Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

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Research Article

Evaluation of genotoxicity (DNA damage and sperm head anomalies) of potentized homeopathic medicines, *Chinchona* and *Arsenicum Album* in laboratory mice

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ABSTRACT

Homeopathic medicines face criticism from the scientific community due to the lack of a clearly defined mechanism of action. The healing process, diagnosis of disorders, and selection of appropriate remedies are often not explained by practitioners. Furthermore, a significant objection to alternative medicines, particularly homeopathy, revolves around the extreme dilution of remedies in water or alcohol, resulting in the absence of detectable active molecules. In the present study the *in vivo* toxicological consequences of mother tincture and 30C and 200C potency of *Chinchona* (Chin.) and *Arsenicum Album* (ars.alb.) were evaluated, using Balb/c mice as an animal model. DNA damage in lymphocytes, bone marrow micronuclei formation, and sperm head anomalies were calculated to measure the potential drug-induced toxic effects of selected medicines. Data from the present study revealed a significant difference among the treatment groups. High damage was caused by positive control and 200C potency of both drugs showing 80.49±0.0 tail DNA % and high frequency of micronucleate bone marrow cells. Further study and research are needed to elucidate the mechanism of action of homeopathic medicines. The current data revealed that there is the least damage caused by negative control and mother tincture while notable damage is caused by positive control and 200C Potency which indicates that excess alcohol may act as a reason for damage.

Keywords: Genotoxicity; homeopathic medicines; *Chinchona*; *Arsenicum Album*; laboratory mice.

OPEN ACCESS

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Article History
Received: December 10, 2024
Accepted: December 26, 2024
Published: December 31, 2024

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INTRODUCTION

The concept of homeopathy was introduced by Hahnemann which employes the principle of “Like cures Like” (Hahnemann, 1796). This principle suggests that a substance that causes certain symptoms in a healthy person can be used to treat similar symptoms in an ill person when administered in extremely diluted doses (Chikramane et al., 2010). Homeopathic medicines are made from the extraction of natural sources, called mother tincture which is further diluted in alcohol solution. This process is called potentization and the dilution thus made is called potency (Muhammad et al., 2024). The practice of homeopathy has been controversial, and the efficacy of homeopathic medicines has been a topic of debate for many years. There is limited scientific evidence to support the claims made by homeopathic practitioners, and many experts argue that homeopathic remedies are no more effective than placebo (Khuda -Baksh., 2006). Homeopathic medicines are criticized by scientists because the mechanism of action of homeopathic remedies is not clearly explained. The therapeutic and curing process of homeopathy, diagnosis of the disorder and reason for the selection of remedy for that disorder is not explained by homeopaths. Apart from this the most critical objection over complementary medicines is the deficiency of active molecules of extract in the high dilution of homeopathic medicines in water or alcohol (Rajan et al., 2017).

Concerns have been raised about the potential toxicity of the excessive alcohol used in homeopathic remedies. Recent studies have been revealed that reactive and toxic metabolite by the degradation of ethanol, has potential genotoxic and mutagenetic effect (Ishikawa et al., 2007). To investigate this, the study tested two widely prescribed homeopathic medicines: Arsenicum Album (Ars. alb). Cinchona (Chin.) is known to cure various forms of low-grade, remittent, intermittent, or malarial fevers and eliminate parasites. It is likely that both Cinchona and malaria share a common "effector toxin" that causes similar symptoms. Homeopathic Cinchona (Chin.) works by neutralizing this common effector toxin, thereby curing malaria (Gonuguntla, 2010). Likewise, Arsenicum Album, prepared from arsenic trioxide through the homeopathic processes of succession and dilution, has been shown to effectively counteract arsenic trioxide-induced toxicity in mice. Additionally, this drug has proven effective as an immunity booster in the treatment of COVID-19 (Bhattacharjee et al., 2021). However, before using any compound or medicine as a therapeutic agent, it is crucial to conduct a thorough toxicological and safety evaluation. There is a significant gap in the literature concerning the safety of homeopathic drugs, emphasizing the need for additional research in this field.

The in vivo toxicological effect of mother tincture(Q), 30C and 200C potency of Chinchona (Chin.Q, Chin.30C and Chin.200) and Arsenicum Album (Ars.alb. Q, Ars.alb. 30C, Ars.alb. 200C) was evaluated through comet assay and Sperm head anomalies, using Balb/c mice as an animal model.

MATERIALS AND METHODS

Animal Selection

Healthy, male Balb/c mice, weighing about 25 gm, aged 3 to 4 months old were selected. Mice were kept and reared in the Animal House at the Department of Pharmacy, University of Peshawar Pakistan and treated according to directives of “2010/63/EU on animal protection of animal utilized for scientific purpose”. Mice were given a standard low-protein diet and water *ad libitum.*

Treatment Groups

A total of 64 mice were assigned 3 different groups i.e., positive control (potentized Alcohol fed), negative control and treatment groups (three groups for each drug, i.e.- Q,30C, 200C). Mice were divided into two batches based on time fixation (i.e., 7 days, and 14 days). Each of the two batches contained 8 different treatment groups and each group had 4 mice.

Dose Administration

Mice were given 0.06 ml or 1 drop/kg of their body weight (Biswas et al., 2010) of each drug orally with the help of a micro-pipette, twice a day. The present study was certified by the “Departmental Ethical Committee” of Women University Swabi under reference number WUS/ZOOL/2020/46.

Samples Extraction

Upon the completion of the experimental duration mice were killed by giving deep anesthesia. The blood samples were taken from the beating heart and transferred to Eppendorf tubes. Bone marrow and testes samples were extracted for micronucleus assay and sperm anomalies respectively.

Comet Assay

Comet assay was performed in the Molecular Biology and Virology Lab, Department of Zoology, The University of Peshawar according to the modified protocol of Singh et al. (1988).

Micronucleus Assay

Femoral bone marrow was chosen for micronucleus test that was performed according to a protocol designed by Schmid et al. (1976).

Sperm Head Anomalies Assay

To get sperm samples, the cauda epididymis of mice was removed after killing. The cauda epididymis was crushed in 2 ml phosphate buffered saline (PBS), and the resulting suspension was filtered via an 80-gm steel mesh to eliminate tissue fragments. A portion of each suspension was taken, and mixed (10:1) in 1% Eosin solution Y (H20). Then smears of this solution were made after 30 minutes. To visualize the sperm anomalies, 1000 sperm cells were examined at 400 magnifications with blue-green filters for each suspension and the result was evaluated based on the ratio of abnormal sperm head to normal sperm head.

Data Analysis

The samples of the comet assay were analyzed through Open Comet v1.3.1 software and four parameters such as tail DNA %, tail length, tail intensity and Olive moment were assessed. Data is presented as mean ± SE. One-way ANOVA was used to measure the difference among treatments, where all values with $P>0.05$ were considered non-significant.

RESULTS AND DISCUSSION

Comet Assay Result of Mice Lymphocyte

Single-cell gel electrophoresis was used to assess DNA damage in mice lymphocytes. The potential DNA damage was assessed through tail DNA percentage, tail length, tail intensity and olive movement. There was no significant difference from the control revealed in the lymphocytes of mice that were given treatment for 7 days. However, there was a significant difference among the lymphocytes of mice treated for 14 days (F= 0.57, df= 14, P= 0.68), tail length (F= 5.61, df= 14, P= 0.01), tail intensity (F= 1.71, df= 14, P= 0.22) and olive movement (F= 4.90, df= 14, P=0.01) between treatments. The comet assay result of mice lymphocytes treated for 7 days revealed that the highest damage was caused in Ars.200 (tail DNA % 61.43±38.1) followed by Chin 200C (tail DNA % 60.51±21.6), Chin 30 (tail DNA % 57.08±21.76) and positive control (tail DNA % 47.25±19.5). The least damage was reported to be caused by negative control (tail DNA % 23.80±19.4), Ars.Q (tail DNA % 31.64±0) and Ars.30 (tail DNA % 31.89±29.3). Likewise, mice treated for 14 days also showed that considerably high damage was caused by Ars.200 (tail DNA % 86.91±12.0), positive control (tail DNA % 80.49±0.0), and Ars.30 (tail DNA % 61.43±38.1). Least tail DNA (3.93±0.0) was reported to be caused in negative control.

Table 1. Comet assay result.

Fixation Intervals	7 Days		14 Days	
Drug group	Tail DNA %	Olive moment	Tail DNA %	Olive moment
Negative control	23.80±19.4	2.41±1.9	3.93±0.0	0.0±0.0
Positive control	47.25±19.5	8.53±3.7	80.49±0.0	63±7.0
Ars Q	31.64±0	3.48±0	26.60±11.0	4.5±1.5
Ars 30	31.89±29.3	5.53±3.4	61.43±38.1	17.5±10.5
Ars 200	61.43±38.1	10.46±10.4	86.91±12.0	33±4.0
Chin Q	42.29±19.10	5.28±2.65	26.60±11.07	4.50±1.50
Chin 30	57.08±21.76	7.74±3.22	14.82±0	0±0
Chin 200	60.51±21.66	7.67±3.22.	34.85±17.00	5.00±5.00

Micronucleus Assay

Micronucleate (MN) bone marrow cells were counted and the ratio of Polychromatocytes vs normochromocytes was analyzed. The frequency of MN was high in positive control of 14 days treatment (45.31±2.06) and 7 days treatment (40.27±2.12), then Ars.200 and Chin 200 (44.56±3.63). The lowest damage was caused by negative control for both fixation intervals (13.96±1.12 and 10.96±0.92). Similarly, a considerably intensified trend in micronucleus formation recorded in mice treated for long fixation intervals i.e., 14 days.

Sperm Abnormalities Assay

A considerably high frequency of sperms having abnormal morphology of head was reported in the influence of treatment. Like other parameters, sperm head abnormalities were reported to be highly influenced by extra diluted drug groups like Chin.200 as well as by prolonged fixation intervals, i.e., 14 days (35.23 ±4.24) than 7 days (51 ± 8.45). This was followed by Chin.30 C (22.07±4.55 and 37.05 ± 2.04 for both intervals). Least damaged sperms were found to be present in negative control (3.60±050) and mother tincture of Ars.Q (6.66±1.22) in 7 days fixation interval and (10.66±2.48) 14 days interval.

Table 2. Micronuclei formation in bone marrow.

Dose	7 days Treatment	14 days Treatment
Negative control	13.96±1.12	10.96±0.92
Positive control	40.27±2.12	45.31±2.06
Ars Q	27.96±3.35	32.53±3.58
Ars 30	30.62±2.90	47.73±4.42
Ars 200	38.65±4.37	57.8±1.47
Chin Q	27.96±4.35	25.33±2.12
Chin 30	29.62±2.90	33.84±4.26
Chin 200	37.65±3.37	44.56±3.63

Table 3. Result of sperm head anomalies.

Dose	7 days	14 days
Negative control	6.60±1.36	3.60±0.50
Positive control	8.75±2.28	17.00±0.91
Chin Q	17.14 ± 4.16	13.05 ± 1.30
Chin 30	22.07±4.55	37.05 ± 2.04
Chin 200	35.23 ±4.24	51 ± 8.45
Ars.Q	6.66±1.22	10.66±2.48
Ars.30	10.50±1.64	11.83±2.53
Ars.200	14.66±2.12	13.00±2.58

The present study revealed that homeopathic drugs have the potential genotoxic effect over mice treated for two fixation intervals i-e 7 days and 14 days. Two potentized homeopathic remedies, Ars. and Chin cause enhanced frequency of Micronucleate bone marrow cell, increase sperm head anomalies and high tail DNA percentage of comet cells. From the current data is clear that high damage was caused by the treatment group with high alcohol dilution, i-e Positive control which was followed in most treatment series by 200 potencies for both Ars. as well Chin. Similarly, an increasing trend was observed in treatment carried for 14 days which indicates that long term exposure to these drugs may increase the potential of damage in treatment groups.

Micronuclei originated from either intact or broken chromosomes that failed to migrate into daughter nuclei during mitosis, and consequently deliver an indirect assessment of the induction of structural chromosomal aberration. Rate of MN in the drug-treated mice in both fixation intervals either 7 days or 14 days, is the result of their effect on the chromosomes in different stages of mitosis. A confirmatory result was reported by Preethi et al (2008) with another homoeopathic drug, where there was also a significant increase in the number of micronucleate erythrocytes in the bone marrow cells of animals after treatment with *Ruta* 200C. However, another study testing the toxicity of *Arsenicum album* (6C and 30C) in mice contrastingly reported a significant decrease in micronuclei formation and chromosome aberration (Banerjee et al., 2008). Shukla et al. (2020) evaluated the safety profile of a homoeopathic Gymnema sylvestre formulation (HPGS) and found that HPGS in vivo had no toxic effect and therefore supported its long-term oral administration in therapeutic settings. Pathak et al (2009) conducted a study in which hepatocellular damage and haematological alterations in mice were assessed. Mice were chronically supplied 0.06% p-dimethylaminoazobenzene (p-DAB) and 0.05% phenobarbital (PB) and it evident that the carcinogens' continuous feeding caused significant toxicity, visible hepatocyte damage, and other changes in the liver's carcinogenetic process. According to Do-Nascimento et al (2016) human cells were used in a comet assay to measure the genotoxic and cytotoxic effects of homoeopathic treatment (CANOVA) and N-methyl- N-nitrosourea (NMU). NMU considerably decreased the frequency of NMU-induced apoptosis, while CANOVA significantly decreased the genotoxic effect. Dayal and Kumar (2017) investigated the toxicity of Nux vomica on the 28 somatic chromosomes of Vicia faba using a homoeopathic medicine, fragmentation during chromatid separation, chromosome disorganization, and chromosome stickiness during orientation were observed (Dayal et al., 2016).

CONCLUSION

Based on the results of the current study it is concluded that the original extracts and mother tinctures of the used drugs are safe however the potential of toxicity increases with increase in the dilution with alcohol.

FUNDING

This study was sponsored by Women University Swabi, Pakistan in December 2020.

ACKNOWLEDGEMENT

The author thanks Administration of Women University Swabi for providing funding and resources for the completion of this work.

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