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Evaluation of cytotoxicity of some common ophthalmic drugs

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Abstract. – OBJECTIVE: The study was aimed at evaluating the *in vitro* cytotoxicity of some commonly used drugs in ophthalmology. Hydrocortisone sodium succinate, Dexamethasone sodium phosphate, 5-Fluorouracil, Tobramycin and Pilocarpine nitrate are frequently used in various indications involving eye care, and the aim was to test the safety of these in cell culture.

MATERIALS AND MATERIALS: The *in vitro* cytotoxicity was carried out on the NIH 3T3 cell line by the Sulforhodamine B (SRB) assay.

RESULTS: With the exception of 5-Fluorouracil, none of the other drugs demonstrated appreciable cytotoxicity up to high conce[illegible]tions of 200 µg/ml at 48 hours of drug expo[illegible]

CONCLUSIONS: Hydrocortisone sodium [illegible]cinate, Dexamethasone sodium phosphate[illegible]bramycin and Pilocarpine nitrate were c[illegible]firmed to be non-cytotoxic whi[illegible]uoroura[illegible] was highly cytotoxic especi[illegible]hours a[illegible] very low concentrations.

Key Words:
Cytotoxicity, NIH 3T[illegible]B, H[illegible]isone sodium succinate, Dexamet[illegible]ne sodium [illegible]sphate, 5-Fluorouracil, Tobram[illegible]ocarpine nit[illegible]

Introduc[illegible]

C[illegible]oxi[illegible] studies form an invaluable facet of safe[illegible]lyses in order to verify whether the investigat[illegible]l co[illegible]und being used as a pharmaceutical [illegible]etic are nontoxic, or whether th[illegible]re intend[illegible] as therapeutic agents for which [illegible]ould play a fundamental role. New chemical entities, food additives and many ac[illegible]es are subjected to comprehensive cytotoxicity [illegible]g before they are made available for use. A large number of cytotoxicity tests have been developed that can help us determine the effects of drugs on a particular cell line[1]. Today a majority of the cytotoxicity experiments are carried out in microtiter plates (96-well format). This approach permits high throughp[illegible]t is al[illegible]onomica[illegible] feasible. An ELISA pla[illegible]ad[illegible]s used[illegible]r experiments based [illegible]colo[illegible]ic and [illegible]inescence assays. C[illegible]oxicity as[illegible]me[illegible]e different parame[illegible] cell mem[illegible] integrity, metabolic [illegible]tivity [illegible] well as morphological[4] and re[illegible]ductive cha[illegible]istics[5] of viable cells. We [illegible]e Sulforhoda[illegible]B (SRB) assay for e[illegible]ation of cytotoxicity.

[illegible]ydrocortis[illegible] Sodium Succinate which is n[illegible]ally secre[illegible] by the adrenal cortex is a gly[illegible]ic ster[illegible] It is used as an anti-rheumatic drug, [illegible]equired in smaller dosage and is more effective when compared to cortisone[6]. For [illegible]st five decades, hydrocortisone has been [illegible]a potent therapeutic tool. It reduces the inflammatory reactions in traumas and allergic symptoms in the eye[7]. Hydrocortisone has also been used in treatment of prostate cancer[8].

Dexamethasone Sodium Phosphate, a glucocorticoid, is a drug most commonly used to reduce postoperative nausea and vomiting[9]. It is an anti-inflammatory drug that functions by inhibiting the action of inflammatory mediators[10]. It has also been used to prevent postoperative swelling and pain[11].

5-Fluorouracil is one of the oldest chemotherapy drugs in use[12] and is a pyrimidine analogue used in the treatment of cancer. Since 1984, it has been used as sub-conjunctival injections after filtering surgery to control high-risk glaucoma although with several side effects. Thus, it is judicious to use this only in the case of eyes with a high risk of scarring rather than for primary normal glaucoma surgeries[13].

Tobramycin an aminoglycoside has been widely used as an antibiotic. Obtained from *Streptomyces tenebrarius* it has been found to be effective against Gram negative bacteria and few *Pseudomonas* infections[14].

Pilocarpine Nitrate is produced from the leaves of a shrub belonging to the genus *Pilocar-*

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pus. Being a non-selective muscarinic receptor agonist, it acts on muscarinic acetylcholine receptor M3. Pilocarpine has been used in the treatment of glaucoma and is responsible for contraction of the iris sphincter and ciliary muscles, resulting in the decrease of intraocular pressure[15,16].

Materials and Methods

Reagents and Chemicals

Culture media Dulbecco's Modified Eagle Medium (DMEM), trypan blue and Sulforhodamine B (SRB) were procured from Sigma St. Louis, MO, USA. Fetal bovine serum (FBS), Biowest, [Cat. No. S1810], penicillin-streptomycin was from Cell clone [Cat No. CC4007]. All other reagents were from Qualigens (Analytical grade) or Merck.

Cell Line

The cell line used in this work was NIH 3T3 procured from the National Centre for Cell Science (NCCS), Pune, India; and propagated in DMEM supplemented with 1% penicillin-streptomycin and 10% heat-inactivated FBS at 37°C in a humidified incubator at 5% CO_2. Cell viability was ascertained by the trypan blue dye exclusion method[17] followed by cytotoxicity experiments by the Sulforhodamine (SRB) assay[18].

SRB Assay

The SRB assay was used to assess cytotoxicity based on total protein content at 24 and 48 hours. Cells were seeded at a density of 1×10^5 cells/well in 96-well flat bottomed tissue culture plates (Tarsons, India) and pre-incubated for 24 hrs.

After treatment with a range of concentrations of Hydrocortisone Sodium Succinate, Dexamethasone Sodium Phosphate, 5-Fluorouracil, Tobramycin and Pilocarpine Nitrate, cells were fixed with 30% TCA, processed for SRB assay and read at 540 nm (Thermo Labsystems; Ascent software). The experiments were set up in triplicates and repeated at least five times.

The calculation of Percent Cell Viability was as follows:

$$\text{Percent Cell Viability} = \frac{\text{O.D. of test} \times 100}{\text{O.D. of control}}$$

The results are expressed as mean ± standard deviation (S.D.) and the IC_{50} (inhibitory concentration) values were calculated from the dose-response curves.

Statistical Analysis

The data was subjected to the single factor analysis of variance (ANOVA) across groups and dose variants where $p < 0.05$ was considered statistically significant.

Results

SRB assay of Pilocarpine Nitrate, Hydrocortisone Sodium Succinate and Tobramycin did not show any significant cytotoxicity up to 48 hrs on the 3T3 cell line and the IC_{50} could be obtained [Figure 2(a) and 2(a)]. For Dexamethasone Sodium Phosphate the 24 hour IC_{50} could not be determined [Figure 2(a)] while the 48 hour IC_{50} was 1 µg/ml [Figure 2(a)]. 5-Fluorouracil was found to be highly cytotoxic on the 3T3 in a time and dose dependent manner with 24 and 48 hour IC_{50} values at 100 and 0.09 µg/ml respectively [Figures 1(b) and 2(b)].

Discussion

Apart from treating the particular eye condition, it is important to establish the safety of commonly used ophthalmic drugs. Most of the drugs available in the market today have already undergone extensive safety and efficacy testing. Nevertheless, we wanted to confirm the same on a simple 3T3-SRB cell cytotoxicity platform. Under the present experimental conditions, Pilocarpine Nitrate, Hydrocortisone Sodium Succinate and Tobramycin were confirmed as safe due to the absence of the IC_{50} values up to 48 hours and at high concentrations of 200 µg/ml. Dexamethasone Sodium Phosphate followed a comparable trend at similar concentrations; the 48 hour IC_{50} was at a very high dose and can thus be considered as non-cytotoxic. Among all the drugs tested, 5-Fluorouracil was the most cytotoxic and it is in keeping with the nature of the drug as it is also used as an anti-neoplastic agent.

Samples et al[19] tested the cytotoxicity of hydrocortisone on human and baboon corneal en-

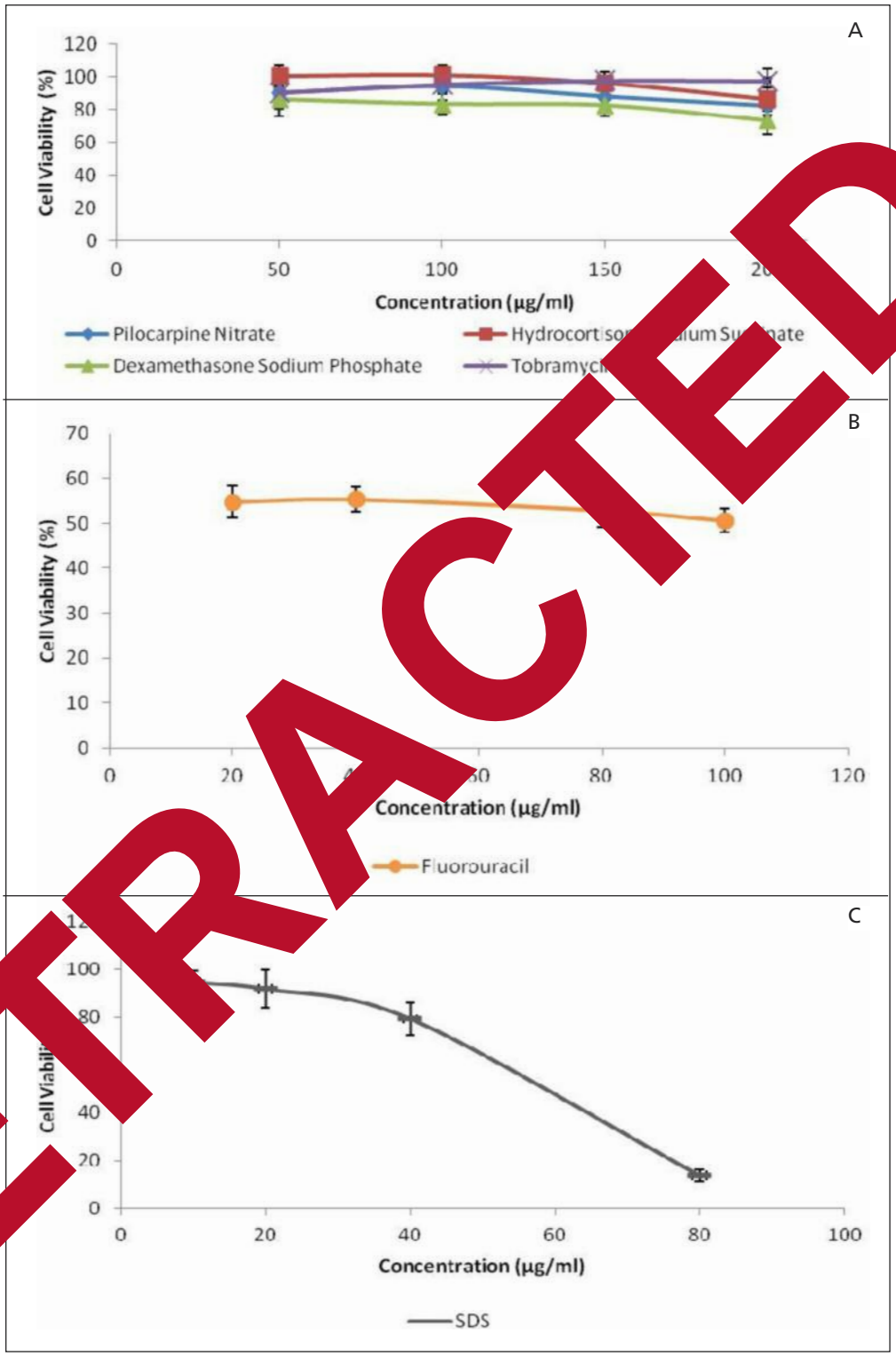

Figure 1. The 24 hour dose-response curves revealed that the difference in inhibition rates due to concentrations and across groups were not significant statistically for Hydrocortisone Sodium Succinate, Dexamethasone Sodium Phosphate, Tobramycin and Pilocarpine Nitrate ($p > 0.05$) 1(a). For 5-Fluorouracil, the inhibition rates were not concentration dependent ($p > 0.05$) 1(b) but they were for SDS ($p < 0.05$) 1(c).

donelial cells and observed that at a concentration of 10^{-4} and 10^{-3}M the cell growth was significantly reduced accompanied with low levels of DNA synthesis. However, in the current study hydrocortisone had minimal cytotoxicity.

In keeping with our findings, that cell viability decreased when exposed to increasing concentrations of dexamethasone, others found that at 24hrs, Human lens epithelial cells (HLE B-3) displayed a low viability at high concentrations

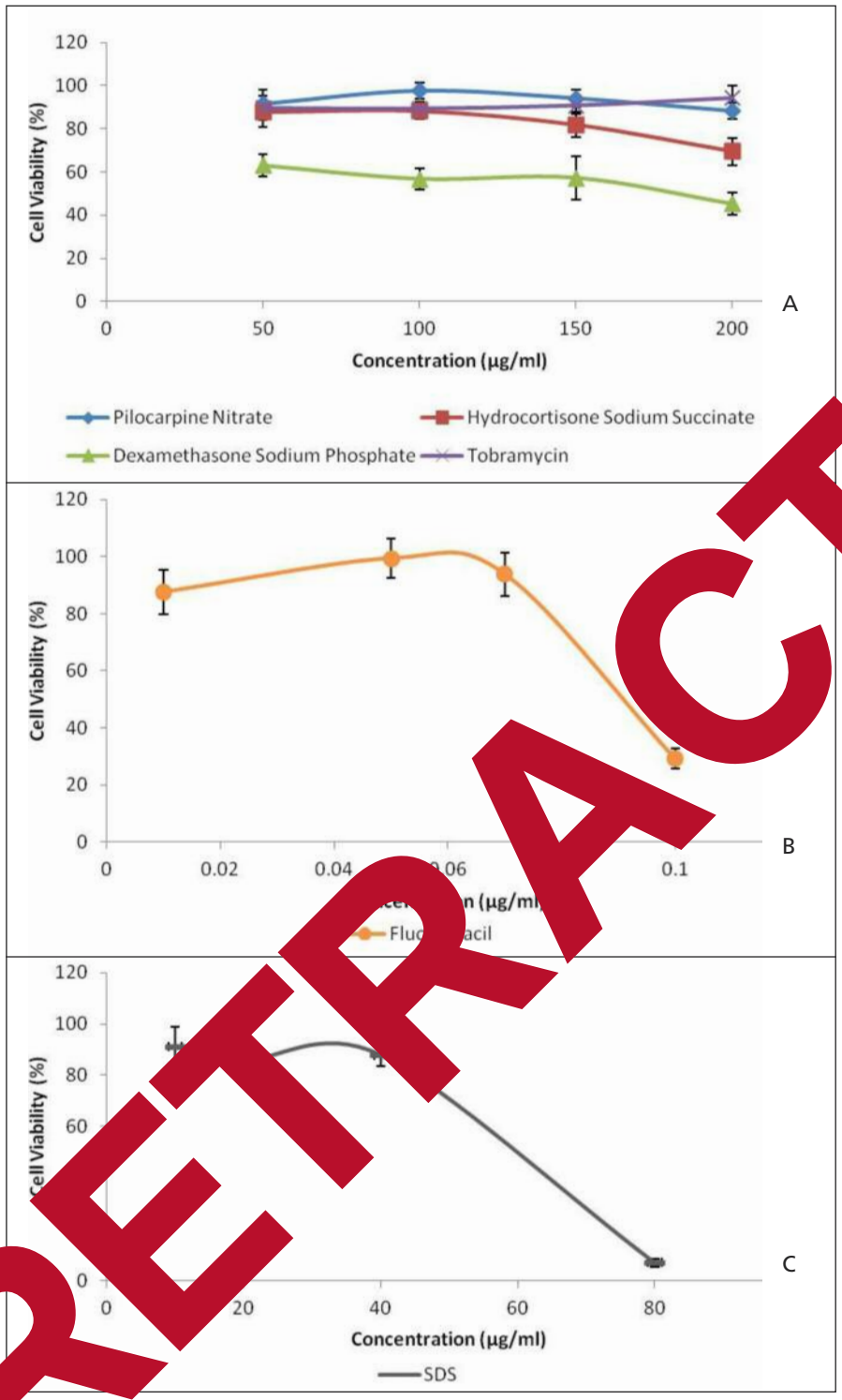

Figure 2. The 48 hour dose response curves revealed that the difference in inhibition rates due to concentrations and across groups were not significant statistically for Hydrocortisone Sodium Succin… bramycin and Pilo… trate ($p > 0.05$) …e differ… ence in inhibi… ates due to Dexamethason… m Phos… phate wa… ignific… istical… ly (p … 5) 2(a). … luo… ro… and SDS the in… …were co… ration d… ($p < 0$… (b) and 2(c).

…and 1 mg/ml; however, lower concentrations did not result in any significant decrease in cell viability[20].

5-Fluorouracil is widely used as an anticancer drug. It has been used in ocular and periorbital surgeries as it is capable of reducing fibrosis and hence has been considered as an important tool for enhanced success rates in ophthalmic surgeries[21]. In their study on the cytotoxicity of 5-fluorouracil on human corneal epithelial cell (HCEC) and human corneal keratocyte (HCK) cultures, Midena et al[22] found that it had a dose and time dependent

effect on both cell lines. However, even at the highest concentration tested, a complete inhibition of cell growth was never observed. This was not entirely in agreement with our results as by the end of 48 hours cell viability was at 29% at a very low concentration of 0.1 µg/ml.

Conclusions

In the present investigation, Tobramycin did not show any cytotoxic effect on the 3T3 cell line. Even at the highest concentration and time point the cell viability was 94% which is in concurrence with other studies[23-25].

The next logical step would be to evaluate these drugs on rabbit corneal epithelial cell line (SIRC) followed by human corneal epithelial cells or the three-dimensional model for testing ocular toxicity. The latter would also compensate for the problems in inter-species variation and co-relation, which were the limitations of the current research.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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