ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Genotoxic Screening of Leaf Extract from *Argyreia nervosa*

***Alan Eapen Roy[1], V S Jose Kumar[1], Leenamma Joseph[1] and Dinesh Roy D[3]**

1. Department of Zoology, Mar Ivanios College, Thiruvananthapuram

2. Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram- 695 024

Manuscript Info

Manuscript History:

Received: 18 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

A. nervosa, Convolvulaceae, Cytotoxic, Genotoxic, CBMN assay

Corresponding Author

Alan Eapen Roy

Abstract

Argyreia nervosa (*A. nervosa*) is plant of native origin, especially found in Western Ghat regions in India. It is a climbing shrub with woody tomentose stem belongs to family Convolvulaceae. The existing evidence on the pharmacological properties of this plant highlights its importance in traditional medicine therapy. However the cytotoxic and genotoxic nature of this plant is not clearly understood. An attempt is made to identify the genotoxicity of plant *A. nervosa*. The leaf extract of the plant is evaluated for the genotoxicity is in this work. The aim of the present study was to determine Genotoxic Screening of Leaf Extract from *Argyreia nervosa* by CBMN assay. So with the aid of CBMN assay it is conclusively proved in the present study for the first time clearly identified that there is no genotoxic effect in the leaf extract from *A. nervosa*.

INTRODUCTION

A. nervosa plant is also known as elephant creeper or woolly morning glory belongs to family Convolvulaceae. This plant is found on river banks, edges of lakes and as undergrowth in semi-deciduous forests. Traditionally, the plant has been used therapeutically for its wide range of clinical effects such as antiviral, antibacterial, anti-fungal and anti-inflammatory properties. It has rejuvenating, age sustainer and spermatogenic activities as well (Gupta, 2008). The seeds contain the highest concentration of psychoactive compounds in the entire plant. In India, usually leaves and root parts of the plant are used as antiseptic and anti-inflammatory drugs (Singhal *et al*., 2011).
Its roots are known to possess aphrodisiac and diuretic properties, and have been used to treat gonorrhea; while its leaves are antiphlogestic, emollient, local stimulant, rubifacient and vesicant. Leaves of *A. nervosa* mainly contain β-sitosterol, 1-tricontanol and quercetin (Gupta, 2008). Internally, the extract of *A. nervosa* leaves has been used to cure the boils and swellings (Dhiman, 2006).

Based on their long-term use by humans, one might expect herbs used in traditional medicine to have low toxicity. Investigations have revealed that many plants used as food or in traditional medicine have mutagenic, cytotoxic and genotoxic effect *in vitro* and *in vivo* assays (Plewa and Wagner, 1993; Higashimoto *et al*., 1993; Schimmer *et al*., 1994; Askın, 2007). Assessment of the potential genotoxicity of traditional medicines is indeed an important issue as damage to the genetic material. It may lead to critical mutations and therefore also at an increased risk of cancer and other diseases (Galloway *et al*., 1998; Galloway, 2000).

Genotoxic substances are those chemical compounds capable of causing genetic mutation. It's contributing to the development of tumors while there are many different factors that can affect DNA, RNA and other genetic materials. The property of genotoxicity only applies to those substances that actually cause harm to the genetic information. A substance that has a property of genotoxicity is known as a genotoxin (Swarnalatha et al., 2014). In most cases, genotoxicity leads to mutations in various cells and other bodily systems. Mutation can lead to host of

other problems, from cancer to a wide variety of different diseases. Some mutations may be completely harmless and can go completely unnoticed. In many other cases, the effects of genotoxins can be deadly. Mutations can come in many different forms; genetic information duplicated, deleted, inserted though there are many mechanisms by which, genotoxicity can affect. One of the most common mechanisms involves the formation of strong chemical bonds between the genotoxins and the molecules that compose genetic information, such as DNA and RNA. In some cases, these bonds do not strongly affect the existing genetic data (Anton et al., 2009; Swarnalatha et al., 2014).

Research laboratories worldwide have found literally thousands of phytochemicals which have *in vitro* inhibitory effects on all types of organisms. These *in vitro* screening programs are important in validating the traditional use of herbal remedies and for providing leads in the search for new active substances. Whereas activity identified by an *in vitro* test does not necessarily confirm that a plant extract is an effective medicine, nor a suitable candidate for drug development. It does provide basic understanding of a plant efficacy and in some cases toxicity. However, more of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organism systems. These include particular toxicity studies as well as an evaluation of their effects on normal microbiota. The non prescription use of medicinal plants is cited today as an important health problem, in particular their toxicity to the kidneys (Mendonca-Filho, 2006). This context make it sense to suggest a study to evaluate the genotoxicity of the *A. nervosa* leaf extracts whether it is recommendable for human consumption.

Material and Methods

The extraction was done by using the soxhlet apparatus, three different leaf extractions of *A. nervosa* was prepared. The solvents were selected by the order of the polarity; methanol as polar solvent, petroleum ether as non polar and ethyl acetate as mid polar solvent.
Leaves of *A. nervosa* was collected, shade dried and 20 g of the powdered leaf was taken, wrapped in filter paper in such a way it will fit into a soxhlet apparatus, placed inside a thimble which is the main chamber of soxhlet extractor. The extraction solvent was taken in the distillation flask equipped with a condenser. After extraction the extracts were collected and evaporated to dryness using warm water bath. The percentage of yield was calculated. The concentrated extracts were stored in refrigerator and were taken later for carrying out CBMN assay. The yields of the extracts are given below:

Solvent	Wt. of leaf taken	Wt. of substance obtained
Pet. Ether	20 g	0.662 g
Ethyl Acetate	20 g	0.317 g
Methanol	20 g	3.752 g

The extracts prepared to be were mixed with respective solvents and was dissolved. Then it was subjected to CBMN assay. Lymphocyte cultures for each subject were prepared in sterile bottles using 10 ml RPMI 1640 medium containing 15% fetal calf serum, 100 units/ml penicillin, 100 units/ml streptomycin, and 1% phytohemagglutinin. At the 44^{th} hr after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, final concentration, 4.5µg/ml). The total incubation time in all cultures was 72 hr. After incubation, the cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored at 100X magnification. The number of micronuclei per 1,000 binucleated cells was recorded.

Result

Genotoxic effect of successive extracts of leaves of *A. nervosa* was tested and the results are reported. The extracts of petroleum ether, ethyl acetate and methanol were subjected to CBMN assay. Two concentrations 5µl/ml and 20µl/ml of the three extracts were tested for the genotoxic assay. The following result was obtained and has been tabulated below (Table 1).
The methanol extract showed a higher CBMN frequency at 20µl/ml when compared to petroleum ether and ethyl acetate extracts. But 5µl/ml concentration of the petroleum ether extract showed a high CBMN frequency compared to respective concentrations of the rest of extracts. The Ethyl acetate extract has a low CBMN frequency when

compared to the other two. The control showed equal frequency values at both concentrations of different solvents viz., methanol, ethyl acetate and petroleum ether.

Table1. Distribution of mean CBMN frequency with respect to type of extracts

Type of extract	Conc of 5µl/ml	Mean CBMN frequency	Conc of 20µl/ml	Mean CBMN frequency
Pet ether	Trail 1	10.8	Trail 1	11
	Trail 2	11	Trail 2	11.1
Ethyl acetate	Trail 1	10.62	Trail 1	10.88
	Trail 2	10.84	Trail 2	10.72
Methanol	Trail 1	10.74	Trail 1	11.2
	Trail 2	10.66	Trail 2	11
Control	Trail 1	10.62	Trail 1	10.62
	Trail 2	10.8	Trail 2	10.8

Discussion

According to Celik and Aslanturk, (2010) mutation is simply a sudden heritable change. Phytochemicals derived from plants or microbes serve as valuable sources for pharmacology, however a good number of plant extracts are genotoxic or mutagenic in nature. The present study examined the genotoxic effect of the different extracts of the leaf of *A. nervosa*. It proved that all the extracts studied are non-toxic and do not satisfy as genotoxic agent. The highest mean CBMN frequency identified as 11.1, which is quite below the range of toxic conditions.

In the extracts with a concentration of 5µl/ml the CBMN frequencies was found between 10-11. This result very well identified that the plant extract is below the limit of genotoxic effect. The concentrations at 20µl/ml the CBMN frequencies for respective extracts lie between 10.7–11.1. It is evident from the result that even the high concentrations of extracts are well below the genotoxic frequencies. This showed that these extracts are advisable for human consumption as it is used in traditional treatment.

A similar observation of genotoxic studies on *Boswellia serrata* (*B. serrata*) is available; anti-inflammatory, anti-pyretic, anti-arthritic properties marking its importance in traditional medicine. It showed zero mutagenicity up to 5mg/plate when tested with *Salmonella typhimurium* and its strains (Magesh *et al*., 2008). When the leaf extract of *A. nervosa* was subjected to genotoxic test, the result was non-toxic up to 5µl/ml. Like *B. serrata*, *A. nervosa* also possess a wide range of medicinal properties. Even when the concentration was increased to 20 µl/ml the effect is non-toxic.

From the present study showed that mean CBMN frequencies of the three extracts, the ethyl acetate extract shows lowest toxicity. An increase in chromosomal aberrations may result from interactions of a great variety of chemical agents with DNA. According to Ishidate *et al*., (1988), the agents which induce an increase in the chromosomal aberrations (CA) frequency by direct or indirect mechanism may also be cytotoxic, for damage to both DNA and other cell targets like enzymes, membranes, structural proteins etc..

The present study conclusively proved the leaf extracts of *A. nervosa* are not cytotoxic in nature as evident from this genotoxic screening test. Hence, the study for the first time identified non-genotoxic nature of leaf of *A. nervosa*. This valuable information along with the earlier studies prove the safety of *A. nervosa* plant for pharmacological application.

Summary

The present study evaluated the genotoxic effect of leaf the extracts of *A. nervosa*. The CBMN assay was followed to screen the genotoxicity. Three different extracts of varying polarity were used in this study. The yield of the extract was higher in methanolic extraction. There were slight variations in the frequency values of the CBMN assay in the three extracts. Two concentrations of the three extracts viz., 5 µl /ml and 20 µl /ml were tested in this assay. A highest CBMN frequency was obtained at 20 µl /ml concentration of methanol (11.2). This value is falling within

the lower limit of the CBMN assay as non-genotoxic. Hence the present study highlights the importance of the plant safety and therefore suggested as nontoxic for pharmacological use. The medicinal properties offered by the plant are widely used in traditional medicines. Since the present investigation identify it as non-toxic at the level of genotoxicity and the different pharmacological properties attributed to this plant signify its importance in traditional medicine. The present study is providing one more pharmacological attributes to this plant. Thus with results of CBMN assay it is proved that there is no genotoxic effect in the leaf extract from *Argyreia nervosa.*

References

A K Singhal, H Gupta and VS Bhati. (2011). Wound healing activity of Argyreia nervosa leaves extract Int J Appl Basic Med Res. Jan-Jun; 1(1): 36–39.

Anton P, Lutz M, Miklos S, Judith KJ, Peter L and David T. (2009). Elevated ethyl methane sulfonate(EMS) in nelfinavirmesylate (viracept, Roche):overview. AIDS Res Ther, 6,18.

Askın Celik T, Aslantürk O.S. (2007). Cytotoxic and genotoxic effects of Lavandula stoechas aqueous extracts.Biologia;62(3):292–296.

Askın Celik, T. & Aslanturk, O.S. (2010). Evaluation of Cytotoxicity and Genotoxicity of Inula viscosa Leaf Extracts with Allium Test. Journal of Biomedicine and Biotechnology, (doi: 10.1155/2010/189252).
Dhiman AK. (2006). Ayurvedic Drug Plants. Delhi: Daya Publication House; pp. 331–2.

G.Swarnalatha, C.Vanitha, V. RajaniSekhar, E. Mounika, I SowkarBaig, B. Vijayakumar. (2014). Review on Genotoxic Impurities in Drug Substances, Indian Journal of Pharmaceutical Science & Research, Vol 4, Issue 4, 210-216.

Galloway S.M., Miller J.E., Armstrong M.J., Bean C.L., Skopek T.R., Nichols W.W, (1998). DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. Mutation Research. 400, 169 –186.

Galloway, S.M. (2000). Cytotoxicity and chromosome aberrations in vitro: Experience inindustry and the case for an upper limit of toxicity in the aberration assay. Environmental and Molecular Mutagenesis 35, 191–201.

Gupta DP. (2008). The Herbs. 1st ed. Indore: Smt. Prem Lata Gupta Prakashak; pp. 57–8.

Higashimoto M, Purintrapiban J, Kataoka K, et al., (1993). Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. Mutation Research;303(3):135–142.

Ishidate, M., Hammois, M.C., Sofuni, T., (1988). A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutation Research, 195, 151–213.

Magesh, V et.al., (2008). Genotoxicity studies of dry extract of Boswellia serrata, Tropical Journal of Pharmaceutical Research, December 2008; 7 (4): 1129-1135.

Mendonça-Filho, R.R. (2006). In Modern phytomedicine turning medicinal plants into drugs Bioactive Phytocompounds: New Approaches in the Phytosciences. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 1-24.

Plewa MJ, Wagner ED (1993). Activation of promutagens by green plants. Annual Review of Genetics.; 27:93–113.

Schimmer O, Kruger A, Paulini H, Haefele F. (1994). An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie.; 49(6):448–451.