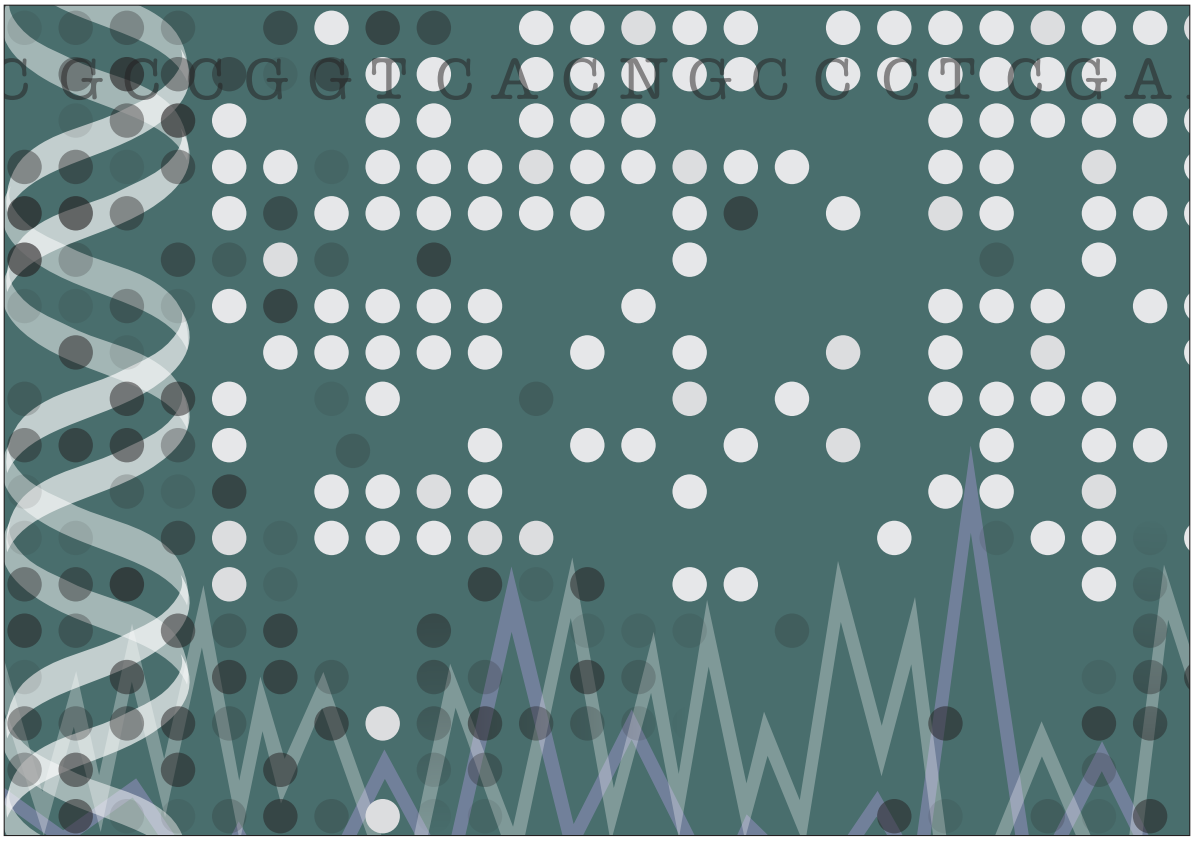

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Research Article
Evaluation of the Mutagenic Potential of Glucocorticoids by *Allium cepa*

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Abstract

Background and Objective: Corticosteroids are drugs used in the therapy of different types of diseases, such as respiratory, inflammatory, allergic, neoplastic and auto-immune diseases. The wide use of these drugs causes certain concerns related to their harmful effects on health. This work has the objective to evaluate the mutagenic potential of three widely used glucocorticoids (Prednisolone, Dexamethasone and Hydrocortisone) in *Allium cepa* meristematic root cells. **Materials and Methods:** The genotoxic potential of the corticosteroid drugs were evaluated using the dosages following the minimum, usual and maximum dosage standards of each of them, the doses being of 100, 1,000 and 2,500 mg for Hydrocortisone, doses of 0.75, 7.5 and 15 mg for Dexamethasone and doses of 5, 20 and 60 mg for Prednisolone, performed by the *Allium cepa* test. For statistical analysis, the ANOVA one-way test was used, with a significance level $\alpha = 0.05$, using the statistical package, GrafPad Prism 5.0. **Results:** The results showed that all doses of the glucocorticoids analyzed induced a significant increase in the index of chromosomal aberrations in a dose-dependent manner, indicating genotoxic potential. **Conclusion:** According to the results, the corticosteroid called "betamethasone" presented genotoxicity and dose-dependent cytotoxicity.

Key words: Cytotoxicity, mutagenicity, glucocorticoids, drugs, genotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Glucocorticoids are steroidal hormones, produced in the cortex of the adrenal gland, which alter carbohydrate metabolism and reduce the inflammatory response[1]. The therapeutic use of steroids began in the early 1930s, even before they observed that the symptoms of arthritis in women were alleviated when they became pregnant, a period in which adrenocortical hypersecretion occurs. They were introduced to the medical practice in 1949 for the treatment of rheumatoid arthritis. However, the side effects were felt in prolonged treatments, in which sodium retention with edema formation predominated[2].

The basic molecular structure of corticosteroids is cyclopentanoperhydrophenanthrene (Fig. 1), a cholesterol derivative consisting of 3 hexane rings and 1 pentane ring. All natural and synthetic steroidal corticosteroids are variations of this basic structure and require an 11-hydroxyl group and are the positions of this group in the structure that differentiate the potency, half-life, metabolism and effects of the drug[4].

The corticosteroids are among the drugs most prescribed for inflammatory medicine, they are often used to treat a variety of diseases such as auto-immune, allergic, respiratory disease or neoplastic diseases and the clinical response to these hormones vary[5-8]. Their anti-inflammatory capacity has not been understood for a long time. They have their own mechanism of action, their main characteristic is that they cross the cell membrane and bind to cytosolic receptors interacting directly with deoxyribonucleic acid (DNA) in the nucleus, activating and inhibiting the transcription of several genes[9].

In the need to obtain synthesized drugs similar to cortisol and with potent anti-inflammatory action, as well as fewer side effects, several compounds were produced and analyzed, among the most used are Prednisolone, Dexamethasone and Hydrocortisone. In addition, drugs with such varied actions end up causing a large number of side effects, particularly intense in the growth stages, directly interfering with growth factors of paracrine and autocrine action in the growth cartilage itself, as is the case with the growth factor (Insulin-like growth (IGF-1)). All caution in the indication and the basic norms of use of these compounds was presented, which may allow the physician greater safety regarding the prescription of a corticosteroid[9].

Most glucocorticoids were purchased by prescription, but prescription is not a mandatory prerequisite for acquiring this group of drugs, which facilitates indiscriminate and sometimes undue use by patients[10]. Already reported in the literature, medications such as Hydrocortisone, Dexamethasone and Prednisolone, can cause the patient some undesirable effects when used in a chronic way and without advice due to/or a lack of a medical or pharmaceutical professional[11].

Some drugs can cause genotoxic effects in the body, which were closely related to carcinogenesis and were therefore capable of causing DNA modifications and can cause

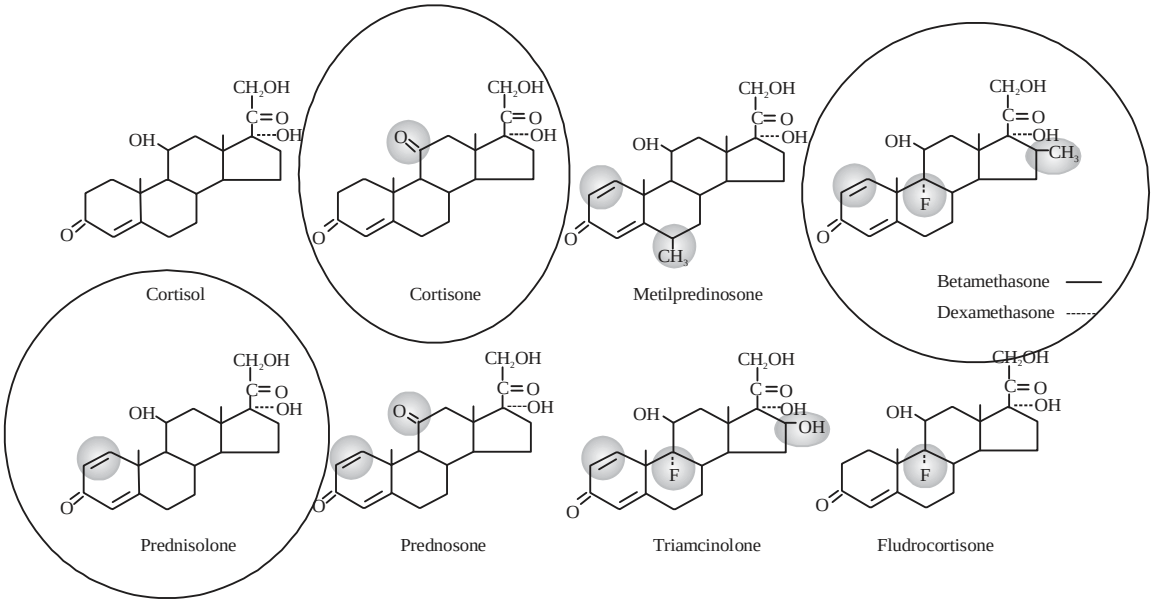

Fig. 1: Molecular structure of some corticosteroids. The red circles are the three drugs used in this study
Reference: Vasconcelos[3]

great damage to cells. The *Allium cepa* test is an inexpensive and reliable test used to evaluate genotoxicity through chromosomal aberrations and toxicity through root growth and the mitotic index of different drugs[12,13]. The research and evaluation on the genotoxic and mutagenic potential is important, since many people use these drugs inappropriately and without follow-up and/or medical prescription. In addition, the literature demonstrates studies that already describe genotoxic and toxic effects with glucocorticoids[14,15]. These studies can predict the ideal dosage and the patient profile can be used in this group of medicines without significant health damage.

Based on these theoretical assumptions, this study has the objective to evaluate the mutagenic potential of three widely used glucocorticoids (Prednisolone, Dexamethasone and Hydrocortisone) in *Allium cepa* meristematic root cells.

MATERIAL AND METHODS

The study was conducted at the Laboratory of Biosciences from the Alfredo Nasser Institute of Health Sciences College (Aparecida de Goiânia, Goiás, Brazil) between February and April, 2017.

The drugs tested in this study were from the following commercial active principle: *Hydrocortisone* (Description: white to almost white crystalline powder, odorless, Internal lot: 16H03-B024-009042, Manufacturer's lot: FS03-1604031W, Origin: China, Date of manufacture: 04/18/2016, Expiration date: 04/18/2018, CAS number: 50-23-7), *Dexamethasone* (Description: white to almost white crystalline powder, odorless, Internal lot: 16H25-B009-009472, Manufacturer's lot: 6TU-8-160601, Origin: China, Date of manufacture: 06/07/2016, Expiration date: 06/06/2019, CAS number: 50-02-2) and *Prednisolone* (Description: white to almost white crystalline powder, odorless, Internal lot: 16H23-B018-010531; Manufacturer's lot: PL-160601, Origin: China, Date of manufacture: 06/08/2016, Expiration date: 06/07/2019, CAS number: 50-24-8).

Organic onion bulbs were purchased locally from a reliable source. The dry external scales were removed without damaging the root area and the central parenchyma of the bud crown was also removed by a circular incision to increase the uptake and uniformity of budding and root growth. These bulbs were washed in running water for about 20 min. Carefully, the roots of the bulbs were exposed with the samples in covered glass beakers to prevent light from entering, so that only the central parenchyma of the bud crown remained in contact with the samples. For each sample analyzed, 5 onion bulbs were used and placed in contact with the samples for 24 h. The negative control was performed in the same way, using distilled water and ethanol in the proportion of 1:1 (solvent)[16,17].

Drug concentrations were adjusted according to the therapeutic window (minimum daily dosage, intermediate daily dosage and maximum daily dosage) of each drug and to the concentrations commonly used by patients (Hydrocortisone 100, 1,000 and 2,500 mg L^{-1}, Dexamethasone 0.75, 7.5 and 15 mg L^{-1} and Prednisolone 5, 20 and 60 mg L^{-1}). The positive control was Paracetamol® at 800 mg L^{-1} concentration.

After growth, the roots immersed in the samples were measured and then fixed in Carnoy's solution (acetic acid and ethyl alcohol, in the concentration of 3:1) for 12 h. After fixation, the roots were washed in distilled water for five minutes and stained on slides. For this, the roots were stained with acetic orcein dye in the dilution of 2% orcein and 45% acetic acid. The root tips were cut and heated for 30 sec and counted with the dye. Then the roots were placed on slides covered by coverslips and one drop of acetic orcein dye was added between the slide and the cover slip. Subsequently, the root was crushed with gentle pressure. The observation of the slides was performed under an optical microscope, with a 100x objective lens, counting 5,000 cells, observing mitotic indexes and chromosomal and mitotic changes[16,18,19].

The calculation of the mitotic index (MI) and the index of chromosomal and mitotic aberrations (MCC) occurred according to the following Eq[20]:

$$MI = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells observed}} \times 100$$

$$NAC = \frac{\text{Number of cells altered}}{\text{Total number of cells observed}} \times 100$$

Statistical analysis: For statistical analysis, the ANOVA one-way test was used[20], with a significance level $\alpha = 0.05$, using the statistical package, GrafPad Prism 5.0.

RESULTS

In relation to the root growth index, there was an inhibition of the growth of roots treated with the drugs in all the tested concentrations when compared with the negative control (C-) as shown in Fig. 2, being Hydrocortisone (Fig. 2a), Dexamethasone (Fig. 2b) and Prednisolone (Fig. 2c). In view of these results, author can conclude that in a specific and targeted study (e.g:. in a carcinoma), it could be shown that

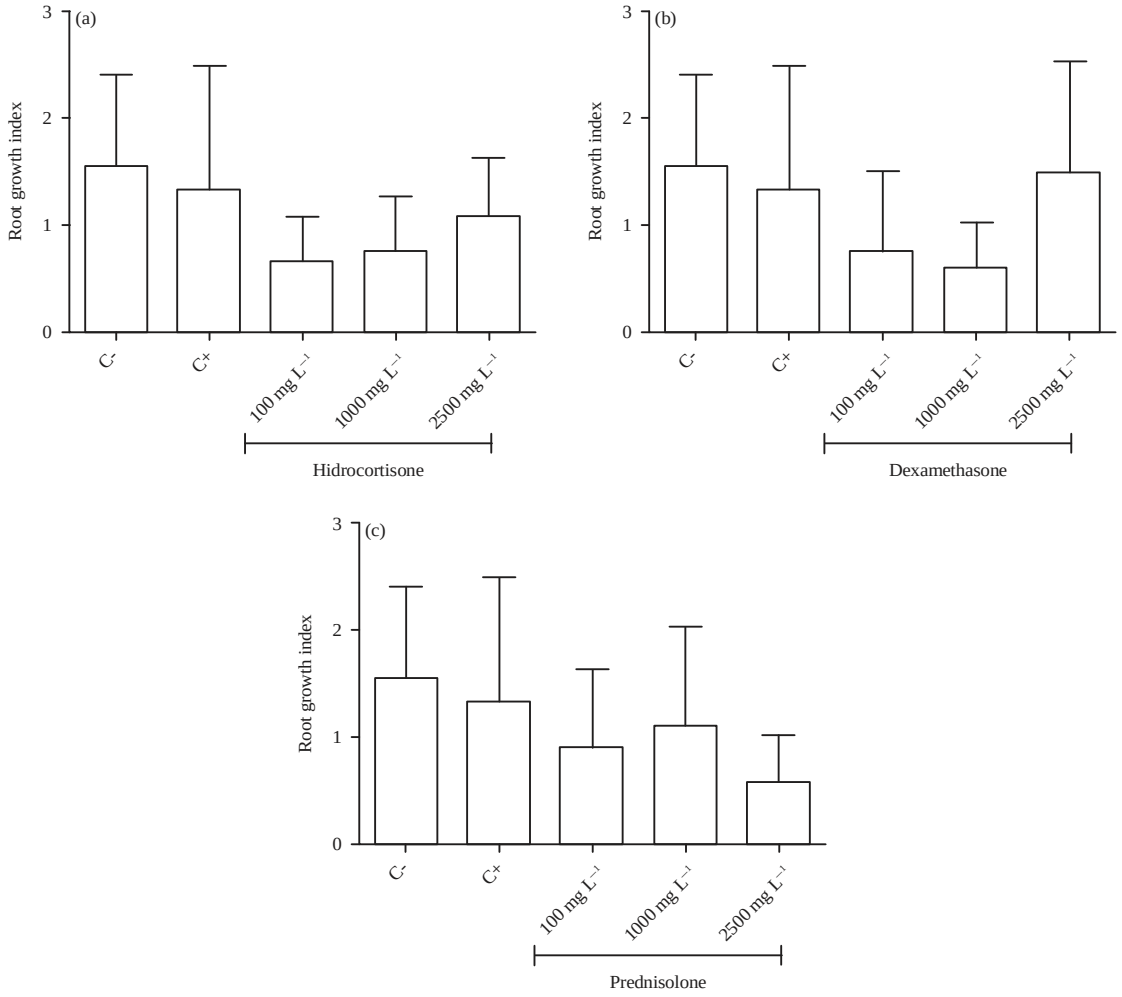

Fig. 2(a-c): Growth index of *Allium cepa* roots treated with (a) Hydrocortisone, (b) Dexamethasone and (c) Prednisolone. Standard bas shows Standard Deviation (SD)

these drugs act positively by inhibiting cell growth, but they inhibited normal plant growth, noting that corticosteroids were already used in neoplastic treatments. The mitotic index of all tested concentrations of each drug decreased relative to the negative control (C-) as shown in Fig. 3. The data are represented in the following chart, being: Hydrocortisone (Fig. 3a), Dexamethasone (Fig. 3b) and Prednisolone (Fig. 3c). The choice of substances used in this research was motivated after observing how much the use of these drugs was made in an indiscriminate way, this research has the intention to make professionals aware of the orientation of their use, for this reason the results of this research are comparative. The mitotic index in these results leads us directly to the mechanism of action where we can observe that inhibition of the synthesis of proteins directly linked with cellular mitosis is occurring.

The decrease in root growth associated with a decrease in MI indicates a cytotoxic potential of the drugs tested in this study. The results with high index of cells with aberrations were already expected because the drugs of choice have in their mechanism of action directly related to the genetic transcription, being able to alter the DNA conformation.

A significant increase ($p<0.05$) in the index of chromosomal aberrations in cells treated with both Hydrocortisone, Dexamethasone and Prednisolone in relation to the negative control (C-) are represented by Fig. 4a-c. Thus, the three drugs tested showed dose-response genotoxicity, that is, as the dosage increased, more chromosomal aberrations were found. With the high inhibition indices and cellular aberrations, observed in this research, we concluded with success that the analyzed drugs have a great mutagenic potential on the cells studied.

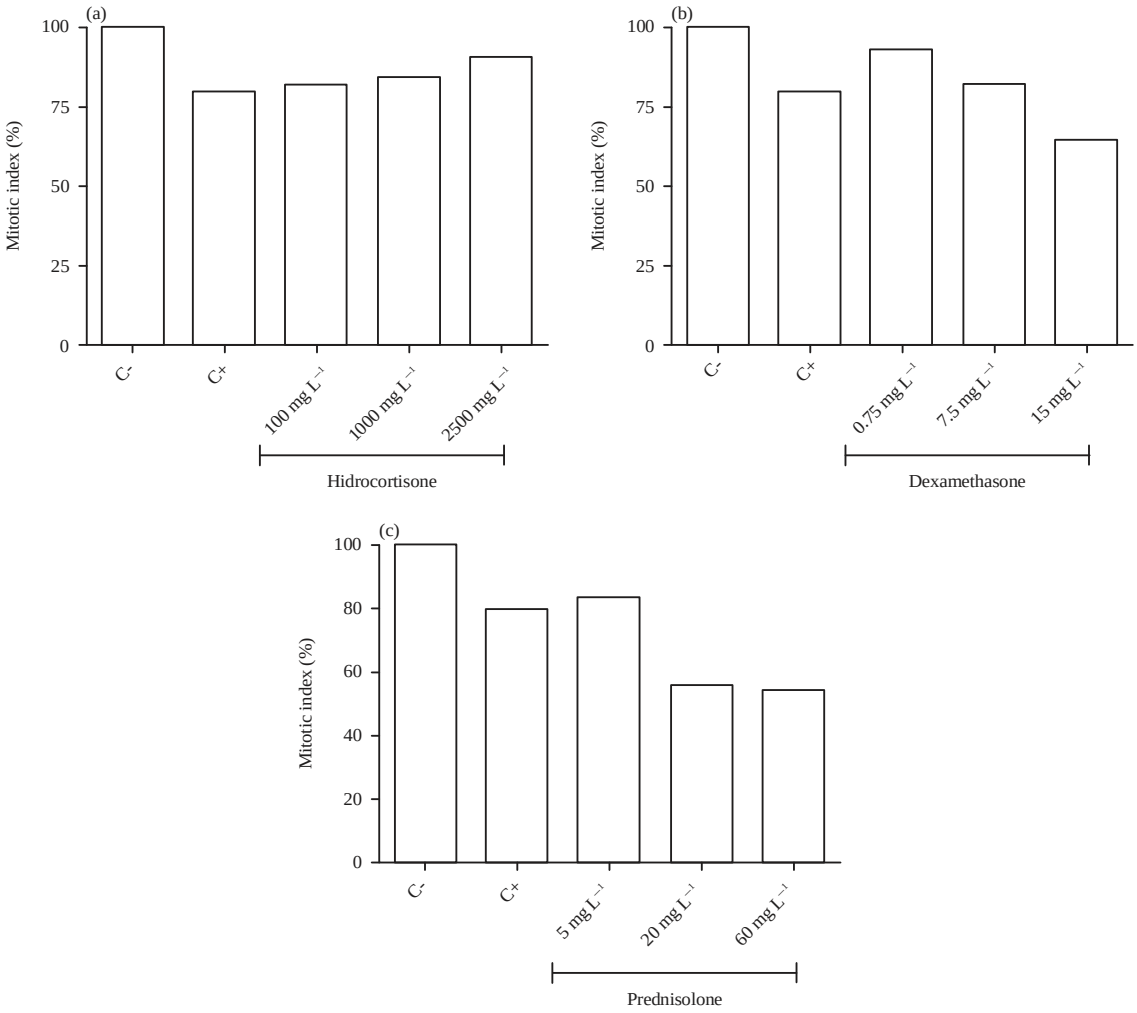

Fig. 3(a-c): Evaluation of mitotic index in *Allium cepa* cells treated with (a) Hydrocortisone, (b) Dexamethasone and (c) Prednisolone

DISCUSSION

Thus, the three drugs tested showed genotoxicity and cytotoxic potential. Genotoxicity studies in human lymphocytes and in the bone marrow of rats showed that Dexamethasone was able to attack the genetic material[21]. In a Comet test performed by Fahmy *et al.*[22] for 14 days with swiss rats (adult males) treated by oral ingestion, using three doses of Dexamethasone 0.03; 0.3 μg L^{-1}, 3.0 g L^{-1}, it showed that in the genotoxicity test, Dexamethasone did not cause genotoxic changes in renal, hepatic and bone marrow cells of mice (the metabolic conditions that are necessary for the formation of metabolites reactive to DNA and radicals). The importance of thresholds in the dose-effect relationship of genotoxicity data and their use in risk assessment, still raises many questions.

In a study conducted to evaluate the genotoxic effect induced by Hydrocortisone, administered intraperitoneally at doses of 26, 39 and 52 mg kg^{-1} in mice, a statistically significant increase in the incidence of aberrations was observed. These findings suggest that Hydrocortisone has genotoxic activity in the bone marrow of mice[23], thus corroborating with this study, that showed a significant increase in the index of chromosomal aberrations, pointing to the genotoxic character of these pharmacists.

In another study conducted by Coondoo *et al.*[24] with the objective of analyzing genotoxic effects in mice, using the micronucleus test in peripheral blood, the authors observed a genotoxic effect for the different topical corticosteroids evaluated. According to the results, the corticosteroid called "betamethasone" presented genotoxicity

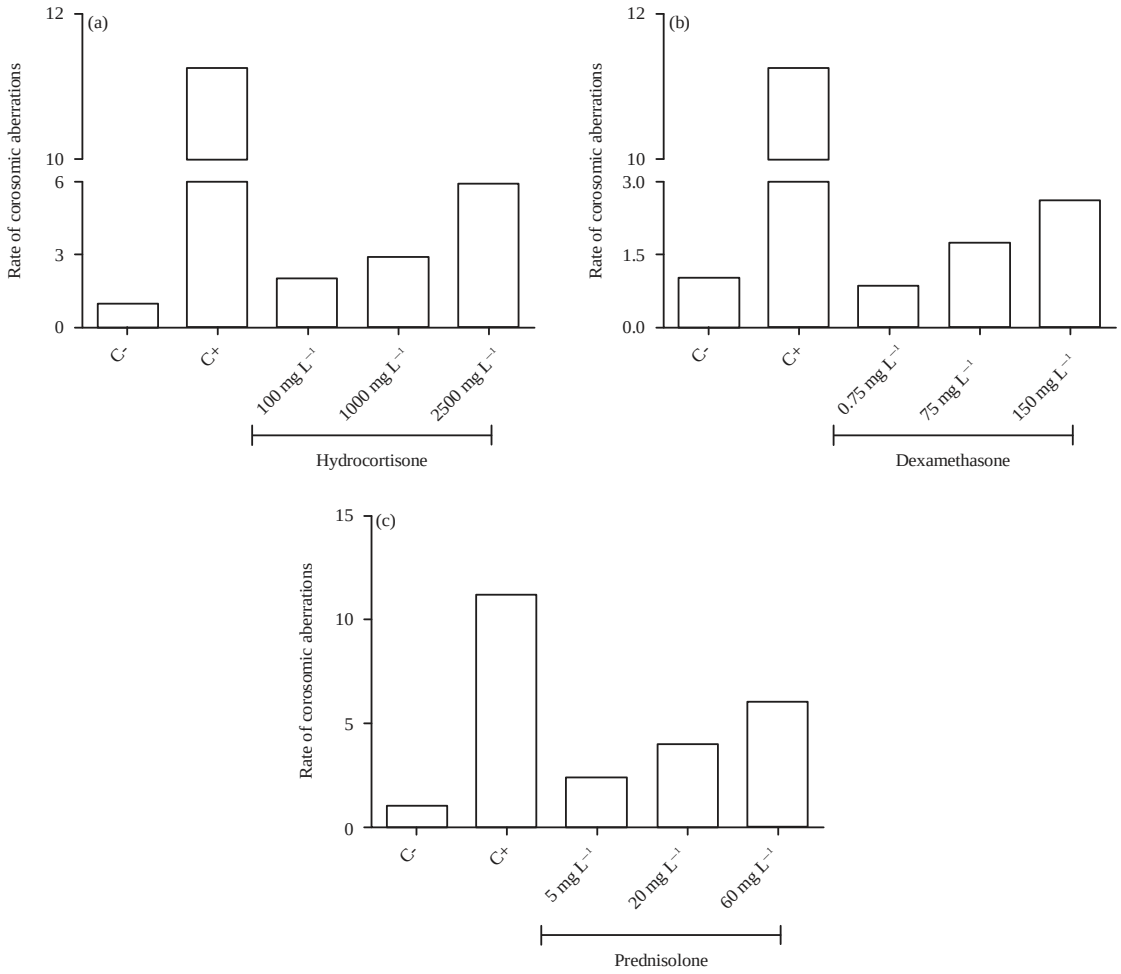

Fig. 4(a-c): Chromosomal aberrations in *Allium cepa* cells treated with (a) Hydrocortisone, (b) Dexamethasone and (c) Prednisolone

and dose-dependent cytotoxicity, which corroborate with the results found by our research group.

Some studies on corticoids indicate its possible mechanism of action, whether it be the inhibition of the transcription of growth genes or the activation of genes that induce apoptosis[25-27]. Corticoids are structurally very similar, it is possible that actions planned for some, also work for other steroids. In addition to the dose-dependence, it becomes necessary to also observe the presence or not of agonist receptors and specific antagonisms in human cells. However, *in vitro* testing, eukaryotes were good for testing possible therapeutic targets and their possible interactions[28].

CONCLUSION AND FUTURE RECOMMENDATIONS

According to the results, the corticosteroid called "betamethasone" presented genotoxicity and dose-dependent cytotoxicity. The three glucocorticoids tests showed cytotoxic and genotoxic potential.

So, further studies with other model organisms are needed to increase the information regarding the genotoxicity of these drugs. Model system research has long contributed to basic biological knowledge and its application to human medicine. Studies using model organism, first identified the action of genetic pathways and the cell cycle regulators, although, this kind of study with model systems are the basic information needed to investigate the population variation and the genetic interactions. Research with model organisms is one of the most productive methods and is the window in understanding life processes.

SIGNIFICANCE STATEMENTS

This study investigates some drugs like glucocorticoids, which have mutagenic potential. Corticosteroids were used in this study, which can introduce genotoxicity in cells. This work is an alert to researchers and health workers.

The authors consider that this work has importance to public health and is a base study for mutagenicity causes.

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