AN EXPERIMENTAL EVALUATION OF POTENTIAL CYTOGENOTOXIC EFFECTS OF *Gymnema sylvestre* R.BR.ON *Allium cepa* L. CELLS.

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ABSTRACT

Over the past few years, the use of plant products as alternative medicine has increased substantially. A common misconception about such products of plant origin is that they are 'natural' and therefore safe. However plants have developed defense mechanisms which includes many chemical metabolites that can have hazardous or lethal effects on humans.The present study was undertaken to evaluate any possibility of genotoxic effects in *Gymnema sylvestre* R.Br.(Gurmar) based homeopathic drug, a widely used herbal medicine for Type II Diabetes by Homeopathic physicians in India. The *Allium* assay was carried out on standardized aqueous alcoholic extract of *Gymnema sylvestre* R.Br. used as mother tincture in homeopathic formulations. The results show that the investigated samples have depressive effects on chromosomes and cell division in *Allium cepa* root cells. The mitotic index is significantly lower and a direct correlation is observed between the exposure time and effect of the test solution. The experiments indicate that the tincture of the *Gymnema sylvestre* R.Br. plant, at the concentrations tested caused both physiological and clastogenic chromosomal abnormalities in the test samples including formation of single and multiple sticky bridges, dispersed chromosomal structure or fragmentation, shorter and thicker chromosomes, clumping, and multipolarity.

Key Words :- *Allium cepa* L.,chromosomal aberrations, cytogenotoxic, *Gymnema sylvestre* R.Br.

INTRODUCTION

Traditional remedies of plant origin have been used for diabetes around the world from time immemorial. Ethnobotanical studies on such herbal formulations with hypoglycemic activity have revealed the existence of about 1,200 species of plants broadly distributed throughout 725 different genera. Among the 20 antidiabetic plants frequently used around the world, many are prescribed in India. *Gymnema sylvestre* R. Br. is commonly used by homeopathic practitioners to treat type 2 diabetes. In India, it is widely used in Ayurvedic formulas for diabetes (Tiwari et al, 2014) and is frequently used as a folk treatment for diabetes (Grover,2002).Traditionally, the leaves are either chewed whole, taken as a powder, or drunk as a water decoction (Nadkarni , 1993).

This plant, of the family Asclepiadaceae, is a woody, climbing vine common in central and southern India. Most important for the treatment of diabetes are the gymnemic acids, which were reportedly first isolated by Hooper in 1889 (Sinsheimer et al.,1970; Gurav et al.,2007). The best studied extract of *Gymnema*, GS4, contains a group of at least 15 triterpene sapinoids (the gymnemic acids) and a polypeptide, gurmarin consisting of 35 amino acids (Sahu et al.,1996; Liu et al.,2004). It has been traditionally observed that chewing *Gymnema* leaves interferes with

the perception of sweet taste, an effect that can last for 1 to 2 hours. The leaf constituents most responsible for this effect are the gymnemic acids and gurmarin, via direct activity on the nerves of the sensory apparatus in the tongue (Spasov et al.,2008; Saneja et al.,2010).

The overall risk for public health is apparently low or negligible as proclaimed by the practitioners of such alternative therapeutic methods but such remedies have also been associated with a number of potentially serious adverse effects. There are observations of nausea occurring in patients taking more than 3 g of *Gymnema* (Gerson, 2000). Although several studies suggest that *Gymnema* can lower blood sugar levels in people with type 1 diabetes (insulin-dependent diabetes) and type 2 diabetes (non-insulin-dependent diabetes), these studies have been small and poor quality, and better research is needed to determine safety and dosing (Cicero et al.,2004).

So in the present work the effects of antidiabetic homeopathic drug obtained from *Gymnema sylvestre* R.Br was studied on the mitotic cells of *Allium cepa* L. The *Allium cepa* chromosomal aberration assay is a simple, eukaryote genotoxicity assay which is relatively rapid and easy to perform(Khanna et. al.,2013) . It provides a sensitive and reproducible method for genotoxicity screening of formulations used in homeopathic medicine (Leme et al.,2009; Gadano et al.,2002). Owing to qualities such as low cost, easy application and good correlation with the mammalian genotoxicity test systems (Fiskesjo,1987), the *Allium cepa* test represents an alternative first tier assay to experiments on animals for preliminary toxicity screening (Ennever,1988; Tedesco et al.,2012).

MATERIALS AND METHODS

The mother tincture or standardized aqueous alcoholic extract of the medicinal plant used in homeopathic formulations was obtained from Hahnemann laboratories. The mother tincture was used as the stock solution from which 5% and 10% concentrated (containing 50000ppm and 100000ppm) solution of the drug were prepared. Distilled water was used as negative control in this experiment.

The onion bulbs were obtained from the local market.The outer scales are removed from young bulbs of onion to expose the root primordial. Bulbs of the onion *Allium cepa* L (15–30 g) were then grown in the dark .The experiments started when meristem roots reached 15–20 mm. Five bulbs were used for each treatment. Root meristems of *Allium cepa* were exposed for 1h , 2h , 3h to test solutions. A 24h recovery set was prepared with the root tip cells pretreated with test solutions. The root tips were exicised and then fixed in a mixture of ethanol/acetic acid (3/1 vol/vol) and 1 M HCl (1 part) overnight. Finally, they were stained with acetic orcein (2%) and macerated in 45% acetic acid for the analysis of mitotic index and chromosomal aberrations according to Grant(1994). Slides were made permanent, mounted in Canada balsam , studied and photographed.

The slides were analyzed in a blind test, using an optical microscope with 45X objective lens. A thousand cells per bulb were analyzed, totaling 5,000 for control, treatment, and respective recovery. Cells were examined for morphological and structural alterations, and the mitotic index (MI) was determined .The proliferative activity (mitotic index) was calculated by counting the number of cells in mitosis within a microscopic field in relation to the total number of cells. The chromosomal abnormality frequencies were calculated from the metaphase and anaphase cells observed.

RESULT AND DISCUSSION

In this study, the tincture of the *Gymnema sylvestre* R.Br. plant, diluted at 100,000ppm and 50,000ppm of the stock solution was observed to have an adverse and toxic effect on the meristematic root cells of *Allium cepa* L. The mitotic index is significantly lower than control in both cases (Direct treatment and Recovery). A correlation was observed between the exposure time and effect of the test solution. The mitotic index of the *Allium cepa* root tip cells decreased with increase in exposure time to treatment doses. Complete recovery does not occur in one cell cycle (24h) as observed from the obtained data .

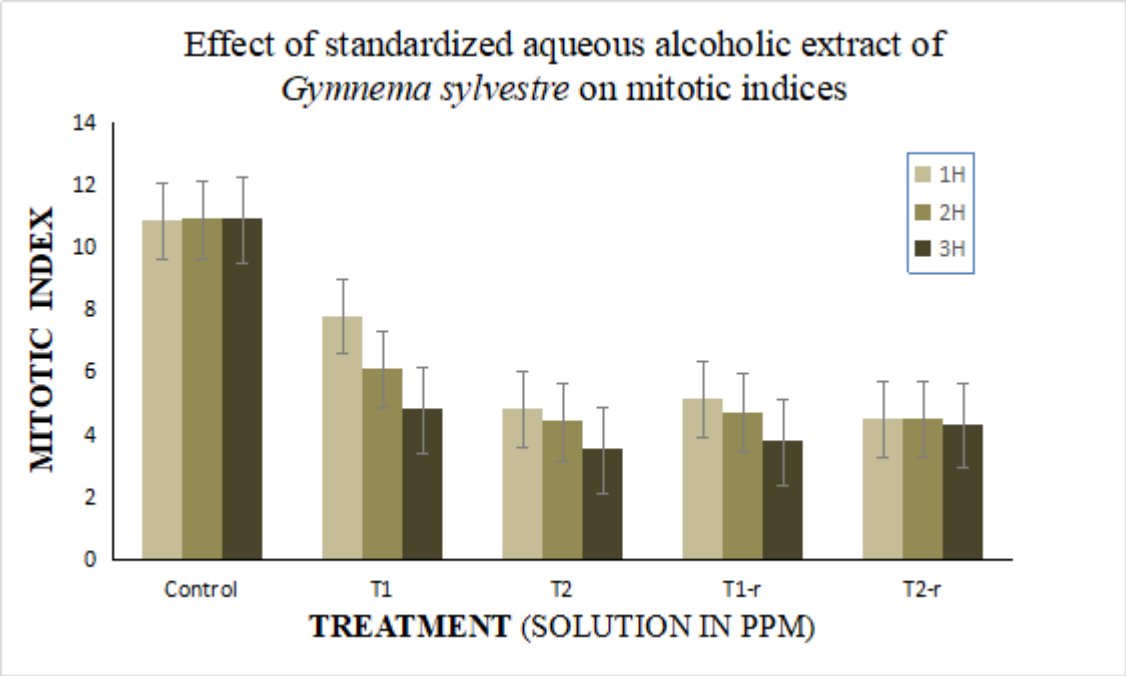


Figure 1. Effect of standardized aqueous alcoholic extract of *Gymnema sylvestre* on mitotic indices of *Allium cepa* L. root tip cells.
C = Control ; T1 = Direct treatment for 24 hours (1Hour,2Hours,3Hours consecutively);
T2 = Direct treatment for 48 hours (1Hour,2Hours,3Hours consecutively); T1-r = 24 h Recovery(1Hour,2Hours,3Hours consecutively); T2-r = 48 h Recovery(1Hour,2Hours,3Hours consecutively).

The meristemetic root tip cells of *Allium cepa* L. treated with *Gymnema sylvestre* test solutions seem to be more in prophase and metaphase state under direct treatment in contrast to the recovered cells which after 1 cell cycle seem to be increasingly in the metaphase - anaphase state of mitotic cell division. This effect of *Gymnema sylvestre* test solution on different phase index in *Allium cepa* L. root tip cells can be seen in the given data.

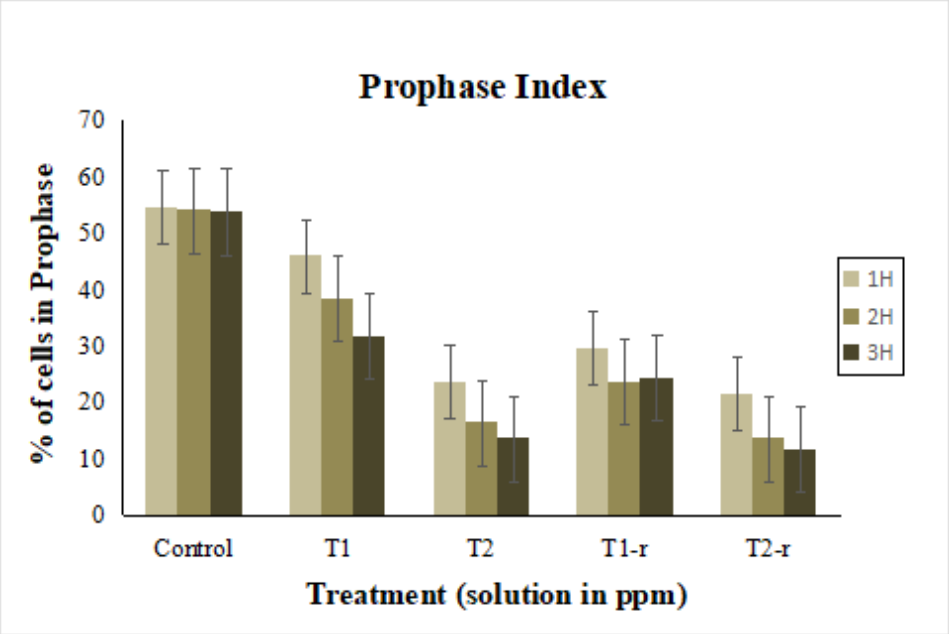


Figure 2. Effect of standardized aqueous alcoholic extract of *Gymnema sylvestre* on Prophase indices of *Allium cepa* L. root tip cells.C = Control ; T1 = Direct treatment for 24 hours (1Hour,2Hours,3Hours consecutively); T2 = Direct treatment for 48 hours (1Hour,2Hours,3Hours consecutively); T1-r = 24 h Recovery(1Hour,2Hours,3Hours consecutively); T2-r = 48 h Recovery(1Hour,2Hours,3Hours consecutively).

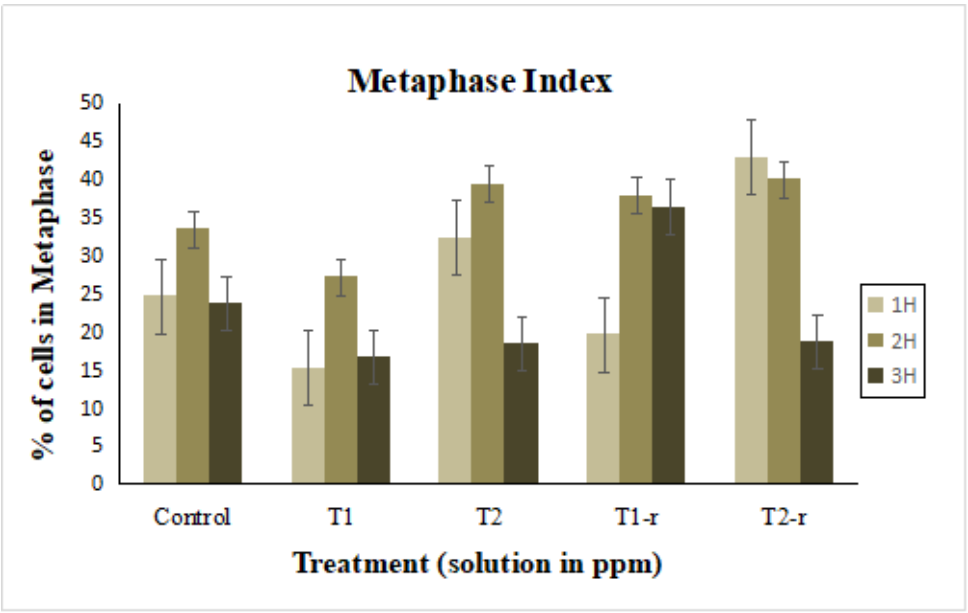


Figure3. Effect of standardized aqueous alcoholic extract of *Gymnema sylvestre* on Metaphase indices of *Allium cepa* L. root tip cells.C = Control ; T1 = Direct treatment for 24 hours (1Hour,2Hours,3Hours consecutively); T2 = Direct treatment for 48 hours (1Hour,2Hours,3Hours consecutively); T1-r = 24 h Recovery(1Hour,2Hours,3Hours consecutively); T2-r = 48 h Recovery(1Hour,2Hours,3Hours consecutively).

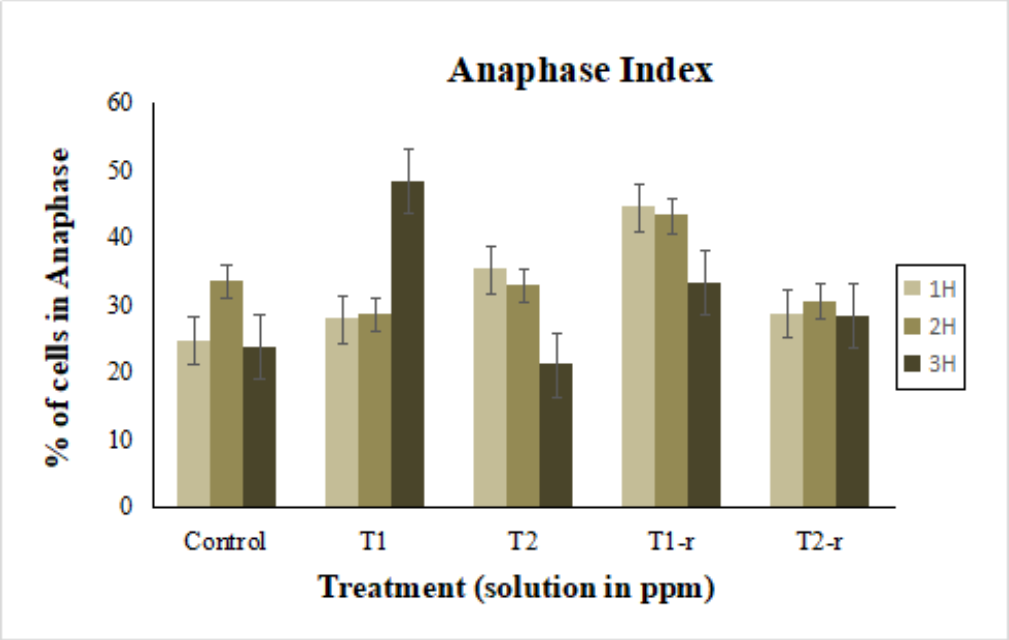


Figure 4. Effect of standardized aqueous alcoholic extract of *Gymnema sylvestre* on Anaphase indices of *Allium cepa* L. root tip cells.C = Control ; T1 = Direct treatment for 24 hours (1Hour,2Hours,3Hours consecutively); T2 = Direct treatment for 48 hours (1Hour,2Hours,3Hours consecutively); T1-r = 24 h Recovery(1Hour,2Hours,3Hours consecutively); T2-r = 48 h Recovery(1Hour,2Hours,3Hours consecutively).

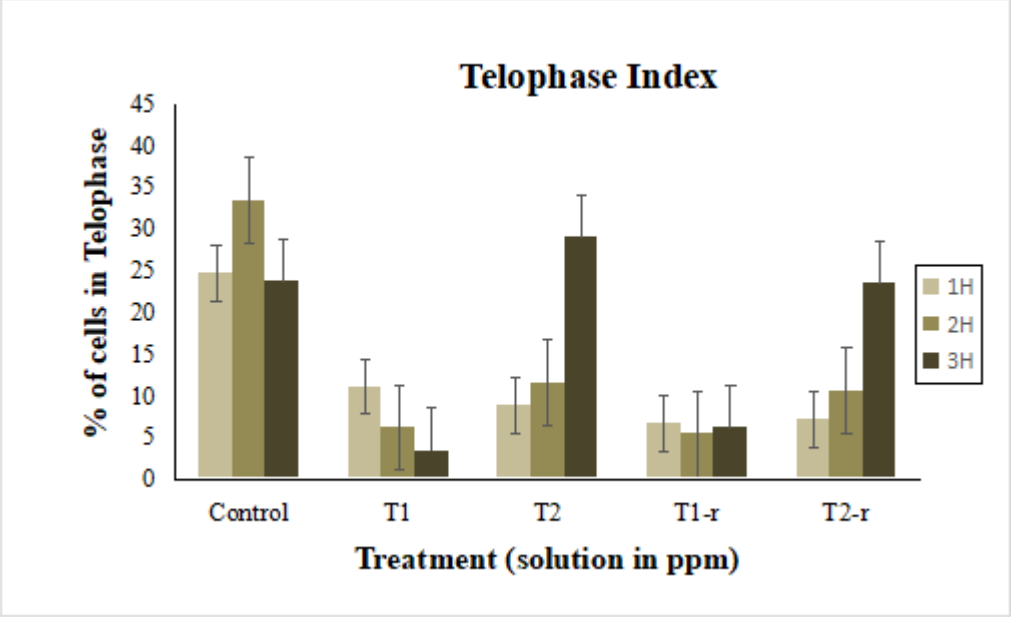


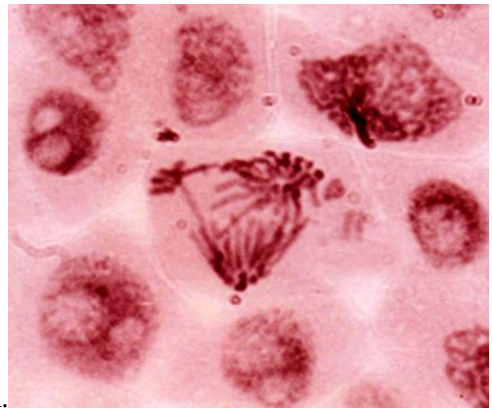
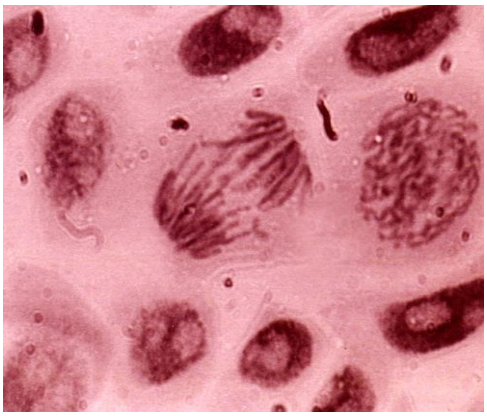
Figure 4. Effect of standardized aqueous alcoholic extract of *Gymnema sylvestre* on Telophase indices of *Allium cepa* L. root tip cells.C = Control ; T1 = Direct treatment for 24 hours (1Hour,2Hours,3Hours consecutively); T2 = Direct treatment for 48 hours (1Hour,2Hours,3Hours consecutively); T1-r = 24 h Recovery(1Hour,2Hours,3Hours consecutively); T2-r = 48 h Recovery(1Hour,2Hours,3Hours consecutively).

A study was further conducted to observe the different types and percentage of metaphase anaphase chromosomal abnormalities induced in *Allium cepa* cells treated with two diluted grades of standardized aqueous alcoholic extract or mother tincture of *Gymnema sylvestre* at different exposure time.The majority of aberrations observed at first metaphase after exposure are lethal to the cell that carries them to the daughter cells. Chromosomal aberrations associated with treatment of the meristemetic cells of *Allium cepa* L. with *G. sylvestre* test solutions included shortening and thickening of chromosomes, delayed chromatid separation leading to laggards, early separation, anaphase bridges, and irregularly

shaped nuclei. Disruption of normal chromosomal structure was also observed after exposure to the different diluted test solutions, including formation of sticky bridges, dispersed chromosomal structure or fragmentation, shorter and thicker chromosomes, clumping, and multipolarity.

Table 1. Types of metaphase anaphase chromosomal abnormalities and its percent in meristemetic root tip cells of *Allium cepa* L. directly treated with standardized aqueous alcoholic extract of *Gymnema sylvestre* at different concentrations and exposure time. Different types of abnormalities are expressed as a percentage of the number of cells in metaphase and anaphase states of mitotic cell division from total dividing cells with mean error respectively.

Concentration (ppm)	Exposure time	METAPHASE Aberrations(%)			ANAPHASE Aberrations (%)	
		Chromosome breaks	Clump formation	Diplochromatid formation	Sticky bridge	Multipolarity
50,000	1hr	1.66 ± 0.2	0.0	0.0	10.52 ± 0.3	0.0
	2hr	1.76 ± 0.3	0.58 ± 0.4	0.58 ±0.3 0.2	12.35 ± 0.3	1.76 ± 0.3
	3hr	0.0	3.73 ± 0.4	1.87 ± 0.3	17.96 ± 0.2	1.86 ± 0.3
100,000	1hr	0.0	7.79 ± 0.3	0.0	3.84 ± 0.4	0.0
	2hr	0.0	16.12 ± 0.2	0.0	6.45 ± 0.3	0.9 ± 0.3
	3hr	2.94 ± 0.3	17.64 ± 0.2	0.0	8.82 ± 0.2	0.57 ± 0.4
Control	1hr	0.0	0.0	0.0	0.0	0.0
	2hr	0.0	0.0	0.0	0.0	0.0
	3hr	0.0	0.0	0.0	0.0	0.0

A.  B. 

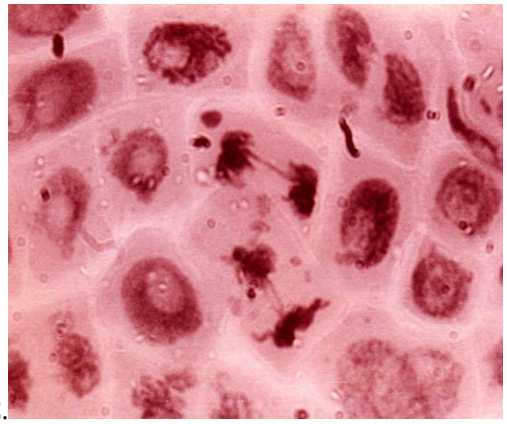
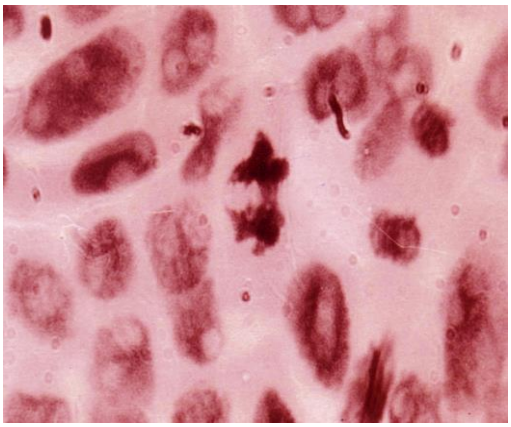
C.  D. 

Plate A) *A.cepa* L. cells showing multipolarity

Plate B) *A.cepa* L. cells showing diplochromatisation and stickiness of chromosomes

Plate C) *A.cepa* L. cells early separation of chromosomes and sticky bridge formation

Plate D) *A.cepa* L. cells showing clumping of chromosomes and columnar sticky bridge

Chromosomal aberrations were not observed in cells of Control or untreated roots. Further, postincubation for 24h in distilled water of directly treated roots of *Allium cepa* L. with standardized aqueous alcoholic extracts of *Gymnema sylvestre* showed partial amelioration and exhibited mitotic recovery to some extent.

CONCLUSION

It is a common misunderstanding of homeopathy that homeopathic medicinal products are always safe because they are diluted. A medicinal product is essentially characterized by its active principles. Many homeopathic products contain active constituents accepted in the homeopathic literature to be safe and effective although there are no standardized clinical or preclinical studies complying with current criteria. In many aspects the diagnostic and therapeutic approach of homeopathy is opposed to that used in the medical community rules and regulations on the practice of medicine. Therefore the assessment of safety and efficacy of homeopathic products is of utmost importance before the said drugs are prescribed for treatment of diseases (Boullata et. al.,2007).The present study was performed on root cells of *Allium cepa* L. which is considered to be a sensitive and reliable test system (Babich et. al.,1997;Oudalova et al.,2017) . Literature on previous tests performed with *Allium cepa* L. root tip cells have shown good correlation with other animal test systems involving genotoxicity (Khanna et. al., 2013; Firbas, 2014).*Gymnema sylvestre* R.Br. is widely recommended for its antidiabetic property (Foster,2002) but there is no substantial data on its cytogenetical effects.Therefore in this study, the effects of mother tincture of *Gymnema sylvestre* R.Br. used in homeopathic formulations was assessed through *Allium cepa* L. test system (Akinboro et.al.,2007).

The data obtained in our experiment showed that sticky bridges were the most common chromosomal aberration observed in all the treatments. Single, double and multiple bridges were recorded which may be the consequence of both chromosome breakage and reunion. Chromosome

stickiness is presumed to be a result of degradation or depolymerization of chromosomal DNA .It reflects irreversible toxic effects which eventually leads to apoptosis or cell death (Khanna et.al.,2013).Stickiness of chromosomes also causes incomplete separation of daughter chromosomes and as a result they remain connected by bridges (Badr *et al*.,1992; Turkoglu, 2007). Moreover the multipolar distribution of chromosomes due to unequal separation of chromosomes may also give rise to multinuclei formation in later stages of cell division (Rank et. al.,1993). A strong correlation between the ability of the chemicals to cause chromosomal aberrations and its capacity to induce point mutation had been reported (Carruyo, 2008).

The results of the present study showed that the investigated samples have depressive effects on chromosomes and cell division in plant (*Allium cepa* L.). Though homeopathic drugs are considered to be safe for treating ailments, further evaluation and constant control through monitoring of their presence in vivo is required for complete assurance in order to safeguard human health.

CONFLICT OF INTEREST

None declared

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