

Original Research Article

Article title: *In vitro* cytotoxic and genotoxic effects of methanol and aqueous extracts of *Gymnosporia montana* plant.

ABSTRACT

Introduction: *Gymnosporia montana* Benth. (GM) is a medicinal herb which has been valued in Ayurvedic medicine for its hepatoprotective effect. The plant has been studied for its pharmacological, antimicrobial, and antioxidant properties, but there are no reports on its genotoxicity.

Aim: Hence, in the present study, two extracts of *G. montana* (70% methanolic and aqueous) at different concentrations were evaluated for the *in vitro* cytotoxicity and genotoxicity in Human peripheral blood lymphocyte cultures (PBLCL) since these are well established techniques for the analysis of the potentially mutagenic and carcinogenic chemicals.

Methodology: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Mitotic index (MI), Sister-chromatid exchanges (SCEs), Cell cycle proliferative index (CCPI), Average generation time (AGT) and Population doubling time (PDT) were scored in cultures set up from 10 different healthy donors. The treatment of the cell culture was done employing different extracts of *G. montana* GM at three concentrations (1.78 µg/mL, 3.57 µg/mL and 7.14 µg/mL) with control and positive control (Ethyl methane sulfonate [EMS (1.93 mM)]).

Results: The MTT results showed the cytotoxic effect in a concentration-dependent manner in both the methanol and aqueous extract and the IC₅₀ value of methanol and aqueous extract was found to be 2.63 µg/mL and 3.63 µg/mL. The MI (p < .001) and CCPI (p < .05) in both the extracts showed significant value at higher concentration, but at lower and mid concentrations both the extracts were non-significant and the total SCEs, AGT, and PDT in all the concentrations showed non-significant results when compared with the control.

Conclusion: These results indicate that the *G. montana* GM plant extracts at lower two concentrations showed no cytotoxicity and genotoxicity effects in cultured human peripheral blood lymphocytes. Therefore, we suggest that the plant extract is safe for use at the lower concentrations in the traditional medicine.

Key words: *Gymnosporia montana*, genotoxicity, cytotoxicity, cell viability, sister chromatid exchanges.

1. INTRODUCTION

Plants produce a diverse range of bioactive molecules making them a rich source of different types of herbal medicines. Plant-derived drugs remain an important resource, especially in developing countries, to overcome serious illnesses. Over 50% of all modern clinical drugs are made up of natural products which play an important role in drug development programs in the pharmaceutical manufacturing [1,2] and many therapeutic substances have been extracted from medicinal plants and used during drug preparations [23,4]. Medicines manufactured from most plants in self-medication or through prescriptions fail to exhibit information on the toxicological profile or their safety and efficacy [35,46]. This is a fact of great concern since several studies have shown mutagenic [57-79], carcinogenic properties [108] and also the property of preventing carcinogenesis [91] in many plant extracts. The interest of the public and the scientific community has increased in the benefit and harmful effect of the natural products and their uses in the last few decades [40,12,13].

Also, green plants in general, are a primary source of antimutagens as well as natural toxic agents [44,14]. Most of the herbal medicines are used without any standard safety and toxicological trials although the common assumption is that these products are non-toxic. However, this assumption is incorrect and dangerous, so toxicological studies should be done for herbal drugs [42,15]. Some investigations have revealed that many plants used as food or in traditional medicine

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have cytotoxic and genotoxic effects *in vitro* and *in vivo* assays [1316,17]. The assessment of the possible toxicity of the medicinal plants, their extracts, or their constituents has been carried out on a relatively small number of plant species [1418]. The claim that natural plant products are safe should be accepted only after the plant product passes through toxicity testing using modern scientific methods [1519].

GymnosporiaG. montana belonging to the family Celastraceae is found in South Africa, Nigeria, Tropical Africa, Mediterranean, Malaya, Australia, Arabia, Afghanistan, and India. It is a shrub or tree growing wild in dry areas and is commonly known as Vikalo in Gujarat, India, where it has been traditionally used in treating the ulcer, jaundice, gastrointestinal disorders, toothache, dysentery, and antispasmodic effect [1620]. The previous study by Patel, et al. [1721] indicates that the extract of *GymnosporiaG. montana* possesses significant hepatoprotective activity. A literature survey also showed that plant extracts can be mutagenic as well as antimutagenic depending on the test system used which indicates that a battery of assays are needed before any conclusion can be reached. Since the *G. montana* plant has not been investigated yet for cytotoxicity and genotoxicity, this study was undertaken to evaluate the plant by using its two different extracts i.e. 70% methanolic extract and an aqueous extract. Since the short-term tests for genotoxicity are typically used to identify potential mutagens and carcinogens, the leaf extracts of *G. montana* were studied for toxicity in cultured human peripheral blood lymphocytes using MTT, mitotic index (MI), sister chromatid exchanges (SCEs), cell cycle proliferative index (CCPI), average generation time (AGT) and population doubling time (PDT), as Peripheral Blood Lymphocyte cultures (PBLC) is well established method to examine the genotoxic damage.

2. MATERIALS AND METHODS

2.1 Collection of plant materials and extract preparation

The leaves of *G. montana* were collected from a nursery in sector-30 of Gandhinagar. The taxonomic identity was authenticated and herbarium sheet with reference No. PH/14/0010 was deposited in Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, India. The leaves of *G. Montana* were extracted according to our previous study [1822] and after performing MTT and MI, the three different concentrations: Low dose (1.78 µg/mL), Mid dose (3.57 µg/mL) and High dose (7.142 µg/mL) were finalized for further studies. Both the methanolic and aqueous extracts were analyzed at these concentrations for their cytotoxic and genotoxic effect using different parameters as mentioned below.

2.2 Chemicals and reagents

All chemicals utilized in the following parameters were procured from Merck, Germany (AR Grade), while media and culture reagent were procured from HiMedia, India and Sigma Aldrich, USA (Culture Grade).

2.3 Protocols

The present study was approved by the Institutional Ethical Committee (IEC) of Gujarat University, Ahmedabad, India and 10 healthy individuals free from any infection, addiction (including tobacco, drug or alcohol abuse and non-smoking) between 20-30 years of age were recruited. Blood collection was carried out according to ethical guidelines and with prior consent of the subjects. Venous blood was collected from each donor in heparinized vacutainer and mixed gently to avoid blood clotting.

2.4 Cell Viability Assay (MTT Assay)

A quantitative colorimetric assay for mammalian cell survival and cell proliferation was assayed by the method of Mosmann [1923] with slight modifications. An equal amount of Hicep and blood was taken in Tarson tubes and the WBC layer was separated for further use. 1.5 mL media, 20 µL PHA, and varying doses ranging from 0.50 µg/mL to 10 µg/mL of the plant extract were added to 100 µL of the sample and EMS (1.93 mM) was used as a positive control. The cultures were incubated for 72 hours and harvesting was done on the 3rd day. After addition of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (30 µL) the cultures were further incubated for 4 hours at 37°C. DMSO was added to the pellet to observe a color change. The samples were centrifuged, and the optical density was measured at 570 nm on Epoch Biotek.

2.5 Mitotic index (MI)

Initially, the cultures were set up as per the standard protocol [2024] at different concentrations and MI was done to finalize the three doses used in this study. By quantifying aspects of a dividing cell population, one can examine how cells differ in their capability to divide under different experimental conditions. Mitotic index is defined as the ratio between the number of cells in mitosis and the total number of cells. A total of 1000 cells were scored for each individual according to the following formula.

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

2.6 Sister chromatid exchanges (SCEs)

Peripheral blood lymphocyte cultures were prepared according to the standard procedure [2024] with slight modifications and for SCEs, 80 µL of Bromodeoxyuridine (1 mg/mL, BrdU) was added at 0th hour [2425] and cultures were incubated in dark at 37°C in an incubator and were harvested at 72 hours after colchicine treatment at the 69th hour and slides were prepared. Cleaned slides were prepared by adding 3 to 4 drops of the cell suspension uniformly on a chilled slide and were flame dried. For Sister Chromatid Differentiation, the slides were stained with Hoechst 33258 for 20 min in the dark and layered with 2X saline sodium citrate buffer. The slides were then kept in UV exposure for 45 min in a moist chamber, washed in water and stained in 2% Giemsa for 5 min and were analyzed for SCEs, CCPI, AGT, and PDT.

2.7 Analysis of sister chromatid exchanges (SCEs)

Metaphases in the second cycle of cell division (M2) were selected for scoring on the basis of the spreading of chromosomes and differentiation of chromatids. Total exchanges were counted in 30 M2 cells to calculate SCE/Plate and SCE/Chromosome to evaluate the level of Genotoxicity [2226] using the following formula:

$$\begin{aligned} \text{SCEs/Plate} &= \frac{\text{Total SCEs scored}}{\text{Total M2 plates scored}} \\ \text{SCEs/Chromosome} &= \frac{\text{SCEs/Plate}}{\text{Total no. of chromosomes (i.e. 46)}} \end{aligned}$$

2.8 Analysis of Cell Cycle Proliferative Index (CCPI)

Differentially stained slides were scored for the proliferative kinetics based on the staining pattern of chromosomes. Total 100 metaphases were analyzed for each individual, classifying them

on the first (M1), second (M2), third (M3) generation cells. At least 100 metaphases from each culture were analyzed for CCPI [2226].

The cell cycle proliferative index (CCPI) for each individual was calculated according to the following formula:

$$\text{CCPI} = \frac{1(\text{M1 plate}) + 2(\text{M2 plate}) + 3(\text{M3 plate})}{100 (\text{Total plates})}$$

2.9 Analysis of average generation time and population doubling time

The cell cycle time AGT is also studied as the ratio of the BrdU time and proliferative index (CCPI)

$$\text{AGT} = \frac{72 \text{ hours (BrdU time)}}{\text{CCPI}}$$

And, the PDT is the time in which the cell divides, i.e. 24 hours

$$\text{PDT} = \frac{24 \text{ hours}}{\text{CCPI}}$$

2.10 Statistical Analysis

Values of all the data are expressed as Mean±Standard Error of the 10 replicates. The statistical analysis was done in GraphPadPrism 6.0 software. A t-test was done to compare two groups while One-way ANOVA was done by Tukey's multiple comparison tests to compare more than two groups and significance was accepted when $p < .05$.

3. RESULTS

In the present study, the cells showed growth inhibition in a dose-dependent manner with a significant increase ($p < .001$) in the percent cell inhibition when treated with two different extracts of *G. montana* at concentrations ranging from $0.50 \mu\text{g/ml}$ to $10 \mu\text{g/ml}$ (Table 1.A and 1.B). The percentage of dead cells in the methanol extract for each concentration was found to be 45.6, 48.77, 53.1, 57.5, 67.2, 68.4, 76.6, 77.8, 79.47, 83.9, 88.3 and in aqueous was found to be 42.2, 47.6, 48.7, 51.7, 51.8, 56.5, 63.5, 71.4, 75.5, 78.262, 78.7. The IC_{50} value of *G. montana* was found to be $2.63 \mu\text{g/ml}$ in methanol and $3.63 \mu\text{g/ml}$ in aqueous extract.

From the prepared slides, dividing and non-dividing cells were counted to determine the mitotic index for the assessment of the cytotoxicity in the control, positive control and plant extracts at different concentrations (Low Dose (LD): $1.78 \mu\text{g/ml}$, Mid Dose (MD): $3.57 \mu\text{g/ml}$ and High Dose (HD): $7.14 \mu\text{g/ml}$). Table 2 shows the mean and standard error of the MI rates of control (3.22 ± 0.167983) and the positive control (1.52 ± 0.099849). The MI of the three concentrations of methanol extract was 2.59 ± 0.114051 , 2.53 ± 0.140774 and 1.65 ± 0.126802 and aqueous extract was found to be 2.56 ± 0.161499 , 2.48 ± 0.11243 and 1.6 ± 0.142307 . For MI, the extracts (both methanol and aqueous) showed a non-significant decrease in LD and MD whereas, highly significant ($p < .001$) decrease was seen in the HD when it was compared with control values. Compared to the MI value of the positive control, a highly significant increase ($p < .001$) in LD and MD and a non-significant increase in HD in methanol extract were seen. On the other hand, the aqueous extract showed a significant increase ($p < .05$) in LD and MD, but a non-significant increase in the HD.

Our analysis showed that the total SCEs were non-significantly increased in all the concentrations when both the extracts (methanol and aqueous) were compared with the control group (Table 3). Also, on comparison with the positive control, both the extract showed highly significant ($p<.001$) decrease in LD, but significant ($p<.01$) decrease in MD and HD. (Table 3). As shown in Table 4, Cell cycle proliferative index (CCPI) showed a non-significant decrease in LD and MD but significant ($p<.05$) decrease in HD when compared with control in both the extracts. Whereas, in comparison with the positive control, significant ($p<.05$) increase in LD and MD and non-significant ($p<.05$) increase in HD was seen. The AGT and PDT were non-significantly elongated in all the concentrations when they were compared with control (Table 4).

TABLE 1. A: Cytotoxicity of *G. montana* in methanol and aqueous extract.

CONCENTRATION ($\mu\text{g} / \text{mL}$)	% CELL INHIBITION (METHANOL EXTRACT)	% CELL INHIBITION (AQUEOUS EXTRACT)
0.50	45.6	42.2
1	48.77	47.6
2	53.1	48.7
3	57.5	51.7
4	67.2	51.8
5	68.4	56.5
6	76.6	63.5
7	77.8	71.4
8	79.47	75.5
9	83.9	78.262
10	88.3	78.7
IC ₅₀ VALUE	2.63	3.63

Each value is represented as Mean \pm SE, $n=10$.

Aqueous Extract vs Methanol Extract: ****= $p<.0001$ (Highly significant)

TABLE 1. B: Cell viability of *G. montana* in methanol and aqueous extract.

CONCENTRATION ($\mu\text{g} / \text{mL}$)	CELL VIABILITY (METHANOL EXTRACT)	CELL VIABILITY (AQUEOUS EXTRACT)
0.50	54.40	57.80
1	51.23	52.40
2	46.90	51.30
3	42.50	48.30
4	32.80	48.20
5	31.60	43.50
6	23.40	36.50
7	22.20	28.60
8	20.53	24.50
9	16.10	21.74
10	11.70	21.30

Each value is represented as Mean \pm SE, n=10.

Aqueous Extract vs Methanol Extract: ****= $p < .0001$ (Highly significant)

Table 2: Mitotic index (M.I %) of control and treated cultures.

GROUP	MITOTIC INDEX OF MEMITOTIC INDEX OF AE	
CONTROL	3.22±0.167983	3.22±0.167983
LOW DOSE METHANOL EXTRACT (1.78µg/mL)	2.59±0.114051 ^{NS, ####}	2.56±0.161499 ^{NS, #}
MID DOSE METHANOL EXTRACT (3.57µg/mL)	2.53±0.140774 ^{NS, ###}	2.48±0.11243 ^{NS, ##}
HIGH DOSE METHANOL EXTRACT (7.14µg/mL)	1.65±0.126802 ^{***, ns}	1.6±0.142307 ^{***, ns}
POSITIVE CONTROL (1.93mM)	1.52±0.099849 ^{***}	1.52±0.099849 ^{***}

Values are Mean±S.E

Control vs LD, MD, HD and PC: ***=p<.001 and NS=Non Significant

Positive Control (Ethyl Methanesulfonate) vs LD, MD and HD: #=p<.05, ##p<.01 and ###=p<.001

LD: Low Dose, MD: Mid Dose, HD: High Dose

Table 3: Showing Total SCEs, SCEs/plate and SCEs/chromosome in control and treated cultures.

EXTRACTSGROUP		TOTAL SCE	SCEs/PLATE	SCEs/CHROMOSOME
	CONTROL	90.1±3.03332	2.999763±0.102211	0.065212±0.002222
	LOW DOSE (1.78µg/ml)	91.2±3.761551 NS, ###	3.042233±0.1242 NS, ###	0.066136±0.0027 NS, ###
	METHANOL EXTRACT			
	MID DOSE (3.57µg/ml)	94.7±4.011352 NS, ##	3.153752±0.124359 NS, ##	0.06856±0.00288 NS, ##
	HIGH DOSE (7.14µg/ml)	107.1±4.062072 NS, ##	3.599726±0.145142 NS, ##	0.078255±0.003155 NS, ##
	LOW DOSE (1.78µg/ml)	91.8±5.603242081 NS, ###	3.062538±0.004093296 NS, ##	0.066577±0.00418228 NS, ##
	AQUEOUS EXTRACT			
	MID DOSE (3.57µg/ml)	97.4±3.739001389 NS, ##	3.245319±0.162222711 NS, ##	0.07055±0.002703465 NS, ##
	HIGH DOSE (7.14µg/ml)	108.1±3.303726066 NS, ##	3.603992±0.110085075 NS, ##	0.078348±0.002393154 NS, ##

Values are Mean±S.E

Control vs LD, MD, HD and PC: ***=p<.001 and NS=Non Significant

Positive Control (Ethyl Methanesulfonate) vs LD, MD and HD: ##p<.01 and ###=p<.001

LD: Low Dose, MD: Mid Dose, HD: High Dose

Table 4: Showing CCPI, AGT, and PDT in control and treated cultures

EXTRACTSGROUPSCCPIAGTPDT				
	CONTROL	2.145± 0.0151	33.5815625± 0.2382	11.19385417± 0.07941
	LOW DOSE (1.78µg/ m mL)	2.13±0.01334 ^{NS, ##}	33.8147±0.2111 ^{NS, ##}	11.2715±0.0703 ^{NS, ##}
METHANOL EXTRACT	MID DOSE (3.57µg/ m mL)	2.063±0.03325 ^{NS, ##}	34.9882±0.6056 ^{NS, #}	11.6627±0.2018 ^{NS, #}
	HIGH DOSE (7.14µg/ m mL)	1.901±0.0621 ^{*, ns}	38.2796±1.3895 ^{NS, ns}	12.7598±0.4631 ^{NS, ns}
	LOW DOSE (1.78µg/ m mL)	2.084±0.0363 ^{NS, #}	34.6549±0.6783 ^{NS, #}	11.5516±0.2261 ^{NS, #}
AQUEOUS EXTRACT	MID DOSE (3.57µg/ m mL)	2.075±0.0251 ^{NS, #}	34.7453±0.4282 ^{NS, #}	11.5817±0.1427 ^{NS, #}
	HIGH DOSE (7.14µg/ m mL)	1.895±0.06102 ^{*, ns}	38.3889±1.3733 ^{NS, ns}	12.7963±0.4577 ^{NS, ns}
	POSITIVE CONTROL (1.93mM)	1.718±0.0693 ^{**}	42.5220±1.7090 ^{**}	14.1740±0.5696 ^{**}

Values are Mean±S.E

Control vs LD, MD, HD and PC: *= $p < .05$, **= $p < .01$ and NS=Non Significant

Positive Control (Ethyl Methanesulfonate) vs LD, MD and HD: #= $p < .05$, ##= $p < .01$ and ns=Non Significant

LD: Low Dose, MD: Mid Dose, HD: High Dose

4. DISCUSSION

Special attention must be paid to the evaluation of the safety, efficacy, and quality of natural products and their components and it is important to determine the toxic effect of plant extracts used as herbal therapy on humans. In our study, we took a step towards exploring the cytotoxic and genotoxic effects of a herbal plant *G. montana*, since it is being used as an alternative medicine locally to cure jaundice and has a hepatoprotective effect [4620,2147]. Previously by Bhavita and coworkers [4620], the leaf of *G. montana* was evaluated for pharmacognostical parameters and phytochemical screening, which indicated that the leaf of *G. montana* was rich in phenol. In our previous study, we have tested the plant for its antioxidant activities in the two different extracts used here, which indicated that the *G. montana* GM-plant is a good antioxidant [4822]. The cytotoxic effect of these two leaf extracts of the plant at different concentrations was checked by doing the MTT assay, which is an appropriate method for screening new substances within a short period of time. The MTT assay has been described as a rapid, simple and reproducible method, widely used in the screening of drugs to measure the cytotoxic properties [2327,2428]. To be a high-quality drug candidate, the IC₅₀ value of the tested compound should be sufficiently low to avoid any possible unspecific effects. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC₅₀ value < 30 µg/ml [2529,2630]. The cytotoxicity tests in the present study showed that IC₅₀ of both the extracts were similar and both the extracts showed a cytotoxic effect in a concentration-dependent manner. In similar studies, Varalakshmi and coworkers [2731] found that neem extract has been cytotoxic to normal lymphocytes just like other chemotherapeutic agents at higher concentrations. Earlier studies hypothesized that the possible cytotoxic effect of plant extracts could be because of the presence of various phytochemicals such as alkaloids, flavonoids, tannins, phenolics, and glycosides [2832-34,29,30]. Our results also showed a similar trend of response; as the concentration is increased, both the extracts showed cytotoxic properties and caused growth inhibition, thus indicating that cytotoxicity is possible in the human lymphocytes upon continued and unregulated consumption of these extracts at higher doses which can be related to a toxic effect on the metabolism of the cell or to DNA damage. Therefore, the traditional healers and patients should be enlightened of the risk of cytotoxicity that might arise from the use of extracts from this plant at high doses.

The Mitotic index (MI) was performed as it is a cytogenetic test, used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression. Results revealed a decrease in MI values, which reflects the inhibition of progression of the cell cycle and/or loss of capacity to proliferate at high doses, which can be correlated with the earlier studies of [23,227,284]. But both the extracts were non-toxic at the LD and MD and only the HD showed significant results. Anything consumed in the higher amount would have a toxic effect. This reduction in MI was more evident at HD could be interpreted as cellular death [3435] and related to the cytotoxic effect of the extracts. Both the extracts of *G. montana* are a mixture of different compounds like phenol, alkaloids, flavonoids, saponins, glycosides, and tannins and excess in any of these components may alter the cell cycle and can cause decreased MI in a concentration-dependent manner.

The genotoxicity study was also performed as it describes the ability of chemical compounds and their metabolites to interact with DNA and/or the cellular machinery controlling the genome integrity [3236]. Previous studies have shown that the genotoxicants interact either directly with DNA or chromosomes to produce DNA damage such as adducts, strand breaks, chromosome breakages, etc., or indirectly, disturbing the genomic integrity through several mechanisms, notably by interaction (1) with proteins involved in DNA replication, transcription, or repair, (2) with components of mitotic spindle, or (3) with protein kinases in charge of cell cycle checkpoints [3337]. Genotoxicants are usually classified according to their mutagenicity, through the transformation of DNA damage into a mutation, clastogenicity, through modification of chromosome structure, and aneugenicity, through changes in the number of chromosomes (loss or gain) [3438,369]. Genotoxicity evaluation of any substance or drug provides us a tool to analyze individual susceptibility to such chemicals and is thus found to have a direct relationship to cancer risk. No previous studies are available on genetic toxicology of the *G. montana* plant extract so present study was undertaken. Sister chromatid exchange analysis in human PBLC has often been applied as cytogenetic testing of potentially mutagenic and carcinogenic chemicals [3640]. The SCE is generally a more sensitive indicator of genotoxic effects than structural aberrations [3741] and it is a classic cytomolecular technique which

provides an easy and accurate index to monitor DNA damage and DNA repair status, hence, this assay was performed to check out the effect of this plant extract on the human chromosomes. In the present study, no significant increases in SCE frequency in lymphocytes were detected in all the concentrations in both the extracts when compared with the control. An increased rate of SCEs could be a sign of persistent DNA damage [3842], but the total SCEs, SCEs/plate and SCEs/chromosomes in the present study were almost similar to that of the control in all the doses (LD, MD, and HD). Therefore, both methanol and aqueous extracts may not promote DNA damage and are non-genotoxic in all the concentrations and they are not likely to affect the frequency of cytogenetic anomalies existing in peripheral lymphocytes *in vitro*. Since sister chromatid exchanges are extremely valuable and highly relevant endpoints for the detection of potential carcinogens, our results clearly indicate that the two extracts are non-carcinogenic. The failure of repair mechanisms to achieve recovery leads to DNA damage and strand breaks, resulting in an increase of SCEs frequencies [39,4043,44]. Haytham and Nasser [445] showed the reduction in sister chromatid exchanges or change in the MI may be due to the direct action of the compounds present in the extract by inactivating it enzymatically or chemically.

The cell cycle kinetics was evaluated by the cell cycle proliferative index (CCPI), a parameter that represents the ratio of cells in the first, second and third mitosis and was gradually decreased in the present study similar to the results of MI for both the extracts, possibly due to an arrest of mitosis which allows the repair of genetic material. Cytotoxic effects occur in cells with a relatively high level of genetic damage [4246]. We propose that the extract might be causing some DNA damage at HD which gets repaired during the G₁ phase and hence no significant difference in SCEs values are observed. The significant decrease in cell cycle progression for HD observed in the present study would allow the DNA repair to take place. We suggest that no DNA damage is taking at the LD and MD, hence no extra time is required for DNA repair to take place in cell cycle and hence the cell cycle progression is similar to control values at these doses. Thus, we can say that the extracts are affecting the cells on higher consumption as MI and CCPI, both at higher doses were affecting the cells by showing antimitotic properties but both LD and MD were having no cytotoxicity, which indicates that both the extracts did not have a significant cytotoxic effect on these parameters at the used lower two concentrations (LD and MD). It can be because medicinal herbs contain complex mixtures of thousands of compounds that can exert their antioxidant and free radical scavenging effect either separately or in synergistic ways [4347]. Also, some bioactive compounds present in the plant can interfere with drug kinetics and produce adverse effects related or unrelated to their pharmacological actions, such as allergic reactions, mutagenic and carcinogenic effects. In this study, both the extracts affect cell proliferation by declining the CCPI and cell progression by elongation of AGT and PDT in a concentration-dependent manner in comparison with control and positive control. The reduction in the mitotic activity and the cell-cycle delay at higher doses could be attributed due to the formation of toxic DNA cross-links. These cross-links inhibit DNA replication and transcription, causing cell cycle arrest and induction of apoptosis [4448]. The data obtained in the present study suggest that the compounds present in the extract of *G. montana* GM are not cytotoxic and not mutagenic at lower doses (LD and MD) and when treated cultures were compared to control and positive control, the values showed mitotic depression in all cultures, however, the values were non-significant. Thus, the plant extract is not harmful at the two lower tested levels (LD and MD) and can be safely used up to these doses and both the extracts are safe and non-toxic at these tested levels.

5. CONCLUSIONS

Gymnosporia G. montana belonging to the family Celastraceae has been traditionally used in treating jaundice and has not been examined for cytotoxicity and genotoxicity till date. Evaluation of the endpoints described here would help to estimate the potential toxicity of this medicinal plant. The MTT results from our study indicate that cytotoxicity was aggravated with the increase in the concentration of both the leaf extracts of *G. montana* GM. Based upon this *in vitro* investigations, we can conclude that the MI and CCPI in both the extracts of *G. montana* GM were almost similar to that of control at lower two concentrations (LD and MD), but at higher doses they produce cytotoxicity. The SCEs showed no genotoxic effect at all the three doses (LD, MD, and HD) in both the extracts. These observations specify that the *G. montana* GM plant extracts showed no cytotoxicity upto MD (3.57 µg/mL) and no genotoxicity effects at all the three doses (LD, MD, and HD) in cultured human

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peripheral blood lymphocytes and it is concluded that it is safe for use in humans and can be used at these doses in herbal medicine. Further *in vitro* and *in vivo* investigations are needed to confirm the results.

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