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IN VITRO STUDY ON HEAVY METAL CONTAINING AYURVEDIC DRUG INDUCED GENOTOXICITY IN HUMAN LYMPHOCYTE

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Abstract

The use of Ayurvedic medicines is common in both adults and children and is increasing in many areas of the world. This paper will discuss the risks of heavy metal poisoning associated with the use of Ayurvedic medicines and illustrate this with some cases managed by the authors. Many Ayurvedic medicines contain heavy metals, including lead, mercury and arsenic, and there have been numerous reports of clinically significant heavy metal poisoning related to their use. However, there have been few studies that allow quantification of the incidence of this problem. There is limited regulation of these products in most areas of the world. Recent European legislation may help to improve safety of products bought in shops, but it is likely to have a relatively limited overall impact as it will not cover personal imports or products prescribed by traditional medicine practitioners. There is an urgent need for studies to quantify the frequency and potential risk of heavy metal poisoning from Ayurvedic medicines and for culturally appropriate education to inform the public of the potential for toxicity associated with these products.

Key words: heavy metals; genotoxicity; MNT; Ayurvedic medicines.

INTRODUCTION

Agents that produce alteration in the nucleic acids and associated components at sub toxic exposure levels, resulting in modified hereditary characteristic or DNA inactivation, are classified as 'genotoxins' (Sprague, 1969). Shugart *et al.*, (1995) defined genotoxins as a substance which produces alteration in the genetic material at non- lethal, non cytotoxic concentrations. However, the use of this term is limited to those compounds which effect the gene expression or replication by reacting chemically with DNA. Heavy metals are also genotoxicant which can enter in human body through various agents like from environment, agricultural land, from various medicines etc. WHO guideline says that heavy metals are necessary for some functions of our body but it becomes toxic when its amount crosses a particular value. Studies have found that heavy metals such as mercury, cadmium, lead, aluminium, and tin affect chemical synaptic transmission in the brain and the peripheral and central nervous system (Lewis *et al.*, 1992). A micronucleus is the erratic (third) nucleus that is formed during the anaphase of mitosis or meiosis. Micronucleus is also the name given to the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. In newly formed red blood cells in humans, these are known as 'Howell-Jolly bodies' (Howell, 1891; Jolly, 1905; Schroeder, 1966).

Micronuclei are expressed in dividing cell that either contain chromosome breaks lacking centromere or whole chromosomes that are unable to travel to the spindle poles during mitosis. At telophase, a nuclear envelope forms around the lagging chromosome and fragments which uncoil and gradually, assume morphology of an interphase nucleus with exception that they are smaller than the main nuclei in the cell, hence assigned the term 'micronuclei'. As per Heddle (1973) and Schmid (1975) micronuclei are formed in cytoplasm through; a) in anaphase, acentric chromatid and chromosomal fragments lag behind when the centric element moves towards the spindle poles. Micronuclei arise from chromosomal fragments or acentric chromosomes that are not incorporated into daughter nuclei at mitosis because they lack a centromere; b) the lagging element may be included in the nuclei of the daughter cells, but a proportion from one or several secondary nuclei that are much smaller than the principal nucleus (1/5 to 1/20), and are called micronuclei.

The nucleoplasmic bridge and binucleated cells provide an additional and complementary measure of chromosome rearrangement. This scored together with the micronucleus. Micronuclei harboring chromosomes fragments results from; a) direct DNA breakage; b) replication on a damaged DNA template, and c) inhibition of DNA synthesis. Micronuclei harboring whole chromosomes are primarily formed from failure of the mitotic spindle, kinetochore, or other parts of the mitotic apparatus or by damage to chromosomal sub-structures, alterations in cellular physiology and mechanical disruption. Elevated frequency of MN could also be related to an overall genetic instability. Cells with an unstable karyotype overtime by eliminating chromosomes at least in part by MN formation (Pedeutour *et al*., 1994). There are many compounds that induce genotoxic effects without directly damaging DNA. An interaction of genotoxic compounds like heavy metals (nickel, zinc, lead etc.) with DNA is not a direct damaging way which can cause a change in DNA conformation (Stopper, 2011). Changes in genetic material due to effect of genotoxic chemicals, generally represents the first step (initiation) of the process of chemical mutagenesis or carcinogenesis. These changes in the genetic material of organisms can be detected at specific level by using various genotoxicity assay systems like chromosomal aberrations, sister chromatid exchange assay, micronucleus assay etc. These assays can detect relatively greater damage to genetic material manifested at cellular, chromosomal and DNA level (Stopper *et al*. 1994).

Ayurvedic drug also contains heavy metals. Ayurvedic drugs are generally combination of herbs, heavy metals, and minerals for manufacturing special formulations to combat chronic diseases. The heavy metals (such as Hg, As, Pb etc.) are used in detoxified state in these medicinal products because of their therapeutic properties. If the detoxification process is not systematically followed during manufacturing, it is possible for the resulting products to contain high levels of heavy metals (such as Hg, As, Pb, Cd etc.) which can be highly toxic for human beings. These heavy metals then pose a particular health risk. Children are most susceptible to the toxic effects of heavy metal poisoning (Singh, 2008). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings (Roberts, 1999). Since, heavy metals can impose a risk of secondary malignancy in human beings therefore; the objective of the present study is to evaluate the concentration at which the ayurvedic drugs can bring about DNA strand break i.e. genotoxicity by micro nucleus test (MNT).

MATERIAL AND METHODS

Some standard marketed ayurvedic drugs which have been banned in USA and Canada due to containing higher amount of heavy metals are selected for the present study (Saper, 2004). The ayurvedic preparations i.e. Mahayograj Guggul & Maha laxmi Vilash Ras were selected for our study. Drug solution were prepared by using the formula $N_1V_1=N_2V_2$ Weight by volume. H_2O_2 was used as a positive control and untreated cells was used as a negative control. Atomic Absorption Spectrometer (AA240FS, Fast sequential Atomic Absorption Spectrophotometer) was used for the assessment of heavy metals (i.e. Zn^{2+}, Ni^{2+}, pb^{2+}) from ayurvedic drugs Mahayograj Guggulu & Mahalaxmivilas ras. Fresh blood of healthy human donor was treated with different compounds of different concentration for 3 hr at 37 ºC. After that lymphocyte were isolated in the whole treated blood with the help of Hi-Sep solution (Hi media). Blood was centrifuged at 800 X g for 20 minutes. The Micro-nucleus test (Schmid, 1975) was performed to assess drug induced genotoxicity. The frequency of micronucleated lymphocytes (MNLs) was evaluate by examining 1000 mature lymphocytes for each person collected from different human and the percentage frequency was expressed as follows-

$$\text{Percentage frequency of MNLs (\%)} = \frac{\text{Total No. of MNLs}}{\text{Total No. of cells examined}} \times 100$$

Only isolated nuclear fragment followed by the morphological criteria described by Tates *et al.*, (1980) was counted as micronucleus (MN) i.e. rounded or ovoid shaped non- refractory particle with color and structure similar to the principal nucleus with a diameter of 1/3 to 1/50 of the main nucleus and clearly detached from it was interrupted as 'micronucleus'. Appropriate statistical method one way analysis of variance (ANOVA) was employed for data analysis.

RESULTS AND DISCUSSION

The result of the present study are subdivided into two parts viz., i) Assessment of heavy metals in ayurvedic drugs and ii) assessment of genotoxicity.

Assessment of heavy metals in ayurvedic drugs

This study was conducted at Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur (M.P.). We had used Atomic Absorption Spectrometer (AA240FS, Fast sequential Atomic Absorption Spectrophotometer) for assessment of heavy metals i.e. Zn^{2+}, Ni^{2+}, Pb^{2+} from ayurvedic drugs like Diabecon, Mahayograj Guggulu, Mahalaxmivilas ras and Makaradhwaj vati. Amount of heavy metals present in these drugs are shown in Table 1. According to WHO (world health organization) and FDA (Federal Drug Administration) average amount of zinc should be 0.6 mg/kg, lead should be 100 mg/kg and nickel should be 50 mg/kg (Singh, 2008). These values may vary due to environmental factors. The present study shows that amount of zinc was found more than the WHO and FDA report in Mahayograj Guggul, Mahalaxmivilas Ras. Amount of lead and nickel was found more than the WHO and FDA report in Mahayograj Guggul. These excess amounts of heavy metals in ayurvedic drugs are due to manufacturing defect, which is responsible for causing genotoxicity (Table 1).

Table 1: Amount of heavy metals in the sampled ayurvedic drugs

Name of ayurvedic drug	Zn (mg /kg)	Pb (mg /kg)	Ni (mg /kg)
Mahayograj Guggul	176.2	112.6	75.2
Mahalaxmivilas Ras	171.9	98.6	32.1

Micronucleus Test

This study was conducted at the Department of P.G. Studies and Research in Biological Sciences, R.D. University Jabalpur (M.P.). The number of micronucleated lymphocytes (MNLs) observed per 1000 cells examined at different concentration of each ayurvedic drugs are shown in Table 2. The micro- nucleus frequency in Mahayograj Guggul treated lymphocytes varies from 3.8 to 4.0% and 3.4 to 3.6% in Mahalaxmivilas Ras treated lymphocytes. Table 3 shows percent frequency vs mean standard deviation and coefficient of variation of the micro nucleated lymphocytes. This result indicates that Mahayograj Guggul is most genotoxic because of presence of high amount of heavy metal and Mahalaxmivilas Ras is least genotoxic because of low amount of heavy metal. The microphotographs of lymphocytes under control and drug treatments are depicted (Figure 1).

Table 2. Percent frequency of micro- nucleated lymphocytes in different concentration of drugs.

S.N.	Name of ayurvedic drug	Replicates	Control			Drug Concentration			Average	% frequency
			(-)ve	(+)ve treated with H_2O_2		10 mg/ml	5 mg/ml	2 mg/ml		
				100 µl	200 µl					
1	Mahayograj Guggul	1	0	50	100	48	41	32	40	4.0
		2	0	52	98	45	39	30	38	3.8
		3	0	52	100	49	40	33	40	4.0
2	Mahalaxmivilas Ras	1	0	45	97	40	37	30	35	3.5
		2	0	43	95	43	35	30	36	3.6
		3	0	42	90	40	35	28	34	3.4

Table 3. Percent frequency vs mean standard deviation and coefficient of variation of the micro nucleated lymphocytes.

S.No.	Drug Name	% Frequency	Mean ± S.D.	Coefficient of Variation
1	Mahayograj Guggul	4.0	3.933 ± 0.00707	0.179
		3.8		
		4.0		
2	Mahalaxmivilas Ras	3.5	3.5 ± 0	0
		3.6		
		3.4		

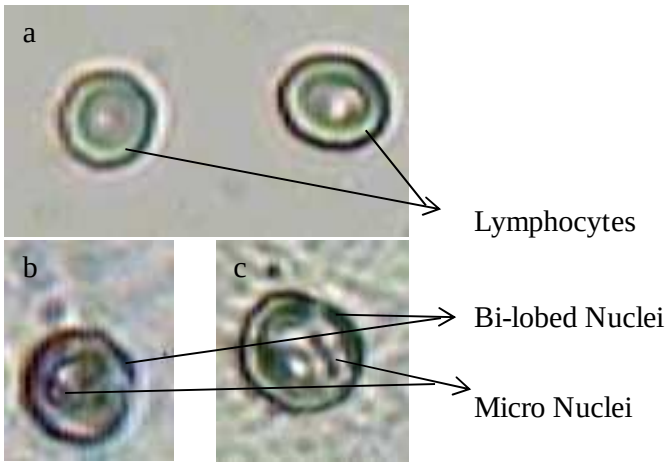

Figure 1. Microphotographs of lymphocytes under control and drug treatment; a) Lymphocytes in control (without treatment); b) Micro nucleated lymphocyte (treated with 10 mg/ml of Mahayograj Guggule); c) Micro nucleated lymphocyte (treated with 10 mg/ml of Mahalaxmi vilash Ras).

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