DISCUSSION
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Mutagenicity of drugs was established after years of intensive research. Mutagenicity screening has become an essential feature of study (Chauhan and Sundaran, 1985). The basis for mutagenicity tests with experimental objects derives from the fact that the genetic material is similar in all living organisms. Furthermore, the transmission of genetic information as well as its transcription basically follows the same mechanism in all eukaryotes.

Exposure of cells to mutagens produce chemical changes (damaged sites) in most macromolecules, including DNA, RNA and proteins; the damaged sites in DNA seem to be paramount in determining the mutagenic effect of the exposure. Once a damage has been induced in DNA, cells experience an acute response in which many biochemical reactions are disturbed. After this acute response, surviving cells recover by virtue of repair processes that eliminate the damage or render it innocuous. Among the survivors some cells show mutations, which are generated by errors that occurred in DNA replication during the recovery period (Witkin, 1968; Witkin and George, 1973).
Chromosomal aberration was the most reliable and recognized end-point, from the time of Muller who used it in the estimation of the effects of physical and chemical mutagens. Hence analysis of chromosome aberration is taken up as the end point in this study. Chromosome aberration analysis was used to overcome any false negative results (Rohrborn, 1982).

Plumbagin, a 2-methyl, 5-hydroxy-1,4-naphthoquinone is used in the present study to prove its clastogenic and teratogenic effect. The clastogenic effect of plumbagin has been studied with respect to both in vitro and in vivo test systems (Santhiya, 1983). It was found to induce acentric fragments in mouse and syrian hamster bone marrow cells. This finds correlation with the present study on the induction of chromosome aberrations in mouse.

Plumbagin was reported to induce aberration of the chromatid type like gaps, breaks, fragments and exchange figures. Plumbagin was also found to induce sister chromatid exchanges in mouse bone marrow and cultured human lymphocytes. Plumbagin at a concentration of 7.5 mg/kg body weight in mice resulted in a significant induction of sister chromatid exchanges amounting to two-folds over the
base line sister chromatid exchanges. In root meristem of *Allium cepa* the type of abnormalities observed were stickiness induced bridges and disintegration of nuclei. Plumbagin was found to have mild clastogenic effects in root meristem too (Santhiya, 1983).

A similar cytological phenomenon was reported by Nils Nyborn and Knutsson (1947) for methyl naphthoquinones at a specific concentration and exposure time. Santhakumari *et al* (1980) observed nuclear and cytoplasmic vacuolization at higher concentrations of plumbagin. It is interesting to record here the mutagenic potential of plumbagin in strain TA 2637 of *Salmonella typhimurium* (Tikkanen *et al*, 1983).

Plumbagin was considered to be a weak inducer of micronucleus which implies cytogenetic damage to bone marrow cells (Ramani, 1989) Plumbagin was found to induce micronuclei apparently at all the doses studied (i.e., 3.75 mg, 7.50 mg and 15.0 mg/kg body weight). Though there is an increase in relation to dose, it is not strictly dose dependent. For instance there is no appreciable increase in the frequency of micronucleated PCEs for an increase in dose from 7.5 mg to 15.0 mg/kg body weight.
Studies on toxicity of plumbagin revealed lethal effects were due to DNA damage rather than membrane damage. Though plumbagin is somewhat mutagenic, the nature of DNA damage produced by plumbagin is yet unclear. It does not cause single strand breaks to a very large extent. These results suggest that single strand breaks may not be a primary lesion caused by plumbagin induced repair. These reports suggest the mutagenicity and toxicity of plumbagin. In the present study also, plumbagin induced toxicity to proliferating bone marrow cells was observed.

The present study on the clastogenicity of plumbagin using chromosomal aberrations as an end point reveals the following observations:

i) There is a significant induction of chromosome aberration in plumbagin treated animals and it is dose dependent (Table 1).

ii) The most frequent type of aberrations observed are gaps and breaks. Isochromatid aberrations were less frequent. Ring and fragments were also observed (Plate I, II).
iii) There is a significant decrease in the number of aberrant metaphases with an increase in duration of exposure at all concentrations (table 2).

iv) There is a significant increase in the number of aberrant plates at increasing concentration of the chemical (table 2).

When cells are treated in S or G2 stage, a very high frequency of gaps are induced. Many investigators do not take gap in to consideration for quantitative comparisons, since it is considered as an artifact. The number and percentage of aberrant plates excluding gaps were also recorded (table 1) and summary of ANNOVA was represented in table 5.

The clastogenic effects were more marked in mouse bone marrow cells. Plumbagin induced aberrations showed a distinct dose and duration dependency. Induction of aberrant cells were observed maximally at 0.015 g/kg body weight (9.7%) and induction of aberrant cells were observed minimally at 0.00375 g/kg body weight (4.7%) (Fig. 2).
Plumbagin could inhibit cell division either by inhibiting DNA synthesis or by participating in oxidative phosphorylation. Quinones interact with the proteins and they inhibit enzymatic reactions in the oxidation of SH groups (Fieser and Fieser, 1956). Protein and sulphur metabolism is of utmost importance in cell division and growth and substances blocking SH groups have a marked effect upon the dividing cells (Kihlman, 1966). Quinone derivations were found to inhibit the synthesis of both DNA and RNA (Lin et al, 1973). Quinones are known to participate in oxidation - reduction reactions of mitochondria, chloroplasts and chromatophore fragments. Substances which inhibit oxidative phosphorylation are known to inhibit cell division (Kihlman, 1966).

The type of aberration observed in metaphase depends on the stage of the cell cycle in which the treatment was made and the type of mutagenic agent used. Based on this criterion, agents have been classified as S - dependent and S - independent agents. The aberrations induced by S - dependent agents are fixed immediately irrespective of the cell stage at which treatment is made and these aberrations are assumed to arise due to non-repair or misrepair of the lesions. In contrast, the
aberrations induced by S-independent agent are assumed to be formed due to misreplication (Evans and Scott, 1969; Kihlman, 1977). And hence to cover all the phases in the cell cycle different durations (6, 12, 24 and 48 hrs) have been employed in the study. The compound tested for mutagenicity must be studied with respect to early, mid and late phases of cell cycle.

Plumbagin showed significant induction of aberrant plates in foetal liver cells of mice treated during pregnancy. Plumbagin at higher concentrations induce severe cytotoxic effects leading to cell death (Santhakumari, 1980). Plumbagin at lower concentrations acts as spindle poison. There was mitotic arrest with an accumulation of cells in metaphase. Hence in this study, plumbagin was administered at 3.75 mg/kg body weight which is the lowest concentration studied for testing the clastogenic potential.

Time of drug administration dramatically affects the incidence of malformations. During the early phase of cell proliferation, malformations are not produced. At this time, the embryo may be killed or if it survives it develops normally. It is also difficult to produce
abnormalities very late in foetal development since the process of organogenesis has been completed. The incidence of malformations among surviving foetuses showed an extremely sharp peak when the drug was given between the seventh through nineteenth days (Harbison, 1980). Hence, in this context plumbagin was administered on 6, 7, 8 day of pregnancy. Plumbagin showed mild significant induction of congenital and skeletal malformations in treated embryos. It induced fusion of ribs and overlapping of ribs in 5 treated embryos. It has brought about reduction in the number and size of treated embryos.

Substances that cause defects of foetal development are called teratogens. Estimates are that from 1 - 5% of congenital defects in the human are drug or chemical related (Wilson, 1973). Since recognition of chemical induced teratogenesis in man, suitable laboratory animal models to determine prenatal safety of drugs have been sought. The teratogenic potential of drugs and chemicals has been primarily tested in mice. Mice like human have a placenta, so chemicals are exposed to maternal tissues and subjected to maternal metabolism before entering the embryo or foetus. Gestation in animal model is short, multiple births are normal so each treated female yields a large
amount of experimental data and housing and maintenance of large numbers of animals are practical because their small size and low cost.

Chandrasekaran and Nagarajan (1982) studied the metabolism of Plumbagin in rats. It could not be detected in blood up to 24 hr. This condition may be due to the fact that distribution of plumbagin in various tissues. Since the metabolism of Plumbagin is as yet unclear, it is difficult to find any correlation between the cytogenetic effects and the level of Plumbagin in blood. The major portion of Plumbagin being excreted at 24 h with traces at 48 h of urine sample.

Thus the present study confirms the earlier observations that Plumbagin is a clastogen. It is interesting to record here that from the view point of chromosomal aberration, plumbagin appears to be a clastogen at all doses and durations studied in mouse bone marrow cells. The effect of Plumbagin on mouse foetal liver cells treated during pregnancy shows the clastogenic potential. The foetotoxic effect of Plumbagin indicated that the transplacental effect has been observed and Plumbagin is found to have mild teratogenic effect.