ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

In Vivo Evaluation of Genotoxic Effects of Sivanar Amirtham Formulation on Rats Using Micro Nucleus Assay

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ABSTRACT:

Traditional medicine may also cause genetic damage that may increase the possibility of cancer and other diseases. Therefore, it is essential to evaluate the genotoxic testing of Siddha drugs. Therefore, a latest take a look at become performed to evaluate the in vivo genotoxic effects of Sivanar Amirtham in mice using micro nucleus assay. Three different doses of 100, 200, and 400 mg / kg b.w were selected in accordance with OECD guidelines. In a micronucleus test, the administration of one oral Sivanar Amirtham at 100, 200 and 400 mg / kg b.w did no longer increase with the mean amount of micronucleated polychromatic erythrocytes or PCE percentage for both sexes showing non clastogenicity. For this reason it could be concluded, Sivanar Amirtham showed no vast genotoxic impact on the rats in both sexes. Sivanar Amirtham was therefore not carcinogenic and therefore could be used safely.

KEYWORDS: Genotoxicity, Sivanar Amirtham, Micronucleus, Carcinogenic.

INTRODUCTION:

Now a day's traditional system of medicine (Indian system of Medicine) became very popular all over the world due to its low toxicity, diminished side effects and medicinal properties[1]. The Siddha medical system, one of Asia's most traditional herbal systems, derives drugs from plants, animal products, minerals and metals[2]. The Siddha medical system, one of Asia's most traditional herbal remedies, derives drugs from plants, animal products, minerals, and minerals from ancient Tamilakam in South India. Sivanar Amirtham is a popular herbo-mineral formula utilized in traditional ayurvedic to deal with a variety of illnesses.

Received on 25.08.2021 Modified on 22.11.2021
Accepted on 26.02.2022
Research J. Pharm. and Tech 2022; 15(11):5017-5020.
DOI: 10.52711/0974-360X.2022.00843

Sivanar Amirtham is a combination of a total of nine ingredients like *Dryopteris filix-max,* Elemental Mercury (purified), *Aconitum napellus*, Elemental sulfur (purified), *Zingiber officinale*, *Piper nigrum*, *Piper longum*, Arsenic disulfide (purified), Borax (purified). Traditionally, Sivanar Amirtham is used to promote the strength of bones and joints by anti-inflammatory and analgesic activity. It is also used to treat stiffness of muscles, coccyx pain, back spasm, and back injury. Partially it is also useful in hypothyroidism, ankylosing spondylitis, sensory neural hearing loss, and avascular necrosis[3].

Siddha medicine aims to make the body perfect, indestructible, and long-lasting. Siddhars have emphasized daily and seasonal routines, as well as dietary, practices, as well as a code of ethics for living a healthy life. Most of the Siddha preparations in clinical

practice currently are prescribed only with the support of ancient literature. Genotoxicity is the capacity to interact with DNA and cellular gadgets that alter genome constancy. Genotoxicity checks were used to expect carcinogenicity[4-8].Checking out for the feasible genotoxicity of traditional medicinal drug is a simply critical issue as genetic harm can lead to genetic mutations and for that reason growth the chance of cancer and different diseases[9-13].

Genetic toxicology research has supplied a number of experimental procedures, each in vitro and in vivo. They are designed to check the results of various genetic tests[14-17]. Examination of mouse micronuclei in vivo has been identified as one of the critical mechanisms to evaluate the toxic genotoxicity of Sivanar Amirtham[18, 19]. Micronucleus testing detects clastogenicity due to chromosome dissociation. Micronucleus is released from cells due to chromosomal harm over the past mitosis[20, 21.]

For many rural populations in underdeveloped nations, traditional medicine may give new chemicals that help counteract the high cost and hazardous effects of existing drugs. Because no research has been done on Sivanar Amirtham intake, a holistic approach to evaluating their efficacy and safety profile is required. As a result, the current study used swiss albino mice and wistar albino rats to test the safety of Sivanar Amirtham in acute and genotoxicity studies.

MATERIALS AND METHOD:

Preparation of suspension:

Suspension formulation was prepared by adding various suspending agents with the drug in that suitable suspending agent were continued.

Particle Size Analysis:

The SAS particle size in the fixed configuration was determined by FE-SEM (ZEISS ULTRA 55, Germany) at a voltage of 20 kV, with the main electrons blown into samples covered with a thin layer of gold produced by QUORUM Q150 RES, UK.

Animals:

Albino rats (Wistar strain) weighing 170-250g of both sexes were used in the study. The animals were bred and housed at the MS Ramaiah University of Applied Sciences, Bangalore. Animal reservation approved by the Institutional Animal Ethics committee of M.S. Ramaiah University of Applied Sciences (IAEC certificate number: XVIII / MSRFPH / M-05 / 08.02.2017).

Genotoxicity study:

Micronucleus test:

Wistar albino rats have been divided into five groups consisting ten rats, five male and five females in each group.

Groups	Dose
Group I	Vehicle control
Group II	Cyclophosphamide (CP) 40 mg/kg b.w., i.p
Group III	SAS 100 mg/kg b.w., p.o
Group IV	SAS 200 mg/kg b.w., p.o
Group V	SAS 400 mg/kg b.w., p.o

Twenty four hours of single-dose treatment, the animals had been sacrificed and bone marrow becomes amassed from each femurs with the aid of flowing with fetal bovine serum. Then, the solution changed into centrifuged at a thousand rpm for 10 mins. The pellet is then collected, smeared and allowed to dry followed by fixation with methanol and stained the use of the May-Grunwald's and Giemsa stains. After staining the slides were dried and viewed under the purpose of oil immersion to find out the frequency of the MN. Percentage of PCE at a 1000 erythrocyte count, number of micronucleus polychromatic cells at 2000 PCE and percent of MNPCE was tested[22].

Percent of PCE = [PCE / (PCE + NCE] × 100
Percent of MNPCE = (MNPCE + PCE) × 100

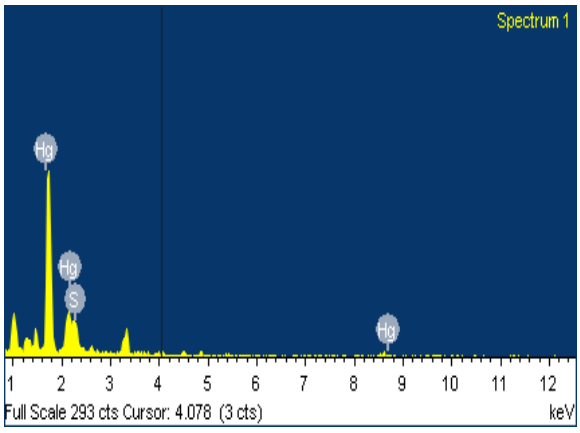
RESULTS:

Preparation of suspension:

The suspension was freshly prepared by mixing 100 mg of drug (Sivanar Amirtham) in 0.1% w/v of supending agent (Carboxy Methyl Cellulose) in distilled water.

Particle size analysis:

The diameter of the particles were found to be within the range of 70-126nm (Figure.1). The particles were found to be spherical in shape. The X-EDS (Figure.2) peaks confirm the absence of impurities.



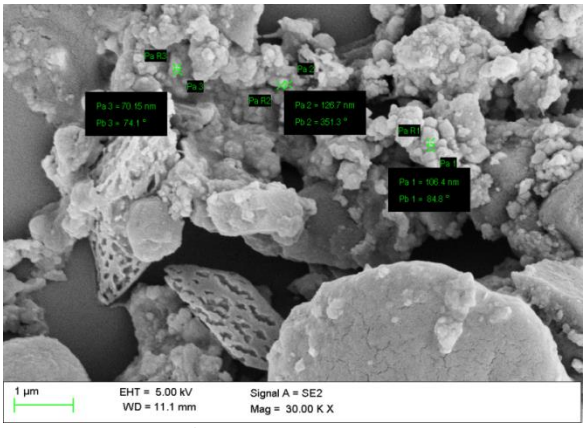


Figure 1: FESEM of Sivanar Amirtham Figure 2. X-EDS of Sivanar Amirtham

Micronucleus assay:

The occurrence of % PCE, % MNPCE and MNPCE were observed in each mice and the effects had been shown in (Table-1). Animals handled with cyclophosphamide i.p showed a significant increase in MNPCE price, % MNPCE and% PCE (P <0.01) in comparison with the control group. Animals when treated at 100, 200 and 400 mg / kg b.w. of Sivanar Amirtham confirmed no extensive increase in MNPCE value, % MNPCE and% PCE when compared with control organization.

Table 1: **Genotoxicity effects of SAS on Wistar albino rats**

Groups	Sex	MNPCE/2000	% MNPCE	% PCE
Vehicle control	**Male**	1.88 ± 0.28	0.84 ± 0.29	21 ± 2.91
	Female	1.81 ± 0.28	0.9 ± 0.22	26.4 ± 3.89
Cyclophosphamide (40 mg/ kg,i.p.)	**Male**	11.42 ±1.028**	1.28 ± 0.18^{ns}	50.3 ± 2.091**
	Female	11.04 ± 1.09**	2.338 ± 0.39**	49 ± 1.81**
SAS 100mg/kg (p.o.)	**Male**	1.46 ±0.22^{ns}	0.70 ± 0.183^{ns}	22.50 ± 1.87^{ns}
	Female	1.26 ± 0.18^{ns}	0.5 ± 0.13^{ns}	23.93 ± 1.23
SAS 200 mg/kg (p.o)	**Male**	1.44 ± 0.23^{ns}	0.79 ± 0.12^{ns}	25.38 ± 1.54^{ns}
	Female	1.42 ± 0.23^{ns}	0.66 ± 0.14^{ns}	37.53 ± 2.74^{ns}
SAS 400 mg/kg(p.o)	**Male**	1.66 ± 0.37^{ns}	0.88 ± 0.23^{ns}	30.62 ± 4.66^{ns}
	Female	1.8 ± 0.37^{ns}	1.46 ± 0.39^{ns}	34.34 ± 5.10^{ns}

Data are expressed as the mean ± SEM. All groups are compared with vehicle control group,** P<0.01,^{ns} non-significant.

DISCUSSION:

According to the OECD guidelines, rodents had been used considerably in animals for widespread pharmacokinetic, toxicokinetic, toxicologic and carcinogenic studies. Genotoxic researches are applicable to become aware of the volume of DNA harm because of the Siddha drug[23]. Genotoxic agents can cause genetic harm to anybody cell. In accordance with OECD 474 suggestions the bone marrow micronucleus test is usually used to test for the genetic toxicity of the take a look at drug in vivo[24].

In micronucleus formation, when the bone erythroblast grows right into a PCE, the primary nucleus is launched, and any artificial micronucleus may additionally continue to be at the back of the anucleated cytoplasm. Visualization of micronuclei is made easier for those cells due to the fact they do not have a core nucleus[25-28]. Micronucleus is a mass of cytoplasmic chromatin with the advent of small nuclei from chromosome delays in anaphase or in acentric chromosomal fragments. They provide a computable measure of recent DNA damage that occurs when fragments of acentric or entire chromosomes are left behind the primary nucleous in telophase. Increased frequency of MNPCEs in treated animals is a sign of chromosome damage. In the three specific Sivanar Amirtham doses there was no increase inside the mean quantity of MNPCE or PCE percent in each intercourse in mice showing non-clastogenicity[29, 30].

CONCLUSION:

It can therefore be concluded that Sivanar Amirtham did not show any Genotoxicity. Sivanar Amirtham was non-mutagenic or carcinogenic and therefore could be used safely.

ACKNOWLEDGEMENT:

Authors are grateful to Dr. V. Madhavan, Dean, Faculty of Pharmacy, M.S.R.U.A.S., Bangalore, for providing all the facilities to carry out this research work.

CONFLICT OF INTEREST:

Authors claim that there's no conflict of interest related to this work.

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