COMPARATIVE STUDY ON THE EFFECT OF SOME DRUGS ON PLANT CELLS

A THESIS

submitted for

Ph. D.

In Botany (Cytology)

By

Zakia Ahmed Abo-El-Khier
(B. Sc., M. Sc.)

Botany Department
University College for Girls
Ain Shams University

1983

ACKNOWLEDGEMENT

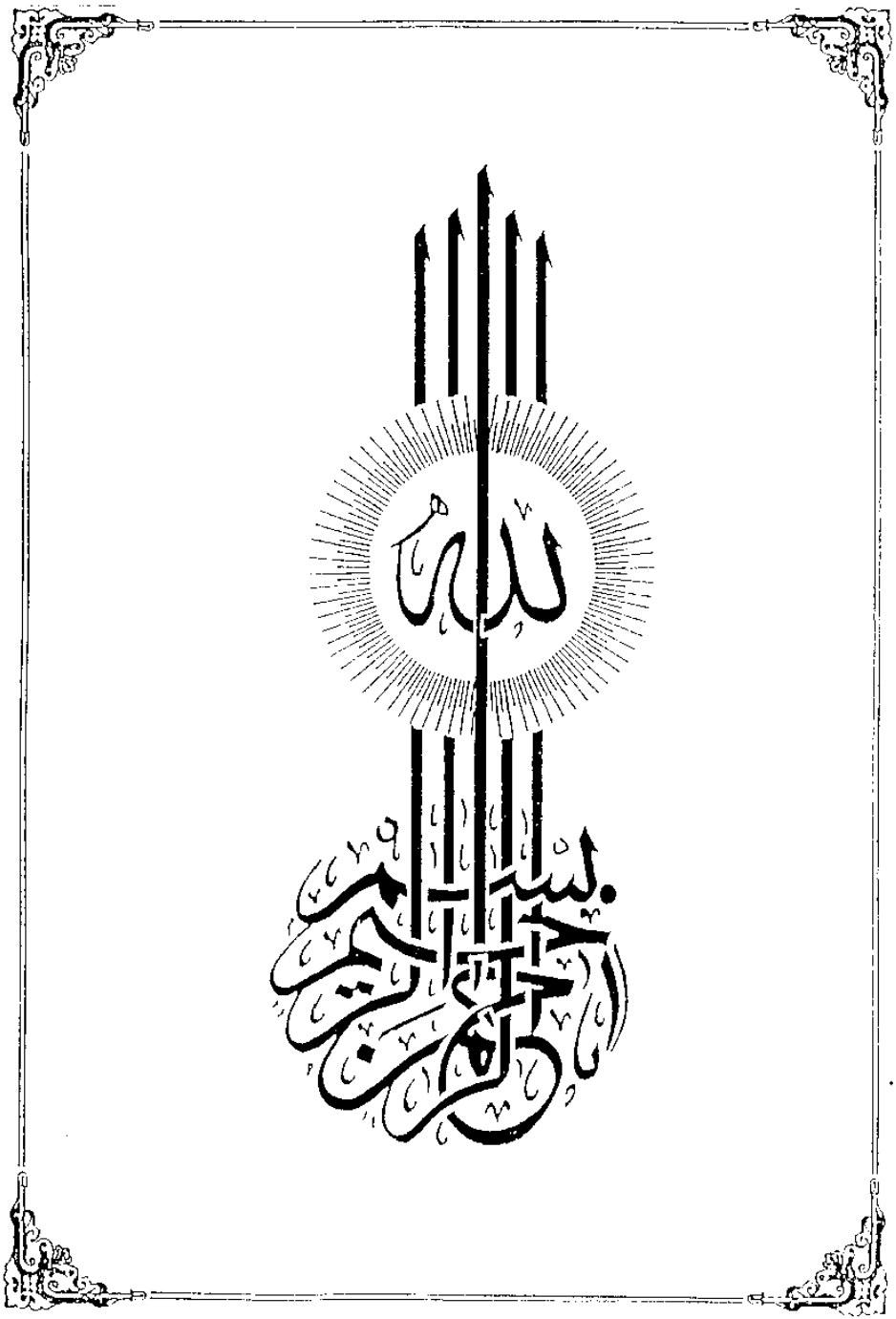
The author wishes to express her gratitude to Professor M. A. Hammouda, Head of the Department of Botany, for his sincere help and guidance.

My deep gratitude is due to Dr. Amal Shehab, Assistant Professor of Botany, for suggesting the subject, continuous advice and kind help during the preparation of the manuscript.

My deep gratitude is expressed to Dr. Zakia Adam, Lecturer of Botany, for her advice and help during the course of this study.

I am deeply indebeted and grateful to my husband for his encouragement and sincere help during the course of this study; to him, I dedicate this manuscript.

My grateful acknowledgement is expressed to the staff-members of the Botany Department for their interest and sincere help.



CONTENTS

INTRODUCTION

Introduction

It has long been known that drugs, besides their medicinal effect, may exhibit untoward harmful side effects. These side effects differ from moderate symptoms as headache, nausea, vomiting, skin rashes to severe ones which may lead to toxicity. Alterations of the process of mitosis or meiosis and chromosomal aberrations are of the prominent side effects which may result from administration of some drugs. These effects would, thus, be more critical during pregnancy since they would lead to a number of mutations.

It was reported that antibiotics may lead to the production of a number of chromosomal aberrations in human subjects and in animals (Obe, 1970, Mana, 1978 and Cilievici et al., 1978). Similarly, a number of antituberculosis drugs caused the appearance of chromosomal aberrations (Obe et al., 1973, Roman and Georgian, 1977).

Studies on animal cells in tissue culture have shown that, as a rule, there appears to be a good correlation between the chromosome breaking activities of chemicals in plant and animal cells. Some chemicals such as hempa, however, are practically inactive in plant cells but are mutagenic in some animal cells. Other agents such as caffeine and FUdR, having mutagenic effect in plant and animal systems but affect the cells at different stages of their cycle or through different mechanisms (Kihlman, 1971).

Drugs as morphine sulphate (Kabarity et al., 1974) and different types of oral contraceptives (Kabarity and Khodari, 1967) were found to induce disturbance in cell division. The effect varied from inhibition of cell division to the production of different types of mitotic abnormalities.

The effect of sulfadiazine, sulfaphenazole and sulfadimidine on plant cells has been studied by El-Shiekh (1980). The study indicated that these drugs induced a mitodepressive effect and this effect increased with increasing drug concentration and duration of treatment. Such effect was attributed to inhibition of DNA synthesis.

Tranquilizing agents were subject to many cytological investigations both in human being as well as in animals. It is recognized clinically that tranquilizers of the phenothiazine group are capable of causing a wide variety of undesirable side effects. A number of workers have investigated their effects on microorganisms and other cell systems. Guttman and Friedman (1963) showed that motility of the ciliates was reduced by each of a series of substituted phenothiazines which varied in effectiveness.

Other workers have studied the effect of chlorpromazine on frog gastrocnemius muscle, isolated mitochondria and human erythrocytes (reviewed by Freeman and Spirtes, 1963).

Rogers (1966) demonstrated that phenothiazine derivatives can influence lipid and nucleic acid levels in tetrahymena, and further suggested that these drugs may interfere directly with

the mechanism of glucose transport into cell.

Cohen et al. (1969) indicated that cytotoxicity to chlorpromazine was evident at concentrations of 10 mg/ml and 1 mg/ml since no mitosis was observed at these concentrations. Drug concentrations of 100, 10 and 1 µg/ml, although yielded dividing cells, exhibited significant antimitotic effect which was linearly related to length of exposure to the drug. At the high concentrations of the drug, non-viable cells with complete "pulverization" of chromatin reflected the cytotoxic effect of the drug.

Adams (1975) illustrated that promazine HCl and chlorpromazine HCl reduced the growth of barely plants by as much as 50%. Polson and Adams (1978) found a growth reduction of 30-40% in barely plants grown in two phenothiazine tranquilizers (prochlorperazine edisylate and trifluperazine HCl). They observed also similar reduction in the number of mid-anaphase cells which may indicate that the reduction of growth of the plants could be the result of a reduced mitotic rate. In addition, the same authors observed several types of chromosomal aberrations.

The effect of tranquilizing agents on the plant cells has been studied also by Barakat (1978). The study showed that valium and phenobarbitone sodium have a great effect on the normal sequence and behaviour of chromosomes during mitotic stages. The drugs induced a wide range of mitotic abnormalities and the frequency of the latter depended on drug concentration and duration of treatment.

The present study was undertaken to determine possible cytological effects of four drugs - representing two groups of drugs having different pharmacological effects, viz., antihistaminic and antipsychotic drugs - on *Vicia faba* and *Allium cepa*.

The antihistaminic drugs are the drugs that block the effects of histamine competitively at various receptor sites. The conditions in which the antihistaminics are helpful include allergic rhinitis, urticaria, some types of asthma and motion sickness. Conditions in which antihistaminics are either not the drugs of choice or should not be used include acute anaphylactic emergencies, most cases of asthma, diseases of the skin, eyes and nose and the common cold.

From the antihistaminic drugs, two examples were selected for the present study. These are promethazine hydrochloride and chlorpheniramine maleate. These two drugs are considered potent antihistaminics. It is to be stated that promethazine HCl, besides being a potent antihistaminic, has also antipsychotic effect. It is a member of the phenothiazine group of antipsychotic drugs.

The antipsychotic drugs are the newer terms for major tranquilizers (Goth , 1974). The pharmacological effects of the phenothiazine tranquilizers are quite complex. These drugs are potent antiemetics and have important actions on the autonomic nervous system at various levels. In large doses they also produce significant toxic side effects such as parkinsonism.

From the antipsychotic drugs, promazine hydrochloride and trifluperazine hydrochloride were selected for this study. These

two drugs have the phenothiazine structure and are considered potent antipsychotic agents.

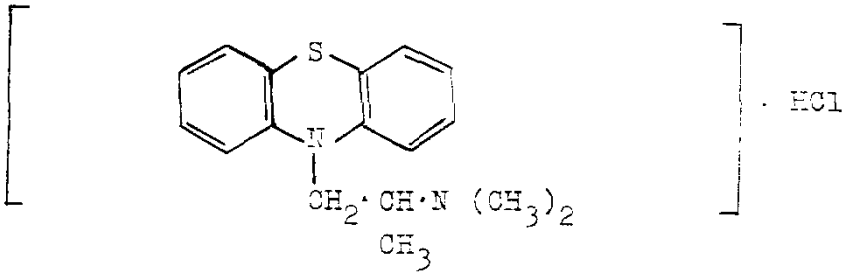
The aim of the present study was to determine the effects of promethazine HCl, chlorpheniramine maleate, promazine HCl and trifluperazine HCl on both meristematic and germ cells (mitosis and meiosis), using different drug concentrations and different times of treatment. The cytological parameters considered in this study were the mitotic index, total percentage of abnormalities, types of abnormalities in addition to pollen viability and pollen length. The plants utilized for these investigations were Vicia faba as a representative plant for the dicotyledons and Allium cepa as a representative one for the monocotyledons.

MATERIALS AND METHODS

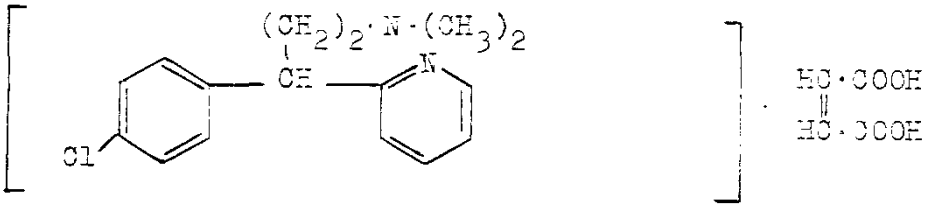
Materials and Methods

In the present study, *Vicia faba* (var. Giza 1) and *Allium cepa* (var. Giza 6) were utilized to investigate possible cytological effects of four drugs, namely:

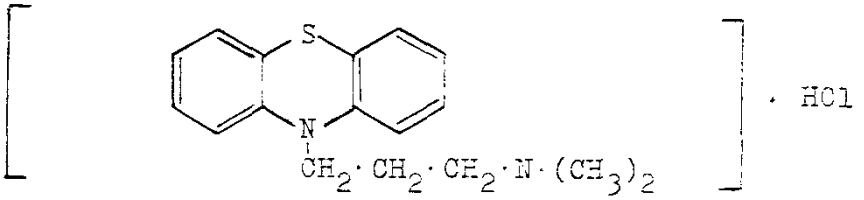
1. Promethazine HCl



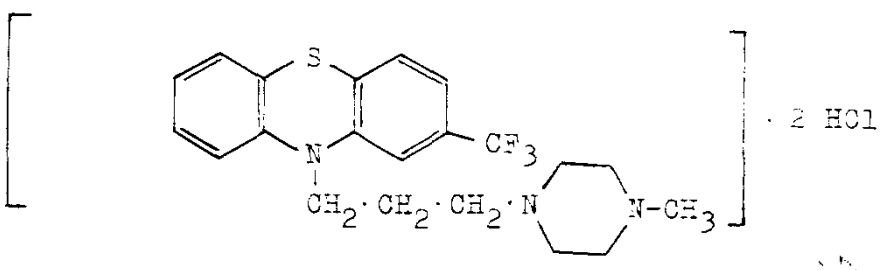
2. Chlorpheniramine maleate



3. Promazine HCl



4. Trifluperazine HCl



The above-mentioned compounds were used in concentrations of 10, 20 and 40 p.p.m. for the mitotic studies and 20 and 40 p.p.m. for the meiotic studies. Fresh aqueous solutions were prepared and kept in dark-coloured bottles since the phenothiazine drugs are sensitive to light.

Cytological studies

I. Mitotic study

Seeds of Vicia faba were soaked in tap water for 24 hr., then sown in saw dust till the roots nearly reached 1.5 - 2 cm. in length. The root tips were immersed in aqueous solutions of the used drugs at concentrations of 10, 20 and 40 p.p.m. for 12, 24 and 48 hr.

In case of Allium cepa, the bulbs of approximately equal size were placed over jars containing tap water for rooting. Water was changed daily to obtain suitable aeration. When the roots attained a length of 2 - 3 cm., they were transferred to the drug solutions of the same concentrations used for the Vicia faba studies and for the same time intervals. Time intervals more than 48 hr. (72 hr.) were found to be unsuitable, since the roots of Vicia faba and Allium cepa soaked in solutions of promethazine HCl, promazine HCl and trifluperazine HCl were found to loose their turgidity and the roots soaked in chlorpheniramine maleate showed great inhibition in the mitotic index.

The roots were then washed thoroughly, cut and fixed in a freshly prepared aceto-alcohol (1:3) fixative for 24 hr. After fixation, the treated material was washed twice and stored in

70% ethyl alcohol in the refrigerator until time of smearing. The roots were hydrolyzed in 1N HCl at 58 - 60°C for 8 min., then washed thoroughly in distilled water and stained for one hour in leuco basic fuchsin and squashed in 45% acetic acid.

For making permanent preparations, the preparations were inverted with their cover-slips downwards in a petri-dish containing 40% ethyl alcohol till the cover-slip falls away the slides. The slides and the cover-slips were then transferred in the following solutions five minutes each:
Absolute alcohol (series of 40, 60, 80 & 95%), absolute alcohol and xylol (9:1, 1:1 & 1:9), and pure xylol, then they were mounted in canada balsam.

The slides prepared as above were examined for both mitotic index and abnormalities.

Number of dividing cells from 10 different root tips for each treatment 10000 - 20000 cells were counted and examined.

II. Meiotic study

Seeds of Vicia faba were soaked in water for 24 hr. before cultivation in soil. On reaching a suitable size (20-30) days after planting, the flower buds were treated with the used drugs. The treatments were done at the morning 7-9 o'clock. Pieces of cotton soaked with the drug solutions (20 or 40 p.p.m.) were placed on the shoot tips having the flower buds and removed after 3 hr. The specimens (treated flower buds) were collected 24 and 48 hr. after treatment at random from 20 plants for each treatment.

In the case of Allium cepa, bulbs of nearly equal size were grown in the soil. At 80-90 days old, flower buds were treated by the drug solutions by the same manner adopted for Vicia faba.

Flower buds which were gathered at random from the drug-treated plants and the control were fixed separately. Fixation of buds in acetic - alcohol 1:3 solutions was followed by aceto-carmine squash preparations.

Photographs were made and scoring was done from the temporary preparations.

Maclintock method was used (Venning 1958) for meiotic cell studies. The method involves the following steps:

1. The stamens were squashed out, each one on a slide in a drop of 45% iron aceto-carmine solution.
2. The contents of the anther were smeared by pressing the slide with its cover gently between filter paper folds.
3. The slides were heated gently for few seconds over an alcohol flame (not boiling), and the cover edges were sealed with paraffin wax to keep it unspoiled for few days.

To make permanent preparations, the slides were passed through the following steps:

1. The slides were inverted on glass triangle in a petri-dish filled with 10% solution of acetic acid.
2. After the glass covers had fallen away the slides, both slides and covers were separately passed through the following solutions:

a. Equal parts of alcohol and acetic acid.
b. Acetic acid : absolute alcohol (1:9).