Safety assessment of Polyhexamethylene biguanide

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ABSTRACT — OBJECTIVE: *The use of antiseptics in the treatment of genital infections has become a systematic alternative to antibiotics. Their use has been also supported for treatment of virus induced genital infections. Cationic compounds, such as polyhexamethylene biguanide (PHMB) and chlorhexidine, represent useful treatment options given their high therapeutic index and broad-spectrum activity. However, concerns have been raised since some compounds have developed bacterial resistance. Contrasting results have been reported on the mutagenic potential of PHMB products and safety concerns have been raised for their clinical use. Given the paucity of data on PHMB mutagenic potential, we performed a mutagenic test on Monogin®-isotonic gynecological solution (Monogin®; Lo.Li. Pharma S.r.l., Rome, Italy), a PHMB solution used for treatment of genital tract infections.*

MATERIALS AND METHODS: *We used the bacterial reverse mutation assay (Ames test), an* in vitro *assay testing the mutagenic potential of new chemicals and drugs, on multiple* Salmonella typhimurium *strains. The test was performed in compliance with the principles of Good Laboratory Practice (GLP).*

RESULTS: *Monogin® solution did not cause any mutation in all the strains tested, compared to positive controls.*

CONCLUSIONS: *Monogin® solution does not carry any mutagenic potential and although further investigations are needed, it can be considered a safe and useful therapeutic approach for treatment of genital tract infections.*

KEYWORDS
Polyhexamethylene biguanide, PHMB, Antiseptics, Polyhexanide, Ames test, Mutagenicity, Genital tract infections.

INTRODUCTION

Different microorganisms live in the genital flora in a fragile balance, which is disrupted when infections arise. Infections can be caused by bacteria, fungi, parasites, viruses and are involved in the impairment of various physiological functions, including reproduction[1,2]. Mycoplasma, gonococcal, Pseudomonas aeruginosa and Gram-positive cocci infections are involved in decrease of fertility in both males and females[2,3]. In females they may also cause ectopic pregnancies and chronic pelvic pain[4]. *Candida albicans* cause infections in males[2], while in females is a common commensal, although, in particular conditions, it may trigger vaginitis and cervicitis[2]. *Enterobacteriaceae*, like *Escherichia coli* or *Enterococcus faecalis* are the most common causes of non-sexually transmitted infections [2]. Bacterial vaginosis is the most common disorder of the vaginal flora, caused by different microbial species, with a prevalence of 5–30% in adult females[5]. Usually, it is treated with antibiotics such as clindamycin or metronidazole[5], however the raising of antibiotic resistance has made antimicrobial chemotherapy increasingly challenging[5,6]. Moreover, new insights into the structure and function of the colonization of the vagina have helped to understand the mechanisms involved in microbial biofilms and the limited efficacy of some antibiotic treatments. Studies have demonstrated that local treatments of genital tract infections show

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less systemic side effects like nausea and vomiting, compared to oral antibiotics[7].

Viral infections of the genital tract are mostly caused by herpes viruses, such as human papilloma viruses (HPV), and human immunodeficiency viruses (HIV). These infections can be sexually transmitted and represent an important public health issue. Genital wart and dysplastic areas of cellular proliferation are the clinical manifestation of genital HPV[8]. HPV infections have been associated to the onset of cervical intraepithelial squamous lesions, cervical cancer in women, and have potentially been related with other anogenital malignancies[4,9]. Current available treatments include cytotoxic, physically ablative, excisional, and immunomodulatory therapies for genital warts. However, none of them can be considered totally effective in eradicating the infection[10,11], and some come with important safety issues[2,12-14].

Among non-surgical options, antiseptics are an excellent alternative to antibiotic treatment, given their broad antimicrobial spectrum, low toxicity, and good applicability. Chlorhexidine digluconate (CHX) has been used as a general vaginal antiseptic for over thirty years[15], but rat experiments highlighted serious adverse effects, including a significant deoxyribonucleic acid (DNA) damage[16,17]. Additionally, cytotoxic and mutagenic effects have been observed in osteoblasts and odontoblast-like cells[18-20], as well as the production of 2-chloroanaline, a well-known carcinogen[21,22].

The antiseptic polyhexamethylene biguanide (PHMB) is structurally similar to CHX, but it does not generate 2-chloroanaline, as by-product, and it is well tolerated when used on skin, wounds, eyes, and vaginal mucous membrane[3,23,24]. No adverse events have been reported so far for PHMB[23], nor indication of mutagenicity or carcinogenicity, *in vitro* or *in vivo*[25,26]. However, few studies have addressed directly the mutagenic effect of PHMB-containing products and contrasting results have been obtained[3]. In order to provide further data on this issue, the mutagenic activity of Monogin® isotonic gynecological solution (for the treatment of infections of the genital tract) was evaluated through the Ames test, a standardized and validated laboratory procedure[3].

MATERIAL AND METHODS

The present experiments were performed at Eurofins Biolab srl and conducted in compliance with the standards of Good Laboratory Practice (GLP) of: OECD Series (Environment Directorate-Organization for Economic Co-Operation and Development, Paris, 1998); Legislative decree n. 50 of March the 2nd, 2007, Enforcement of Community Directives 2004/9/CE and 2004/10/CE, concerning the inspection and verification of GLP and drawing of the legislative, regulatory and administrative dispositions relative to the application of GLP rules, to the control of their application on the assays performed on chemical substances (GU n. 86 of April the 13th, 200); United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments; Certification N. 038/2013 released by the Italian Ministry of Health on November 19th, 2013.

Materials

Monogin®-isotonic gynecological solution, pH 4.0 (Monogin®; Lo.Li. Pharma S.r.l., Rome, Italy) consists of hydroxyethylcellulose, lactic acid, glycerol, potassium chloride, sodium edetate, PHMB, purified water; Phosphate Buffer 0.2 M, pH 7.4 (PBS, Moltox, Boone, NC, USA); Sodium Azide, 9-Aminoacridine, 2-Nitrofluorene, Mitomicyn C, 2-Aminoantracene, Ciclophospamide (Moltox, NC, USA); Nutrient Broth, Top agar supplemented (0.05 Mn Biotin and Histidine), S9, Regensys A (45 ml) and Regensys B and crystal violet disks (Moltox, USA); QUAD PC plates, Nutrient agar plate (TSA) (Oxoid, Moltox, USA plate and Minimal Glucose Agar (MGA) plate (Moltox, USA).

Methods

Cell strains preparation and validation

Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and TA102 were used. Strains were kept as lyophilized discs at 5 ± 3°C. One day before the experiment the strains were suspended in 100 ml of cultural medium (Tryptic soy Broth, TSB, Moltox, USA), and incubated at 37 ± 1°C for 15-18 hours, in order to allow cultures to reach exponential growth and expose a high number of cells to mutagens. During the test, cultures were kept on ice in order to avoid their vitality reduction.

Each strain was validated for the following genetic characteristic: histidine requirement; presence or absence of R-factor plasmid, when appropriate (ampicillin resistance for TA98 and TA100 strains; ampicillin + tetracycline resistance for TA102 strain); the presence of mutations for UVrB and rfa genes. The range of number of spontaneous revertant frequency was also validated.

Assay validity criteria

Microbiological controls were performed on minimal glucose agar (MGA) and tryptic soy agar (TSA) plates with samples at the highest concentrations; full medium (top agar supplemented, solvent, PBS and S9mix) and no more than two colonies were allowed

as contaminants. On average, for TA1535, TA1537 and TA98 strains, the number of revertant colonies developed by the positive control was at least 300% higher than the respective negative control (lower limit for positive control/negative control ratio for strains with lower mutation frequency); for TA100 and TA102 strains, the number of revertant colonies developed by the positive control was at least 200% higher than the negative control (a 20% variation is accepted at the lower limit of the positive control/ negative control ratio in strains with high mutation frequency). The number of spontaneously revertant colonies per plate in negative controls was included between the validated limits. These criteria were internal method validation only, since OECD 471 guideline does not provide any reference thereof[27].

Ames Test

The Ames test is a bacterial reverse mutation test that is formally used to assess the mutagenic potential of chemical compounds. It uses several strains of bacteria (*i.e. Salmonella Typhimurium*) that carry mutation[21]. These mutations revert the ability of tested bacterial strains to produce the amino acid. To allow microorganism cell survival and show possible mutations, MGA and an overlay agar (top agar) containing small amounts of histidine and biotin, were used. Particularly sensitive bacterial strains were used in the test. Their features included: responsive DNA sequences at the reversion sites, increased cell permeability to large molecules, elimination of DNA repair systems or enhancement of error prone DNA-repair processes. The revertant bacteria were detected by their ability to grow in the absence of the aminoacid required by the parental strain [21]. The whole assay was performed twice.

Preparation of the assay sample

The solvent was tested for chemical reaction with the test substance and for being compatible with bacterial survival and S9 activity. The test substance was examined as a solution in water at the equivalent concentration of 50 mg/ml and 4 subsequent dilutions of semi-log intervals between them, were prepared at the concentration of 15 mg/ml, 4.5 mg/ml, 1.35 mg/ml and 0.41 mg/ml.

Plates without metabolic activation

0.1 ml of assay sample, 0.1 ml of the bacterial cell suspension and 0.5 ml of PBS were added to the aliquoted tubes containing the top agar supplemented, then briefly stirred and poured into MGA plates. Negative control (water and frequency of mutation), solvent controls and positive controls were also prepared. The plates hence incubated at 37 ± 1°C for 48 hours.

Plates with metabolic activation

0.1 ml of assay sample, 0.1 ml of bacterial cell suspensions and 0.5 ml of the enzymatic system for metabolism activation were added to the aliquoted tubes containing the top agar supplemented, then briefly stirred and poured into MGA plates. Negative control, solvent controls and positive controls were also prepared. The plates then incubated at 37 ± 1°C for 48 hours.

Afterwards, the reverted colonies of the assay samples at the different concentrations (with and without activation), as well as those of negative controls and positive controls were counted in each plate. Plate test with metabolic activation. Three replicates were performed for all samples, including negative and positive controls.

Enzymatic system for metabolism activation

The metabolism activation is an enzymatic system based on the addition to the culture medium of the S9 mix (Moltox, USA, a hepatic homogenate obtained from the liver of adult male rats, previously induced with Aroclor 1254 soybean oil solution) to Regensys A and to Regensys B containing respectively PBS and Glucose-6 phosphate and 1153 mg nicotinamide adenine dinucleotide phosphate (NADP) for the activation. S9 mix was previously tested for sterility to exclude any possible contamination.

Statistical analysis

Data are expressed as a mean number of revertant colonies per plate and the mean number of spontaneous revertant colonies counted on solvent plates, with the standard deviation (SD). One-way ANOVA with uncorrected Fisher's LSD test was performed and *p*-values less than 0.05 (Confidence Interval 95%) were considered statistically significant. The analysis was conducted using GraphPad Prism version 7.00d for Mac OS X (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). The result was considered negative when no statistically significant difference between the negative control and test groups was noted.

RESULTS

Preliminary tests

The verification of genetic characteristics showed that the tested strains maintained the required genetic properties in both assay repetitions (data not shown). The microbiological control performed on the assay sample, solvent, Top agar, PBS and S9 mix did not show any contamination (data not shown). Not toxic effects of PHMB were observed, either in the presence or absence of the enzymatic system for metabolism activation.

Safety assessment of PHMB

The mutagenic potential of PHMB was evaluated through the Ames test (Table 1). The number of revertant colonies obtained for negative, positive controls and for each sample, was similar in the two experimental repetitions (Table 1), demonstrating the test reproducibility.

The number of spontaneous revertant colonies obtained during the test, either with or without S9 metabolic activation, did not exceed the established limits, whereas the positive controls displayed a significant increase (Table 2).

The analysis of the revertant colonies counted in all the sample plates, either with or without S9 activation, revealed no significant increase compared to the negative controls (Table 2). No significant difference was detected among the four PHMB dilutions used in the test (Table 1 and 2).

DISCUSSION

The increasing development of antibiotic resistance has emerged as a crucial challenge in treating microorganisms. Antiseptics, in particular cationic compounds, have demonstrated to be valuable options for antimicrobial treatments. Among cationic compounds, PHMB and CHX represent broad-spectrum antiseptics, active against fungi and protozoa, bacteria and viruses[24].

PHMB directly damage the cytoplasmic membrane of microorganisms, resulting in a non-specific, immediate, and irreversible loss of selective permeability[24]. Specifically, PHMB causes a disorganization and consequently high fluidity, and permeability of the bacterial cytoplasmic membrane[28], while it is less active against mammalian cell membranes[29,30]. Moreover, other authors reported a specific disruption of the bacterial metabolism[28,31,32]. These results explain the high therapeutic index attributed to this compound[3,24].

Studies have also demonstrated that PHMB kills methicillin-resistant *Staphylococcus aureus* within keratinocytes by direct interactions inside the host cells, through a dynamin-dependent uptake mechanism[33]. Moreover, recent results proved that PHMB enters bacterial cells preventing cell division and chromosome condensation, and resulting in intracellular foci of DNA[25]. Furthermore, PHMB selectively binds to bacterial DNA, since it is confined inside endosomes and excluded from mammalian cell nuclei[25]. Ex vivo experiments also demonstrated that PHMB, along with other antiseptics, prolongs the lag phase for bacterial regrowth[34]. Finally, PHMB blocks the microbial attachment to surfaces and effectively removes biofilms *in vitro* and *in vivo*[23].

CHX has been used for decades as antiseptic for treatment of the genital tract diseases[35]. However, CHX can cause hyperkeratosis, ulceration, dysplasia and an increase of DNA damage in rats[16,36]. Furthermore, CHX inhibits wound healing in a concentration-dependent fashion[18]. Moreover, CHX may generate 2-chloroanline, which multiple studies reported owning a mutagenetic potential[22,37]. It emerged that resistant bacteria mutants may present low susceptibility to biocides, including CHX, along with antibiotics resistance[38,39]. The non-anchored cell wall (NCW) gene might play a role in the tolerance to PHMB during fungal infections[40], but no acquired resistant mutants to PHMB have been reported[24].

As mutations can result in cancer, mutagens are directly related to carcinogenesis[41]. Other Ames tests results on a PHMB product[42], used as an antibacterial preservative, showed no evidence of mutagenic, genotoxic or neurotoxic effects[3,32]. Primary screening tests, such as Ames test, play a central role in prevention by identifying mutagenic chemicals in the environment[21]. In utero exposure studies failed to find any effects on the offspring in a two-generation reproductive study[32].

Here, we report for the first time the results of a mutagenicity test on a product containing PHMB, Monogin®-isotonic gynecological solution, in order to determine whether it has a mutagenic potential. Our results proved that PHMB has no mutagenic effect on any of the *Salmonella typhimurium* strains used, since no spontaneous revertant colonies have been found, either with or without metabolic activation.

Surgical treatments in the genital tract include electrosurgery, surgical excision, cryotherapy, and laser surgery. Nonsurgical physician-prescribed and -applied therapies include podophylla resin, interferon (IFN), and bi- and tri-chloroacetic acid (BCA/TCA). Patient-applied treatments include podophyllotoxin, imiquimod, and 5-fluorouracil (5-FU)[43]. Imiquimod is approved by the Food and Drug Administration (FDA) for the treatment of external genital warts in patients aged 12 years and older, however safety and efficacy have not been evaluated in pregnant, breastfeeding, immunosuppressed patients, or in patients with intravaginal, cervical, rectal, or intra-anal warts[44]. Adverse events related to podophyllin resin misuse included fetal loss and even death[14,45]. Given these safety concerns, the use of antiseptics, represent a non-invasive and safe approach for treating genital infections. Our results represent an important step forward since Monogin®-isotonic gynecological solution is patented for the genital area. Specifically, PHMB products for genital treatments have contributed to the regression of HPV infections, particularly in patients with external genital warts[46] and cervical lesions[47]. Vaginal solutions containing PHMB are effective in the treatment of women affected by vaginal candidiasis[48] and bacterial vaginosis[47]. No safety issues, regarding mutagenic and carcinogenic potential were raised, even when PHMB solution were administered in combination with other products[47,49,50].

Table I. PHMB safety activity. Results obtained from two repetitions of Ames test on TA1535, T1537, TA98, TA100 and TA102 strains, without and with metabolic activation. Highlighted in bold samples with revertant colonies growth.

Strain Mean ± SD	Validated MFs	Control (MFs)	Negative Control (H_2O)	Positive Control	5 mg/ plate	1.5 mg/ plate	0.45 mg /plate	0.135 mg /plate	0.041 mg/ plate
Without activation									
TA1535	14±6	15.83±5.49	13.5±2.63	**636.3±166.62 (NaN_3)**	29.66±7.63	27.00±6.29	22.34±4.08	28.00±7.80	25.66±5.43
T1537	14±6	13.5±4.13	13.5±2.88	**636.3±83.31 (9-AAc)**	14.83±3.82	13.5±3.15	11.17±2.04	14±3.90	12.83±2.71
TA98	23±8	24.67±5.68	25±3.578	**603.2±97.7 (2-NF)**	21.5±5.01	21.5±4.76	23±5.86	23.33±3.61	26.17±3.66
TA100	124±48	119.2±22.86	127.2±29.59	**918.2±97.21 (NaN_3)**	139±20.90	124.2±21.74	134.7±19.66	129.2±35.97	129.8±35.85
TA102	247±47	258.3±33.57	249.5±25.41	**1270±219.9 (MMC)**	236.3±23.22	262.5±28.6	236.5±21.32	271±12.21	249±27.55
With activation									
TA1535	14±6	16.83±3.31	13.67±4.08	**624.2±64.46 (CP)**	10.33±2.50	14±3.03	15±3.74	13.67±3.20	14±3.79
T1537	14±6	12.83±3.97	15.67±2.87	**570.5±80.66 (2-AA)**	14.33±3.59	13.33±3.01	13.33±2.73	15.5±1.64	15.5±3.39
TA98	23±8	23.83±5.81	20.83±4.35	**623.7±96.28 (2-AA)**	21±3.41	22.83±6.05	22.83±5.95	22.17±3.19	22.17±3.76
TA100	124±48	122.7±24.55	110.5±31.42	**1066±248.80 (CP)**	129.3±34.92	131.7±34.92	121.2±15.35	119.2±27.75	114.5±20.15
TA102	247±47	243.8±24.37	247.8±33.52	**1287±211.10 (2-AA)**	258±24.48	253.7±35.7	226±20.34	242.2±29.93	253.2±28.51

Abbreviations: SD, standard deviation; MFs, Spontaneous mutation frequency; H_2O, water; NaN_3, sodium azide; 2-NF, 2-nitrofluorene; 9-AAc, 9-aminoacridine HCl; MMC, mitomycin C; 2-AA, 2-aminoanthracene; CP, cyclophosphamide.

Table II. Abbreviations: CI, confidence interval; Mean diff, mean difference; MFs, Spontaneous mutation frequency, internal validated reference; H_2O, water; NaN_3, sodium azide; 2-NF, 2-nitrofluorene; 9-AAc, 9-aminoacridine HCl; MMC, mitomycin C; 2-AA, 2-aminoanthracene; CP, cyclophosphamide; DF, degrees of freedom. One-way ANOVA with uncorrected Fisher's LSD test was performed; *p*-value <0.05 was considered statistically significant. Significant results are highlighted in bold.

Strain	Uncorrected Fisher's LSD	Mean Diff.	95% CI of diff.	Individual *p*-value
Without activation				
TA1535	Control (negative, H_2O) *vs.* Control (MFs)	-3167	-30.02 to 23.69	0.8128
	Control (negative, H_2O) *vs.* Control (positive, NaN_3)	-610.5	-637.4 to -583.6	<0.0001
	Control (negative, H_2O) *vs.* 5 mg/plate	3.33	-23.52 to 30.19	0.8032
	Control (negative, H_2O) *vs.* 1.5 mg/plate	-0.33	-27.19 to 26.52	0.9801
	Control (negative, H_2O) *vs.* 0.45 mg/plate	-1333	-28.19 to 25.52	0.9206
	Control (negative, H_2O) *vs.* 0.135 mg/plate	0	-26.85 to 26.85	>0.9999
	Control (negative, H_2O) *vs.* 0.041 mg/plate	-0.33	-27.19 to 26.52	0.9801
TA1537	Control (negative, H_2O) *vs.* Control (MFs)	-2333	-40.04 to 35.37	0.9011
	Control (negative, H_2O) *vs.* Control (positive, NaN_3)	-629.7	-667.4 to -592	<0.0001
	Control (negative, H_2O) *vs.* 5 mg/plate	-0.17	-37.87 to 37.54	0.9929
	Control (negative, H_2O) *vs.* 1.5 mg/plate	-1833	-39.54 to 35.87	0.9222
	Control (negative, H_2O) *vs.* 0.45 mg/plate	-1	-38.71 to 36.71	0.9575
	Control (negative, H_2O) *vs.* 0.135 mg/plate	0.17	-37.54 to 37.87	0.9929
	Control (negative, H_2O) *vs.* 0.041 mg/plate	-0.67	-38.37 to 37.04	0.9717
TA98	Control (negative, H_2O) *vs.* Control (MFs)	0.33	-40.3 to 40.96	0.9869
	Control (negative, H_2O) *vs.* Control (positive, 2-NF)	-578.2	-618.8 to -537.5	<0.0001
	Control (negative, H_2O) *vs.* 5 mg/plate	3.5	-37.13 to 44.13	0.8627
	Control (negative, H_2O) *vs.* 1.5 mg/plate	3.5	-37.13 to 44.13	0.8627
	Control (negative, H_2O) *vs.* 0.45 mg/plate	2	-38.63 to 42.63	0.9212
	Control (negative, H_2O) *vs.* 0.135 mg/plate	1667	-38.96 to 42.3	0.9343
	Control (negative, H_2O) *vs.* 0.041 mg/plate	-1167	-41.8 to 39.46	0.954
TA100	Control (negative, H_2O) *vs.* Control (MFs)	8	-42.06 to 58.06	0.7484
	Control (negative, H_2O) *vs.* Control (positive, NaN_3)	-791	-841.1 to -740.9	<0.0001
	Control (negative, H_2O) *vs.* 5 mg/plate	-11.83	-61.89 to 38.22	0.6354
	Control (negative, H_2O) *vs.* 1.5 mg/plate	3	-47.06 to 53.06	0.9042
	Control (negative, H_2O) *vs.* 0.45 mg/plate	-7.5	-57.56 to 42.56	0.7636
	Control (negative, H_2O) *vs.* 0.135 mg/plate	-2	-52.06 to 48.06	0.936
	Control (negative, H_2O) *vs.* 0.041 mg/plate	-2667	-52.72 to 47.39	0.9148
TA102	Control (negative, H_2O) *vs.* Control (MFs)	-8833	-103.7 to 85.99	0.8516
	Control (negative, H_2O) *vs.* Control (positive, MMC)	-1021	-1115 to -925.7	<0.0001
	Control (negative, H_2O) *vs.* 5 mg/plate	13.17	-81.66 to 108	0.7804
	Control (negative, H_2O) *vs.* 1.5 mg/plate	-13	-107.8 to 81.82	0.7831
	Control (negative, H_2O) *vs.* 0.45 mg/plate	13	-81.82 to 107.8	0.7831
	Control (negative, H_2O) *vs.* 0.135 mg/plate	-21.5	-116.3 to 73.32	0.6492
	Control (negative, H_2O) *vs.* 0.041 mg/plate	0.5	-94.32 to 95.32	0.9915

Continued

Table II *(continued).* Abbreviations: CI, confidence interval; Mean diff, mean difference; MFs, Spontaneous mutation frequency, internal validated reference; H_2O, water; NaN_3, sodium azide; 2-NF, 2-nitrofluorene; 9-AAc, 9-aminoacridine HCl; MMC, mitomycin C; 2-AA, 2-aminoanthracene; CP, cyclophosphamide; DF, degrees of freedom. One-way ANOVA with uncorrected Fisher's LSD test was performed; *p*-value <0.05 was considered statistically significant. Significant results are highlighted in bold.

Strain	Uncorrected Fisher's LSD	Mean Diff.	95% CI of diff.	Individual *p*-value
With activation				
TA1535	Control (negative, H_2O) vs. Control (MFs)	-3167	-39.1 to 32.77	0.8128
	Control (negative, H_2O) vs. Control (positive, CP)	-610.5	-646.4 to -574.6	<0.0001
	Control (negative, H_2O) vs. 5 mg/plate	3333	-32.6 to 39.27	0.8032
	Control (negative, H_2O) vs. 1.5 mg/plate	-0.33	-36.27 to 35.6	0.9801
	Control (negative, H_2O) vs. 0.45 mg/plate	-1333	-37.27 to 34.6	0.9206
	Control (negative, H_2O) vs. 0.135 mg/plate	0	-35.93 to 35.93	>0.9999
	Control (negative, H_2O) vs. 0.041 mg/plate	-0.33	-36.27 to 35.6	0.9801
TA1537	Control (negative, H_2O) vs. Control (MFs)	2833	-30.61 to 36.28	0.8649
	Control (negative, H_2O) vs. Control (positive. 2-AA)	-554.8	-588.3 to -521.4	<0.0001
	Control (negative, H_2O) vs. 5 mg/plate	1333	-32.11 to 34.78	0.9362
	Control (negative, H_2O) vs. 1.5 mg/plate	2333	-31.11 to 35.78	0.8886
	Control (negative, H_2O) vs. 0.45 mg/plate	2333	-31.11 to 35.78	0.8886
	Control (negative, H_2O) vs. 0.135 mg/plate	0.17	-33.28 to 33.61	0.992
	Control (negative, H_2O) vs. 0.041 mg/plate	0'.17	-33.28 to 33.61	0.992
TA98	Control (negative, H_2O) vs. Control (MFs)	-3	-43.06 to 37.06	0.8805
	Control (negative, H_2O) vs. Control (positive. 2-AA)	-602.8	-642.9 to -562.8	<0.0001
	Control (negative, H_2O) vs. 5 mg/plate	-0.17	-40.23 to 39.9	0.9933
	Control (negative, H_2O) vs. 1.5 mg/plate	-2	-42.06 to 38.06	0.9201
	Control (negative, H_2O) vs. 0.45 mg/plate	-2	-42.06 to 38.06	0.9201
	Control (negative, H_2O) vs. 0.135 mg/plate	-1333	-41.4 to 38.73	0.9467
	Control (negative, H_2O) vs. 0.041 mg/plate	-1333	-41.4 to 38.73	0.9467
TA100	Control (negative, H_2O) vs. Control (MFs)	-12.17	-119 to 94.65	0.8191
	Control (negative, H_2O) vs. Control (positive. CP)	-955.3	-1062 to -848.5	<0.0001
	Control (negative, H_2O) vs. 5 mg/plate	-18.83	-125.6 to 87.98	0.7234
	Control (negative, H_2O) vs. 1.5 mg/plate	-21.17	-128 to 85.65	0.6909
	Control (negative, H_2O) vs. 0.45 mg/plate	-10.67	-117.5 to 96.15	0.8411
	Control (negative, H_2O) vs. 0.135 mg/plate	-8667	-115.5 to 98.15	0.8706
	Control (negative, H_2O) vs. 0.041 mg/plate	-4	-110.8 to 102.8	0.94
TA102	Control (negative, H_2O) vs. Control (MFs)	4	-88.5 to 96.5	0.9308
	Control (negative, H_2O) vs. Control (positive. 2-AA)	-1039	-1131 to -946.3	<0.0001
	Control (negative, H_2O) vs. 5 mg/plate	-10.17	-102.7 to 82.33	0.8253
	Control (negative, H_2O) vs. 1.5 mg/plate	-5833	-98.33 to 86.66	0.8992
	Control (negative, H_2O) vs. 0.45 mg/plate	21.83	-70.66 to 114.3	0.6359
	Control (negative, H_2O) vs. 0.135 mg/plate	5667	-86.83 to 98.16	0.9021
	Control (negative, H_2O) vs. 0.041 mg/plate	-5333	-97.83 to 87.16	0.9078

CONCLUSIONS

Monogin® solution has no mutagenic potential and can be considered a safe and useful therapeutic approach for treatment of genital tract infections. Further experiments, both *in vitro* and *in vivo*, might support our results on the safety of PHMB solutions[21].

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Conflicts of interest:
B.O., P.L. and V.U. are employee at LO.LI. Pharma, Rome, Italy. The other author declares no conflict of interest.

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