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Cytotoxic response of SMA-DMSO complex along with other test chemical on the HEPA RG and CHO cell line

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ABSTRACT

Safe, non-toxic and well accepted contraceptive has a great demand to control the increasing human population. In this connection, a lot of hormonal and non-hormonal contraceptives have been developed. SMA-DMSO complex is one of the remarkable break-through in the research of male contraceptive. Lot of researches has been made to prove the safe and non-toxic nature of this non hormonal polymeric compound. Present study demonstrates the non-cytotoxic nature of SMA-DMSO complex as compared to the DMSO and SDS (as negative control) and H2O2 (as positive control). The result shows that SMA-DMSO complex significantly decreases the MTT in both culture (CHO & HEPA RG). The concentrations of the test chemicals ranging between 2.5 to 10 µg were observed to decrease the % cytotoxicity in contrast to lower and higher concentration levels. SMA-DMSO complex has been observed to have least effect on the cellular viability.

INTRODUCTION

The increasing world human population is of great concern for policy makers and for medical researchers. Despite, a decrease of human population growth rates owing to the collective efforts made worldwide, countries such as India, China, Pakistan, US and several other south east Asian countries are worst affected because of higher natality rate and life expectancy. Several family planning efforts social and scientific have been made to control the increasing population culminated into the development of various cost effective and safe contraceptives. Some of the contraceptive measures are reversible such as use of contraceptive pills and devices and some are non-reversible such as female tubectomy and male vasectomy. These measures however; have been observed to be non-preferable in most of the human community therefore, safe, time saving, and popular methods are being developed. . Indeed, the past few decades have brought great advances in the hormonal and non-hormonal male contraceptives (Anderson and Baird, 2002; Lyttle and Kopf, 2003; Kamischke and Nieschalf, 2004).

SMA-DMSO complex has revolutionized this study to greater extent because of its highest successful rate. SMA-DMSO complex rendering reversible inhibition of sperms under guidance (RISUG) is a potent anti-fertility compound developed by Prof. S. K Guha of IIT Kharagpur India (Guha, 1996). This non-hormonal contraceptive is expected to provide valuable additions to the currently available limited options of male contraception (Ananthaswamy, 2002). RISUG consists of a co-polymer styrene maleic anhydride (SMA) dissolved in 99.9% pure dimethylsulphoxide (DMSO) (Guha, 1996) forming a non-sclerosing porous polymer that disrupts the sperm cell membranes by lowering the pH soon after it is injected in vas from where the sperms traverse through, producing damaged, nonviable sperm. Singh et al. (2014) recently reported no damage to DNA content of animal cells as a result of RISUG application however; the toxic influence on the animal cells are yet to study. Although the human application is all set to start in USA under Vasalgel trademark (Gifford, 2011). Present study has been designed to understand the toxicological manifestations of SMA-DMSO compound in terms of toxic manifestations on animal cells using HEPA RG and CHO cell line.

MATERIALS AND METHODS

To conduct the overall toxicological profiling two commercially available cell lines viz., HEPA RGTM (human hepatocellular carcinoma) and CHO (Chinese Hamster Ovary) were used. The entire experiment was carried under biosafety standards at the Department of Biotechnology, IILM Greater Noida.

Cell culture and maintenance

Cell line (CHO and HEPA RG) are more convenient, predictable and possess karyotypic stability over a number of passages. HEPA-RG do not undergo transfection of genomic modification, they retain the range of hepatic cell responses. These cells are also useful for in-vitro analysis of compound more rapidly as compared to other cell line and shows distinguished results like as change in Golgi apparatus and membrane analysis by using certain dyes. In the present research work cells of HEPA RG and CHO cell lines were cultured in phenol red free Williams Medium E and MEM (minimum essential medium) respectively provided by Sigma Aldrich. Cells along with the medium were incubated in CO2 incubator (Thermo Scientific Make) at 370C with a regular replacement of the medium at an interval of 5 days. The viability of the cells were also monitored on a regular basis by using tetrazolium vital dyes.

MTT assay for cell proliferation and survival

The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, by the action of dehydrogenase enzymes, resulting in the intracellular purple formazan which is quantified by spectrophotometrically. In the present study the improvised method of Twentyman and Luscombe (1987) was applied.

High density cells (100,000 cells/well) were plate and incubated (at 370C) for 6 hr followed by the addition of 10 µl MTT reagent (Sigma Aldrich) (12 mM stock concentration). The cells were further incubated for 2 to 4 hr till the appearance of purple precipitates. 100 µl of commercially available detergent reagent was added and microtitre plates were left as such for about 2 hr in dark at room temperature 270C ± 20C. The absorbance was recorded using a microtitre plate reader at 570 nm. The test chemicals viz., SMA-DMSO (dimethylsulfoxide), SDS (sodium dodecyl sulfate) (as a negative control), DMSO and H2O2 (hydrogen peroxide as appositive control) were added in the plates in 10, 5.0 and 2.5 µg to study the response of the cells for these compounds. Prepared SMA-DMSO was provided by the CSIR-CDRI laboratory Lucknow, whereas rest of the chemicals was provided by the Hi-Media Inc. Cell viability was observed under hemocytometer and the values were calculated using following procedure:

Cell Viability (%) = total viable cells (unstained) / total cells (stained and unstained) × 100

Cytotoxicity calculation

Percentage toxicity to the cell was calculated by using the formula:

Cytotoxicity % = (a –b)*100/ (c-b)

Where a) is OD at 570 nm obtained from a well added with a test chemical; b) is the mean OD at 570 nm of blank wells; and c) is the mean OD at 570 nm derived from control wells (added culture medium as a test sample).

RESULTS AND DISCUSSION

MTT Assay has been commonly used as a test of cytotoxity in order to evaluate the toxic responses to chemical exposure. In the present study MTT assay revealed the toxic manifestation of the test chemicals viz., SMA-DMSO, SDS, DMSO and H2O2 although with a varying extent.Table 1 presents the overall impact of the test chemicals on the cells of corresponding cell line. As per the results, the time intervals and concentration influenced the cell viability to a greater extent which is indicative by higher values of optical density recorded.

These values as shown in the table were observed to vary in all the concentrations viz., 10, 5.0 and 2.5 µg of each chemical. The higher values in the 10 µg SMA DMSO treated experimental sets where the HEPA RG and CHO cell viability was highest > 2.2 and > 3.0 followed by the negative control SDS > 2.0 and > 2.1 respectively in 24 hr. exposure. However, toxic manifestation was observed continued in all sets except in control. Out of the four tested chemicals, the HEPA RG cell viability was found to follow the order SMA-DMSO>SDS>DMSO>H2O2 whereas the CHO cell viability was found to follow the order SMA-DMSO>>SDS>DMSO>H2O2. The CHO cells were found to survive better in SMA-DMSO complex exposition in 24 hr. with a value 3.12 treated with 10 µg SMA-DMSO followed by a decreasing trend of cell viability. From the results it is indicative that the higher concentrations of the test chemicals except SMA-DMSO complex are lethal which has earlier been reported for DMSO (Cao et al., 2007); for SDS (Rosety et al., 2001) and for hydrogen peroxide (López and Calvo, 2011). The result of the present study demonstrated that the % of cytotoxicity are higher in HEPA RG cell line as compared to CHO cell line after 72 hr. at concentration of 10 µg, at the concentration of 5 µg are in HEPA RG cell line and in CHO cell line and at concentration of 2.5 µg the % of cytotoxicity as shown in the figure 1 and 2 was observed in HEPA RG and in CHO cell line. The toxic response showed by SDS is subjected to anionic surface activity which disrupts the mammalian cell membrane integrity (Inácio et al., 2011). The SMA-DMSO compound has the potential to internalize by the cell and have the potential to interact with DNA causing the death of sperms. In addition to the reports, demonstrating the toxicity of SMA DMSO complex on the spermatozoa, present study prove no ill effects of the complex on the mammalian cells.

CONCLUSION

In the present study, SMA DMSO complex significantly decreases the MTT in both culture (CHO & HEPA RG). The concentration ranges of 2.5 to 10 µg were observed to decrease the % cytotoxicity in contrast to lower and higher concentration levels. The cytotoxicity of test compound was tested at different concentrations started from 2.5 microgram to 10 µg. From the present study it is quite clear that SMA-DMSO complex is safe for the use as male non-hormonal contraceptive.

Table 1: Influence of SMA/DMSO/SMA-DMSO and H2O2 on the growth of HEPA RG and CHO Cells: Toxic profiling made on the total suppression of growth in 24 hr.

Time Intervals →	24 hr		48 hr		72 hr	
Sample ↓	HEPA RG	CHO	HEPA RG	CHO	HEPA RG	CHO
SMA DMSO (10µg)	2.24 ± 1.17	3.12 ± 0.27	1.42 ± 0.15	1.41 ± 0.22	1.31 ± 0.18	1.08 ± 0.096
SMA DMSO (5µg)	2.15 ± 0.80	2.34 ± 0.53	1.95 ± 0.32	1.51 ± 0.38	1.58 ± 0.37	1.49 ± 0.08
SMA DMSO (2.5µg)	1.61 ± 0.55	1.76 ± 0.63	1.98 ± 0.86	1.35 ± 0.16	2.71 ± 1.20	1.51 ± 0.66
SDS (10µg)	2.10 ± 0.86	2.19 ± 1.05	1.57 ± 0.68	1.19 ± 0.09	1.39 ± 0.44	1.01 ± 0.25
SDS (5µg)	1.50 ± 0.25	1.68 ± 0.53	1.79 ± 0.56	1.51 ± 0.61	1.68 ± 0.76	1.61 ± 0.91
SDS (2.5µg)	1.10 ± 0.29	2.47 ± 0.23	1.92 ± 0.61	1.71 ± 0.58	2.42 ± 1.19	1.89 ± 0.47
DMSO (10µg)	1.36 ± 0.35	1.53 ± 0.83	1.22 ± 0.61	2.54 ± 0.16	1.75 ± 0.90	1.26 ± 0.44
DMSO (5µg)	1.01 ± 0.27	1.20 ± 0.19	1.10 ± 0.86	1.66 ± 0.53	1.55 ± 0.32	1.39 ± 0.16
DMSO (2.5µg)	1.13 ± 0.77	1.79 ± 0.26	1.47 ± 0.59	1.90 ± 0.23	1.05 ± 0.17	1.41 ± 0.30
**H_2O_2 (10µg)	0.28 ± 0.11	0.20 ± 0.004	0.09 ± 0	0.092 ± 0.01	0.06 ± 0.003	0.067 ± 0.001
H_2O_2 (5µg)	0.51 ± 0.03	0.60 ± 0.50	0.51 ± 0.04	0.59 ± 0.17	0.075 ± 0.012	0.074 ± 0.001
H_2O_2 (2.5µg)	1.22 ± 0.66	1.33 ± 0.19	0.29 ± 0.06	1.40 ± 0.08	0.078 ± 0.001	0.168 ± 0.017
Control*	2.67 ± 0.25	3.32 ± 0.15	2.33 ± 0.25	2.60 ± 0.18	2.82 ± 0.112	2.33 ± 0.11

** H2O2has been taken as positive control.

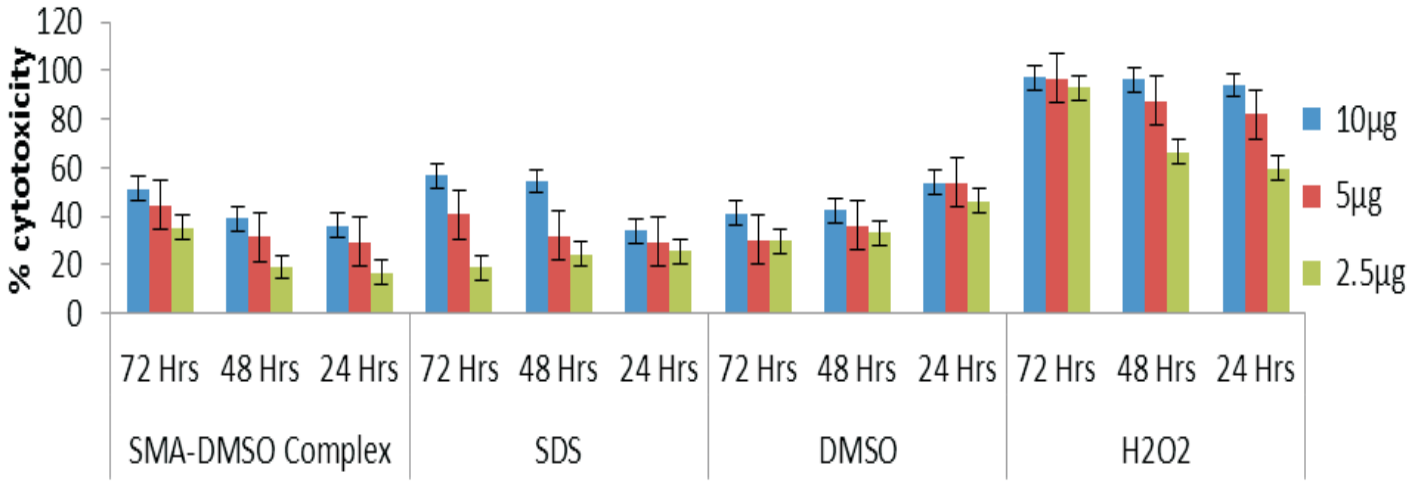

Fig1. % Cytotoxicity as MTT assay of various concentrations of SMA-DMSO/SDS/DMSO and H2O2 on the CHO cells.

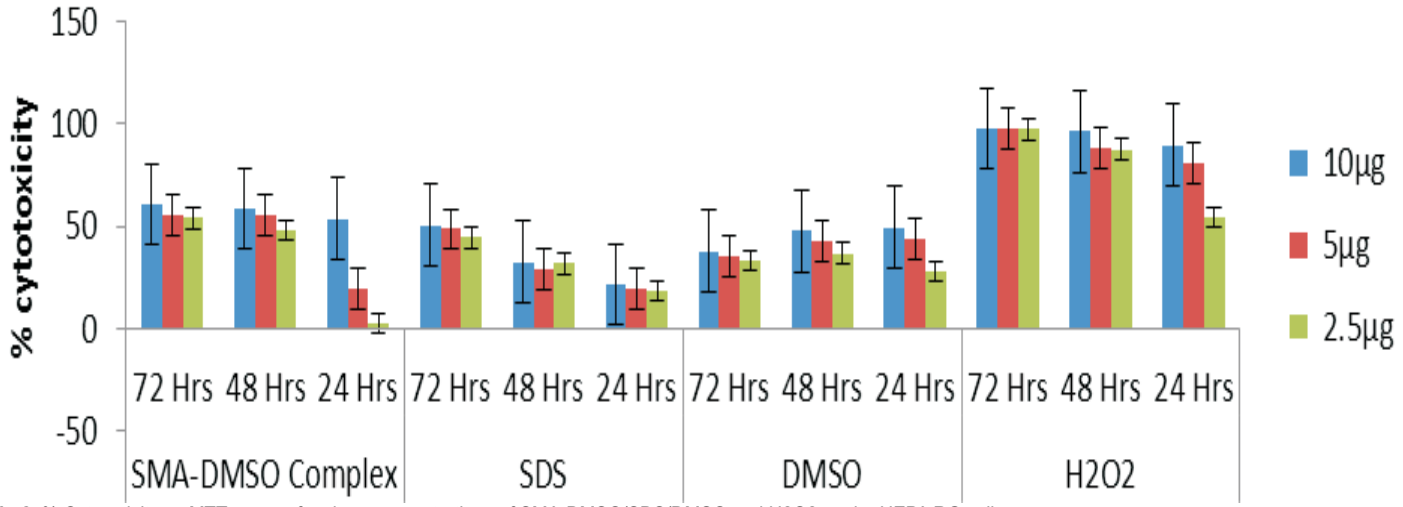

Fig 2. % Cytotoxicity as MTT assay of various concentrations of SMA-DMSO/SDS/DMSO and H2O2 on the HEPA RG cells

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SMA - styrene maleic anhydride
DMSO – di-methyl-sulphoxide
SDS – sodium dodecyl sulfate
MTT - 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
HEPA - human hepatocellular carcinoma cell line
CHO – Chinese Hamster Ovary cell line
MEM – Minimum essential medium .

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