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Research Article

ANTIMUTAGENIC EFFECT OF TRIPHALA ON CHROMOSOMAL ABERRATIONS IN SWISS ALBINO MICE

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ABSTRACT

Triphala is a herbal formula used in the ancient Science of Ayurveda. Triphala, a composite mixture of *Terminalia bellerica*, *Terminalia chebula* and *Emblica officinalis*, has been used in traditional system of medicine for the treatment of many malaises such as heart ailment and hepatic disease. The present set of investigation is designed to evaluate the antimutagenic potential of aqueous extract of Triphala using in vivo chromosomal aberration assay in Swiss albino mice. Cyclophosphamide(CP), a well known mutagen was given by intraperitoneal injection at a dose of 40mg/kg body weight. Triphala was given at the dose of 125,250 and 500mg/kg body weight orally for seven consecutive days prior to Cyclophosphamide treatment. The animals were sacrificed at the sampling time of 24hrs after treatment and their bone marrow tissues were analysed for chromosomal damage and mitotic index. In Cyclophosphamide treated animals, a significant induction of chromosomal aberration was recorded with decrease in mitotic index. However, in Triphala supplemented animals, no significant induction in chromosomal damage or change in mitotic index was recorded. In different Triphala supplemented groups, a dose dependent significant decrease in Cyclophosphsmide induced clastogenicity was recorded. The incidence of aberrant cell was found to be reduced by the doses of Triphala when compared to Cyclophosphamide treated group. The study revealed the antimutagenic potential of Triphala against Cyclophosphamide induced chromosomal mutations. Analysis of the individual constituents of Triphala is shown to be rich in tannins as well as other chemical agents like carbohydrates, saponins, gallic acid, ellagic acids. Presence of these active principles in Triphala is likely to make it an effective antimutagenic agent.

Keywords: Triphala, Chromosomal aberrations, Cyclophosphamide, Swiss albino Mice, Antimutagenic.

INTRODUCTION

Naturally occurring phytochemicals are widely used in the traditional Indian medicinal system of Ayurveda for treatment of a variety of diseases. Triphala is a herbal formulation consisting of the dried and powdered fruits of three plants, *Terminalia chebula, Emblica officinalis* and *Terminalia bellerica* in equal proportions. It is an important medicine of the 'rasayana' group and is believed to promote health, immunity and longevity[1]. This formulation, rich in antioxidants, is a frequently used Ayurvedic medicine to treat many diseases such as anemia, jaundice, constipation, asthma, fever and chronic ulcers. Most people practicing Ayurvedic medicine consume Triphala as a 'health tonic'. Recent studies have reported anticlastogenic and anti-tumor properties of *Emblica officinalis*[2] and anti-proliferative effect of *Terminalia chebula*[3]. The pharmacological functions of Triphala including antioxidant and antidiabetic effects have been well described. Triphala has got radioprotective effect in the mice exposed to ♈ radiation. Turmeric exhibits cytotoxic and apoptotic activity. The present study investigates in vivo antimutagenic effect of Triphala on cyclophosphamide (CP) induced chromosomal aberrations and mitotic index in the mice bone marrow cells.

MATERIALS AND METHODS

Chemicals

The aqueous extract of Triphala powder was prepared as described in the Ayurvedic text. About 100 grams of the powder was boiled in 1000 ml of distilled water till the volume was reduced to one fourth of the original (250 ml). The extract was cooled, and filtered and was concentrated by evaporating its liquid contents. An approximate 26% yield of the extract was obtained. Rest of the chemicals used in the study was of analytical grade purity and obtained locally.

Animals

Swiss albino mice of *Mus musculus* species weighing between 20-30g body weights were selected for the experimental study. In this model animals were treated with three different doses of Triphala and the animals were divided into 6 groups each consisting of 6 mice and kept under standard laboratory conditions. They had free access to commercial pellet diet and water ad libitum.

Acute toxicity studies

The toxicity study was carried out in mice weighing 25-30 gms of either sex, bred in our animal house. Acute toxicity studies were conducted to determine the median lethal (LD_{50}) of the aqueous extract of Triphala. The acute toxicity studies were conducted as per "Up and Down" method of OECD 425 guidelines. The overnight fasted animals were treated with a dose of 1000 mg/kg of extract, orally. The animals were observed continuously for 2-3 hours for general behavior, neurological and autonomic profile and finally for death after 24 hours. There were no mortality or signs of toxicity were observed at this dose. So a higher dose of 2500 mg/kg of extract was given and was observed for next 2-3 hours and then for 24 hours.[5]

Treatment protocol

The control group I received intraperitoneal injection of distilled water. The standard group II received single dose of intraperitoneal injection of Cyclophosphamide only. Mice were pre-administered Triphala, suspended in distilled water, at the dose of 125, 250 and 500mg/kg per day to groups III, IV, V of animals through oral intubation for 7 days. Cyclophosphamide was used as positive mutagen and was given intraperitoneal injection at single dose of 40mg/kg body weight, 2hr after last dose of Triphala on 7th day in the above group of animals. The group VI received per oral Triphala at dose of 250mg/kg body weight per day for 7 days.

Group	Pre-treatment	Treatment
I	-	0.4 ml d.w.,i.p.
II	-	CP (40mg/kg b.w.,i.p.)
III	Triphala (125mg/kg b.w.,p.o)	CP (40mg/kg b.w.,i.p.)
IV	Triphala (250mg/kg b.w.,p.o)	CP (40mg/kg b.w.,i.p.)
V	Triphala (500mg/kg b.w.,p.o)	CP (40mg/kg b.w.,i.p.)
VI	Triphala (250mg/kg b.w.,p.o)	-

90 minutes before sacrifice, the animals were injected intraperitoneally with colchicines at the dose of 4mg/kg body

weight. Bone marrow was flushed from both femur using 0.56% KCl. Bone marrow suspension was mixed and incubated and then centrifuged at 1000 rpm for 8 min. The pellet was dispersed in the fixative. Finally, using a dropper, 2-3 drops of suspension was dropped on a pre-chilled slides and were flame dried. The slides were stained with buffered Giemsa of pH 6.8. After staining the excess stain was washed with buffer and slides were air dried and observed under microscope.[6]

Statistical Analysis

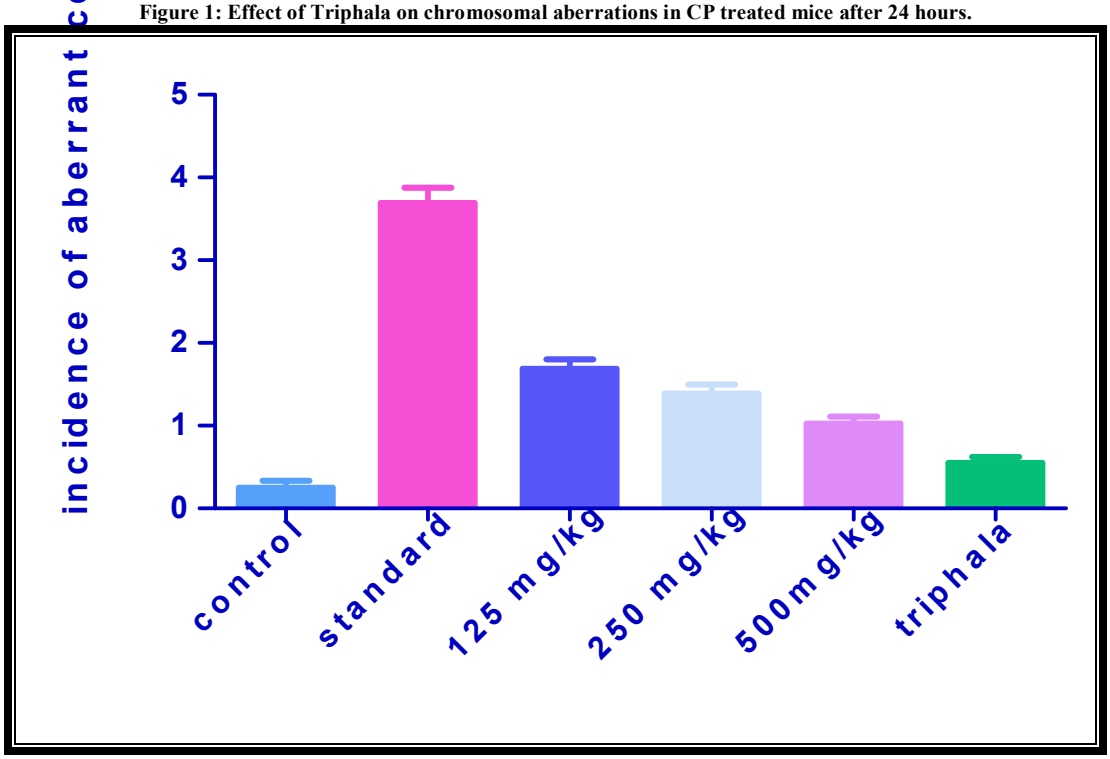
The results of biochemical estimation were expressed as mean ± SEM. The total variation present in the data was analysed by one way analysis of variance (ANOVA) with $P<0.05$ as a minimum level of significance followed by Post hoc Dunnett's test by using the software SPSS Version 15.

Table 1: Effect of Triphala on Chromosome Aberration Test after 24 hours

Group	Dose in mg/kg	Mitotic index±SEM	Breaks	Exchange	Fragments	Incidence of aberrant cells (%)	Suppression (%)
I	Control	4.89 ± 0.09	1.66± 0.17	0.00± 0.00	0.83± 0.31	0.25± 0.08	-
II	Standard	1.57± 0.11^{+}	5.66± 0.49	3.17± 0.47^{+}	3.83± 0.31^{+}	3.69± 0.18^{+}	-
III	TRP125+CP (p.o)	2.60± 0.07**	2.83± 0.30	1.83± 0.31*	1.67± 0.21**	1.69± 0.11**	54.20
IV	TRP250+CP (p.o)	3.12± 0.12**	2.16± 0.31	1.33± 0.21**	1.50± 0.34**	1.39± 0.10**	62.33
V	TRP500+CP (p.o)	3.88± 0.09**	1.33± 0.21	1.16± 0.31**	1.17± 0.30**	1.03± 0.08**	72.08
VI	TRP250 (p.o)	4.71± 0.06**	0.66± 0.21	0.83± 0.17**	0.83± 0.16**	0.55± 0.07**	-

$^{*}P<0.05$ compared to control, $^{**}P<0.01$ compared to control, $^{+}P<0.01$ compared to Cyclophosphamide

Figure 1: Effect of Triphala on chromosomal aberrations in CP treated mice after 24 hours.



RESULTS AND DISCUSSION

The mitotic index was found to be 4.89±0.09 in water alone treated control group I. The cytotoxicity of Cyclophosphamide was also evident as the mitotic index was found to be declined (1.57±0.11) in group II. However, in Triphala alone treated group VI, insignificant decrease in mitotic index (4.71±0.06) was observed, indicative of mild cytotoxicity. Moreover, in groups III, IV and V, influence on increment of mitotic index was noticed when compared to group II indicating anticytotoxic effect of Triphala towards cyclophosphamide induced damage. In groups III, IV and V, the mitotic index was found to be increased by value 2.60±0.07, 3.12±0.12 and 3.88±0.09 respectively by the administration of Triphala. Triphala significantly inhibits the Cyclophosphamide induced chromosomal aberrations which may be due to inhibition of Cyclophosphamide induced chromosomal damage. Cyclophosphamide alone treated animals showed a total aberration of 3.69±0.18 after 24 hours. However, combination of Triphala and Cyclophosphamide showed aberration of 1.69±0.11, 1.39±0.10, 1.03±0.08 after 24 hours for 125 mg/kg, 250 mg/kg and 500 mg/kg respectively, which means there is a dose dependent antimutagenic effect.

A combination of 125 mg/kg Triphala and Cyclophosphamide showed 54.2% of suppression after 24 hours. Combination of 250 mg/kg Triphala and Cyclophosphamide showed 62.33% of suppression of aberrations and a combination of 500 mg/kg Triphala and Cyclophosphamide showed 72.08% of suppression after 24 hours.

Thus, the study implies that oral administration of Triphala has potential in inhibiting cytotoxic and clastogenic damage produced by cyclophosphamide.

CONCLUSION

Considerable emphasis has been laid down on the use of dietary constituents preventing mutagen induced cytogenic damage due to their relative non-toxic effects. In the present study, Triphala has been found to inhibit the incidence of Cyclophosphamide induced chromosomal aberrations in mice in a dose dependent manner, suggesting its potential as an antimutagenic agent.

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