Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (2), 2023; 409-414

Genotoxicity Evaluation of *Vaishvanara Churna* - A classical Ayurvedic Formulation

Research Article

Rishi Kumar[1], Rupa Mazumder[2], Amrish Chandra[3*]

1. Research Scholar, 3. Professor, Amity Institute of Pharmacy,
Amity University Uttar Pradesh, Sector-125, Noida, Uttar Pradesh. India.
2. Professor, Department of Pharmaceutical Technology,
Noida Institute of Engineering and Technology, Knowledge Park-II, Institutional Area, Greater Noida. India.

Abstract

Aims: The current study aimed to evaluate the genotoxicity of the *Vaishvanara Churna*, a polyherbal formulation by using Peripheral Blood Mononuclear Cells. The study was supported by the study of the content of heavy metals in the ayurvedic formulation. Background: *Vaishvanara Churna* is a classical ayurvedic formulation that is used for treating conditions, such as flatulence with gurgling sound (Adhmana), abdominal lump (Gulma); Duodenal ulcer (Parinamasula), Rheumatism (Amavata); heart disease (Hrdroga). Objective: The objective is to study the genotoxicity of *Vaishvanara Churna* on Peripheral Blood Mononuclear Cells using alkaline Single-cell Gel Electrophoresis and correlate the possible association of genotoxicity to the number of heavy metals present in the polyherbal formulation. Methodology: The Alkaline Single Cell Gel Electrophoresis was used to see the effects on the Peripheral Blood Mononuclear cells using 2% Hydrogen Peroxide as a standard oxidizing agent. Observation: Peripheral Blood Mononuclear Cells, isolated from a fresh human blood sample, were treated in vitro with varying concentrations of *Vaishvanara Churna* (0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml of aqueous extract) using a standard protocol. It was found that none of the assessed concentrations of the ayurvedic formulation was toxic to Peripheral Blood Mononuclear Cells. Conclusion: Single-cell Gel Electrophoresis is a simple technique to evaluate the genotoxicity of any drug to Human Beings, and it can be used as an alternative to animal genotoxicity studies.

Key Words: Genotoxicity, Single Cell Gel Electrophoresis, Heavy Metals, *Vaishvanara Churna,* Ayurvedic formulation, Peripheral Blood Mononuclear Cells.

Introduction

Heavy Metals have been reported to cause various ailments in living organism. The toxicity of heavy metals depends on several factors, including the concentration of the metal, the duration of exposure, and the route of exposure. For example, inhaling airborne particles containing heavy metals can be particularly harmful, as they can easily enter the lungs and blood stream. One of the main mechanisms by which heavy metals exert their toxicity is through the generation of reactive oxygen species (ROS). ROS can damage cellular structures such as DNA, lipids, and proteins, leading to oxidative stress and inflammation. This can contribute to a range of adverse health effects, including neurotoxicity, cardiovascular disease, and cancer. It is important to reduce exposure to heavy metals, this might include avoiding certain types of seafood that may be high in mercury, using water filtration systems to reduce heavy metal contamination in drinking water, and avoiding contact with contaminated soil or dust. In addition, maintaining a healthy lifestyle, including a balanced diet and regular exercise, can help to support your body's natural detoxification processes and reduce the risk of heavy metal toxicity (1).

Arsenic is one of the most toxic heavy metals that can cause various health problems, including cancer, skin lesions, cardiovascular disease, and neurotoxicity. Cadmium exposure is associated with renal dysfunction, osteoporosis, and lung cancer. Mercury toxicity can lead to neurotoxicity, developmental delays, and kidney damage. Lead exposure is associated with developmental delays in children, anemia, and neurological disorders. Chromium is toxic to the respiratory system and can cause lung cancer (2).

Heavy metals can also interfere with DNA repair mechanisms, leading to mutations and chromosomal aberrations. This can result in the development of cancer and other chronic diseases. Heavy metals may also affect the immune system, leading to an increased susceptibility to infections (3).

Prevention of heavy metal exposure requires a multi-faceted approach. This includes the regulation of industrial and agricultural practices that lead to heavy metal contamination, testing of water and food supplies for heavy metal contamination, and education of the public on the risks associated with heavy metal

*** Corresponding Author:**
Amrish Chandra
Professor,
Amity Institute of Pharmacy,
Amity University Uttar Pradesh, Sector-125,
Noida, Uttar Pradesh. India.
Email Id: chandra.amrish@gmail.com

exposure. Additionally, it is important to consume a healthy diet rich in antioxidants to mitigate the effects of oxidative stress.

In conclusion, heavy metal exposure can have detrimental effects on human health, and the public must take measures to limit their exposure to heavy metals. The understanding of the mechanisms of heavy metal toxicity is critical in developing effective strategies to prevent and treat heavy metal-related diseases (4).

Vaishvanara Churna, an Ayurvedic polyherbal Churna formulation, is prescribed by the Ayurvedic Physician for treating conditions, such as flatulence with gurgling sound (Adhmana), abdominal lump (Gulma), Duodenal ulcer (Parinamasula), Rheumatism (Amavata) and heart disease (Hrdroga) [1]. It contains five ingredients, Manimantha (Saindhava Lavana Active Pharmaceutical Ingredient (API)), Rock salt, Yamani (Yavani API), *Trachyspermum ammi (L.)* Sprague., Ajmoda API *Trachyspermum roxburghianum* (DC.) Wolf., Nagara (Sunthi API) *Zingiber officinalis* Rosc., Haritaki API *Terminalia chebula* Retz. mixed in quantity as mentioned in Table 2.

It is well known that there are number of toxic contaminants and residues that may cause harm to the consumers of herbal medicines. Heavy metals and microorganisms are considered important contaminants. Some contaminants used for cultivation of herbal drugs may percolate into the herbal drugs e.g. pesticides etc. It is also observed that heavy metals may absorb by plants in process of their vegetative growth. This is the reason; during the quality check, the heavy metals concentration needs to be checked. The risk of contamination can be reduced if the quality of herbal raw drugs verified before formulating them in finished goods products. Heavy metals such as lead, mercury, cadmium, and arsenic can be found in some Ayurvedic formulations due to their use in traditional preparations. However, these metals can have genotoxic effects on the body, meaning they can cause damage to DNA and potentially lead to cancer.

The presence of heavy metals in Ayurvedic formulations is a concern, particularly if the metals are present in high concentrations or if the products are used over an extended period of time. Some studies have reported that heavy metals in Ayurvedic products can cause liver damage, kidney damage, and neurological disorders (5).

To minimize the risk of genotoxicity due to heavy metals in Ayurvedic formulations, it is important to purchase products from reputable manufacturers who follow strict quality control measures. It is also essential to use these products in moderation and under the guidance of a qualified healthcare professional.

Additionally, regulatory agencies like the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have issued guidelines on heavy metal content in herbal medicines, including Ayurvedic formulations, to ensure their safety and quality. Consumers should be aware of these guidelines and choose products that comply with them.

Some of the countries prescribed the limits of heavy metals as depicted in Table 1 (5,6).

Table 1: Examples of national limits for arsenic and toxic metals in herbal medicines and products

		Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Mercury (Hg)	Copper (Cu)	Total Toxic metals as Lead
	For Herbal medicine							
India		3 ppm	10 ppm	0.3 ppm	-	1 ppm	-	-
Canada	Raw herbal material	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
	Finished Herbal Products	0.01 mg/ day	0.02 mg/ day	0.006 mg/ day	0.02 mg/day	0.02 mg/ day	-	-
China	Herbal Material	2 ppm	10 ppm	1 ppm	-	0.5 ppm		20 ppm
Malaysia	Finished herbal Products	5 mg/ kg	10 mg/kg			0.5 mg/kg		
Republic of Korea	Herbal Material							30 ppm
Singapore	Finished Herbal Products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand	Herbal Material and Finished Herbal Products	4 ppm	10 ppm	0.3 ppm				
WHO Recommendation			10 mg/kg	0.3 mg/kg				

In this study, the genotoxicity and cytotoxicity of the ayurvedic formulation was studied and its correlation with the data of heavy metals was evaluated (7).

Materials and Methods

The herbs used for the preparation of *Vaishvanara Churna* were procured from the local market of Ghaziabad. The individual herbs were authenticated by the Council of Scientific and Industrial Research-National Institute of Science, Communication and Information Resources (CSIR-NISCAIR) The voucher specimen of these ingredients were deposited in the Raw Material Herbarium and Museum, Delhi (RHMD) for future reference. All the chemicals used in the study were purchased from Merck. The standards of heavy metals were purchased from Merck. Vaisavanara churna was prepared in house as per the standard method of preparation available in the Ayurvedic Pharmacopoeia of India and mixed in quantities as mentioned in Table 2 (8).

Table 2- Formulation of *Vaishvanara Churna*

Sr. No.	Common Name of Ingredients	Ingredient	Part Used	Qty
1	Manimantha (Saindhava lavana API)	Rock Salt	-	20 g
2	Yamani (Yavani API)	*Trachyspermum ammi* Fr.	Dried Fruit	20 g
3	Ajmoda API	*Apium leptophyllum* Fr.	Dried Fruit	30 g
4	Nagara (Sunthi API)	*Zingiber officinale* Rz.	Dried Rhizome	50 g
5	Haritaki API	*Terminalia chebula* P.	Dried Fruit pulp	120 g

The Single Cell Gel Electrophoresis (Comet Assay) is the technique of identifying DNA damage. It is simple method of identifying the DNA damage of cells at high pH. The results appear as structures resembling comets observed by fluorescence microscopy and due to this reason, this technique is also called as Comet assay.

The standard protocol given by Dhawan *et al*. was used with slight modification. Peripheral Blood Mononuclear Cells (PBMCs) were used to determine the genotoxicity caused by the aqueous extract of *Vaishvanara Churna* Formulation. The PBMCs cells were isolated as per the standard protocol mentioned by Dhawan *et al*. The brief description of the protocol followed for this study is given below-

A 0.5% low-melting-point agarose solution is prepared in phosphate-buffered saline (PBS) and kept at 37°C. A cell suspension of the desired concentration, usually ranging between 1x10^4 and 1x10^5 cells/ml, is prepared in PBS. Equal volumes of the cell suspension and the 0.5% agarose solution are mixed together. Then, 75 µl of the mixture is pipetted onto a pre-coated glass slide. The agarose-cell mixture is gently spread with a coverslip and allowed to solidify at 4°C for 10-15 minutes. The coverslip is removed, and the slide is immersed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, 1% Triton X-100, and 10% DMSO, pH 10.0) for 1-2 hours at 4°C. Next, the slide is placed in an electrophoresis tank filled with cold electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH >13.0) and allowed to stand for 20 minutes to allow DNA unwinding. The slide is then electrophoresed for 20 minutes at 25 V (0.7 V/cm) and 300 mA. To neutralize the DNA, the slide is rinsed three times for 5 minutes each in neutralization buffer (0.4 M Tris-HCl, pH 7.5). The slide is stained with a DNA-specific fluorescent dye, such as 80 µl of 1X ethidium bromide. Under a fluorescence microscope, the slide is examined, and images of the comets are captured using appropriate software. The images are then analyzed to determine the amount of DNA damage in individual cells, typically by measuring the length of the comet tail or the amount of DNA in the tail compared to the head. It is important to note that this protocol may need to be modified based on specific experimental conditions and cell types. However, following a standardized protocol like these helps ensure reliable results that can be compared across different studies. For the evaluation of DNA damage, stained DNA with ethidium bromide is observed using a fluorescence microscope with a 40x objective lens, such as the Nikon Inverted Microscope. For quantification of SCGE data, an image analysis system like ImageJ, linked to a CCD camera, is used to analyze DNA damage in the peripheral blood mononuclear cells (PBMCs). The damage was measured by measuring the length of tail formed during the migration of DNA and was compared with controlled cells (9).

Determination of Heavy Metal Content in *Vaishvanara Churna*

The heavy metal content of the formulation was estimated by using ICP Mass Spectrometer, Model NexION 300X of Perkin Elmer. The Standard Protocol was followed for the estimation of heavy metals. The standards of heavy metals selected for the study were Cd, Pb, Cr, As, Ni, Cu, Co, Zn, Hg and purchased from Merck. The Chemical used in the Single Cell Gel Electrophoresis were purchased from Merck and Himedia (10-11).

Results and Discussion

Comet assay is a very simple and sensitive technique to identify genotoxicity and DNA damage and repair. The Comet Assay has many applications in Research and development like identification of radiation toxicity, effects of chemicals/pharmaceuticals/drugs on the cells etc. (12).

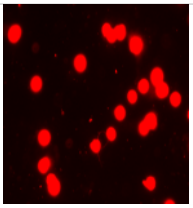
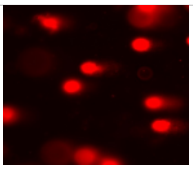
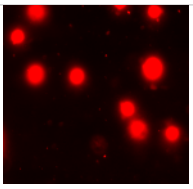
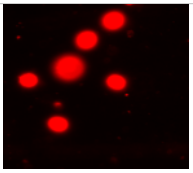
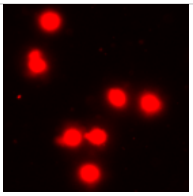
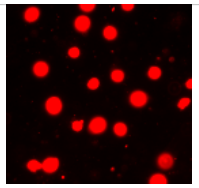
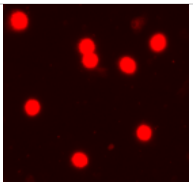
The Heavy metals are also responsible for genotoxicity such as Cd and As are reported, the effect of contaminated land, water, and various pollutants on the ecosystem is well known to the scientists (13). In the present study, the effects of heavy metals and oxidizing agents on PBMCs were observed and presented. Heavy metals are available naturally but due to irresponsible activities of humans and other unnatural activities lead them to enter in the water bodies, air, soil and at present it has become a global issue. These are equally toxic to plants. (14)

Sathya *et al.*, also tested some herbomineral ayurvedic preparations for genotoxicity due to traces of heavy metals using comet assay (15).

In the current study, the amount of Cd, Pb, Cr, As, Ni, Cu, Co, Zn, and Hg was found to be 0.005, 0.875,0.008, 0.008, 0.599, 21.233, 0.003, 12.041, 0.003, and 0.001, 0.702, 0.054, 0.002, 0.604, 9.277, 0.004, 0.331, 0.001 ppm respectively in Marketed formulation and In-house formulation of *Vaishvanara Churna*. All the values were found to be within limits as mentioned in Ayurvedic Pharmacopoeia.

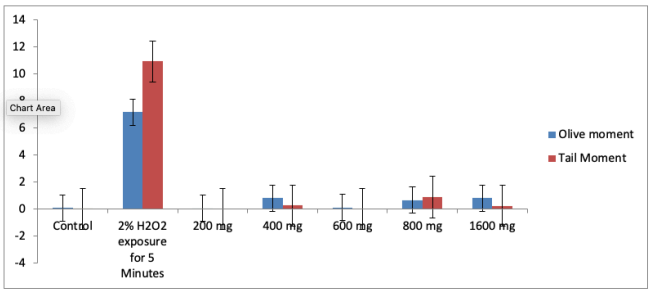
The results of single cell gel electrophoresis (Alkaline Comet Assay) showed that the *Vaishvanara Churna* is not toxic for Peripheral Blood Mononuclear Cells (PBMCs) as depicted in Figure1. Alkaline Comet Assay was evaluated with Open Comet Image J software and the results obtained are shown in Figure 2(17-20).

Figure 1: The photographs of the slides following Single Cell Gel electrophoresis

	**(a)Control Cells**
	**(b)2% H_2O_2 Exposure to Cells for 5 Minutes**
	(c) 200 µg amount of Aqueous extract was added in 10000 Cells suspended in 500 µL PBS
	(d) 400 µg amount of Aqueous extract was added in 10000 Cells suspended in 500 µL PBS
	(e) 800 µg amount of Aqueous extract was added in 10000 Cells suspended in 500 µL PBS
	(f) 1600 µg amount of Aqueous extract was added in 10000 Cells suspended in 500 µL PBS
	(g) 3200 µg amount of Aqueous extract was added in 10000 Cells suspended in 500 µL PBS

(a) Control Cells, (b) PBMCs with 2% H_2O_2 exposure for 5 minutes, (c) PBMCs with 200µg of aqueous extract, (d) PBMCs with 400µg of aqueous extract, (e) PBMCs with 800µg of aqueous extract, (f) PBMCs with 1600µg of aqueous extract, (g) PBMCs with 3200µg of aqueous extract.

Figure 2. - Olive moment and Tail moment of PBMCs after Alkaline Comet Assay



Statistical Analysis of Olive Moment

1. Control vs. 2% H_2O_2 exposure for 5 Minutes:
   - Null hypothesis (H0): There is no significant difference in the means of the control and 2% H_2O_2 exposure groups for "Olive Moment."
   - Alternative hypothesis (Ha): There is a significant difference in the means of the control and 2% H_2O_2 exposure groups for "Olive Moment."
2. Control vs. Formulation at different concentrations:
   - Null hypothesis (H0): There is no significant difference in the means of the control and formulation groups at each concentration for "Olive Moment."
   - Alternative hypothesis (Ha): There is a significant difference in the means of the control and formulation groups at each concentration for "Olive Moment."

Performing t-tests for the "Olive Moment" data:

Control vs. 2% H_2O_2 exposure for 5 Minutes (Olive Moment): t-value: -4.819 p-value: 0.001 Control vs. Formulation at 200 µg (Olive Moment): t-value: -1.968 p-value: 0.092 Control vs. Formulation at 400 µg (Olive Moment): t-value: -2.068 p-value: 0.079 Control vs. Formulation at 800 µg (Olive Moment): t-value: -0.857 p-value: 0.434 Control vs. Formulation at 1600 µg (Olive Moment): t-value: -2.066 p-value: 0.08 Control vs. Formulation at 3200 µg (Olive Moment): t-value: -2.442 p-value: 0.055

Based on the t-tests for the "Olive Moment" data, the comparison between the control and 2% H_2O_2 exposure group yields a t-value of -4.819 and a p-value of 0.001, indicating a significant difference.

Comparing the control group to the different concentrations of the formulation, none of the p-values for the formulation groups are less than the common significance level of 0.05. This suggests that there is no significant difference between the control group and the formulation groups at each concentration for the "Olive Moment" data.

Statistical Analysis of Tail Moment

Performing t-tests for the "Tail Moment" data:

1. Control vs. 2% H_2O_2 exposure for 5 Minutes:
   - Null hypothesis (H0): There is no significant difference in the means of the control and 2% H_2O_2 exposure groups for "Tail Moment."
   - Alternative hypothesis (Ha): There is a significant difference in the means of the control and 2% H_2O_2 exposure groups for "Tail Moment."
2. Control vs. Formulation at different concentrations:
   - Null hypothesis (H0): There is no significant difference in the means of the control and formulation groups at each concentration for "Tail Moment."
   - Alternative hypothesis (Ha): There is a significant difference in the means of the control and formulation groups at each concentration for "Tail Moment."

Performing t-tests for the "Tail Moment" data:

Control vs. 2% H_2O_2 exposure for 5 Minutes (Tail Moment): t-value: 9.098 p-value: 0.0001 Control vs. Formulation at 200 µg (Tail Moment): t-value: -0.418 p-value: 0.702 Control vs. Formulation at 400 µg (Tail Moment): t-value: -1.992 p-value: 0.103 Control vs. Formulation at 800 µg (Tail Moment): t-value: -0.812 p-value: 0.46 Control vs. Formulation at 1600 µg (Tail Moment): t-value: -1.79 p-value: 0.145 Control vs. Formulation at 3200 µg (Tail Moment): t-value: -0.592 p-value: 0.583

Based on the t-tests for the "Tail Moment" data, the comparison between the control and 2% H_2O_2 exposure group yields a t-value of 9.098 and a p-value of 0.0001, indicating a significant difference.

However, when comparing the control group to the different concentrations of the formulation, none of the p-values for the formulation groups are less than the common significance level of 0.05. This suggests that there is no significant difference between the control group and the formulation groups at each concentration for the "Tail Moment" data.

Therefore, based on the statistical analysis, the formulation does not show significant differences compared to the control group for the "Tail Moment" at different concentrations, indicating that the formulation may be considered non-toxic in comparison to the 2% H_2O_2 exposure.

Conclusion

In conclusion, the results indicate that genotoxic potency measured by the comet assay of *Vaishvanara Churna* in PBMCs may predict genotoxicity in exposed humans However it can strongly provide a supportive data that may support that the formulation is not showing any genotoxicity in human cells. The observation in current study revealed that there is no damage to DNA of Peripheral Blood Mononuclear Cells, due to the presence of *Vaishvanara Churna* extract. It was not found toxic to Peripheral Blood Mononuclear Cells. Further Authors recommends that single cell gel electrophoresis may be used to study the genotoxic effect of other traditional formulations on human cells. This technique will improve the public confidence in the use of traditional formulations like Ayurveda, Unani, Siddha, Sowa rigpa, and other traditional system of medicines.

Ethics approval and Consent of participate

Not applicable

Human and Animal Rights

No human or animals were used for studies that are base of this research.

Consent for Publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

None.

Conflict of Interest

Nil.

Acknowledgements

The Authors are thankful to Dr Raj Kumar, Scientist 'F', Defence Research and Development Organization-Institute of Nuclear Medicine and Allied Sciences, SK Mazumder Road, Delhi-110054, for providing laboratory facilities to carry out Single Cell Gel Electrophoresis. We are equally thankful to Indian Pharmacopoeia Commission for providing facilities for ICP-MS analysis of heavy metals.

References

1. Jadoon S, Malik A. DNA damage by heavy metals in animals and human beings: an overview. Biochem. Pharmacol. December, 2017; 6(235); 2167-0501.
2. Chang L.W, Magos L, Suzuki T, editors. Toxicology of metals. Taylor & Francis US; 1996.
3. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Molecular and cellular biochemistry. June, 2001;222(1-2); 3-9.
4. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insights into molecular and cellular mechanisms. Archives of toxicology. August, 2008;82(8); 493.
5. WHO guidelines for assessing the quality of herbal medicines with reference to contaminants and residues, 2007, 24p.
6. The Ayurvedic Pharmacopoeia of India, Part-II, Volume-II, First Edition (Formulations), Published by Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy (AYUSH), New Delhi, 2008, 168p.
7. Kopp B, Zalko D, Audebert M. Genotoxicity of 11 heavy metals detected as food contaminants in two human cell lines. Environmental and molecular mutagenesis. April, 2018;59(3);202-10.
8. The Ayurvedic Pharmacopoeia of India, Part-II, Volume-I, First Edition (Formulations), Published by Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy (AYUSH), New Delhi, 2007, 59p.
9. Dhawan A, Bajpayee M.M, Pandey A.K, Parmar D. Protocol for the single cell gel electrophoresis/ comet assay for rapid genotoxicity assessment. Sigma. 2003;1077(1).
10. Olive P.L, Banáth J.P. The comet assay: a method to measure DNA damage in individual cells. Nature Protocols. 2006 Jun 1;1(1):23.
11. Singh N.P, McCoy M.T, Tice R.R, Schneider E.L. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental cell research. March, 1988;175(1):184-91

12. Gunasekarana V, Raj G.V, Chand P. A comprehensive review on clinical applications of comet assay. Journal of clinical and diagnostic research: JCDR. March, 2015; 9(3):GE01.
13. Ghiani A, Fumagalli P, Van T.N, Gentili R, Citterio S. The combined toxic and genotoxic effects of Cd and As on the plant bioindicator Trifolium repens L. PloS one. June, 2014; 9(6); e99239.
14. Tchounwou P.B, Yedjou C.G, Patlolla A.K, Sutton D.J. Heavy metal toxicity and the environment. Exp Suppl. 2012; 101;133-64.
15. Sathya T, Murthy B, Vardhini N. Genotoxicity evaluation of certain Bhasmas using micronucleus and comet assays. Internet J Altern Med., June, 2009; 8;7:1.
16. Kumar R, Samajdar S, Mazumder R, Chandra A. Formulation, standardization, and evaluation of Vaiśvānaracūrn.a tablet. Ancient Sci Life 2018; 38; 59-67
17. Gyori B.M, Venkatachalam G, Thiagarajan P.S, Hsu D, Clement M.V. OpenComet: an automated tool for comet assay image analysis. Redox biology. January, 2014; 2; 457-65.
18. Mishra RP, Mishra S. In Vitro Assessment of Genotoxicity of Some Ayurvedic Drugs in Human Lymphocytes by Using Single Cell Gel Electrophoresis. J Drug Metab Toxicol. 2013; 4:139.
19. Sathya T, Murthy B, Vardhini N. Genotoxicity evaluation of certain Bhasmas using micronucleus and comet assays. Internet J Altern Med., June, 2009; 8;7:1.
20. Malhotra P, Gupta A.K, Singh D, Mishra S, Singh S.K, Kumar R, N-Acetyl-tryptophan glucoside (NATG) protects J774A.1 murine macrophages against gamma radiation-induced cell death by modulating oxidative stress. Mol Cell Biochem. October, 2018;447(1-2); 9-19.

*****