CYTOLOGICAL EFFECTS OF ARGEMONE OIL ON THE MITOTIC CELLS OF ALLIUM CEPA

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ABSTRACT

The cytological effects of Argemone oil, which is one of the adulterants of edible oils, on the mitotic cells of Allium cepa, have been investigated in detail following a treatment for one hour at different concentrations and also after recovery for 24, 48 and 72 hours. Large number of cells have been scored and aberrations such as chromosome erosions, breakages, fragmentations, gaps, ana- and telo-phase bridges, stickiness and groupings have been analysed. Among the two types of Argemone oil employed, the screw-pressed oil was more effective in inducing these changes than the solvent-extracted one. The recovery from the effects of treatment caused by the former was extremely slow compared to that of the latter. The implications of these results and the possible mutagenic action of the oil have been discussed. Representative photomicrographs are presented.

INTRODUCTION

Argemone mexicana Linn., which is a member of the family Papaveraceae, is a prickly annual with bright yellow flowers and capsules containing many seeds resembling black mustard. The seeds yield a nauseous, bitter and non-edible oil, which causes vomittings and purgings at higher doses. The oil is often mixed with mustard and other vegetable oils consumed by human beings (Bhatnagar et al., 1948).

It has been well established that epidemic dropsy is caused by the consumption of edible oils adulterated with the Argemone oil (Lal and Roy, 1937; Lal and Das Gupta, 1941; Lal, 1951; Patwardhan, 1952). Sarkar (1948) has demonstrated that the toxicity of the oil is due to the presence of an

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alkaloid, Sanguinarine, in it. Recent investigations have indicated that a fatty acid factor is essential to potentiate the toxic principle of Sanguinarine. These also revealed that among the two types of oils, viz., the screw-pressed and the solvent-extracted, the former which contained the above factor had produced the toxic manifestations in the experimental animals. These experiments further showed that while the oral administration of oil did not demonstrate any toxicity, intraperitoneal injections of the same to rats and mice produced the toxic symptoms (Ramasastri and Babu, 1969, 1970; Shenolikar, 1971; Rukmini, 1971). Svejda et al. (1969) described some of the effects of Sanguinarine on the L-cells in inducing few chromosome aberrations.

In their recent and intensive analysis of testing the carcinogenicity of edible oils, Ranadive et al. (1973) have proved the co-carcinogenic effects of Argemone oil in combination with samples of mustard oil from the market. Few of the vegetative and mineral oils have also been demonstrated to produce some chromosome aberrations in addition to inducing viable mutations (Swaminathan and Natarajan, 1956, 1959; Patel, 1959). Since the vegetable oils constituting part of our diet are subject to adulteration with Argemone oil and as there is a lack of knowledge on the cytological effects of the latter, the present investigations have been carried out.

**MATERIAL AND METHODS**

Germinating bulbs of *Allium cepa* with their roots were immersed for one hour at room temperature in screw-pressed and solvent-extracted samples of Argemone oil at concentrations of 100% and as 5% and 10% well-emulsified oils in distilled water. They were washed thoroughly in running water to free the roots of the traces of oil. While in one set the roots were fixed soon after washing, the others from the same bulbs belonging to the different series were grown in distilled water replaced at 12 hourly intervals for 24, 48 and 72 hours, to see whether the roots which had received an initial treatment with the oils would recover from their effects before fixation. They were fixed in acetic-alcohol and processed as haematoxylin squashes as described elsewhere (Subramanyam and Subramaniam, 1970). Some preparations were also made by the Feulgen squash method as detailed by Darlington and La Cour (1960).

**OBSERVATIONS**

The distribution pattern of the various cytological changes induced by the two types of oils during treatment and recovery are summarized in
Tables I and II. There was no over-contraction of the chromosomes. On the other hand, an inhibitory effect of the oil on the mitotic cycle was seen. The radiomimetic effects in the form of fragmentation and breakages were also observed. Representative photomicrographs are presented.

I. Experiments with Screw-pressed Oil

(a) One hour treatment.—A comparison of the mean mitotic index (MI) of experimental with that of the control shows that there is a considerable lowering of it. The percentages of abnormal cells increased with the concentration of the oil. The individual as well as mean percentages were significantly high (see Table I). Figure 1 is from a control metaphase. The induced chromosome fragments are indicated by arrows in Figs. 2 and 3 which are from squashes of roots treated with 10% and 100% Argemone oil respectively. While the bridge can be seen in the late anaphase in Fig. 4 exhibiting a tendency for stickiness, an extreme case of the latter is observable in Fig. 5. In some instances the oil seems to have caused an irreversible damage in the form of chromosome erosion (Fig. 6) which might even lead to a loss of its morphological integrity.

(b) Recovery experiments.—A perusal of Table I would reveal some fluctuations in the MI during the recovery period as compared with that of the control. When a comparison is made between the treatment and the recovery for 5% and 10% oils there is a corresponding fall in the MI. This may be due to an inhibitory effect on the cells from undergoing divisions and also due to the reversion of divisions at the time of treatment to interkinesis. In the case of 100% oil the MI is comparatively constant and higher at corresponding times which seems to be due to the poor penetrability of the oil and the application of quick recovery treatment before it could wield its influence. The increase in MI from 24 to 48 hours and a decline after 48 hours for any concentration may be due to the slow and delayed effects of the lingering active principle on the surface of the roots being carried to the sites of division during recovery and its waning effect later.

The percentages of aberrations show an increase for any concentration up to 48 hours and then a decline of the same for 5% and 10% oils. It virtually maintains the same values after that period with 100% oil (Table I). These are obvious from Fig. 19 wherein the total percentages of abnormalities for the different concentrations are plotted against the treatment and recovery periods. These would be discussed later.
Fig. 19

Screw-pressed oil

Total abnormalities (%)

1 Hr. Treatment

Hours of recovery

100%

10%

5%

Fig. 20

Solvent-extracted oil

Total abnormalities (%)

1 Hr. Treatment

Hours of recovery

100%

10%

5%

Fig. 21

- Screw pressed
- Solvent extracted

Figs 19-20. Chromosomal abnormalities during treatment and recovery.

Fig. 21. Differential induction of abnormalities by the two types of Argemone oil.
The illustrations of chromosomes seen after treatment with screw-pressed oil followed by recovery in distilled water for various periods include stages from mild to extreme cases of fragmentation at metaphase (Figs. 7 and 8) and anaphase (Fig. 9) to a number of chromosome fragments lagging at anaphase (Fig. 10), grouping of restituting nuclear material at telophase (Fig. 11) and a dicentric anaphase bridge (Fig. 12).

II. Experiments with Solvent-extracted Oil

(a) One hour treatment.—In contrast to the controls there is a drop in the mean MI after one hour treatment (see Table II). The total percentage of abnormal cells increases with the concentration of oil with the mean, however, remaining at 6.24 which is significantly high. Chromosomes possessing a tendency for stickiness and bridge formation are illustrated in Figs. 13 and 14.

(b) Recovery experiments.—The recovery of roots during the 72 hour period seems to be of a higher order and faster than that with the screw-pressed oil. This is true for the values of the mean MI of both series for the corresponding recovery periods (see Tables I and II). However, there is a decline in the MI with the solvent-extracted oil during the recovery. While the treatment for one hour had produced a higher percentage of abnormalities, the recovery of the treated roots had showed a tendency for considerable decrease from 24 to 72 hours (Table II). Figure 20 indicates the percentage of total abnormalities plotted against the treatment and recovery periods for the various concentrations. Whereas the trend of decline follows the same pattern for 5% and 10% oils, the same for 100% is gradual up to 48 hours. A comparison of the mean percentage of abnormalities produced by the screw-pressed and solvent-extracted oils reveals a higher percentage by the former for the period of treatment or the corresponding periods of recovery (Tables I and II). Figure 21 also demonstrates this feature. A binucleate cell produced after 24 hour recovery is seen in Fig. 15. The arrows A and B in the anaphase (Fig. 16) represent a lagging chromosome fragment and break respectively, seen after recovery for 48 hours. Occasionally chromosomal groupings could also be observed (Figs. 17 and 18, arrows).

DISCUSSION

The role of Argemone oil in causing epidemic dropsy by its adulteration with other edible oils and in possessing the co-carcinogenic activity with mustard oil has been fully demonstrated (Lal and Roy, 1937; Lal and Das Gupta, 1941; Lal, 1951; Patwardhan, 1952; Ramasastri and Babu, 1969, 1970;
Argemone Oil on Mitotic Cells of Allium cepa

Shenolikar, 1971; Rukmini, 1971; Ranadive et al. 1973). The need for a study of its cytological effects was, therefore, felt inevitable. The results reported in this paper and summarized in the two tables fully substantiate its action in causing chromosome aberrations which are highly significant.

A careful perusal of Table I shows that a higher percentage (mean as well as individual) of abnormalities are produced not only after treatment but even during the various periods of recovery at all concentrations of screw-pressed oil. This is particularly interesting when the analysis is extended to those produced by the solvent-extracted oil (compare Tables I and II; Figs. 19, 20 and 21). The drastic action of screw-pressed oil is also evidenced by the production of chromosomal erosions which might lead to the loss of their structural integrity. In this context it is relevant to recall the observations of Rukmini (1971) who had extensively studied the effect of Argemone oil in animals and pointed out that the screw-pressed oil was more effective in causing the toxic symptoms than the solvent-extracted one. The cytological data reported here agree with the above contention.

Table II indicates the reduction in the aberrations with the passage of time. This is directly related to the higher MI during the recovery although there is a reduction for the respective periods. A comparison of the figures with those of screw-pressed oil (Tables I and II) shows that while the MI is correspondingly higher in solvent-extracted oil, the same for the former is low. The recovery from the phenomenally high damage produced by the screw-pressed oil is extremely slow while that from the solvent-extracted oil is relatively quicker. Further the lower toxic nature of the solvent-extracted oil is evident from the reduction in the abnormalities which is gradual from 24 to 72 hour recovery. This differential response may possibly be due to the presence of the additional potentiating factor activating the Sanguinarine in the screw-pressed oil and its absence in the solvent-extracted one (Rukmini, 1971).

In quoting the experiments of Rossi (1955) with the alkaloid, berberine sulphate, Sharma and Sharma (1960) observed the manifestation of the radiomimetic nature which was distinct after a certain period of recovery in water due to the removal of the toxic effect. The increase in the percentage of aberrations upto 48 hour recovery in water following treatment with the screw-pressed oil reported here (see Table I and Fig. 19) may be explained on this basis. However the reduction seen after 48 hour recovery (see Fig. 21) may be either due to the waning effect of the toxic principle or due to the reversion from divisions at the time of treatment to interkinesis during recovery.
### Table I

**Argemone oil (screw-pressed) induced chromosomal aberrations in A. cepa**

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<th>Concentration of oil (%)</th>
<th>Period of recovery (Hrs)</th>
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<th>Cells in division</th>
<th>Mitotic Index (MI)</th>
<th>Mean (MI)</th>
<th>Breakages</th>
<th>Fragments</th>
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**Table II**

*Argemone oil (solvent-extracted) induced chromosomal aberrations in A. cepa*

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<th>Concentration of oil (%)</th>
<th>Period of recovery (Hrs.)</th>
<th>Total No. of cells</th>
<th>Cells in division</th>
<th>Mitotic index (MI)</th>
<th>Mean MI</th>
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* Others include lagging chromosomes and binucleate cells.
and this accounts for the fall in the frequency of the division figures and hence in the cells with aberrations (see also Venkateswarlu and Srinivasan, 1960).

The mutagenic action of many vegetable oils has been thoroughly investigated by Swaminathan and Natarajan (1956, 1959). The results enumerated above indicate that Argemone oil could perhaps be a mutagenic agent especially in view of its chromosome breaking property. Since its co-carcinogenic activity has also been described and as some carcinogens are also mutagens, there is a need for systematic investigation of this property of the oil. The present observations also point out the possible hazardous nature of the oil on the genetic material as it is used as an adulterant of the vegetable oils often consumed by the human beings.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. O. S. Reddi, Head, Department of Genetics, Osmania University, Hyderabad, for his keen interest and encouragement and to Dr. I. S. Shenolikar of the National Institute of Nutrition, Hyderabad, for the supply of Argemone oil. One of them (P. V. R.) is indebted to Sri. G. S. Chary, Principal, Government College, Jagtial, A.P., for his encouragement.

REFERENCES


Figs. 1—18
Argemone Oil on Mitotic Cells of Allium cepa


EXPLANATION OF PLATE III

Figs. 1-18.—Chromosome aberrations in root tip cells of A. cepa following one hour treatment with Argemone oil. Haematoxylin squashes except Fig. 8, a Feulgen squash.

Fig. 1. Control.
Fig. 2. Control.
Fig. 3. Screw-pressed oil.
Fig. 4. Solvent-extracted oil.

(All from 100% oil except Figs. 2 and 8 from 10% oil series.)
Magnifications: Fig. 6, × ca. 650; Figs. 1-3, 8-10, 12, 17 and 18, × ca. 750; Figs. 4, 5, 7, 11, 13-16, × ca. 950.

Fig. 1. Metaphase.
Fig. 2 and 3. Chromosome breakage, Metaphase.
Fig. 4 and 5. Stickiness and bridge formation.
FIG. 6. Chromosome erosion.
7. Chromosome fragments (24 hr. recovery).

FIG. 8. Extreme fragmentation (28 hr. recovery).

FIG. 9. Anaphase fragments (24 hr. recovery).

FIG. 10. Anaphase. Lagging chromosome fragments (24 hr. recovery).

FIG. 11. Telophase chromosome grouping (72 hr. recovery).

FIG. 12. Anaphase bridge (72 hr. recovery).


FIG. 15. Binucleate cell (24 hr. recovery).

FIG. 16. Lagging chromosome fragment and breakage (48 hr. recovery).

FIGS. 17 and 18. Chromosome groupings (72 hr. recovery).