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Evaluation of the Antigenotoxic Potential of Methanolic Leaves Extract of *Triticum aestivum* in Mice

Gaurav Jain[1*], Shailesh M. Kewatker[2], Govind Nayak[3] and Amit Nayak[4]

[1]IES Institute of Pharmacy, IES University Campus, Kalkheda, Ratibad Main Road, Bhopal, 462044, Madhya Pradesh, India.
[2]Rajarshi Shahu College of Pharmacy, Buldana, 443001, Maharashtra, India.
[3]Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal, 462022, Madhya Pradesh, India.
[4]GD Goenka University, Sohna-Gurgaon Road, Sohna Gurgon, 122103, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mutations are changes to the nucleotide sequence of the genetic material of an organism. Reactive oxygen species (ROS) play an important role in process like mutagenesis, carcinogenesis and aging by their ability to damage cellular DNA. Inhibition of mutagenesis or carcinogenesis is generally not based on one specific mechanism. Protection against cancer can occur at different stages of the complicated processes of carcinogenesis. Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidation. *Triticum aestivum (T. aestivum)* have revealed its medicinal potential for some human diseases; therefore, this study aimed to evaluate the genotoxic and antigenotoxic potential of methanolic extract. To accomplish this, the methanolic extract of *T. aestivum* was evaluated for its antigenotoxic effect using the chromosomal aberrations and micronucleus assay of bone marrow cells of mice.

**Corresponding author: E-mail: drgaurav.jain@iesbpl.ac.in, gauravjain20us@gmail.com;*

T. aestivum extract has shown significant protection against Cyclophosphamide induced micronucleus formation and chromosomal aberrations.

Keywords: Triticum aestivum; cyclophosphamide; chromosomal aberrations; micronucleus assay; antigenotoxic; genotoxicity; carcinogenesis.

1. INTRODUCTION

Oxidative deoxy ribonucleic acid (DNA) damage is caused by mutagen which leads to mutagenesis. It is crucial step in the development of cancer and other degenerative processes such as cardiovascular and neurodegenerative disease as well as premature ageing [1]. The mutation in the DNA leads to the formation of faulty DNA that leads to cancer. Classical treatment for cancer in is radio or chemo therapy. But the therapies work by targeting the DNA and damaging both the malignant as well as the normal cells [2–5].

The mutagens can be prevented by many herbal therapies by maximizing the pharmacological effects and minimizing the side-effects. Therefore, many types of research have been performed to find out plants having secondary metabolites which act as antimutagens and nontoxic to the normal cells [6]. It is found that green plants are generally the primary source of antimutagens as well as toxic agents. Many plants contain mutagenic or carcinogenic substances and their use has been diagnosed for tumor formation in some patients. Recent research has affirmed that many plants used as food or in traditional medicine have mutagenic and genotoxic effects [7,8,9,10]. Genotoxicity testing is a significant part of the threat estimation of chemicals for regulatory purposes [11,12]. It is undertaken for two main reasons: a) to detect chemicals that might cause genetic damage, including point mutations in germ cells and thus increase the burden of genetic disease in the human population and b) to detect chemicals that might be carcinogenic (based on the assumption that a mutagenesis is a key event in the process of carcinogenesis) [13,14]. In crude preparations, other components are also present in addition to the active components which may be counteracting the toxic effects of the active components. These known and unknown components may act as synergists for the therapeutic effects and antagonists for the side effects of the active components as well as for other toxic components in the crude preparation [15]. In the past few years, there has been considerable interest in natural products endowed with antimutagenic and anticarcinogenic properties.

Triticum aestivum (T. aestivum) has been a primary source of food from ancient times, therefore it commemorates to check whether *T.aestivum* leaves extract itself is mutagenic and also to ascertain its antigenotoxic potential. Many studies have been performed on its leaves in this context. It has been used for its antioxidant, apoptotic, antiproliferative activities on CML (K562) and MCF-7 breast cancer cell lines [16,17]. Its leaves extract was also evaluated for its chemopreventive action against DBMA and croton oil [18]. Its sprouts have also been used for antimutagenicity and its mechanism of action is also identified [19,20]. Chlorophyllin a potent antioxidant compound is extensively used for its chemopreventive, antimutagenic, anticytotoxic, antiproliferative, anticarcinogenic, antitumorigenic, apoptotic activity and its mechanism of *in vitro* action against benzo(a)pyrene is also reported [21,22,23,24–35]. Chlorophyllin is also used as a protector of mitochondrial membranes against γ-radiation and photosensitization [36]. Wheat sprout extract also induces apoptosis in human cancer cells and also induces changes in 20S proteasomes functionality [37]. The sprout is also reported for its antioxidant activity and inhibits DNA oxidative damage [38]. The nutritional relevance of its sprouts containing high levels of organic phosphates and antioxidant compounds was also studied [39]. Cytotoxic activity on HL60 and the antiproliferative effect of local health supplement wheatgrass and the mixture of fibers were investigated *in vitro* using a cancerous cell line and normal blood cell culture [40].

The objective of the work was to evaluate the antigenotoxic potential of methanolic extract of *T.aestivum* young leaves using cyclophosphamide as an inducer of genotoxicity. Cyclophosphamide is an alkylating agent possessing the clastogenic effect which produces the formation of micronuclei in bone marrow cells [41]. The Antigenotoxic effects were evaluated by microsomal aberrations and micronucleus assay models.

2. MATERIALS AND METHODS

2.1 Procurement of Seeds and Authentication of the Plant Material

The *T.aestivum* seeds for the research were purchased from Breeder Seed Production Unit Field crops, Department of Plant Breeding and Genetics, Jawahar Lal Nehru Krishi Vishwavidyalaya, Krishinagar, Jabalpur M.P. and the release order number DFP2219 was obtained. The whole plant of TA was collected in December and authenticated at Safia college of Science Bhopal, Madhya Pradesh. The herbarium of the plant was prepared and the voucher specimen number 236/BOT/SAFIA/2011 was obtained.

2.2 Preparation of Powder

T.aestivum was cultivated, and the leaves were collected on the ninth day. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in a labeled air-tight container for further studies.

2.3 Preparation of Extract

The maceration process involves the separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in the bulk of solvent or menstrum. Solid drug material was taken in a stoppered container with about 750 mL of the methanol and allowed to stand for seven days in a warm place with frequent shaking. The mixture of crude drug containing solvent was filtered until most of the liquid drains off. The filtrate and the washing were combined to produce 1000 mL of the solution. This solution was kept aside for solidification and was dried on a hot air oven at 40 ℃. The extract is then collected in a dark colored bottle [42].

2.4 Animal Care and Handling

The procurement of the animals and the studies were performed at Sapience Bioanalytical Research Lab Bhopal and the animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2 ℃ relative humidity 44–56% and light and dark cycles of 12:12 hours, fed with standard pellet diet and water *ad libitum* during experiment.

2.5 Micronucleus assay

Grouping: In the experiment, 24 mice were used. The mice were divided into 4 groups comprising of 6 animals in each group as follows:
Group I: Normal control, vehicle treated.
Group II: Negative control, cyclophosphamide (50 mg/kg i.p) treated.
Group III: *T.aestivum* extract (500mg/kg p.o.) for 7 days and cyclophosphamide.
Group IV: Only *T.aestivum* extract for 7 days.

2.6 Bone Marrow Samples

After completion of 7 days of treatment, the animals were sacrificed by cervical dislocation and bone marrow cells were harvested. Bone marrow cells were aspirated by flushing with Hank's balanced salt solution (HBSS) with the help of a syringe. The tubes were centrifuged at 1000 rpm for 5 min. The supernatant was removed. The cells in the sediment were carefully mixed by aspiration and a small drop of the viscous suspension was put on the end of a slide and spread by pulling the material behind a polished cover glass held at an angle of 45 degrees. The composition was then dried and fixed for 2-5 mins. Staining was carried out in ordinary vertical staining jars. The slides were stained for 10 mins in a may-Gruenwald giemsa solution. Then slides were rinsed in distilled water, blotted, cleaned and then dried on the slide warmer. Polychromatic erythrocytes (PCE) were scored for micronuclei under the microscope, at least 1000 PCE per animal were scored for the incidence of micronuclei. The ratio of PCE to non-chromatic erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes [43–45].

2.7 Chromosomal Aberration Test

Grouping: In the experiment, a total of 24 mice were used. The mice were divided into 4 groups comprising of 6 animals in each group as follows:
Group I: Normal control, vehicle treated.
Group II: Negative control, cyclophosphamide (50 mg/kg i.p.) treated.
Group III: *T.aestivum* extract (500 mg/kg p.o.) for 7 days and cyclophosphamide (50 mg/kg p.o.).
Group IV: Only *T.aestivum* extract **(**500 mg/kg p.o.) for 7 days.
cyclophosphamide was injected 24 hrs before sacrificing in group II and III and colchicine (25 mg/kg i.p.) were injected to all groups before 1hr to sacrifice.

2.8 Bone Marrow Samples

After completion of 7 days of treatment, the animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The suspension was flushed in the tube properly to get good cell suspension and centrifugation for 10 min at 1000 rpm. The supernatant was discarded. Pellet was treated with pre-warmed (37ºC) KCl on cyclomixer. The suspension was left in a water bath (37ºC) for 20 min. It was centrifuged and the supernatant was discarded. Pellet was treated with freshly prepared cornoy's fixative on cyclomixer, centrifuged and the supernatant was discarded. The above step of treatment with Cornoy's fixative was repeated 3 times to get debris free white pellet. To pellet Cornoy's fixative (quantity sufficient) was added to get a good cell suspension. Slides were made with Air Drop Method. Stained (Giemsa-3 min, Methanol-3 min and DDW-1 Dip) and observed under the microscope in 40x10X and then in 100x10X magnifications. No. of cells having aberration and the particular aberrations were scored. A total of 1000 cells were counted [46].

3. RESULTS AND DISCUSSION

3.1 Effect of TA Extract on Micronucleus Formations in Mouse Bone Marrow Cells

PCE, NCE and micronucleus (MN) are the forms of micronucleus formation produced by administration of cyclophosphamide in the bone marrow cells of mice given in Table 1 and Figs. 1-2.

Table 1. Effect of TA extract on micronucleus formations in mouse bone marrow cells

Group	Treatment	Dose	MN/PCE ± S.E.M.	PCE/NCE± S.E.M. Ratio
I	Control	1 mg/kg p.o.	0.78±0.54	0.38±0.23
II	Cyclophasphamide	50 mg/kg i.p.	1.84±0.30a*	0.78±0.53
III	*T.aestivum* Extract + cyclophasphamide	500mg/kg p.o. + 50 mg/kg i.p.	0.97±0.26a*,b*	0.55±0.34
IV	*T.aestivum* Extract	500mg/kg p.o.	0.90±0.23	0.41±0.21

*n= 6 in each group,*P<0.05, compared with multiple groups using One-way ANOVA followed by Dunnett's multiple comparison test*

a = significant difference when compared with vehicle treated group

b = significant inhibition, when compared with cyclophosphamide, treated group

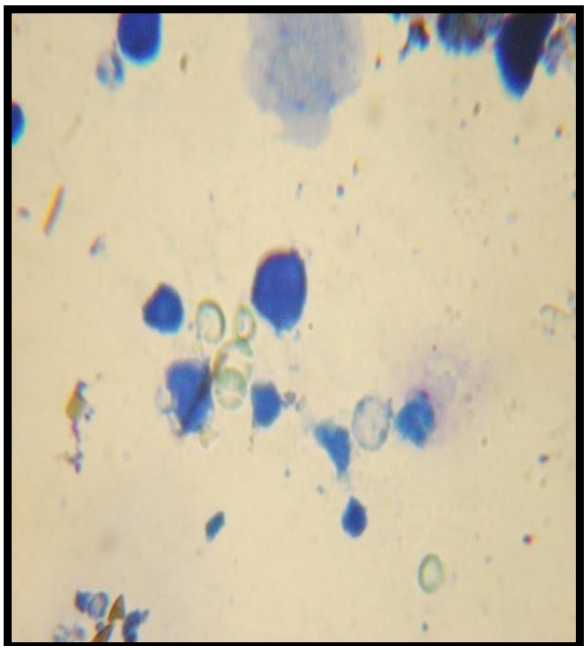

Fig. 1. Normal bone marrow cells

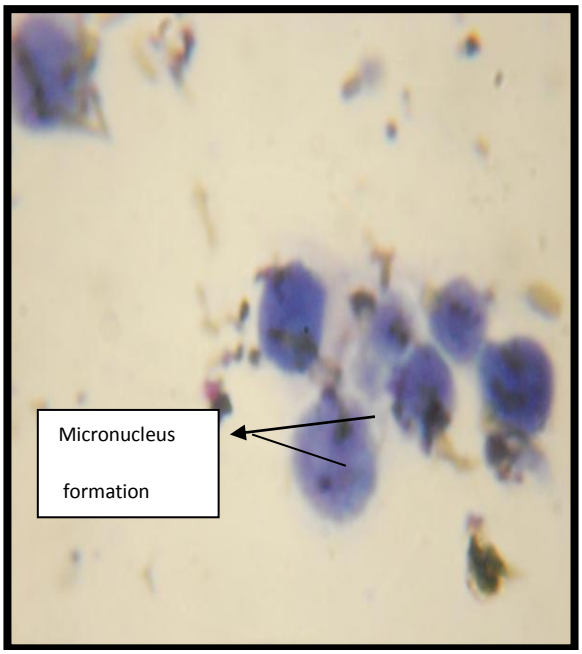

Fig. 2. Bone marrow cells with micronucleus

3.2 Effect of *T. aestivum* Extract on Chromosomal Aberration in Mouse Bone Marrow Cells

Chromosomal aberration (C.Ab.), chromatid break (C.B.), chromatid fragment (C.F.), chromatid gap (C.G.), ring formation (R.F.) and centromeric association (C.A.) are the different types of chromosomal aberrations found in the bone marrow cells shown in Fig. 4-6. They serve as an important tool in assessing the genotoxicity produced by the drugs as their side effect. The administration of *T.aestivum* extract at the dose of 500 mg/kg body weight, 24 hours before i.p. administration of cyclophosphamide (50mg/kg) have significantly prevented the micronucleus formations (Fig. 1& 2) and chromosomal aberration in bone marrow cells of mice as compared to cyclophosphamide (Table 2).

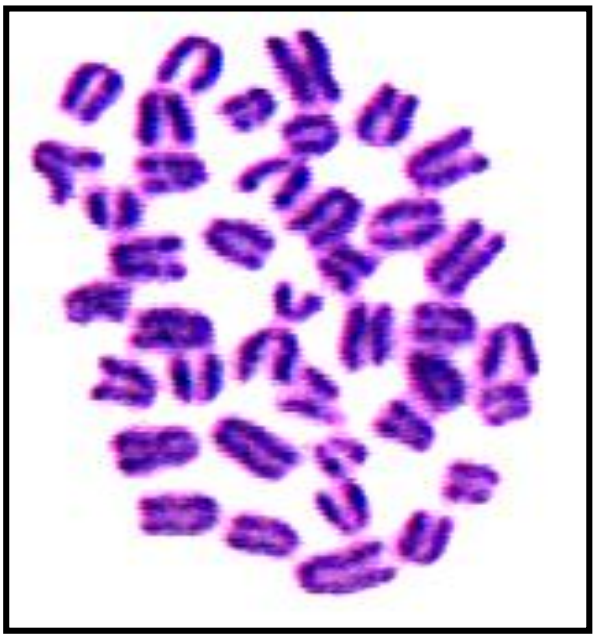

Fig. 3. Normal

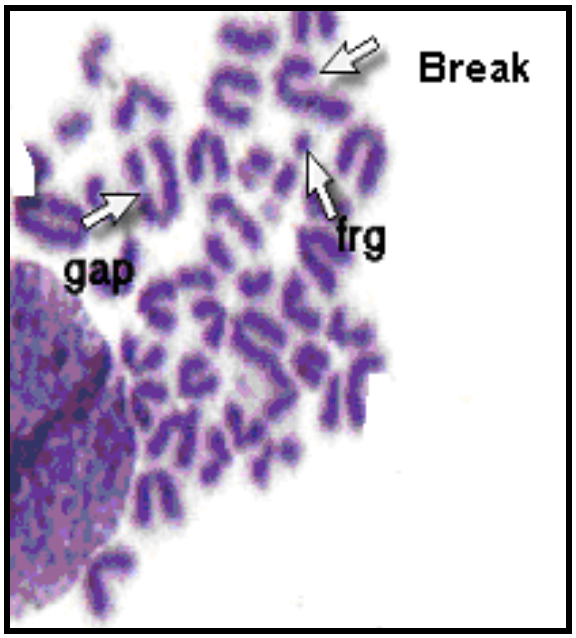

Fig. 4. Break, fragment, gap

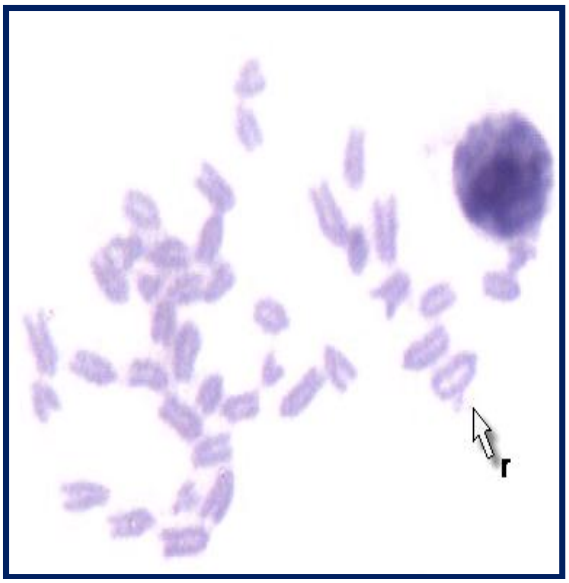

Fig. 5. Ring formation

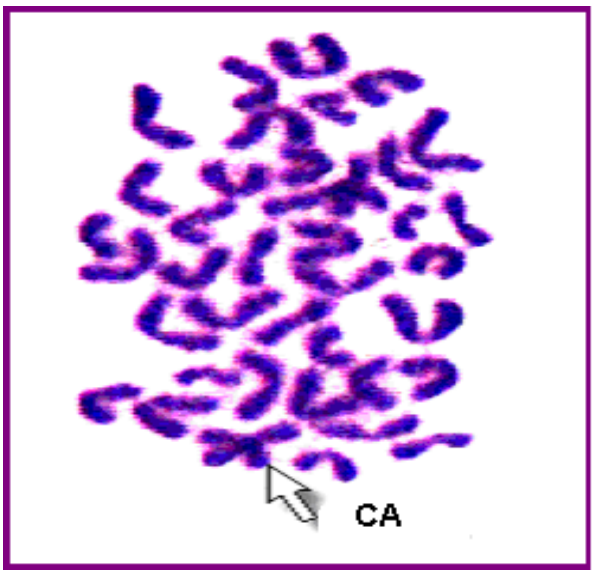

Fig. 6. Centromeric association

Fig. 3-6. Different types of chromosomal aberrations

Table 2. Effect of *T.aestivum* extract on chromosomal aberration in mouse bone marrow cells

Group	Treatment	Mean ±S.E.M. (C. Ab.)	% C.B.	% C.F.	% C.G.	% R.F.	% C.A.
I.	Control 1 mg/kg p.o.	29.0±3.10	14	12	3	Nil	Nil
II.	Cyclophosphamide 50 mg/kg i.p.	51.3±4.48a*	24	20	7.3	4	2
III.	*T.aestivum* Extract + Cyclophosphamide 500mg/kg p.o. + 50 mg/kg i.p.	39.7±2.59a*,b**	17.7	16	4	2	Nil
IV.	*T.aestivum* Extract 500mg/kg p.o.	31.8±3.43b**	14.8	13	2	2	Nil

*Data are expressed in mean ± SEM, n= 6 (no. of six animals) in each group,*p<0.05, **p<0.01compared with multiple groups using One-way ANOVA followed by Dunnett's multiple comparison test. a = significant difference in compared with vehicle treated group*
b= significant protection in comparison with Cyclophosphamide treated group

4. CONCLUSION

T. aestivum extract has shown significant protection against cyclophosphamide induced micronucleus formation and chromosomal aberrations. It may be concluded that the antimutagenic effects of *T. aestivum* young leaves may be due to the presence of phenolic and flavonoids in the extract. Quercetin may be the key flavonoid of ethanolic extract of *T. aestivum* which may be engaged reduce the harmful effects of cyclophosphamide. These findings confirmed the health benefits of *T. aestivum* as a medicinal plant to reduce mutagenicity. Further studies are needed to check the role of phenolic and flavonoids in the antigenotoxic activity of *T. aestivum*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiment was approved by the institutional ethical committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/ CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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