Original article:

Mutagenic properties of modified hydrothermal nanotitania extract

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Abstract:

Backgrounds*:** The mutagenic properties of modified hydrothermal nanotitania extract were carried out using the Ames test (genotoxicity). ***Materials and methods*:** The Ames test was performed on Salmonella strains (TA98, TA100, TA1535, TA1537 and TA 102) which contain mutations in several genes with and without S9 metabolic activation from rat liver using the standard assay. The materials were extracted in distilled water and the serial dilutions of concentration ranging from 313 to 5000 µg/mLwere used after the incubation period of 24 h at 37° C. ***Results: These results suggested that all tested concentrations of the material extracts did not produce mutagenic effect in all the strains tested. ***Conclusions:*** Findings from this study showed that the modified hydrothermal nanotitania extract was non-mutagenic under present conditions.

Keywords: *Mutagenicity; Ames test; genotoxicity*

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Introduction

Ames *et al*.[1] developed the assay in an early 1970s. The test principle is the usage of a number of *S. typhimurium*strainswith the pre-existing mutations whichprevent the bacteria to synthesize the required amino acid (histidine) and therefore powerless to grow and forming colonies in the absence of the amino acid.[2] The Ames test is routinely used to assess the biocompatibility property and is applied worldwide as initial screening to determine the mutagenic potential of new chemical or drug.[3] This assay is often used to screen and predict whether substances might express mutagenic potential due to its rapid screening technique[3] together with sensitivity of its ability to induce mutations in DNA, which is the indicative of adverse changes at a cellular level.[3,5,6] Thus, the identification of substances capability of inducing mutations has become an important procedure in pre-commercialization of new materials. The aim of the present study was to determine the mutagenic properties of modified hydrothermal nanotitania extract, by testing it against*Salmonella* strainsby using Ames test.

Materials & methods

Chemical, test materials and preparation of test substance

All chemical reagents, media and solutions were prepared using sterilized deionized water in an ion-free and dust free laboratory environment. The materials were extracted with distilled water (5mg/mL) and were incubated at 37°C for 24 hours. The extraction substances were then collected into sterile

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syringes at the end of this period and filtered through a 0.45 µm filter (Millipore, UK). The tubes were then positioned in ultrasonic bath (Branson Ultrasonics, Danbury, CT, USA) for 30 minutes to break up the modified hydrothermal nanotitania extract compounds. Subsequently, these original extracts were then serially diluted (5000 µg, 2500 µg, 1250 µg, 625 µg, 313 µg) prior to assay.

Mutagenicity Screening

The Ames test was performed according to Maron and Ames[2]and fulfilling the criteria specified in OECD Guidelines 471: Bacterial Reverse Mutation Test.[7] The tester strains used in the assay were TA98, TA100, TA1535, TA1537 and TA 102. [5] Two separate experiments were performed, using triplicate plates in the presence or absence of S9 metabolic activation (S9 mix). Pure distilled water was used as negative control and different positive controls were used for different bacterial strains in both experiments. Fresh cultures of tester strains were grown to approximately 10^8 cells/ mL in 5 mL nutrient broth (Oxoid Ltd, Hampshire, UK). The cultures were shaken in the incubator for 12 h at 37° C to ensure adequate aeration. The single colony from each strain was checked for the genetic markers. Different concentrations of test material were plated triplicate with 0.1mL of overnight bacterial cultures per plate. The solutions were prepared in the absence (0.5 mL/ plate phosphate buffer 1M, pH=7.4) and presence of S9 mixture (0.5 mL/plate). The mixture containing chemicals and bacteria with or without S9 were mixed and added to 2 mL of top agar and pre-incubated at 37°C for 30 minutes. The vehicle and an appropriate mutagenic chemical were used as the negative and positive controls for each individual tester strains. Top agar was added to the treatment mixture, which was then poured onto glucose-supplemented minimal agar(GMA). After the top agar hardened, the plates were incubated in the dark at 37C for 48 hours. After incubation, the plates were observed and the revertant colonies were counted manually.

Statistical and data analysis for determining the mutagenicity

The data was presented as the number of revertant colonies per plate. The numbers of revertant colonies on both negative and positive control plates were also counted and were defined as described previously[8, 10]. Ifthe treatments caused dose-related increase and at least in one strain induced two-fold or more change in the number of revertant colonies over the negative control, the testing material were considered as positive. Negative response was defined as no dose-dependent increment in the number of revertant colonies.

Data for mean and standard deviation was generated from three independent experiments. All the data were analysed for statistical significance by one-way analysis of variance ($_{ANOVA}$). All analysed were one-tailed, and $P < 0.05$ was considered statistically significance differences.

Ethical clearance: Prior the submission the study was approved by the ethics committee of the Universiti Malaysia Sabah, Jalan UMS

Results

Table 1: Mutagenicity of modified hydrothermal nanotitania extract in Salmonella typhimurium tester strains in the absence of metabolic activation system (S9 mix)

Table 1. Blood glucose concentration post STZ-NA inductioG GrouM **Mean of blood glucose concentratio(** (mg/dL	**I** 7 75,47±1,				
	TA 98	**TA 100**	**TA 1535**	**TA 1537**	**TA 102**
313	13 ± 3	138 ± 29	18 ± 4	8 ± 2	120 ± 12
625	12 ± 3	160 ± 14	16 ± 3	10 ± 2	127 ± 8
1250	12 ± 3	139 ± 8	18 ± 3	10 ± 3	132 ± 19
2500	14 ± 5	141 ± 37	18 ± 3	16 ± 2	127 ± 25
5000	15 ± 0	128 ± 15	15 ± 4	9 ± 4	131 ± 10
Negative control	12 ± 3	136 ± 10	17 ± 4	21 ± 7	125 ± 10
Positive control	226 ± 34[a]	1344 ±300[b]	475 ± 21[c]	794 ± 27[d]	1311 ± 390[e]

Note: Three independent assays were performed with SD represents standard deviation. The chemicals for positive controls are [a]4-Nitro-o-phenylenediamine (2.5 µg/plate), [b]SodiumAzide (0.5 µg/plate), [c]SodiumAzide (0.5 µg/plate), [d]9-Aminoacridine(50 µg/plate), [e]Mytomycin (5 µg/plate). * and *** indicates P< 0.05 and 0.01 vs. control.

Table 2: Mutagenicity of modified hydrothermal nanotitania extract in Salmonella typhimurium tester strains in the presence of metabolic activation system (S9 mix)

Concentrations (µg/plate)	Number of revertant colonies per plate (mean ± SD, n = 3)				
	TA 98	TA 100	TA 1535	TA 1537	TA 102
313	22 ± 4	151 ± 14	16 ± 2	8 ± 2	148 ±6
625	13 ± 2	139 ± 4	16 ± 4	10 ± 2	152 ±7
1250	18 ± 3	146 ± 16	18 ± 4	10 ± 3	156 ±8
2500	13 ± 2	141 ± 5	10 ± 2	16 ± 2	156 ±9
5000	20 ± 6	174 ± 12	17 ± 3	9 ± 4	161 ± 11
Negative control	22 ± 6	165 ± 30	16 ± 2	10 ± 3	151 ± 17
Positive control	[a]216 ± 6	[b]976 ± 181	[c]349 ± 22	[d]191 ± 32	[e]2313 ± 470

Note: Three independent assays were performed with SD represents standard deviation. The chemicals for positive controls are [a] 2-Aminoanthracene(1 µg/plate), [b]2-Aminoanthracene (1 µg/plate), [c]2-Aminoanthracene (2 µg/plate), [d]2-Aminoanthracene (2 µg/plate), [e]2-Aminoanthracene (2 µg/plate). * and *** indicates $P< 0.05$ and 0.01 vs. control.

Table 1 and Table 2 are the summary of the mutagenicity properties (Ames test) with *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 102 in the absence(**Table 1**) and presence (**Table 2**) of a metabolically active microsomal fraction from rat liver (S9 mix). Five different concentrations of nanotitanium powder (313-5000 µg/plate) were used and it was found that they were no significance changes of mutagenic effect on *S. typhimurium* strainsfrom the spontaneous reversion rate (negative controls) in the absence and presence of S9 mix. According to the OECD Guidelines no. 471[7] a negative control should be confirmed using the highest concentrations up to 5 mg per plate. The highest concentration in this study was 5000 µg/plate, which was not significantly induced any more revertants than the negative controls.Whereas, the positive control chemicals for the five strains were significantly increased the mutant frequencies over the negative controls. From the results, strain TA 100 and TA 102 showed a high colony counting because of a spontaneous background and all above 100 colonies with and without S9 mix. The spontaneous revertants count might be higher due to contamination or excessive histidine added to top agar; in which the top agar is the most critical medium components in Ames test. The reason for this is it contains biotin at a concentration of 0.05 mM which is exceeded from the required of the growth of *Salmonella* strains. Thus, the materials are non-mutagenic in the Ames test in the present conditions.

Discussion

Mutagenicity refers to any potential damage of a substance to the genetic material.Ames test is often used to identify and characterize the mutagenicity of chemicals in basic research, and to examine the safety of industrial products prior to approval by regulatory agencies[4, 11].As mentioned above, *Salmonella* strains contain various types of genomic mutations specifically the most important is TA 1535 for its ability to detect even weak mutagenic potential.This investigation testedmodified hydrothermal nanotitania extractwith the result showed no mutagenic potential on *S. thiphymurium* TA 1535. Single strain is sufficient to demonstrate a mutagenic response, with a possibility of false negative result.However, the false negative result is verified with usage of four to five strains[12].Here is the reason of this study conducted using a few strains of Salmonella; and were always considered necessary. Metabolic activation system is absent in *Salmonella*, and in order to improve the potentiality of bacterial test systems, liver extracts of Swiss albino mice are used (S9 mix). This serves as a rich source in converting carcinogens to electrophilic chemicals that are incorporated to detect *in vivo* mutagens and carcinogens[13].

In the present study, no any dose related increment in the number of revertants in all strains. Indeed, we observed a similarity in the revertant numbers compared to negative control even at the highest dose of the test material with absence (**Table 1**) and presence (**Table 2**) of S9 mix. This means that it was not produce bacterial mutation at any concentration tested by using *Salmonella* strains, applying the two-fold rule proposed by Maron and Ames.[2]

Furthermore, to speed up dissolution of the tested material, ultrasonic bath was used by breaking intermolecular interactions because it was not possible to stirred and dissolved the sample in the distilled water. Ultrasonic bath is commonly used in nanotechnology and useful for uniformly dispersing

nanoparticles in liquid. In addition, the fresh extracts of the materials used in the study were not produced mutagenic effect on all *Salmonella* strain implies that the fresh extract do not lead to base pair substitution in TA 100 and TA 1535 and frame shift mutation in TA 98 and TA 1537. Sterile distilled water was used for the materials extractionfor the reason that itis non-toxic compared to other solvents. Comparing to other researches in the literature detailing aspects of the mutagenicity, their results showed that the material using ethanol or DMSO extracts may induce mutagenicity under the chosen study conditions[12].

Conclusion

Within the limitation of this study it can be concluded that modified hydrothermal nanotitania extractexhibited no mutagenic potential on five strains of *S. typhimurium*with absence presence of S9 mix. Appropriate positive and negative controls were employed in each assay producing the expected results, demonstrating the validity of the methodologies used in the study.Further research needs to be conducted to validate these findings such as, toxicity in animal (*in-vivo*) and clinical trial in human.

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Competing, financial interests:

The author declares no competing financial interests.

Conflict of Interest:

The author declares that he has no conflict ofinterest.

Author's contribution:

Data gathering and idea owner of this study:AMH, NBG, NFN, RA and MKA

Study design: AMH, NBG, NFN

Data gathering: AMH, NBG, NFN, RA

Writing and submitting manuscript: AMH, NBG, NFN, RA and MKA

Editing and approval of final draft: AMH, NBG, NFN, RA and MKA

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