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Studies on the Anticlastogenic Effect of *Terminalia chebula* Extract on Cyclophosphamide-Induced Micronucleus Formation and Chromosome Aberrations in *Swiss albino* Mice

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Abstract: The present investigation was undertaken to explore the Antimutagenicity activity of *Terminalia chebula* on micronucleus formation and chromosomal aberration assay in bone marrow cells of *Swiss albino* mice. The protective effect of *Terminalia chebula* extract is reported against cyclophosphamide (CP)-induced micronuclei formation and chromosomal aberration in mouse bone marrow cells. The doses namely 50, 100 and 150 mg/kg body weight of *Terminalia chebula* extract provided protection when given 24 hr prior to the single i. p. administration of cyclophosphamide (50 mg/kg body weight). A dose dependent inhibition of micronuclei formation and chromosomal aberration was observed which was statistically significant ($p<0.05$) as compared to the cyclophosphamide group. It was observed that *Terminalia chebula* extract alone could not induced micronuclei formation and chromosomal aberrations at the test dose 50 mg/kg body weight. Thus in mutagenicity assay, *Terminalia chebula* extract protective potential against cyclophosphamide induced micronuclei formation and chromosomal aberration in mouse bone marrow cells.

Key words: Antimutagenicity % *Terminalia chebula* % Chromosome % Cyclophosphamide % Bone marrow % Micronucleus

INTRODUCTION

Terminalia chebula is a plant species belonging to the genous *Terminalia,* family Combretaceae. The fruit of the tree has been used as traditional medicine for household remedy against various human ailments, since antiquity [1-5]. *Terminalia chebula* has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. *Terminalia chebula* exhibited antibacterial activity against a number of bacterial species [6]. One group of researchers found that it is effective in inhibiting the urease activity of *Helicobactor pyroli,* an ubiquitous bacterium implicated in the development of gastritis, ulcers and stomach cancers [7]. Antibacterial activity of *Terminalia chebula* against both Gram positive and Gram negative human pathogenic bacteria has also been reported [8]. An aqueous extract of *Terminalia chebula* exhibits antifungal activity against a number of dermatophytes and yeasts [9, 10]. *Terminalia chebula* fruits afforded four immunodeficiency virus type 1 (HIV-1) integrase inhibitors, gallic acid and three galloy glucoses. Their galloyl moiety plays an important role in inhibition against the 3'-processing of HIV-1 integrase of the compounds [11]. Antimutagenic activity of hydrolyzable tannins from *Terminalia chebula* in *Salmonella typhimurium* has been documented [12]. A group of researchers have reported the inhibitory action on cancer cell growth by the phenolics of *Terminalia chebula* fruit and found that chebulinic acid, tannic acid and ellagic acid were the most growth inhibitory phenolics of *Terminalia chebula* [13]. Six extracts and four compounds of *Terminalia chebula* fruit exhibited antioxidant activity at different magnitudes of potency [14]. Its fruit exerts antioxidant and radioprotective activity in rats [15]. It exhibited the development of duodenal ulcers and appeared to exert a cytoprotective effect on the gastric mucosa *in vivo* [16]. Cytoprotective effect on oxidative stress and inhibitory effect on cellular aging of its fruits have also been documented [17]. The administration of *Terminalia chebula* extract prior to whole body irradiation

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of mice resulted in a reduction of peroxidation of membrane lipids in the mice liver as well as a decrease in radiation induced damage to DNA. It also protected the human lymphocytes from undergoing the gamma radiation-induced damage to DNA exposed *in vitro* [18]. Similar types of study were also found that cyclophosphamide induced different type of chromosomal aberration in bone marrow cells of *Swiss albino* mice [28, 29]. We have therefore undertaken antimutagenic effect of *Terminalia chebula* extract in bone marrow cells of *Swiss albino* mice.

MATERIALS AND METHODS

Animal: The study was conducted on random bred, 6-7 weeks old and 24-28 gm body weight male *Swiss albino* mice. They were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water *ad libitum*.

Chemical: Cyclophosphamide was purchased from Sigma Chemical Co. (St Louis, MO, USA). Other Reagent grades chemical were procured locally.

Extract Preparation: The *Terminalia chebula* were collected from the local garden and dry for few days. Then make powder with the help of grinder. Take 50 gms powder in a separating funnel and add 50% Methanol, then mix it gently. After few hours two separate layers to be seen. Collect the upper layer in a beaker and collect until transparent form appears. The extract was dried into powder at 60°C using water bath. The total weight of powder was weighed. On the day of experimentation, the desired amount of powder was suspended in double distilled water for the final administration.

Micronucleus Assay: For the micronucleus assay, the extract at the volume of 0.2 ml at different doses level such as 50, 100 and 150 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide, to six animals. The positive control group received single i. p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid, [19] and modified by Aron, *et al.* [20]. After staining with May-Gruenwald and Giemsa, a total 1000 cells were scored at the magnification of x1000 (100 x 10x) for each group. The data are expressed as the average number of micronucleated cells/thousand polychromatied erythrocytes cells/animal (±SE) for a group of six animals. The results were compared with the vehicle control group using Student's't' test with significance determined at $p<0.05$.

Chromosomal Aberration Assay: Different doses of *Terminalia chebula* namely 50, 100 and 150 mg/kg body weight were administered. *Terminalia chebula* extract dissolved in double distilled water and administered as single dose in 0.2 ml per mouse 24 hours prior to cyclophosphamide (CP) administration. Control mice were administered an equal volume of vehicle alone. The positive control group also received a single i.p. injection of 50 mg /kg CP in 0.9 % saline. The animals were sacrificed by cervical dislocations and bone marrow cells harvested. Colchicine (4 mg/kg b. wt.) was administered intraperetonally 2 hrs before the harvest of the cells. The slides are prepared essentially as per modified method of Preston, *et al*. [21]. Briefly, femur bones were excised and the bone marrow extracted in 0.56 % KCl. The harvested cells were incubated at 37°C for 20 minutes and then centrifuged for 10 minuets at 1000 rpm. Cells were fixed in Carnoy's fixative (Methanol: Acetic acid 3:1) and bursed opened on clean slides to release chromosome. The slide were stained with 5% Giemsa solution for 15 min and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 x 10) for each groups. Different types of chromosomal aberrations such as chromatid breaks, gaps, pulverization, centromeric association etc. were scored and expressed as % chromosomal aberrations. The statistical significance was determined using Student's't' test.

RESULTS

Single application of *Terminalia chebula* extract at the dose of 50, 100 and 150 mg/kg body weight, 24 hours prior to i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide. However, *Terminalia chebula* extract alone has not induced any micronucleus formations in bone marrow cells as compared to control group. It was noteworthy that different doses of *Terminalia chebula* and CP used in the present experiments were not cytotoxic for PCE/NCE (normochromatid erythrocytes)

Table 1: Effect of *Terminalia chebula* in Micronucleus formation in mice bone marrow cells

S. No.	Groups	MNPCE ± SE	PCE/NCE RATIO
1.	Cyclophosphamide (CP) 50 mg/kg	3.36 ± 0.56	0.455 ± 0.219
2.	*Terminalia chebula* 50 mg/kg + CP	1.4 ± 0.55*	1.38 ± 0.31*
3.	*Terminalia chebula* 100 mg/kg + CP	0.8 ± 0.83*	1.33 ± 0.18*
4.	*Terminalia chebula* 150 mg/kg +CP	0.6 ± 0.54*	1.64 ± 0.36*
5.	*Terminalia chebula* 50 mg/kg alone	0.2 ± 0.66*	1.11 ± 0.66*

*denotes statistically significant as compared to cyclophosphamide group at p<0.05.

Table 2: Effect of *Terminalia Cebula* in Chromosomal aberration in Mice Bone Marrow cells

			Different aberration in %					
S.N.	*Groups*	*Mean± SE*	*Chro. Frag.*	*Chro. Break*	*Chro. Gap*	*Chro. Ring*	Chro. Asso.	*% Inhib-ition*
1.	Cyclophosphamide 50 mg/kg	68.80 ± 3.16	18.64	16.4	19.8	11	-	-
2.	*Terminalia chebula* 50 mg/kg + CP	52.72 ± 0.17*	25	8	9	4	12	15.94%
3.	*Terminalia chebula* 100 mg/kg + CP	44.95 ± 0.22*	19	7	8	6	9	28.33%
4.	*Terminalia chebula* 150 mg/kg +CP	34.23 ±0.31*	14	6	6	3	9	45.42%
5.	*Terminalia chebula* 50 mg/kg alone	4.23 ± 0.56	1	2	2	-	-	-

* denotes statistically significant as compared to cyclophosphamide group at p<0.05.

ratio (Table 1). The test doses level of *Terminalia chebula* extracted protected against cyclophosphamide induced micronucleus formations in bone marrow cells of *Swiss albino* mice.

In another set of experiment, the single administration of the various doses of *Terminalia chebula* that is 50, 100 and 150 mg/kg body weight was given 24 hrs Prior to administration of cyclophosphamide (50 mg/kg) have produces the dose dependent protection and drug alone has not showed any chromosomal aberration against the cyclophosphamide alone group. The degree of protection was increased with increase the concentration of *Terminalia chebula* extract. The degree of protection was 15.94, 28.33 and 45.42% for various doses of *Terminalia chebula* respectively. A statistically significant (p<0.05) protection was observed with all the dose levels tested. All kinds of observed aberrations like a Breaks, Gaps, Fragmentations, Ring formation and Associations were found to be protected.

DISCUSSION

Several natural compounds and antioxidant agents, such as vanillin [22], garlic acid [23], humic acid [24], fatty acids [25], squalene [26] and Tochu [27], have shown antimutagenic properties against chromosomal damage in mice. Similar type of study was also done using some other herbal compound such as *S. lycopersicum* [28], *B. variegata* [29]. As oxidative damage in biological systems is considered to cause aging, degenerative diseases and cancer, particular attention has been focused on the possibility of modulating these effects through the use of free-radical scavengers to minimize cellular injury.

The present data demonstrate that *Terminalia chebula* extract was dose dependent inhibition of micronucleus formation and chromosomal aberration induced by CP in the bone marrow cells of mice. *Terminalia chebula*, when tested for mutagenic effect at various test dose levels, failed to induce micronucleus formation and chromosomal aberrations. It was statistically significant as compared with positive control.

Terminalia chebula was found to be non-mutagenic in mice and rats. This extract exhibited antioxidant activity at different magnitudes of potency [14]. Its fruit exerts antioxidant and radioprotective activity in rats [15]. It exhibited the development of duodenal ulcers and appeared to exert a cytoprotective effect on the gastric mucosa *in vivo* [16]. Antimutagenic activity of hydrolysable tannins from *Terminalia chebula* in *Salmonella typhimurium* has been documented [12].

The potentiating effect of known antioxidant compounds combined with clastogenic compounds has also been reported by others. Chromosome aberrations induced by alkylating agents in cultured Chinese hamster cells were enhanced in the presence of vanillin, an isomer of vanillin [30]. Aruoma, (1993) [31] found a pro-oxidant action of vitamin E in an assay for DNA damage with BLM and iron. Anderson, *et al.* (1994) [32] showed that ascorbic acid produced exacerbating effects using Comet assay with human lymphocytes when it was combined with BLM [33].

On the basis of these data, we may conclude that *Terminalia chebula* extract have shown significant protection against cyclophosphamide-induced micronucleus formation and chromosomal aberrations. It may be concluded that antimutgenic effects of *Terminalia chebula* due to hydrolysable tannins and flavonoids present in the extract.

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