In-vitro Evaluation of mutagenic and anti-mutagenic effects of Siddha formulation Mathan thailam.

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Abstract:
Many Siddha formulations are used for the treatment of infectious diseases but literature search revealed very less number of scientific studies on mutagenic properties of Siddha formulations.
In India the use of traditional herbal remedies plays as alternative medicine, and the effectiveness and harmful properties are based on the previous data.
The present study performs the evaluation of the mutagenic and anti-mutagenic effects of the Siddha formulation Mathan thailam. Mathan Thailam contains Datura leaf juice, Coconut oil and Copper sulphate. This commercially available formulation is used for treating various skin diseases in traditional clinical practice in India The drug was screened for mutagenicity and antimutagenicity using the Salmonella mutagenicity assay (Ames test).
This study results explains the Genotoxicity of Mathan Thailam will provide information and evidence for additional research. Biological and statistical results are considered for evaluation. This test results reveals that Mathan Thailam not induced any gene mutations in TA 1537, TA 1535, TA 98, TA 100 and TA 102 Salmonella typhimurium tester strains at the test doses 5, 2.5, 1.25, 0.625, 0.312 ml/plate, in the presence and absence of metabolic activation system.

Key words:
Ames test, Mathan Thailam, Siddha formulations, mutagenic and anti-mutagenic tests

Introduction:
Herbal medicines from the basis of healthcare throughout the world since time immemorial are still widely being used and have considerable importance in National and International health sector. Many herbal medicines are directly used as therapeutic agents, besides there are several medicinal plants act as a starting material for the synthesis of drugs or as models for pharmacologically active compounds.
With the tremendous expansion in the use of herbal medicines at National and International level, quality control and standardization of herbal medicines have become important concern for both health authorization and public. Even World Health Assembly in a number of resolutions has emphasized the need to have proper regulatory measures and the need to ensure the quality of medicinal plants by using modern control techniques and applying suitable standards.
Quality control is multistep process and covers all the stages from the growing of botanical material to the final control of the finished product and the evaluation of its stability and quality over time. It is essential at all stages of production of botanical material which includes transportation, extraction, processing and the elaboration of the finished pharmaceutical products.
Several Ayurvedic and Siddha formulations are described in the ancient texts, against various pathological conditions. However, modern scientific literature regarding standardization and clinical applications of this formulation is scanty.
Many Siddha formulations are used for the treatment of infectious diseases but literature search revealed very less number of scientific studies on the antimicrobial properties and mutagenic properties of Siddha formulation.
The present study performs the evaluation of the mutagenic and anti-mutagenic effects of the Siddha formulation Mathan thailam. The drug screened for mutagenicity and antimutagenicity using the Salmonella mutagenicity assay (Ames test).
Ames test is a test to determine the mutagenic activity of chemicals by observing whether they cause mutations or not. Mutations means any sudden permanent change in the sequence of DNA. Any agents that

cause mutations are called "mutagens." Mutagens may be I) chemical agents like 1-4,dichlorobenzene or 1-3, dichloro-2 propanol II) physical agents like sun, microwaves or X-rays.

Ames test is widely employed method used to test whether a given chemical can cause mutations in DNA in test organism. It is also called "bacterial reverse mutation assay". It is based on the Principle of back mutation or reverse mutation. Ames test was brought forwarded by Bruce Ames in 1970. He was a professor in biochemistry department in the University of California. He developed this method as the previous methods or assay's are expensive and time consuming.

Examples of chemicals that gives positive responses to Ames test : 2-aminofluorene, Ethylenedibromide(EDC), Ziram, Safrole, Saccharine, Aflotoxin.

Mutations are one of the normal causes of many human genetic diseases. Mutations plays a vital role in cancer has proved from the molecular studies of oncogenes and tumour suppressor genes. Mutational alteration of proto-oncogene can lead to over expression of their growth-stimulating activity whereas mutation that inactivate tumour suppressor genes. Bacterial reverse mutation test is universally used for the detection agents that cause mutation which leads to genetic disorders.

Materials and method

Authenticated Mathan Thailam was procured from IMPCOPS pharmaceuticals, Chennai. This Siddha formulation was prepared by using standard formulation prepared by an ancient Siddhars, and mentioned in the Formulary of Siddha medicines. As per the Formulary of Siddha medicines Mathan Thailam contains Datura leaf juice, Coconut oil and Copper sulphate. This commercially available formulations were used for treating various skin diseases in traditional clinical practice in India. It is generally prescribed to apply on affected parts of skin and used only externally.

Ames test is one of the widely used short term bacterial test method for the recognition of Geno toxicity in bacteria. Mainly used the Salmonella typhimurium bacterial strains. The mutant gene is unable to biosynthesis the amino acid histidine. As a result, they are impotent to form colonies in lacking histidine medium after they are treated with chemicals cause back mutation to attain its function and regrow in lacking histidine medium.

In the presence of metabolising system mutagenic potential can be investigated in the Ames test. To recognize pro-mutagens as well directly acting mutagens Aroclor 1254 induced rat liver s9 fraction).

In Ames testing the 2 common strains TA98(Frameshift mutation) & TA100(base pair substitution) of *Salmonella typhimurium* strains are assessed. Both strains have: rfa mutations, defective polysaccharides make bacteria more permeable to larger molecules. eliminate excision repair of DNA damage by Uvrb mutations. pKM101 plasmid mutations- increases error-prone repair of DNA damage.

As a substitute to Ames testing, commercial system is Greenscreen ^{TM} HC genotoxicity test is recommended. This Geno toxicity test offers improved effiency, lower compound requirements and faster turnaround.

Mutagenicity Test Plate incorporation method was used for the mutagenicity test along with 5 tester strains. In the absence and presence of metabolic activation system (TA 1537, TA 1535, TA 98, TA 100 and TA 102), at doses 5, 2.5, 1.25, 0.625, 0.312 ml/plate in triplicates are utilized. For each strain positive and negative control plates are there. Negative control plates are treated with 100 µL of DMSO/plate. In the absence and presence of metabolic activation system each strains of positive control are treated with known positive controls in triplicates. Once the treatment was over plates were incubated in an inverted position for a period of 48 hours at 37±2°C. Colony counts were matched with negative control. Determining a positive outcome, the criteria should be dose dependent increase or a replicable expansion at one or more concentration. In the absence or presence of metabolic activation system number of revertant colonies per plate is at least one strain. At 1 or more concentration Two fold (TA 98, TA100, TA 102) or >2 fold (TA 1537, TA 1535) at one or more concentrations increase in revertant colonies corresponding to negative control will be considered as positive results. The results not meet these criteria considered as non-mutagenic.

Results:

This study results explains the Genotoxicity of Mathan Thailam will provide information and evidence for additional research. Biological and statistical results are considered for evaluation. This test results reveals that Mathan Thailam not induced any gene mutations in TA 1537, TA 1535, TA 98, TA 100 and TA 102 Salmonella typhimurium tester strains at the test doses 5, 2.5, 1.25, 0.625, 0.312 ml/plate, in the presence and absence of metabolic activation system.

Table 10.2: Mutagenicity Test Result for Mathan thailam
(Values indicate Mean + Standard Deviation).

DOSE*	***In the absence of metabolic activation (-S9)***				
	TA 1537	TA 1535	TA 98	TA 100	TA 102
Negative control	6.0±1.0	13.0±2.0	25.0±1.0	149.3±16.9	315.7±18.6
0.3125	6.6±1.1	12.7±2.1	24.3±2.5	147.0±13.1	282.3±30.9
0.625	5.3±1.5	14.3±1.5	25.3±5.5	158.7±14.0	315.3±8.4
1.25	5.3±0.6	11.0±1.0	25±8.9	152.0±19.2	325.3±15.5
2.5	9.6± 3.5	9.7± 3.5	24.7±5.5	145.3±9.3	312.0±17.8
5.0	8.6±0.6	8.0±1.0	27.3±3.1	158.3±16.1	325.3±17.9
Positive Control	155±32.6	274.7±26.8	395±41.6	951.3±71.0	1291.3±33.0
DOSE*	***In the presence of metabolic activation (+S9)***				
	TA 1537	TA 1535	TA 98	TA 100	TA 102
Negative Control	7.3±1.5	13.0±2.6	26.7±1.5	141.3±3.2	343.3±13.6
0.3125	7.7±3.1	10.0±2.0	26.0±2.0	149.0±4.6	341.7±21.5
0.625	6.7±2.1	10.7±2.1	25.3±2.5	144.3±7.2	345.3±22.3
1.25	6.3±2.1	12.0±2.0	22.7±10.7	153.0±20.3	374.7±8.7
2.5	7.0±1.7	11.3±3.1	23.0±6.2	137.0±10.0	340.0±18.0
5.0	7.7±2.1	11.7±3.0	27.0±1.7	136.7±33.1	323.3±32.1
Positive control	115±5.0	267.3±45.0	481.7±50.3	951.3±62.7	1224.0±174.1

Conclusion: Based on the study it is concluded that, Mathan Thailam, is a non-mutagenic and non-cytotoxic in Salmonella typhimurium tester strains present and absent in the metabolic activation system up to the dose 5mg/plate. Negative results in the bacterial reverse mutation test suggesting that Mathan Thailam is potentially safe to use.

References:

1. Mortelmans Kand Zeiger E. (2000) Mutation Research 455; 29-60
2. ICH guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use S2(R1) November 2011
3. C.P. Prince *et al. International Journal of Drug Research and Technology* 2016, Vol. 6 (1), 01-06
4. C.P. Prince et al., J. Pharm. Res. 2016, 5(1), 1-2
5. Van de Ven, W. J. M. (1999). Proto-Oncogenes and tumor suppressor genes. Introduction to Tumor Biology, 6, 29.
6. Chandrasekhar, P., Lakshmi, T., & Suresh, s. (2013). Bacterial reverse mutation test with Nardostachys jatamansi. International journal of pharmacy & pharmaceutical sciences, 5, 262-266.
7. Tejs,s.(2008). The Ames test: a methodological short review. Environmental biotechnology, 4, 7-14.
8. Mortelmans, K., & Zeiger, e. (2000). The Ames Salmonella/microsome mutagenicity assay. Mutation research/fundamental and molecular mechanisms of mutagenesis, 455(1), 29-60.
9. Birrell, L., Cahill, P., Hughes, C., Tate, M., & Walmsley, R. M. (2010). GADD45a-GFP GreenScreen HC assay results for the ECVAM recommended lists of genotoxic and non-genotoxic

chemicals for assessment of new Geno toxicity tests. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 695(1), 87-95.

10. Hughes, C., Rabinowitz, A., Tate, M., Birrell, L., Allsup, J., Billinton, N., & Walmsley, R. M. (2012). Development of a high-throughput Gaussia luciferase reporter assay for the activation of the GADD45a gene by mutagens, promutagens, clastogens, and aneugens. Journal of biomolecular screening, 17(10), 1302-1315.
11. Hastwell, P. W., Webster, T. W., Tate, M., Billinton, N., Lynch, A. M., Harvey, J. S.,& Walmsley, R. M. (2009). Analysis of 75 marketed pharmaceuticals using the GADD45a-GFP 'GreenScreen HC'genotoxicity assay. Mutagenesis, 24(5), 455-463.
12. Maron, D. M., & Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. Mutation Research/Environmental Mutagenesis and Related Subjects, 113(3-4), 173-215