SUMMARY AND CONCLUSIONS

1. A new group of chemicals belonging to amides and isobutylamides were used in this investigation. The primary aim was to evaluate their antimitotic and radiomimetic potential. This is the first study involving a study of the effects of amides on plant tissues.

2. Some compounds were synthetically prepared while others are of natural origin, having been isolated from the plant Piper peeploides. Some of the compounds used in this study are new.

3. The compounds were of diverse chemical structure. Some were open chained, some aromatic and a few heterocyclic. Such compounds alone were selected which had reactive groups present at different positions of the molecule.

4. Some of the compounds were insoluble in water. Therefore, a suspension was made in a few drops of alcohol and later on the required solutions were made in water.

5. pH of all the test solutions was maintained at 7, using citric acid buffer, as amides are weakly basic.

6. Young growing roots of Allium cepa were treated with various concentrations of the compounds for varying durations. Fixations were made either

immediately after the treatment was over or following different recovery intervals. Mitotic index and chromosome breakage was studied in all the fixations.

7(a). Pellitorine: Treatment last for 1-4 hrs. A sudden reduction in mitotic index was noted even after one hour's treatment with 0.005% solution, while 0.02% fully inhibited the cell division after 4 hrs. of treatment. A prophase to anaphase ratio showed that there is an appreciable amount of prophase inhibition and inhibition of the spindle mechanism which resulted in polyploid nuclei. Cytological studies revealed abnormalities like bridges with or without fragments and a considerable number of polyploid cells. The treated roots were allowed to recover for duration upto 120 hours. It was seen that the inhibition of frequency of division lessened as the period of recovery increased and the number of normal cells increased correspondingly.

In barley seeds treated with 0.05% of pellitorine for 8 hours germination was fully inhibited. Cytological abnormalities induced in barley root-tips included dicentric bridges and fragments. Pyknotic masses and sticky chromosomes etc. were also noted.

7(b). N-isobutyl capramide also showed mitotic suppression but it was only 20% as efficient as pellitorine. There was a reduction in the frequency of cells in post metaphase stage. This indicates that the chemical either effects the centromeric movements or

induces some spindle abnormalities. The effect was non-toxic and temporary. At lower concentration, no cytological abnormalities were seen and at higher concentration major abnormalities included chromosomal bridges with fragments and free fragments alone. The breakage points were generally at the centromeric region.

7(c). N-iscbutyl veratamide: The compound reduces mitotic index significantly and as the concentration of the treatment increases, the index falls. A positive prophase inhibition was also observed. The effect was not permanent and normal rate of cell division was achieved after 72 hours of recovery. The chromosomes presented a well condensed shape. The major abnormalities seen were chromatid bridges, fragments, binucleate cells and nuclear disintegration. Re-union index was high.

7(d). N-isobutyl cinnamide: Treatment with this compound had very little effect on the frequency of dividing cells. For short period treatments, no cytological damage was seen, but on prolonging the treatments, nucleo toxic effects like multipolar spindle, stickiness and a few micronuclei were observed.

7(e). Formamide and dimethyl formamide: Both of these compounds reduced the mitotic index in onion roots as well as barley seeds. While iM formamide for 2 hours reduced mitotic index from 5.20 to 0.94, only 0.1M dimethyl formamide reduced mitotic index from 5.31 to 1.63. This indicates that DMF is about ten times

more effective than formamide. Chromosomes were differentially stained. There was polyploidy and multipolar formation. Chromosome breakage and erosion were the major abnormalities. However, only a few exchanges were found. Germination as well as seedling height of barley seeds showed a significant reduction following a treatment with Dimethyl formamide in addition to chromosome aberrations.

7(f). Hexamine: This compound had no effect on the frequency of dividing cells. But 0.07% for 4 hours induced nearly 43% abnormal cells. A significant observation was the induction of chromosomal fragments as the main abnormality. There were no exchanges.

7(g). Nikathamide; There was an immediate cessation of cell division even after a treatment with 0.125% for 8 hours while a treatment of 0.5% proved completely lethal to the treated tissue. Nuclear abnormalities included endomitosis, somatic segregation and polyploidy.

7(h). PPA-amide: There was no antimitotic effect due to this compound, but showed radiomimetic property. However, the major abnormality was bridges with few or no fragments. That means most of the breaks rejoined to form bridges.

7(i). Acetamide, piperine and P.O.-amide: had no effect at all either as an antimitotic or radiomimetic agent.

8. Normal mitotic cycle time appears to

have been disturbed by the treatment with amides and isobutyl-amides. This was deduced from the lack of synchronization of division cycle of cells in division as compared to control. The general effect was a sort of prophase poisoning and spindle disturbances.

9. On cytological ground, these compounds induced both nucleotoxic, as well as cyto-toxic effects. Among nucleotoxic effects are stickiness, multipolar spindles and nuclear disintegration. Among cyto-toxic effects were true chromatid or chromosome breaks, dicentric bridge formation and fragments. However, among the breaks induced by Hexamine there was a complete lack of interaction while in the material treated with PPA-amide reuinion index was very high.

10. Amides are important biological molecules and there is evidence that they have an effective hydrogen bond breaking capacity.

11. The group of chemicals used here represented an array of structural analogues. Some of them were very active, some less active and a few devoid of any activity. From the relationship between structure and activity some deductions could be made regarding their mode of action. Most probably, these compounds act through:

i. alkylation of genetic material.

ii. breakage of -H bonds.

iii. due to enzymatic inhibition.

iv. by the introduction of cross links.

12. From the observations made, it can be concluded that activation of amide group by various electron donating substituents enhance the biological activity of the compound. It has also been shown that mere presence of active centres is not so important as the absolute geometry of the organic compound.

13. Most of these compounds including pellitorine, NIB-veratamide, NIB-capramide, nikathamide etc. induce considerable mitotic inhibition along with appreciable distortion and breakage of chromosomes, without killing the organism, it seems, therefore, logical to test the carcinogenetic potential of these compounds. At higher concentrations, they may also prove useful mutagens.

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