested from the above study that Phyllanthus emblica plays a key role in inhibition of heavy metal mutagenesis in mammals.*

Key words: *Phyllanthus emblica, Lead nitrate, Antimutagenicity, Mice, Germ cells*

Introduction

The development of modern technology and industrialization are some of the factors responsible for boom in the environmental pollution in last two decades. Among heavy metals, lead (Pb), a major environmental pollutant is known for its mutagenic potential in different test systems including plants like *Allium sativum, Vallisneria spiralis* (Dhir *et al.*,1986), *Tradescantia* species (Dryanovska,1987) and animals like mice (Dhir *et al.*,1985 a, Devi and Kameshwari, 1988; Swamy *et al.*,1990 and 1993) and mosquito (Sharma *et al.*,1988). Dietary inhibitors of mutagenesis and carcinogenesis are of particular importance since they reduce the toxic effects of known carcinogens, mutagens and clastogens (De flora and Ramel, 1988; Hayatsu *et al.*, 1988; Sharma, 1990).

The fruits of *Phyllanthus emblica* (*Emblica officinalis*) Gaertn family Euphorbiaceae have been used extensively in medicine. It is highly regarded due to its magnificent vitamin C (L-ascorbic acid) content (Gopalan *et al.*, 1991). *Phyllanthus emblica* dried fruit extract has been observed to reduce cytotoxic effects of zinc chloride (Giri *et al.*, 1986), lead and aluminium salts (Dhir *et al.*, 1990 a,b) and Nick (Agarwal *et al.*,1989). The inhibition of cytotoxocity is because of vitamin C and anticlastogenic properties (Gebhardt *et al.*, 1985; Mirvish, 1981; Irene *et al.*, 1999). It has been shown to antagonise the toxic activity of metallic salts (Chakraborty *et al.*, 1977).

Experiments were designed to evaluate the protective effect of PFE on the genetic damage induced by lead nitrate in the germ cells of swiss albino male mice.

Materials and Methods

Eight to ten weeks old inbred swiss albino mice with an average body weight of 22-24 g were utilized. *Phyllanthus* fruit extract doses *viz.* 171.25, 342.25 and 685 mg/kg and lead nitrate dose *viz.* 40 mg/kg were selected.

Cameron and Pauling (1979) suggested that the daily intake of vitamin C is 1-10 g/day for human being. Data based on maximum ascorbate concentrations in human body suggest a maximum body pool of around 5000 mg, which is approximately 70mg/kg body weight in man. (Counsell and Horning, 1981). In the present study a corresponding amount of an aqueous extract of PFE containing the same amount of vitamin C was used for mice, as calculated from a daily 1 g intake for a 60 kg person. The fruits were procured in bulk, cut into pieces and dried in sunlight. Known quantities weighed and kept in distilled water for 24hr. The AA content of the decoction was estimated by the 2,6-dichlorophenol indophenol method (Pearson, 1952) and it amounted to 685mg/kg body weight.

In the first experiment the animals were administered lead nitrate orally for five days. Six animals were used for the dose. Parallel controls and mitomycin-C as positive control were maintained.

In the second experiment the three doses of PFE were selected on therapeutic basis. Each set was administered orally with one dose for 35 days. All the animals were sacrificed on 35th day after treatment. Epididymal sperms were collected in physiological saline and stained with 1% eosin following the method described by Wyrobek and Bruce (1975).

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For priming experiments, the animals were orally fed with 3 doses of PFE (171.25, 342.25 and 685 mg/kg) for 35 days, on every 7th day after priming with PFE 40 mg/kg lead nitrate was fed orally. All the animals were sacrificed on 35th day; slides were prepared according to the method described above.

The prepared slides were screened for the presence of various types of sperm head abnormalities such as amorphous, banana, hammer, hook like *etc.* A total of 2000 sperms per animal were scored for the incidence of sperm head abnormalities in controls and treated animals.

Results and Discussion

The results for the incidence of sperm head abnormalities in PFE treated animals are depicted in Table 1. The frequency of aberrant sperms after the administration of 171.25, 342.25 and 685 mg/kg PFE were 3.25%, 3.35% and 3.50% as against 3.15% in control animals and the difference was statistically insignificant ($p>0.05$).

The results on the incidence of abnormal cells in germ cells of mice administered with lead and Pb+PFE are depicted in Table 2. The percentage of sperm head abnormalities in controls was 3.60 and it increased to 10.9 in 40 mg/kg in Lead treated group ($p>0.01$). The frequency of sperm head abnormalities was 5.9, 5.3 and 3.3 after the treatment with 40 +71.25, 40 + 342.25 and 40 + 685 mg/kg Pb+PFE treated animals. This was significantly less ($p<0.05$) when compared with the lead treated group.

Table - 1: Frequency of sperm head abnormalities in mice treated with various doses of *Phyllanthus* fruit extract (PFE)

Treatment	Normal sperms (%)	Aberrant sperms (%)
Control	7744 (96.8)	256 (3.20)
171.25mg/kg	7736 (96.7)	264 (3.30)
342.5mg/kg	7724 (96.55)	276 (3.45)
685mg/kg	7712 (96.4)	288 (3.60)

The values in parenthesis are percentages
*p> 0.05

Sperm morphology assay is also said to provide a quantitative method for locating genetic damage in male germline cells. In our laboratory the drugs like Metepa, Pyrantel paomaote, Asthalin and Thiotepa have been tested for the incidence of sperm head abnormalities and published else where (Devi and Reddy,1983; Reddy and Devi,1990; Devi and Kameshwari,1997). In the present investigation mice were treated with doses of lead nitrate (40 mg/kg). The results clearly indicate a dose effect relationship. The results are comparable with those of Heddle and Bruce (1977) who tested lead nitrate intraperitonially. Such a phenomenon in the incidence of sperm abnormalities was reported with other chemicals also (Wyrobeck and Bruce, 1975; Devi and Reddy,1986). The results obtained in the present study indicate the lead induced cytogenetic damage in germ cells of mice.

The protective effects of *Phyllanthus* fruit extract (PFE) against heavy metal genotoxicity were not reported earlier and it is the first report in the mammalian system.

However, the results suggest that PFE did not induce sperm head abnormalities in mice and the results are comparable with that of Irene *et al.*,1999, who observed that AA and PFE showed inhibitory effects against the cytotoxicity induced by lead. Vitamin C is a well known antioxidant, able to suppress chemically induced transformation (Benedict *et al.*,1980). Earlier the antioxidant activity of PFE was reported elsewhere (Rao and Siddique,1964). Further the higher protective action of the PFE extract against known clastogen Caesium Chloride has been demonstrated (Gosh *et al.*,1992). The present work is of

Table - 2: Frequency of sperm head abnormalities in lead treated mice primed with *phyllanthus* fruit extract

Treatment	Primed with PFE							
	Non primed		171.75mg/kg		342.15mg/kg		685mg/kg	
	Normal sperms (%)	Aberrant sperms (%)	Normal sperms (%)	Aberrant sperms (%)	Normal sperms (%)	Aberrant sperms (%)	Normal sperms (%)	Aberrant sperms (%)
Control	7712 (96.4)	288 (3.60)						
MMC	7096 (88.7)	904 (11.3)						
40mg/kg	7128 (89.10)	872 (10.90)*	7528 (94.1)	472 (5.90)**	7576 (94.7)	424 (5.3)*	7736 (96.7)	264 (3.3)*

The values in parenthesis are percentages, *p > 0.01, **p < 0.05

importance as PFE can be used as natural dietary supplement to counteract the cytotoxic effects of exposure to lead compounds.

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