In vitro mammalian cell micronucleus test of *XYX001* in V79 cell lines

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Abstract: The study aimed to assess the genotoxic (*In vitro* mammalian cell micronucleus test) and cytotoxic potential of the compound *XYX001* using the V79 rodent cell line as a model system. The investigation was conducted in accordance with OECD Guideline 487 and included both metabolic and non-metabolic conditions through the incorporation of an S9 mix. *XYX001* was tested at various concentrations (5, 2.5, 1.25, 0.625, and 0.313 mg/ml) for its cytotoxic and clastogenic effects. Positive controls included Benzo(a)pyrene and Mitomycin C, while Dimethyl Sulfoxide (DMSO) served as the negative control. The study employed the in vitro micronucleus test to evaluate clastogenicity and cytotoxicity, quantified through parameters such as Cytokinesis-Block Proliferation Index (CBPI), Replication Index (RI), and % Cytostasis. The positive controls exhibited expected increases in micronuclei formation, validating the assay. *XYX001* did not induce cytotoxicity or clastogenicity at the concentrations tested. Specifically, at the highest concentration of 2.5 mg/ml, *XYX001* was found to be non-cytotoxic and non-clastogenic. *XYX001* demonstrated a favorable safety profile in the V79 rodent cell line under the conditions of this in vitro study. The compound was non-cytotoxic and non-clastogenic at the highest tested concentration of 2.5 mg/ml. The study's findings are considered valid and reliable, given the consistency with historical control data and adherence to international guidelines.

Keywords: V79 Rodent Cell Line, OECD Guideline 487, Cytotoxicity, Clastogenicity, Micronucleus Test, S9 Mix, Genotoxicity

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INTRODUCTION

The quest for novel therapeutic agents has led to the synthesis and investigation of a myriad of compounds, each with unique chemical structures and potential biological activities [1]. Among these, XYX001 (coded drug) has emerged as a compound of interest due to its promising pharmacological properties. However, like any new compound, it is imperative to assess not only its therapeutic efficacy but also its safety profile, particularly its genotoxic potential. Genotoxicity is a critical parameter in drug development, as substances that interact adversely with cellular DNA can lead to mutations, and consequently, a range of diseases including cancer [2].

The micronucleus test in mammalian cells serves as a robust and reliable assay for detecting genotoxic effects. It is based on the principle that genotoxic agents can induce micronuclei—small, additional nuclei—in cells, which are indicative of chromosomal damage or whole chromosome loss during cell division [3]. The V79 cell line, derived from Chinese hamster lung cells, is commonly employed in this assay due to its well-characterized genetics and ease of culture. Moreover, the V79 cell line is particularly sensitive to chromosomal aberrations, making it an ideal model for micronucleus tests [4].

The present study aims to investigate the genotoxic potential of XYX001 using the in vitro mammalian cell micronucleus test in V79 cell lines [5]. This research is not only crucial for understanding the safety profile of XYX001 but also contributes to the broader scientific understanding of genotoxicity assays. By employing a well-established cell line and a standardized method, the study aims to provide reliable and replicable results that could serve as a cornerstone for future research and potential clinical applications [6].

In summary, the objectives of this study are twofold: first, to assess the genotoxic potential of XYX001, thereby contributing to its safety profile, and second, to validate the efficacy of the V79 cell line as a model for genotoxicity testing. The outcomes of this research could have far-reaching implications, from drug development and regulatory approval to clinical practice and public health.

METHODOLOGY

Dose Range Finding Study [7]

The initial phase of the study involved a dose range finding assessment to determine

the appropriate concentrations of XYX001 for subsequent cytotoxicity evaluations. V79 cells, a well-characterized cell line derived from Chinese hamster lung cells, served as the experimental model. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum and 1% penicillin-streptomycin, and maintained in a controlled environment at 37°C with a 5% CO2 atmosphere.

Cytotoxicity Assessment [8]

For the cytotoxicity assessments, both short-term and extended treatments were conducted. In the short-term study with S9 activation (S9+), the treatment groups included a 1% DMSO control, a 5µg/ml Benzo(a)pyrene positive control, and varying concentrations of XYX001 ranging from 0.313mg/ml to 5mg/ml. A parallel short-term study without S9 activation (S9-) included a 1% DMSO control, 0.05µg/ml Mitomycin, and 0.1 µg/ml Colchicine as positive controls, along with the same range of XYX001 concentrations. An extended treatment was also conducted without S9 activation, employing the same controls and XYX001 concentrations as in the short-term S9- study.

Slide Scoring Protocol [9, 10]

Slide scoring was conducted in a coded manner to ensure unbiased results. Four slides were prepared for each culture concentration. Scoring was performed under an inverted microscope using a DLC counter. Only binucleate cells with intact cell membranes and cytoplasm were scored. A minimum of 500 cells per slide were assessed to calculate the cell proliferation, using metrics such as the Cytokinesis-Block Proliferation Index (CBPI) and Replication Index (RI). Cytostasis was also evaluated by counting slides containing mononucleate, binucleate, and multinucleate cells. For assessing micronucleus frequency, a total of 2000 binucleated cells per concentration in a single culture were counted. These 2000 binucleated cells were equally divided among the replicates for scoring the presence of micronuclei.

Main Study

Main Study Methodology

Test Tube Preparation [11]

A total of 24 test tubes were prepared for the study. These were divided into three categories: 12 tubes for the test item XYX001 at concentrations of 5mg/ml, 2.5mg/ml, and 1.25mg/ml (4 tubes each for -S9, +S9, and extended treatment); 6 tubes

for the negative control (2 tubes each for -S9, +S9, and extended treatment); and 6 tubes for the positive control (2 tubes each for -S9, +S9, and extended treatment).

Cell Culture and Treatment [12]

Each test tube contained 3.95 ml of DMEM media. The V79 cell lines were cultured for 48 hours in a CO2 incubator at 37±1°C with 5% CO2. Post-incubation, the cells were exposed to 100µl per tube of XYX001 for all test concentrations. Additionally, 500µl per tube of S9 mix was added for metabolic activation, and 500µl per tube of Phosphate Buffer Saline (PBS) 1x was added for tubes without metabolic activation. The tubes were then incubated for an additional 3 hours at 37±1°C for short-term treatment. No medium change was performed for extended treatment tubes.

Harvesting and Slide Preparation [13]

Three hours before harvesting, cells were exposed to 0.1µg/ml of Cytochalasin B, an anaphase arresting agent. Post-incubation, the tubes were centrifuged at 1000 rpm for 10 minutes. Cell smears were prepared on slides, air-dried, fixed with ice-cold methanol, and stained with 10% Giemsa stain. The slides were then air-dried again and mounted with DPX.

Scoring [14]

The prepared slides were used for scoring the Cytokinesis-Block Proliferation Index (CBPI), Replication Index (RI), and % cytostasis for each slide.

Compliance with Guidelines [15]

The methodology was designed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines for the testing of chemicals 478, adopted on 29 July 2016.

1. **Cytokinesis-Block Proliferation Index (CBPI)**: CBPI = (M1 + 2*M2* + *3*M3) / (M1 + M2 + M3)

   Where M1, M2, M3 are the numbers of mono-, bi-, and multinucleated cells, respectively.

2. **Replication Index (RI)**: RI = (N2 + 2*N3* + *3*N4) / (N1 + N2 + N3 + N4)

   Where N1, N2, N3, N4 are the numbers of cells with 1, 2, 3, and 4 nuclei, respectively.

3. **% Cytostasis**: % Cytostasis = 100 * (1 - (CBPI_treated / CBPI_control))

RESULTS

Dose Range finding Study

The results section presents the data obtained from the cytotoxicity assessments, which were conducted according to the OECD guidelines for the testing of chemicals 478. The study involved three different treatment conditions: short-term treatment without S9 activation (S9-), short-term treatment with S9 activation (S9+), and extended treatment without S9 activation (S9-).

Cytotoxicity Assessment - Short-Term Treatment without S9 Activation (S9-)

In the DMSO 1% control group, the CBPI was 3.850 and the RI was 1.85. For the Mitomycin 0.05µg/ml positive control, the CBPI was 2.844 and the RI was 0.844, resulting in 35.291% cytostasis. The Colchicine 0.1 µg/ml group had a CBPI of 2.978 and an RI of 0.978, with 30.587% cytostasis.

For the test item XYX001, the CBPI and RI values varied with concentration. At 5mg/ml, the CBPI was 2.744 and the RI was 0.744, resulting in 38.799% cytostasis. At 2.5mg/ml, the CBPI was 3.14 and the RI was 1.14, with 24.903% cytostasis. At 1.25mg/ml, the CBPI was 3.152 and the RI was 1.152, with 24.505% cytostasis. At 0.625mg/ml, the CBPI was 3.06 and the RI was 1.06, with 27.706% cytostasis. Finally, at 0.313mg/ml, the CBPI was 4.298 and the RI was 2.298, with 13.58% cytostasis.

Cytotoxicity Assessment - Short-Term Treatment with S9 Activation (S9+)

In the DMSO 1% control group, the CBPI was 4.028 and the RI was 2.028. For the Benzo (a) pyrene 5µg/ml positive control, the CBPI was 2.99 and the RI was 0.99, resulting in 33.55% cytostasis.

For XYX001, the CBPI and RI values were as follows: at 5mg/ml, the CBPI was 2.738 and the RI was 0.738, with 42.262% cytostasis; at 2.5mg/ml, the CBPI was 3.158 and the RI was 1.158, with 27.686% cytostasis; at 1.25mg/ml, the CBPI was 3.118 and the RI was 1.118, with 29.625% cytostasis; at 0.625mg/ml, the CBPI was 3.412 and the RI was 1.412, with 18.968% cytostasis; and at 0.313mg/ml, the CBPI was 4.29 and the RI was 2.29, with 7.85% cytostasis.

Cytotoxicity Assessment - Extended Treatment without S9 Activation (S9-)

In the DMSO 1% control group, the CBPI was 3.596 and the RI was 1.596. For the Mitomycin 0.05µg/ml positive control, the CBPI was 3.156 and the RI was 1.156, resulting in 16.568% cytostasis. The Colchicine 0.1 µg/ml group had a CBPI of

3.1 and an RI of 1.1, with 19.146% cytostasis.

For XYX001, the CBPI and RI values were as follows: at 5mg/ml, the CBPI was 2.788 and the RI was 0.788, with 31.142% cytostasis; at 2.5mg/ml, the CBPI was 3.028 and the RI was 1.028, with 21.558% cytostasis; at 1.25mg/ml, the CBPI was 3.204 and the RI was 0.986, with 14.968% cytostasis; at 0.625mg/ml, the CBPI was 3.19 and the RI was 1.19, with 15.458% cytostasis; and at 0.313mg/ml, the CBPI was 4.029 and the RI was 2.092, with 16.05% cytostasis.

Table 1- Cytotoxicity Assessment - Short-Term Treatment with and without S9 Activation (1000 cells)

Treatment	Condition	Mono-nucleate	Bi-nucleate	Multi-nucleate	CBPI	RI	% Cytostasis
DMSO 1%	S9- (ST)	434	202	364	3.85	1.85	NA
	S9+ (ST)	380	352	268	4.028	2.028	NA
Mitomycin 0.05µg/ml	S9- (ST)	628	346	26	2.844	0.844	35.291
Benzo(a)pyrene 5µg/ml	S9+ (ST)	576	304	120	2.99	0.99	33.55
Colchicine 0.1µg/ml	S9- (ST)	632	280	88	2.978	0.978	30.587
XYX001 5mg/ml	S9- (ST)	684	286	30	2.744	0.744	38.799
	S9+ (ST)	684	274	42	2.738	0.738	42.262
XYX001 2.5mg/ml	S9- (ST)	552	356	92	3.14	1.14	24.903
	S9+ (ST)	510	302	188	3.158	1.158	27.686
XYX001 1.25mg/ml	S9- (ST)	488	388	124	3.152	1.152	24.505
	S9+ (ST)	566	320	114	3.118	1.118	29.625

XYX001 0.625mg/ml	S9- (ST)	630	258	112	3.06	1.06	27.706
	S9+ (ST)	428	290	282	3.412	1.412	18.968
XYX001 0.313mg/ml	S9- (ST)	218	422	360	4.298	2.298	13.58
	S9+ (ST)	202	430	368	4.29	2.29	7.85

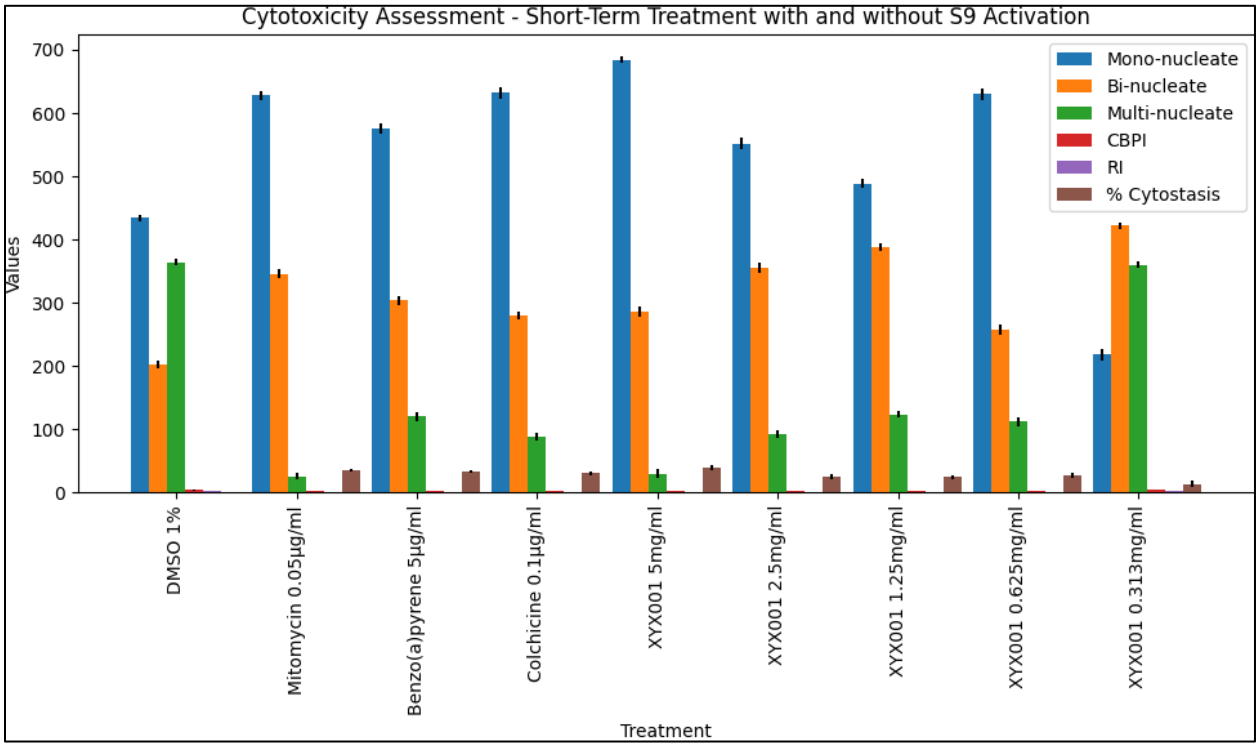

Fig.1- Short-Term Treatment with and without S9 Activation

Table 2- Cytotoxicity assessment- (s9-) extended treatment (1000 cells)

Treatment	Mono-nucleate (Mean±SD)	Bi-nucleate (Mean±SD)	Multi-nucleate (Mean±SD)	CBPI (Mean± SD)	RI (Mean± SD)	% Cytostasis (Mean±SD)
DMSO 1%	309±15.0	584±12.0	107±27.1	3.596±0	1.596±0	NA
Mitomycin 0.05µg/ml	537±73.2	348±28.3	115±45.3	3.156±0	1.156±0	16.568±0
Colchicine 0.1µg/ml	494±39.6	462±8.5	44±31.1	3.1±0	1.1±0	19.146±0
XYX001 5mg/ml	647±43.8	312±36.8	41±7.1	2.788±0	0.788±0	31.142±0

XYX001 2.5mg/ml	580±36.8	326±62.2	94±99.0	3.028±0	1.028±0	21.558±0
XYX001 1.25mg/ml	503±57.9	392±67.8	105±9.9	3.204±0	0.986±0	14.968±0
XYX001 0.625mg/ml	500±45.2	405±38.2	95±7.1	3.19±0	1.19±0	15.458±0
XYX001 0.313mg/ml	274±36.8	406±11.3	320±25.5	4.029±0	2.092±0	16.05±0

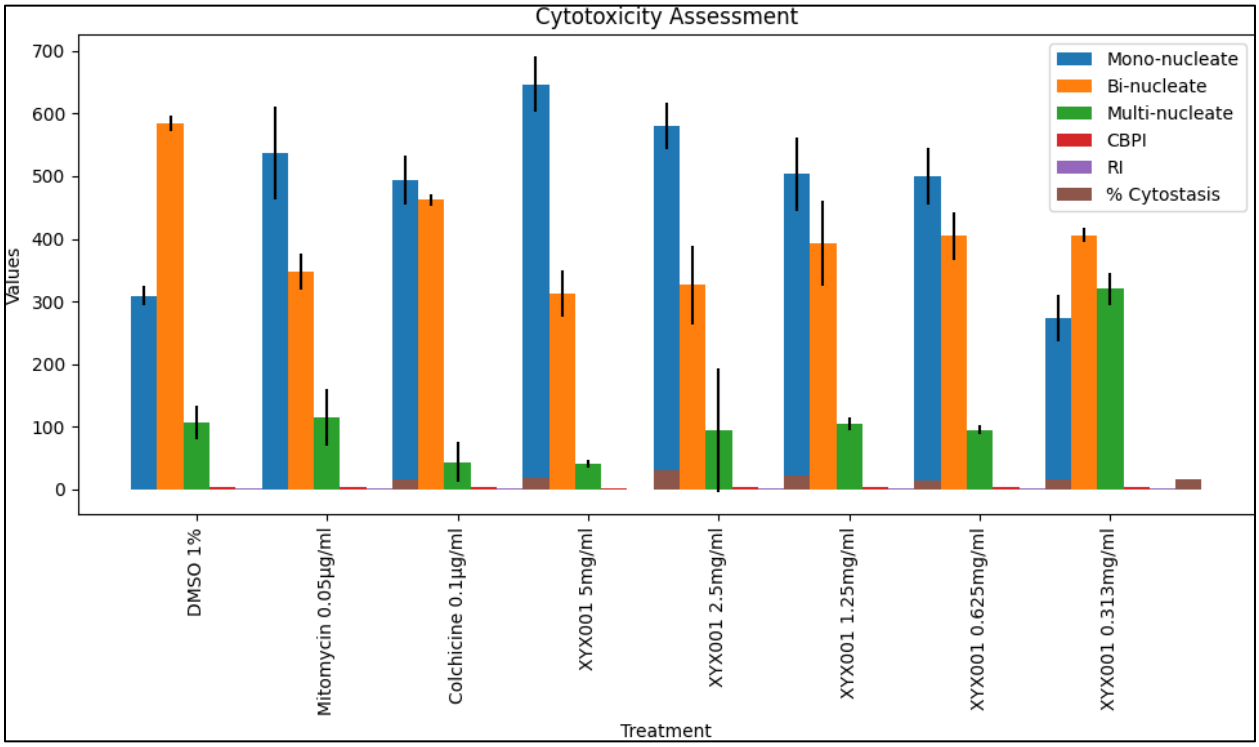

Fig.2- Cytotoxicity assessment- (s9-) extended treatment

Main Study Results

The main study was conducted to assess the cytotoxicity and genotoxicity of the test compound *XYX001*. The study was designed in accordance with the OECD guidelines for the testing of chemicals 478. The results are presented in a series of tables, each corresponding to different treatment conditions and endpoints.

Cytotoxicity Assessment - Short-Term Treatment without S9 Activation (S9-)

The DMSO 1% control group exhibited a CBPI of 1.90 and an RI of 0.90. Mitomycin at 0.05µg/ml, used as a positive control,

showed a CBPI of 1.43 and an RI of 0.44, resulting in 51.46% cytostasis. Colchicine at 0.1µg/ml had a CBPI of 1.48 and an RI of 0.49, with 46.09% cytostasis.

For XYX001, the CBPI and RI values were as follows:

- At 2.5mg/ml, the CBPI was 1.52 and the RI was 0.52, with 41.58% cytostasis.
- At 1.25mg/ml, the CBPI was 1.53 and the RI was 0.55, with 41.49% cytostasis.
- At 0.625mg/ml, the CBPI was 1.56 and the RI was 0.56, with 37.18% cytostasis.

Table 3- ***Cytotoxicity Assessment - Short-Term Treatment without S9 Activation (S9-)***

Treatment	**Mono-nucleate (Mean±SD)**	**Bi-nucleate (Mean±SD)**	**Multi-nucleate (Mean±SD)**	**CBPI (Mean±SD)**	**RI (Mean ±SD)**	**% Cytostasis (Mean±SD)**
DMSO 1%	216±10.4	116.75±18.8	167.25±11.8	1.9±0	0.9±0	NA
Mitomycin 0.05µg/ml	311.25±12.9	156.5±27.2	32.25±21.8	1.43±0	0.44±0	51.46±0
Colchicine 0.1µg/ml	289.25±17.6	176±20.8	34.75±8.7	1.48±0	0.49±0	46.09±0
XYX001 2.5mg/ml	285.5±24.8	165.75±16.9	48.75±16.2	1.52±0	0.52±0	41.58±0
XYX001 1.25mg/ml	283.5±24.2	154.25±6.9	62.25±7.9	1.53±0	0.55±0	41.49±0
XYX001 0.625mg/ml	252±6.7	212±17.8	36±22.2	1.56±0	0.56±0	37.18±0

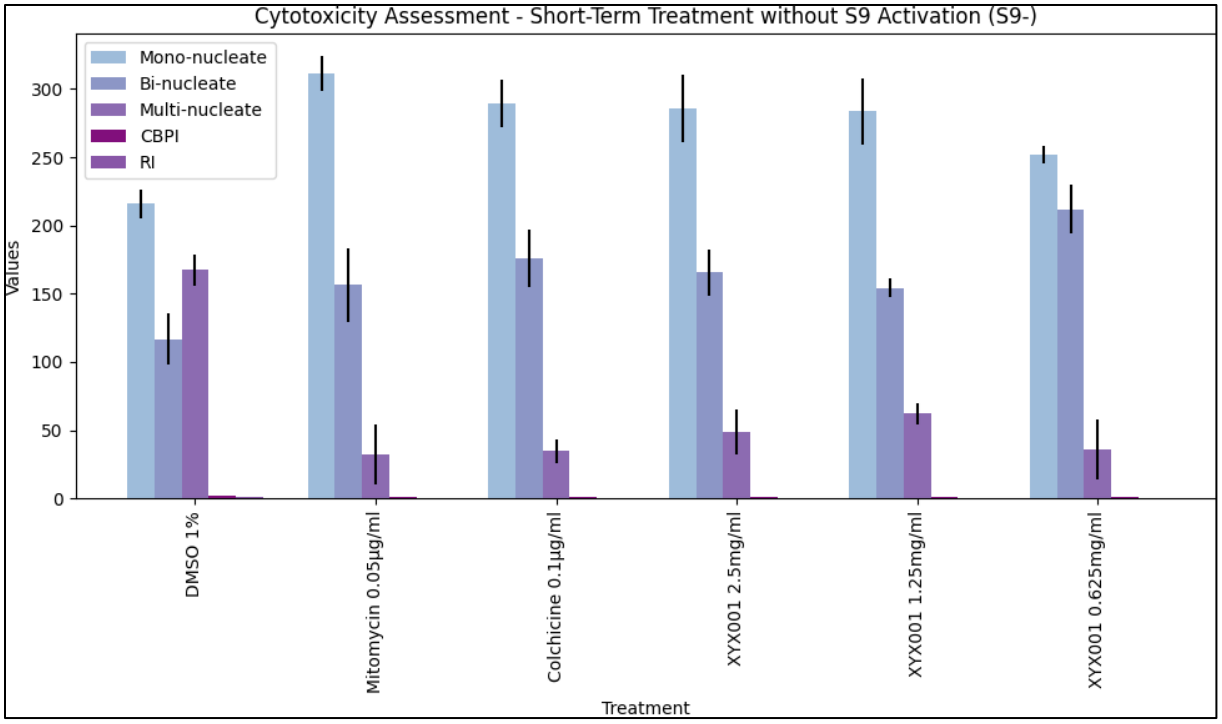

Fig.3- Short-Term Treatment without S9 Activation (S9-)

Cytotoxicity Assessment - Short-Term Treatment with S9 Activation (S9+)

The DMSO 1% control group had a CBPI of 2.02 and an RI of 1.03. Benzo (a) pyrene at 5µg/ml showed a CBPI of 1.52 and an RI of 0.53, resulting in 47.96% cytostasis.

For *XYX001*, the CBPI and RI values were as follows:

- At 2.5mg/ml, the CBPI was 1.57 and the RI was 0.58, with 42.61% cytostasis.
- At 1.25mg/ml, the CBPI was 1.59 and the RI was 0.59, with 40.75% cytostasis.
- At 0.625mg/ml, the CBPI was 1.62 and the RI was 0.63, with 37.13% cytostasis.

Table 4- Cytotoxicity Assessment - Short-Term Treatment with S9 Activation (S9+)

Treatment	Mono-nucleate (Mean ± SD)	Bi-nucleate (Mean ± SD)	Multi-nucleate (Mean ± SD)	CBPI (Mean ± SD)	RI (Mean ± SD)	% Cytostasis (Mean ± SD)
DMSO 1%	165.75 ± 25.48	152.25 ± 17.05	182 ± 35.44	2.02 ± 0	1.03 ± 0	NA
Benzo(a)p	282.5 ± 12.58	169.5 ±	48 ± 24.49	1.52 ± 0	0.53 ±	47.96 ± 0

yrene 5µg/ml		15.51			0	
XYX001 2.5mg/ml	268.5 ± 10.61	172.75 ± 20.41	58.75 ± 31.23	1.57 ± 0	0.58 ± 0	42.61 ± 0
XYX001 1.25mg/ml	264 ± 19.21	172.5 ± 17.68	63.5 ± 31.5	1.59 ± 0	0.59 ± 0	40.75 ± 0
XYX001 0.625mg/ml	257.25 ± 26.3	169.5 ± 22.2	73.25 ± 47.83	1.62 ± 0	0.63 ± 0	37.13 ± 0

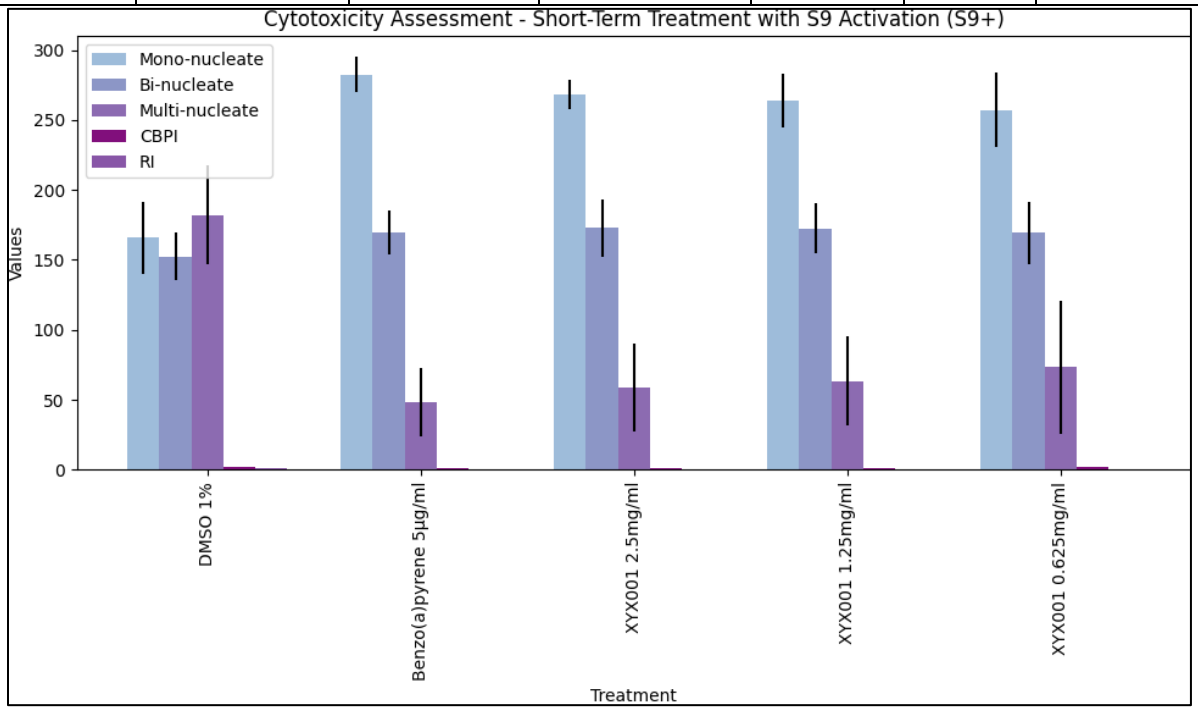

Fig.4-Short-Term Treatment with S9 Activation (S9+)

Cytotoxicity Assessment - Extended Treatment without S9 Activation (S9-)

The DMSO 1% control group had a CBPI of 1.89 and an RI of 0.89. Mitomycin at 0.05µg/ml showed a CBPI of 1.59 and an RI of 0.59, resulting in 31.86% cytostasis. Colchicine at 0.1µg/ml had a CBPI of 1.54 and an RI of 0.54, with 37.97% cytostasis.

For XYX001, the CBPI and RI values were as follows:

- At 2.5mg/ml, the CBPI was 1.55 and the RI was 0.56, with 35.33% cytostasis.
- At 1.25mg/ml, the CBPI was 1.55 and the RI was 0.60, with 36.54% cytostasis.
- At 0.625mg/ml, the CBPI was 1.57 and the RI was 0.57, with 34.28% cytostasis.

Table 5- Cytotoxicity Assessment - Extended Treatment without S9 Activation (S9-)

Treatment	Mono-nucleate (Mean ± SD)	Bi-nucleate (Mean ± SD)	Multi-nucleate (Mean ± SD)	CBPI (Mean ± SD)	RI (Mean ± SD)	% Cytostasis (Mean ± SD)
DMSO 1%	144 ± 20.74	264.75 ± 27.67	91.25 ± 50.42	1.89 ± 0	0.89 ± 0	NA
Mitomycin 0.05µg/ml	265.75 ± 29.79	170.5 ± 11.26	63.75 ± 28.92	1.59 ± 0	0.59 ± 0	31.86 ± 0
Colchicine 0.1µg/ml	255.25 ± 18.5	217.25 ± 14.16	27.5 ± 13.44	1.54 ± 0	0.54 ± 0	37.97 ± 0
XYX001 2.5mg/ml	267.5 ± 24.35	185 ± 34.64	47.5 ± 29.07	1.55 ± 0	0.56 ± 0	35.33 ± 0
XYX001 1.25mg/ml	256.5 ± 14.43	184.5 ± 17.68	59 ± 20.59	1.55 ± 0	0.60 ± 0	36.54 ± 0
XYX001 0.625mg/ml	256.5 ± 15.51	198 ± 18.44	45.5 ± 9.19	1.57 ± 0	0.57 ± 0	34.28 ± 0

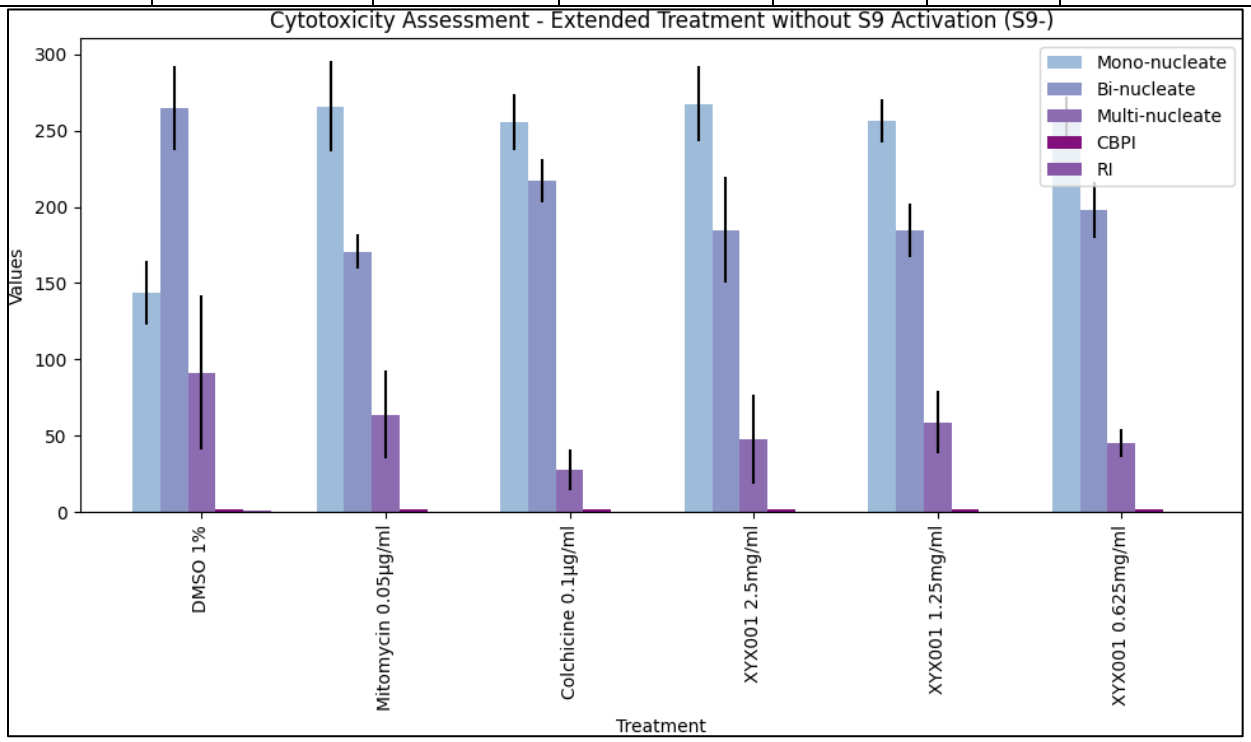

Fig.5- Extended Treatment without S9 Activation (S9-)

Micronucleus Scoring Summary

The micronucleus scoring summary tables indicate the frequency of micronuclei in bi-nucleated cells for each treatment group. The percentage of micronuclei was calculated and expressed as mean±SD. The significant criteria were fixed as P = <0.05.

For example, the DMSO 1% control group had 0.9±2.645% micronuclei, while Mitomycin at 0.05µg/ml had 6.25±2.986% micronuclei. For *XYX001* at 2.5mg/ml, the percentage of micronuclei was 28.15±2.645%.

Table 6- Micronucleus Scoring Summary

Treatment	Condition	Bi-nucleate (Mean ± SD)	Bi-nucleate with micronucleus (Mean ± SD)	% MN (Mean ± SD)
DMSO 1%	S9- (ST)	495.5 ± 2.38	4.5 ± 2.38	0.9 ± 2.645
	S9+ (ST)	495.25 ± 2.87	4.75 ± 2.87	0.95 ± 2.986
	S9- (ET)	496 ± 1.41	4 ± 1.41	0.8 ± 1.825
Mitomycin 0.05µg/ml	S9- (ST)	468.75 ± 2.63	31.25 ± 2.22	6.25 ± 2.986
	S9- (ET)	472.75 ± 2.38	27.25 ± 2.38	5.45 ± 3.201
Colchicine 0.1µg/ml	S9- (ST)	479 ± 3.74	21 ± 3.74	4.2 ± 4.082
	S9- (ET)	477.75 ± 1.89	22.25 ± 1.89	4.45 ± 2.217
Benzo (a) pyrene 5µg/ml	S9+ (ST)	466 ± 1.83	34 ± 1.83	6.8 ± 2.160
XYX001 2.5mg/ml	S9- (ST)	495.5 ± 2.38	4.5 ± 2.38	28.15 ± 2.645
	S9+ (ST)	495.5 ± 1.73	4.5 ± 1.73	0.9 ± 1.914
	S9- (ET)	495.25 ± 2.38	4.75 ± 2.38	0.95 ± 2.986
XYX001 1.25mg/ml	S9- (ST)	494 ± 2.16	6 ± 2.16	1.2 ± 2.943
	S9+ (ST)	492.75 ± 2.22	7.25 ± 2.22	16.55 ± 2.217
	S9- (ET)	494.75 ± 2.22	5.25 ± 2.22	1.05 ± 2.5
XYX001 0.625mg/ml	S9- (ST)	495.75 ± 2.63	4.25 ± 2.87	0.85 ± 3.304
	S9+ (ST)	495 ± 2.16	5 ± 2.16	1.00 ± 2.581
	S9- (ET)	495.5 ± 1.73	4.5 ± 1.73	0.9 ± 2.886

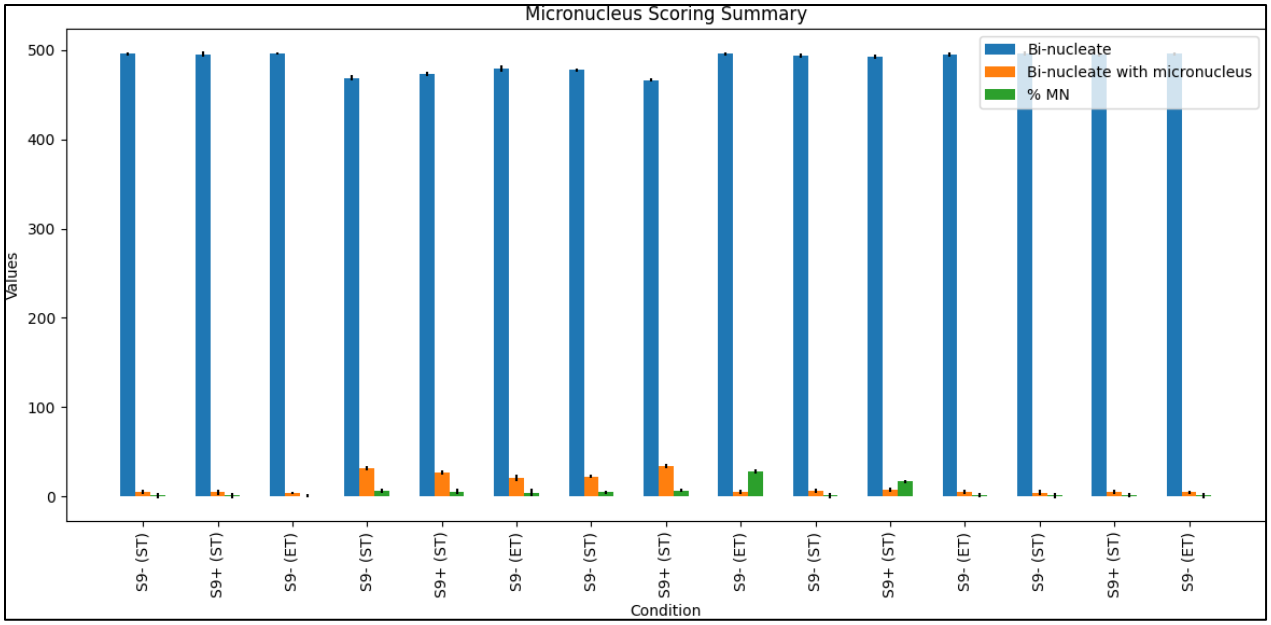

Fig.6- Micronucleus Scoring Summary

DISCUSSION

Study Design and Objectives

The study was meticulously designed to assess the genotoxic and cytotoxic potential of the test compound *XYX001*, in compliance with OECD Guideline 487. The V79 rodent cell line was chosen as the test system, and the study was conducted both in the presence and absence of an exogenous metabolic activation system (S9 mix). The objective was to evaluate the clastogenicity and micronucleus formation induced by *XYX001*.

Dose Range Finding (DRF) Study

The DRF study was pivotal in identifying the appropriate concentrations for the main study. Five concentrations were initially tested, ranging from 0.313mg/ml to 5mg/ml. The cytotoxicity assessment revealed that *XYX001* at 5mg/ml was cytotoxic both in the presence and absence of S9 mix, with %cytostasis values of 42.26 and 38.79, respectively. Concentrations of 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, and 0.313mg/ml did not exhibit cytotoxicity, thereby making them suitable candidates for the main study.

Main Study: Cytotoxicity Assessment

The main study was conducted at three selected concentrations: 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml. These concentrations were found to be non-cytotoxic in both the presence and absence of S9 mix, corroborating the findings from

the DRF study. The %cytostasis values were comparable to those of the positive controls, Benzo(a)pyrene and Mitomycin C, thereby confirming the validity of the test system.

Main Study: Micronucleus Assessment

The micronucleus assay was conducted under three different conditions: short-term without S9, short-term with S9, and extended-term without S9. The results were intriguing. While the positive controls (Benzo(a)pyrene and Mitomycin C) showed a significant increase in micronucleus formation, *XYX001* did not induce any statistically significant increase in micronucleus frequency at any of the tested concentrations. This was consistent across all three exposure conditions.

Validation of the Study

The positive control results were within the historical range, confirming the sensitivity of the test system and the effectiveness of the S9 mix. This adds an extra layer of validity to the study. The negative controls also fell within the historical range, further corroborating the study's validity.

Conclusions and Implications

The study provides compelling evidence that *XYX001* does not induce cytotoxicity or micronucleus formation at the concentrations tested, under the conditions of this study. The absence of genotoxicity and cytotoxicity in *XYX001* is a promising indicator of its safety profile, although it is crucial to note that these results are specific to the V79 rodent cell line and the conditions under which the study was conducted.

The study's robust design, adherence to OECD guidelines, and the consistency of the results with historical controls add to the reliability of these findings. However, it would be scientifically prudent to conduct further in vivo studies to corroborate these in vitro results, as well as to explore the compound's potential effects in different biological systems and under varying conditions.

In summary, *XYX001* appears to be non-genotoxic and non-cytotoxic under the conditions of this study, but further research is warranted to confirm these findings and to explore the compound's safety profile more comprehensively.

Conclusion

Summary of Findings

The study was executed with scientific rigor, adhering to OECD Guideline 487, to

evaluate the genotoxic and cytotoxic potential of the test compound *XYX001*. The V79 rodent cell line served as the experimental model, and the study was conducted both in the presence and absence of an exogenous metabolic activation system (S9 mix).

The positive controls, Benzo(a)pyrene and Mitomycin C, exhibited the expected increase in micronuclei formation, thereby validating the sensitivity of the test system and the effectiveness of the S9 mix. These results were within the historical range, adding an additional layer of confidence in the assay's validity.

In stark contrast, *XYX001* did not induce cytotoxicity or clastogenicity at the concentrations tested. Specifically, at the highest concentration of 2.5mg/ml, *XYX001* was found to be non-cytotoxic and non-clastogenic in the in vitro micronucleus test.

Interpretation and Implications

The absence of cytotoxic and clastogenic effects in *XYX001* is a promising indicator of its safety profile, at least under the specific conditions of this study and in the V79 rodent cell line. This could have significant implications for the further development and potential applications of *XYX001*, particularly if these findings are corroborated by additional studies.

Validation and Reliability

The study's robust design, the consistency of the positive and negative control results with historical data, and the adherence to international guidelines collectively contribute to the reliability and validity of these findings.

Future Directions

While the results are promising, it is scientifically prudent to recommend further in vivo studies to validate these in vitro findings. Additional research should also explore the compound's effects in different biological systems and under varying conditions to establish a comprehensive safety profile.

Final Conclusion

In summary, *XYX001* was found to be non-cytotoxic and non-clastogenic at the highest tested concentration of 2.5mg/ml in the V79 rodent cell line under the conditions of this study. The study is valid, and the findings are reliable, serving as a foundational step for future research into the safety and potential applications of *XYX001*.

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