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Original article

Genotoxic potential of a novel PDE-4B inhibitor Apremilast by chromosomal aberration and micronucleus assay in mice

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ABSTRACT

Objective: Researchers have confirmed that chronic administration of drugs at high doses causes genotoxicity which serve as first step in development of cancers. Apremilast, a phosphodiesterase-4 inhibitor is Food and Drug Administration (FDA) approved drug for Psoriatic Arthritis. The present study designed to conduct genotoxicity testing using the genotoxic study which give simple, sensitive, economical and fast tools for the assessment of damage of genetic material.
Methods: To conduct genotoxicity study of Apremilast, 60 Swiss albino male mice divided into 6 groups (n = 10). Group1 served as a normal control group without any treatment, Group 2 treated as a disease control and administered with cyclophosphamide 40 mg/kg, IP. Group 3, 4, 5 and 6 treated as test groups and received 10, 20, 40 and 80 mg/kg/day Apremilast respectively. The total duration of study was 13 weeks. At termination day animals were sacrificed and chromosomal aberration assay (BMCAA) and micronucleus assay (BMMNA) were performed to know the genotoxicity potential of Apremilast.
Results: The results indicates significant rise in chromosomal aberrations (CA) frequency in bone marrow cells and decrease in the MI of the disease control animals as well as Apremilast treated groups. Further significant ($p < 0.001$; $p < 0.0001$) increase in score of micronucleated polychromatic erythrocytes (MNPCEs) and percentage of micronucleated PCEs per 1000 PCEs and decrease in the ratio of polychromatic/normochromatic erythrocytes (PCE/NCE) was observed in micronucleus assay. Genotoxic effect increases with the increase of Apremilast dose. *Conclusion:* Finding of present indicates that Apremilast shows genotoxic potential on high administration although further detailed toxicity studies required for confirmations.

1. Introduction

Advancement in various scientific fields assisted human beings to meet the demand of fast growing world and also to improve quality of life significantly. Every year massive quantities of different chemical substances are being added to environment due to rapid and progressive growth of urbanization and industrialization which is a threat to human race due to genetic degeneration. Genetic disease effects human health in a variety of styles, from adaptation to mortality. Data analysis reflects 25% ill health is associated with genetic origin (Zubair et al., 2019). Nearly 10% of all disease has genetic association (Toh et al., 2019). Significant

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exposure of our society to synthetic chemicals resulted due to industrialization, requires to standardize the impact of such chemicals on health and economy of our community despite of their undoubted benefits. Presently massive genetic hazards to humans are from some chemicals due to their extensive use and this lead to production of genetic injuries in somatic cells which progress to cancer, or induction inheritable genotoxicity and mutations in germ cells and alterations in chromosomal morphology and number, which affect coming generations (Long et al., 2019). More than millions of injuries occur to DNA daily to each cell due to external and regular processes inside the cell (Shi et al., 2018). Data available depicts that occurrence of chromosomal aberrations spontaneous abortions in 12 weeks is 614/1000, while in new born this is 6–7/1000. Assumptions are that 9/1000 newborn are affected from genetic diseases due to gene mutations. This elevation in prevalence of mutation was in turn increase genetic disorders and a significant decline in quality of life (Jiang et al., 2019).

More than 75,000 of natural; chemical or medicinal substances can cause irreversible genetic injuries. More than 60,000 of them are used 1000 added annually to this list (Panahi et al., 2018). Few of them cause genotoxicity and mutations by direct DNA damage and can change expressions or may cause both at separate instance (Khan et al., 2019). Among all chemicals exposed to humans drugs occupy major place. Annually centenary of chemical substances are introduced in pharmacotherapy of various diseases. Excessive use of drug leads to drugs accumulation in tissue which play significant role in health with respect to genetic trait, which may be inherited to offspring by genome (Tanyous, 2019).

Apremilast is a FDA approved phosphodiesterase 4 inhibitor for the treatment of plaque psoriasis and psoriatic arthritis (Maloney et al., 2019). It is usually administered for long periods as it is a part of Disease-modifying Anti-rheumatic Drugs (DMARDs) regimen for psoriatic arthritis and peripheral arthritis (Gossec et al., 2016). In spite of beneficial effect in psoriasis and arthritis Apremilast shows wide range of toxicity including reduced viability, dystocia, litter size and fetal weight, increases in death of embryo-fetal and abortion (Torres and Puig, 2018). So evaluation of genotoxic potential of this drug especially important. Considering widespread use of this drug and risk to humans due to toxicity, there is a need of genotoxic study by standard procedure and assessment of their genotoxic potential. Therefore, current experimental study was performed for investigation of genotoxic potential of PDE-4enzyme inhibitor, anti-psoriatic drug at four separate increasing dose for 13 week study as per standard guidelines for genotoxic testing study.

2. Methods

2.1. Drugs and chemicals

Apremilast and Cyclophosphamide obtained as a gift sample from Mr. Naved Qasim, Deputy Manager, Quality Assurance, Accent Pharmaceuticals, Jammu and Kashmir, India. Various stains and other chemicals were bought from Sigma-Aldrich Chemical, USA. All the drugs/chemicals used in experiment were of analytical quality and from commercial source.

2.2. Animals

Albino Swiss mice of 6–8 weeks were obtained from central animal house facility of Pharmaceutical Technology, Jadavpur University, India. Research work was conducted in Pharmaceutical Technology Lab, Jadavpur University, India. Mice were kept for seven days under standard environmental conditions for adaptation. Temperature of room was maintained at 26 ± 3 °C with optimum relative humidity with free access to water *ad libitum* and commercial food pellets throughout experiments. Pellets are complete food with appropriate proportion of carbohydrates, proteins, fats, minerals, vitamins for growth of body. For conducting experiments on animals, project was approved from Animal Ethics Committee of College, before starting the experimental work. All experiment procedures were conducted with adherence to guidelines and ethical rules mentioned by OECD guidelines (ICH-S2B, 1997; OECD-452, 2008; OECD-471, 2008B; ICH-S2A 2008) and CPCSEA, guidelines.

2.3. Induction of genotoxicity

For induction of genotoxicity in disease control (Group 2) animals, cyclophosphamide was dissolved in saline. Intraperitoneal injection of 40 mg/kg body weight administered in animals (Ehling and Neuhäuser-Klaus, 1988).

2.4. Design of study

Male Mice of weight around 18–20 g were separated randomly into six separate groups with each group containing 10 animals. The study was designed for total duration of 13 weeks. Group1 served as a normal control group and left untreated throughout experimental study, Group 2, was a disease control group and administered with cyclophosphamide 40 mg/kg, *i.p.* Group 3, 4, 5 and 6 treated as test groups and received 10, 20, 40 and 80 mg/kg/-day dose of Apremilast respectively. As per, Imam et al. (2018) Apremilast dose up to 20 mg/kg is safe. Hence dose up to 80 mg/kg selected for study of genotoxic effect. This dose was selected on the basis of previous at termination day animals were sacrificed and chromosomal aberration assay (BMCAA) and micronucleus assay (BMMNA) were performed to know the genotoxic effect of Apremilast.

2.5. Mice bone marrow chromosomal aberration assay

At the end of protocol, mice were intraperitoneally injected 0.05%, 0.4 ml solution of colchicine 1.5 h before the sampling of tissue. Mice were killed at the end of protocol by cervical decapitation. Femurs of both limb were isolated safely and muscles were removed. Femurs end were cut for opening bone marrow and washed with distal side with 0.56%, 1 ml hypotonic solution of potassium chloride and forcefully aspirated in the centrifugation tube till content of bone become empty. Cell suspension after rinsing was then vortexed and left for 20 min. Then suspension of cell was centrifuged for 8 min at 1000 rpm/min speed. After centrifugation the obtained supernatant was discarded. The cells were stirred by addition of drop wise cold fixative solution of Glacial acetic acid and Methanol in a ratio of 1:3 and after that it was left aside for 1 h for stabilization purpose. Suspension of cell was centrifuged again for at 1000 rpm for the period of 8 min and obtained supernatant after centrifugation was discarded. Cell was again stirred by addition of drop wise freshly prepared cold fixative (1 ml) and left aside for 20 min. The process was repeated 5 times. After last and fifth centrifugation process, the supernatant obtained was discarded. Sediment obtained was then mixed with fixative (10 drops) and with the help of cell suspension slide was prepared. Prepared slides dipped in alcohol and then with the help of tissue paper it is polished for making it free of grease. Slides were stored in freezer for two to 3 h before the use. Suspension of cells was poured drop wise with the height of 30 cm on pre-chilled slides. After that slides heated and a drop of fixative was added for clarification of chromosomal pictures. After that slides were dried in air. 1 g of stain of giemsa was dissolved in glycerin (56 ml) with stirring and then heated till the dissolution completes. Then kept in the oven for

the period of 2 h at 60 °C. Then after methanol (84 ml) was added, mixed and then filtered. Obtained filtrate was utilized for the staining. 2.366 g of Disodium hydrogen phosphate dissolved in distilled water (250 ml). 2.27 g of dihydrogen Potassium phosphate was dissolved in distilled water (250 ml). 50 ml of each of above solution was mixed with 1000 ml of distil water and 6.8 pH was readjusted. Staining of slides was done after spend of 24 h of their preparation. Staining jars was filled with 50 ml solution of buffer and Giemsa stain (20 drops) was added in 1st jar. Previously heat fixed slide were dipped in 1st jar for the period of 20 min. Slides were carefully removed without any shaking and then dipped in 2nd jar for 5 min which contains solution of buffer for wash of extra stain. Washing were repeated in buffer solutions for 3–4 times for removal of excess stain. Stained slides were dried in air and permanently fixed by DPX mount and 22 × 60 mm histopathology cover slips. Stained slides microscopic analysis were done by 100X oil immersion lens magnification. 100 clear metaphase was analyzed for each animal (five hundred metaphase per treatment group animals). Aberration types were scored and calculated. In metaphase observation different aberration types, like chromosomal and chromatid gaps and ring, breaks, stickiness, deletion, dicentric, fragmentation, exchange, and acentric fragments were analyzed. All the aberrations were counted equal irrespective of the number of breakages involved in it. Chromosome aberrations per cells (CA/cell) were calculated excluding and including gaps. Chromosome aberration (CA) tests were performed with 3 different doses of Apremilast. Finally animals were sacrificed at the end of 13 weeks by dislocation of cervical vertebra (Tjio and Whang, 1962).

2.6. Mitotic index (MI)

Slides prepared for chromosomal aberration assay was also utilized for MI calculation. MI were calculated among 2500 cells by using the following formula (Preston et al., 1981).

$$MI = A/(A + B) \times 100$$

A = Dividing cells number (Metaphases + Anaphases).
B = Non-dividing cell number.

2.7. Bone marrow micronucleus assay

Mmicronucleus assay was performed as per standard procedure. Saline of Phosphate buffer was made by adding 41 ml disodium hydrogen phosphate (A) in 9 ml sodium di-hydrogen phosphate (B), then A + B added to make 50 ml and then adjust at 7.2 pH. The 5% bovine serum albumin was prepared. For this 500 mg of albumin of bovine serum was mixed with saline of phosphate buffer (10 ml). After preparation of these samples, mice were sacrificed and complete femurs were isolated and all the muscular tissues removed. Muscles attached with bones was removed by fingers and gauze. With traction, epiphyseal section was peeled off with the remaining surrounding muscles and tibia. Distal and proximal both femur ends was successfully cleansed by scissor till a small opening to the canal of marrow seen. With the needle, approximate 5% (0.2 ml) of albumin of bovine serum drawn into a plastic syringe. This serum was centrifuged. Centrifugation was done at 1000 rpm for the period of 5 min, and smear was obtained through this process. Supernatant obtained after centrifugation were removed with the addition of 2–3 serum drops. Then sediment cells was mixed by aspirating into the capillary portion of a pipette. To prepare smear, a drop of the suspension was placed on a slide end and by pulling it was spread on the material behind a glass slide (polished) held at 45° angle. The prepared slides dried in air. For staining purpose, stock solution was prepared. For this May-Gruenwald stain was prepared as a 0.02% methanol solution. Buffer of Phosphate with pH-6.8 prepared carefully. Staining process of slide was done for 15 min in May-Gruenwald stain diluted with buffer of Phosphate in 1: 1 ratio. Quickly these slides were transferred in another stain jar which contains another stain-Giemsa which is diluted with buffer of phosphate solution in 1:6 ratio and for 15 min staine. These slides washed in phosphate buffer for 4 min and blot was dried with filter paper. Back side of dyed slides were cleansed with methanol, then dried in air and permanently fixed by using DPX mounting and 22 × 60 mm cover slips. Stained slides were observed in microscope with 100X oil immersion lens magnification. Objective was cleaned with oil-xylene. For evaluation of toxicity of bone marrow, erythrocytes (polychromatic)/erythrocytes (normochromatic) ratio was calculated by the count of 1000 total erythrocytes. Approximate 1000 erythrocytes (polychromatic) were analyzed for micronuclei presence in group of each animal. Micronucleus assay was completed with 3 different doses of Aprelimast. All group mice were sacrificed at 13 weeks after daily drug administration by dislocation cervical bone (Schmid, 1975).

2.8. Statistical analysis

Data were expressed as mean ± standard mean error. Significance of results in between more than two groups was measured via one way analysis of variance and then Student's *t*-test was used with Graph Pad Prism 5.0 software. The differences of ($p < 0.05$) were considered to be statistically significant.

3. Results

3.1. Bone marrow chromosomal aberration assay

The findings observed for bone marrow chromosomal aberration assay are depicted in Table 1.

In disease control an animal, the frequency of bone marrow chromosomal aberrations was significantly increased by cyclophosphamide to 13.1 ± 0.02 including gaps and 7.2 ± 0.07 excluding gaps respectively in comparison to normal control animals. This increased the abnormal chromosomal frequency in the disease control mouse significantly ($p < 0.001$) in comparison to normal control mouse (0.4 ± 0.02 with gaps and 0.3 ± 0.01 without gap respectively).

In different treatment groups, mouse administered with four different dose of Apremilast shows genotoxic effect. Genotoxic effect increased with increasing dose of Apremilast in a dose dependent manner. In Group 3, at 10 mg/kg Apremilast dose, frequency of chromosomal aberration was 3.2 ± 0.03 including gaps and 2.2 ± 0.03 with significant value $p < 0.001$. In Group 4, at 20 mg/kg Apremilast dose, chromosomal aberration frequency was 6.6 ± 0.05 including gaps and 4.8 ± 0.09 with significant value $p < 0.01$ and $p < 0.001$ respectively. In Group 5 animals, at 40 mg/kg. Apremilast dose, chromosomal aberration frequency was 9.4 ± 0.06 including gaps and 6.8 ± 0.08 with significant value $p < 0.001$ and $p < 0.01$ respectively. In Group 6 animals, at 80 mg/kg Apremilast dose, chromosomal aberration frequency was 10.8 ± 0.09 including gaps and 7.9 ± 0.06 with significant value $p < 0.001$ (Table 1).

3.2. Mitotic index (MI)

Bone marrow mitotic index of cells is generally used for the measurement of the cytotoxic potential of drugs. Table 1, also gives MI data of study. In normal control mouse, no changes were found in MI (7.53 ± 0.08).MI was a significantly reduced ($p > 0.001$) in Group 2, cyclophosphamide treated animals (2.23 ± 0.03). In Group

Table 1
Effect of Apremilast on Bone marrow chromosomal aberration assay and mitotic index.

Abbreviated cells (%)						
Groups	Dose (mg/kg)	No. of analyzed metaphase	Including gaps	Excluding gaps	No. of cells observed for MI	MI (%)
Group 1	40	500	0.4 ± 0.02	0.3 ± 0.01	2500	7.53 ± 0.08
Group 2	10	500	13.1 ± 0.02###	7.2 ± 0.07##	2500	2.23 ± 0.03###
Group 3	10	500	3.2 ± 0.03***	4.8 ± 0.09***	2500	6.13 ± 0.05***
Group 4	20	500	6.6 ± 0.05**	4.8 ± 0.09***	2500	5.24 ± 0.04***
Group 5	40	500	9.4 ± 0.06***	6.8 ± 0.08**	2500	3.57 ± 0.06**
Group 6	80	500	10.8 ± 0.09***	7.9 ± 0.06***	2500	2.12 ± 0.02***

Results are mean ± SEM (n = 6).
MI: mitotic index.
Significance: (#) Group 2 (cyclophosphamide control) as compared to normal control. (*) Groups as compared to disease control.
*P < 0.05;**P < 0.01; ***P < 0.001.

3, MI is slightly reduced (6.13 ± 0.05) with significant value p > 0.001. MI was a significantly reduced (p > 0.001) in Group 4, Apremilast treated animals (5.24 ± 0.04). In Group 5, MI is significantly decreased (3.57 ± 0.06) with significant value p > 0.01. While in Group 5 animals, MI is significantly decreased (2.12 ± 0. 02) with significant value p > 0.001 comparable to disease control animals. Value of MI decreases with increase of Apremilast dose in a dose dependent manner (Table 1).

3.3. Bone marrow micronuclear assay

In Group 2 (positive control) score of micronucleated polychromatic erythrocytes (MNPCEs) in in 1000 PCEs was significantly (p < 0.001) increased to 31.6 ± 8.3 as compare to Group 1 (negative control) group PCEs was 3.83 ± 0.3. Score of micronucleated PCEs were also significantly (p < 0.001) increased in all the treatment groups in a dose dependent pattern (Fig. 1).

In the Group 1, percentage of MNPCEs/1000 PCEs was found to be 0.40 ± 0.12 while in Group 2 (positive control) animals value of PCEs was found to be 3.24 ± 0.91, which was significantly (p < 0.001) increased in comparison to Group 1 animals. In treatment group 4, 5 and 6 percentage of MNPCEs/1000 PCEs were also increased significantly (p < 0.001) in comparison to Group 2 in a dose dependent pattern. Only result of Group 3 was non-significant (Fig. 2).

The ratios of polychromatic/normochromatic erythrocytes (PCE/NCE) in Group 1 was 1.19 ± 0.23. In Group 2 it was reduced significantly (p < 0.001) to 0.65 ± 0.08 in comparison to Group 1 animals. In Group 4, 5 and 6 it was also reduced significantly ((p < 0.001; p < 0.0001) as compare to Group 1 animals (Fig. 3).

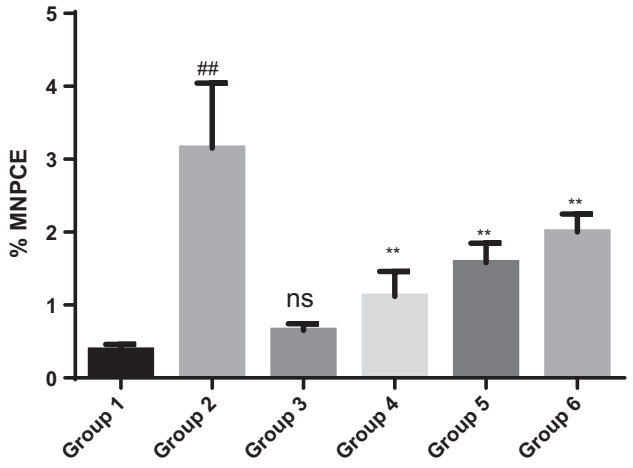

Fig. 2. Effect of Apremilast on percentage of micronucleated PCEs/1000 PCEs. Results are mean ± SEM (n = 6). Significance: (#) Group 2 (cyclophosphamide control) as compared to normal control. (*) Groups as compared to disease control. *P < 0.05;**P < 0.01; ***P; ns: non-significant.

4. Discussion

Drugs can damage DNA by conjugating with it or can perturb DNA physiology by binding irreversibly. Both interactions can

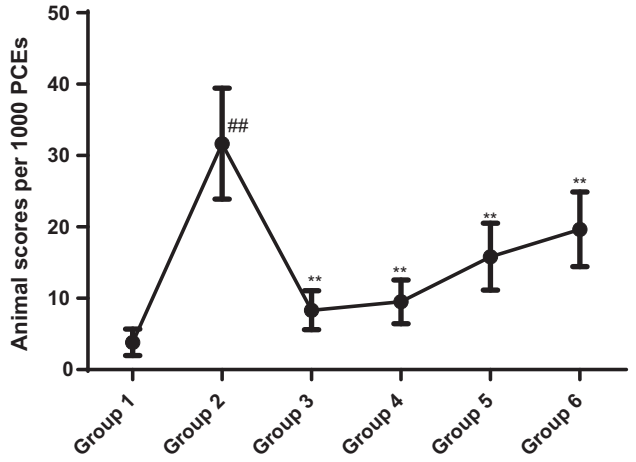

Fig. 1. Effect of Apremilast on score of micronucleated PCEs in 1000 PCEs. Results are mean ± SEM (n = 6). Significance: (#) Group 2 (cyclophosphamide control) as compared to normal control. (*) Groups as compared to disease control. *P < 0.05; **P < 0.01; ***P < 0.001.

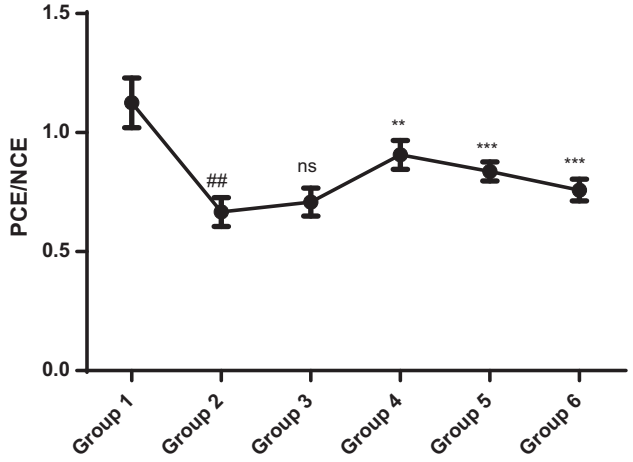

Fig. 3. Effect of Apremilast on the ratio of polychromatic/normochromatic erythrocytes (PCE/NCE). Significance: (#) Group 2 (cyclophosphamide control) as compared to normal control. (*) Groups as compared to disease control. *P < 0.05; **P < 0.01; ***P; ns: non-significant.

cause genetic alterations i.e. replication arrest, frustrated cell cycle, cellular damage, following genomic instability which contributes to cancers (Montagner et al., 2018). Genotoxicity is a destruction of genetic material inside the cell by chemical or radiations which affects integrity of cell while mutagenicity is permanent changes in the structure or amount of the transmissible DNA of cells/organism. Genotoxic effect is similar to mutagenic effect except genotoxicity is not always linked with mutations. All mutagens have genotoxic effect while not all genotoxic chmical have mutagenic potential (Turkez et al., 2017). Chronic administration of drugs with high dose can lead to genotoxicity which serve as first mile stone of cancers journey (Liu et al., 2015). Apremilast is also used chronically for long time for the treatment of psoriasis. On long term use, it is linked with rare side effects including chronic tearing, depression, lichenoid reactions, suicidality, hyperpigmentation, peripheral neuropathy, Fanconi syndrome and lentigines appearance on resolving plaques of psoriasis (Maloney et al., 2019). Cyclophosphamide used in this study as genotoxin in disease control animals. It is generally used for the treatment of different kind of cancer, immunosuppressive agent in transplantation of organs and rheumatoid arthritis (Imamura and Shigematsu, 2019). However, it is also known as a carcinogen and produce tumors in human (Alshahrani et al., 2019). It is also associated with chromosome aberrations, genetic mutations, sister chromatid and micronuclei exchanges (Anderson et al., 1995). In our study, cyclophosphamide at a dose of 40 mg/kg shows genotoxic effect significantly ($p < 0.001$) in comparison to normal control animals which indicate chromosomal aberrations were successfully induced in disease control animals.

Chromosome abnormality is an extra, missing or irregular abnormal portion of DNA of chromosome (Ma et al., 2016). It leads to different kind of genetic disorders which may affect life style of individual. Chromosomal aberrations is an important biological effect of exposure of genotoxic chemicals and drug and ionizing radiation (Zepeda-Mendoza et al., 2017). Different class of cancers are linked with specific types of chromosomal aberrations. Frequency of Congenital chromosomal defect is common and high in human and important to knowhow chromosomal aberration originate and transmitted to offspring.

Bone marrow chromosomal aberration assay in animals and humans is a most important method for detection of genotoxic potential of chemicals, drugs, radiation and viruses (Bagri and Jain, 2019). This assay is prescribed by WHO for routine analysis in order to detect genotoxicity at early stage (Moon et al., 2018). Therefore, bone marrow chromosome aberration assay and MI parameters chosen for evaluation of Apremilast genotoxic potential with long time use in a dose dependent manner. At lower dose 10 mg/kg of Apremilast, percentage abbreviation in cells was 3.2 ± 0.03 including gaps and 2.2 ± 0.03 without gaps. It indicates that at low dose Apremilast shows slight genotoxic effect on chromosome and increases in dose dependent manner. At a dose of 20 mg/kg of Apremilast, percentage abbreviation in cells was 6.6 ± 0.05 including gaps and 4.8 ± 0.09 without gaps. It reflects 20 mg/kg dose of Apremilast is sufficient to initiate genotoxic effect in mice. Genotoxic effect on this dose is higher than 10 mg/kg dose. Significant genotoxic effect observed at 40 mg/kg and 80 mg/kg dose of Apremilast. At 40 mg/kg percentage cell aberration significantly high 9.4 ± 0.06 with gaps and 6.8 ± 0.08 without gaps. Highest percentage of cell aberration effect observed on high dose 80 mg/kg. At this dose cell abbreviation percentage was 10.8 ± 0.09 with gaps and 7.9 ± 0.06 without gaps which is comparable to genotoxic data of disease control animals. Results of all the Apremilast treated animals were significant $p > 0.01$ and $p > 0.001$ which indicates experimental data were compatible with a experimental statistical model. Possible mechanism of genotoxicity of Apremilast at higher dose may be due to structure similarities of Apremilast with thalidomide (Schafer et al., 2014). Thalidomide have already human cereblon binding capacity and shows genotoxic effect due to this property (Gemechu et al., 2018). Cereblon is a functional protein found humans produced by genetic expression of CRBN gene (Bila et al., 2016). At low concentration Apremilast does not show cereblon binding.

MI is the ratio of the number of cells undergoing mitotic division to the total number of cells (Wheless et al., 2018). MI is the measure of proliferation of cells (Bedekovics et al., 2018). It is very crucial prognostic tool for prediction of response to chemotherapy and survival in different types of cancer (Patlolla et al., 2015). The relation between progression of cycle of cell and cell proliferation inhibition was evaluated by the determination of MI (Muñoz-Barrera and Monje-Casas, 2017). Decrease the MI percentage in cyclophosphamide administered animals give the idea that bone marrow cell proliferation is decreased. Further percentage MI is decreased in Apremilast treated animals in a dose dependent manner. It reflects that Apremilast exhibit anticancer effect and inhibits cell proliferation at higher dose.

Micronucleus assay is main test used in the screening for genotoxic potential of chemicals (Smart et al., 2019). This test is considered as a one of the most successful and reliable test for carcinogens screening (Díez-Quijada et al., 2019). Further for the confirmation of results of chromosomal aberrations assay, micronuclear assay was conducted in order to evaluate the genotoxic efficacy of Apremilast. Results of this study clearly indicate that administration of Apremilast in animals significantly raised PCEs frequency in the cells of bone marrow of animals when administered per oral at different doses 10, 20, 40 and 80 mg/kg/day for 13 weeks (Figs. 1–3). The increase of score of micronucleated PCEs in 1000 PCEs in Group 2 cyclophosphamide positive control group clearly indicate genotoxic effect of Apremilast. Further, from micronuclear assay cytotoxic potential of Apremilast found from PCE/NCE ratio. With the exposure to genotoxic chemicals polychromatic erythrocytes (immature erythrocytes) PCE number decreases in comparison to the mature erythrocytes (NCE). Hence, decreased ratio of PCE/NCE ratio with the exposure of chemicals is indication of cytotoxic potential of chemical (Çelik et al., 2019). In all the treatment groups PCE/NCE ratio was significantly decreased which indicates that Apremilast also shows cytotoxic effects in a dose dependent pattern.

5. Conclusion

Mice chronically exposed to Apremilast exhibited a dose dependent genotoxic effect comparable to cyclophosphamide. Apremilast induces chromosomal aberrations and cytotoxic effect which opens new scope for further clinical investigation on genotoxic effect of Apremilast.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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