wjpmr, 2023, 9(11), 232-235

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

IN-VITRO STUDY ON EVALUATION OF THE ANTIMUTAGENIC ACTIVITY OF AQUEOUS EXTRACT OF BRAHMARASAYANA IN HUMAN LYMPHOCYTES

Ashim M.K.[1] and Menakshi Pachori[2*]

[1]Associate Professor, Department of Swasthavritta, Sanskriti Ayurvedic Medical College & Hospital, Mathura, Uttar Pradesh, India.

[2*]Assistant Professor, Biostatistics, Sanskriti Ayurvedic Medical College & Hospital, Mathura, Uttar Pradesh, India.

***Corresponding Author: Menakshi Pachori**

Assistant Professor, Biostatistics, Sanskriti Ayurvedic Medical College & Hospital, Mathura, Uttar Pradesh, India.

Article Received on 13/09/2023 **Article Revised on 03/10/2023** **Article Accepted on 24/10/2023**

ABSTRACT

Introduction- Identification and evaluation of antimutagenic compounds and their mechanisms of action are of great significance in protecting human health. However, the genotoxic potential of many rasayanas remains to be evaluated. **Aims and objectives-** The aim of this study is to verify the antimutagenic activity (i.e., effect on genotoxicity) of *Brahmarasayana* against some standard mutagens in human lymphocyte cultures. As it will be beneficial for further in vivo studies and clinical studies regarding the prevention and treatment of diseases like cancer. **Material and methods-** Antimutagenic study was done in human lymphocytes. CBMN assay was performed in all the cultures and the level of genetic damage was assessed. In this study the antimutagenic activity of test drug *Brahmarasayana* was assessed by counting the MN frequency in not less than 1000 binucleated cells. **Observations and results-** The experimental study was carried out in lymphocytes of a healthy individual. The blood samples collected was divided into 22 and separate lymphocytic cultures were prepared viz 1,2,3,4,5,…,22. In Culture 1, no extracts of study drug *Brahmarasayana* was added which is the positive control, and in Culture 22 500 µg/ml aqueous extract of Brahmarasayana was added and no bleomycin (a mutagenic substance) was added. While in Culture 2, 3, 4, 5,…,21 the aqueous extract of *Brahmarasayana* was added at a concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml, 350 µg/ml, 400 µg/ml, 450 µg/ml, 500 µg/ml, 550 µg/ml, 600 µg/ml, 650 µg/ml, 700 µg/ml, 750 µg/ml, 800 µg/ml, 850 µg/ml, 900 µg/ml, 950 µg/ml, and 1000 µg/ml respectively. **Discussion-** There are many Ayurvedic herbal products explained for natural rejuvenation. Among them, *Brahmarasayana* stands as the choicest natural remedy for chronic stress and tiredness and very useful to increase life span. *Brahmarasayana* should be consumed daily equal to one time food (Ahara thulya mathra). The dosage of avaleha kalpana is *1 pala* (48g). The statistical analysis of the sample collected after testing showed that *Brahmarasayana* has antimutagenic effect. **Conclusion-** The study revealed that *Brahmarasayana* is effective in reducing the micronuclei frequency thereby helping in preventing the genetic damage.

KEYWORDS: Antimutagenic activity, *Brahmarasayana*, CBMN assay, Micronuclei.

INTRODUCTION

Ayurveda is considered holistic, since it takes into consideration body, mind and spirit as a whole. Indian healthcare consists of Medical pluralism and Ayurveda still remains dominant compared to Modern medicine, particularly for treatment of a variety of chronic disease conditions.[1] The aim is to first maintain the health of an individual and then cure the disease thus emphasizing upon the principle "Prevention is better than cure". *Rasayana chikitsa* stresses upon the above principle. *Rasayana* replenish the vital fluids of our body, thus keeping us away from diseases.[2]

Ayurveda, an ancient Indian system of medicine, has a plethora of herbal products that are known to have rejuvenating properties. One such product is *Brahmarasayana*. It is considered to be the choicest natural remedy for stress and chronic tiredness, and is believed to be very useful in increasing lifespan.

It rejuvenates the body and fights against fatigue, early grey hairs, wrinkling etc. It also improves intelligence, memory, and immune power. It is a good natural rejuvenating and anti-aging formulation. *Brahmarasayana*[3,4] combines the immunity supporting and antioxidant herbs used in the mind for increased action on mental faculties such as memory and intelligence. It provides excellent protection year-round, gently detoxifies and promotes healthy cellular regeneration throughout the body.

Mutations are the cause of innate metabolic defects in cellular systems, triggering morbidity and mortality in living organisms. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents are known to act as mutagenic, cocarcinogenic and/or carcinogenic agents.There is increasing evidence that mutation in somatic cells are not only involved in the carcinogenesis but can also cause genetic disorders like atherosclerosis, heart diseases and several other degenerative disorders.[5] Chemicals that reduce the mutagenicity of physical and chemical mutagens are referred to as antimutagens.[6]

More than 500 compounds belonging to at least 25 chemical classes have been recognized as possessing antimutagenic or protective effects.[7] A Micronucleus is the erratic (third) nucleus that is formed during the anaphase of mitosis or meiosis. Micronuclei (the name means 'small nucleus') are cytoplasmic bodies having a portion of acentric chromosome or whole chromosome which was not carried to the opposite poles during the anaphase.[8] The combination of the micronucleus assay with fluorescence in situ hybridisation (FISH) with a probe labeling the peri- centromeric region of the chromosomes (FISH assay) allows discrimination between micronuclei containing a whole chromosome (centromere positive micronucleus) and an acentric chromosome fragment (centromere negative micronucleus).[9]

AIMS AND OBJECTIVES

- To evaluate the antimutagenic/ genoprotective role of aqueous extract of *Brahmarasayana* on human lymphocytes by CBMN assay.
- To assess whether *Brahmarasayana* in the permitted dose may cause any mutagenecity/ genotoxicity to human lymphocytes.
- To correlate the antimutagenicity with the concentrations of *Brahmarasayana*.

MATERIAL AND METHODS

Aqueous extract of Brahmarasayana

The test drug *Brahmarasayana* was prepared according to the classical method (*Ashtanga Hridaya*). Aqueous extract was prepared by taking 10 g of rasayana in 10 ml of distilled water. It was shaken uniformly and filtered through a 0.2-micron porous membrane filter. Aqueous extract prepared was induced in the lymphocytic cultures in different concentrations.

Blood sample

Blood sample was collected from healthy individual (investigator) and divided it into twenty two cultures. Small sample size (only from investigator) is selected because genetic test results can generate anxiety in affected individuals and family members, and there is possibility of discrimination on the basis of test results.

Culture Groups

22 parallel Cultures (Culture 1, 2, 3, 4, 5, 6, 7…22) of lymphocytes are set up. In Culture 1, no extracts of study drug *Brahmarasayana* was added which is the positive control, and in Culture 22, 500 µg/ml aqueous extract of *Brahmarasayana* was added and no bleomycin was added, which is the negative control. While in Culture 2, 3, 4, 5, …, 21 the aqueous extract of *Brahmarasayana* was added at a concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml, 350 µg/ml, 400 µg/ml, 450 µg/ml, 500 µg/ml, 550 µg/ml, 600 µg/ml, 650 µg/ml, 700 µg/ml, 750 µg/ml, 800 µg/ml, 850 µg/ml, 900 µg/ml, 950 µg/ml, and 1000 µg/ml respectively.

METHOD

8-10 ml of fasting venous blood was collected aseptically from the healthy subject by venepuncture. 4ml of blood was transferred aseptically in heparinized vacutainers and was used for lymphocyte separation, tissue culture preparation and Cytokinesis block micronuclei CBMN Assay.[10]

STATISTICAL ANALYSIS

Study Design: Experimental research design.

Duration of study: Total duration of study was two months.

The present study evaluated the antimutagenic/genoprotective effect of *Brahmarsayana* on to human lymphocytes by CBMN assay. Even though many methods are available for evaluating the same, CBMN assay was taken because of its simplicity and accuracy. The purpose was to analyse the antimutagenic property of *Brahmarasayana* on human lymphocytes. The antimutagenic property was compared with that of a control culture maintained with and without Bleomycin (mutagen). The study also evaluated the antimutagenic property of *Brahmarasayana* with various concentrations.

The study demonstrated that the increasing concentration of *Brahmarasayana* showed an increasing genoprotective role against mutagenic (bleomycin) substance. The frequency of micronuclei among 1000 binucleated cells were enumerated, recorded, and analysed in all the samples. Higher the number of micronuclei, higher will be the genetic damage. The positive control; culture 1 (lymphocytes exposed to Bleomycin) showed a mean CBMN frequency of 13.15 and the negative control; culture 22 (without bleomycin exposure) showed a mean CBMN frequency of 10.96. This difference has statistical significance.

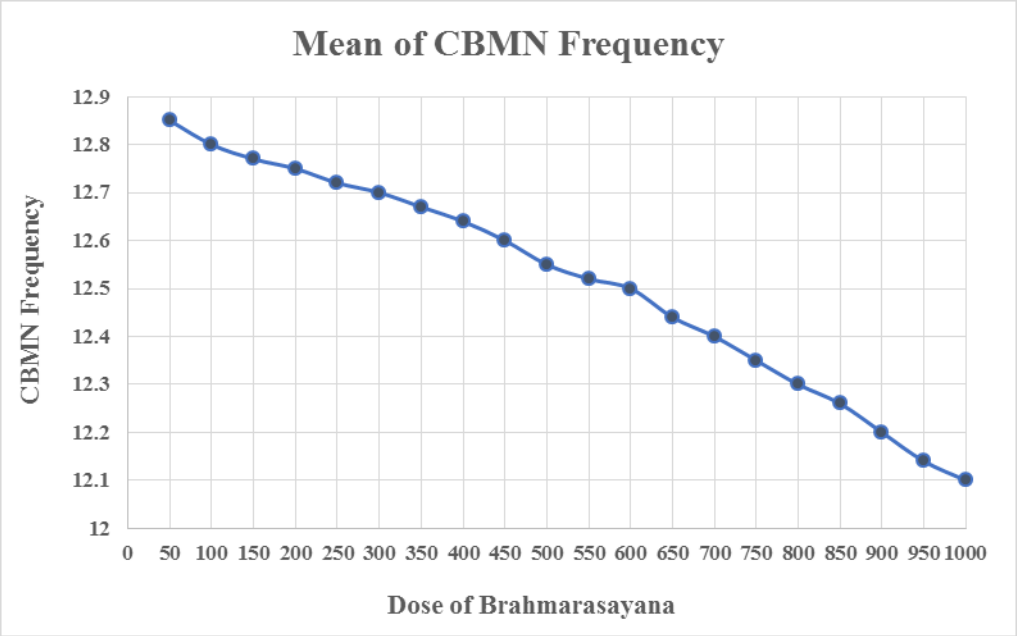

Fig. 1: Distribution of Mean CBMN Frequency according to different concentration of Brahmarasayan.

Hypothesis

Null hypothesis - *Brahmarasayana* does not possess antimutagenic activity

Alternative hypothesis - *Brahmarasayana* possess antimutagenic activity.

From this study, it can be summarized that supplementation of *Brahmarasayan* to human lymphocyte culture showed antimutagenic property against a mutagenic substance (bleomycin). Moreover, the antimutagenic property increases with increasing concentration of *Brahmarasayana*.

As this is a pre and post evaluation type of study, the data can be compared and analysed using the paired 't' test. As there is no test drug in positive control culture 1 and no mutagen in negative control culture 22, they are ignored for the convenience of the calculation. As the cultures were made from the blood of same individual and the quantity of bleomycin (mutagen) added to all cultures are same, let us assume the pre-test value as 13.15 (mean CBMN frequency of positive control) for all the twenty cultures taken for calculation.

The description of the post-test values for all the twenty cultures are given in the following table 1.

Table 1: Descriptive Statistics for the Post-test values.

	n	Mean	SD	Min	Max	SE (mean)	Median	IQR
Post-Test	20	12.513	0.23029	12.10	12.85	0.05149	12.535	0.3675

Table 2: Result of the Paired t- Test.

Paired t-test
Data: Pre-test and Post-test
t = 12.3704**,** **df** = 19**,** **p-value** = 0.0000000001548
95 percent confidence interval: 0.52922; 0.74478
Sample estimates: Mean of the differences = 0.637

Since, p-value is less than 0.05 ($p < 0.05$), at 19 degrees of freedom, the test is statistically significant. Hence the null hypothesis is rejected and alternate hypothesis can be accepted, i.e., the mean difference noted between pre and post values are due to the anti-mutagenicity of *Brahmarasayana*.

RESULTS AND DISCUSSION

The present study was to determine the antimutagenic potential of *Brahmarasayana* in peripheral human blood lymphocytes. The study is a broadly based on comprising of assessing the antimutagenic activity by evaluating test drug effect on decreasing frequency of MNi.

From evaluating the data of the experiment, it can be inferred that the supplementation of *Brahmarasayan* to human lymphocyte culture showed an antimutagenic property against a mutagenic substance (bleomycin). Moreover, the antimutagenic property increases with increasing concentration of *Brahmarasayana*. Thus, this can be assumed that *Brahmarasayana* will be effective in preventing mutation. Vata dosha controls the very basic body processes such as cell division in the form of vyana vayu. Mutations may be due to viguna/ vimarga vyana vayu. Rasayanas provide health and stability to dhathus. As doshas and dhathus are ashraya and asrayi, healthy

dhathus will coexist with healthy doshas. Thus by enhancing the samavastha of doshas like vyana vayu rasayanas provide protection against mutation (kosha vikruthi).

Statistical analysis of the data collected after experiment revealed that *Brahmarasayana* has antimutagenic potential. It is suggested for further detailed studies of the same for proving *Brahmarasayana* in the form of an effective and popular anti cancerous medicine.

CONCLUSION

Based on this study, it can be concluded that, Drug *Brahmarasayana* was found to be antimutagenic at cellular level and is effective in reducing the genetic damage. *Brahmarasayana* in the permitted dose do not cause any mutagenecity/ genotoxicity to human lymphocytes. Statistical analysis showed that there was reduction in micronuclei in *Brahmarasayana* incorporated CBMN assay. *Rasayana* therapy can provide some immediate sort of gene protective effect.

Since, this is a small sample in vitro study and it is found relevant, further in vitro studies with higher sample size, in vivo studies and human clinical trials are required for establishing *Brahmarasayana* as an effective and popular anti cancerous herbal preparation.

ACKNOWLEDGEMENT

Genetika, Centre for Advanced Genetic studies, Pettah, Thiruvananthapuram.

REFERENCES

1. M.Waxler. Plural medicine in India and Sri Lanka: Do Ayurvedic and Western medical practices differ. Soc. Sci. Med., 1988; 27: 531-544.
2. D.R. Ray. Ayurdiya Kriyasharir, Ist Edition, Sh.Vednath Ayurved Bhawan Ltd, 1953; 547-675.
3. Charaka Samhita– chikithsasthana 1/8 By Chakrapani with Ayurveda Dipika commentary, Nirnaya Sagar Press - Bombay.
4. Ashtanga Hridaya– utharashtana 39/2 With Sarvanga Sundari commentary Nirnaya Sagar press.
5. De Flora, S., A. Izzoth and C. Benniceili. Mechanisms of Antimutagenesis and Anticarcinogenesis, Role in Primary Protection. In: Antimutagenesis and Anticarcinogenesis Mechenisms, Bronezetti, G., M.D. Waters and D.M. Shanket (Eds.) Plenum Press, New York, 1992; pp: 162-178.
6. Mitscher, L.A., S. Drake, S.R. Gollapuri, J.A. Harris and D.M. Shankel. Antimutagenesis and Anticarcinogenesis Mechanisms. Plenum Press, New York, 1986.
7. Edenharder, R. and X. Tang. Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitrtopyrene by flavonoids, coumarin, quinones and other phenolic compounds. Food Chem. Toxicol, 1997; 35: 357-372.
8. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E; Human Micron Nucleus project. "HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures." Mutat Res, 2003; 534(1-2): 65-75.
9. Kirsch-Volders M, Fenech M. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. Mutagenesis, 2001; 16(1): 51-58.
10. Thomas, P., Fenech, M. Cytokinesis-Block Micronucleus Cytome Assay in Lymphocytes. In: Didenko, V. (eds) DNA Damage Detection In Situ, Ex Vivo, and In Vivo. Methods in Molecular Biology, 2011; vol 682. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-60327-409-8_16.