International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 07-05-2020; Accepted: 23-05-2020: Published: 12-06-2020
www.botanyjournals.com
Volume 5; Issue 3; 2020; Page No. 213-218

In vitro and *In vivo* antimutagenic activity of *G. montana* stem and leaf extracts

Dhru Bhavita[1*], Maitreyi Zaveri[2], Lakshmi B[3]
[1, 2] KB Institute of Pharmaceutical Education and Research, Sector-23, GH-6, Gandhinagar, Gujarat, India
[3] Department of Biotechnology, KSV University, Gandhinagar, Gujarat, India

Abstract
The use of dietary antimutagens has been seen as a promising approach to the protection of human health. *Gymnosporia montana* is a well-known ethnomedicinal plant. The antimutagenic activity of the stem and leaf extracts of the *Gymnosporia montana* was investigated. The activity was assayed by Ames *Salmonella* mutagenicity test using histidine mutants of *Salmonella typhimurium* tester strains, TA 98, TA 100 and TA 102. Against all the mutagens 70 % methanolic and aqueous extracts of leaf of *G.montana* showed significant antimutagenic activity in a dose dependent manner. 70 % methanolic extract of leaf showed significant antimutagenic activity against all mutagens. Highest antimutagenic activity showed against benzo [*a*] pyrene (64.04 % (TA 98), and 58.48 % (TA 100) and against sodium azide 58.48% (TA 100), 58.51 % (TA 102). *In vivo* antimutagenic activity of extract was also assayed by determining the mutagenicity of the urine of rats administrated with B [*a*] P as a mutagen. The prior administration of extract markedly inhibited mutagenicity induced by B [*a*] P. The results indicated that the 70 % methanolic and aqueous extract of leaf of *G.montana* possessed significant antimutagenic activity. The results revealed that *G.montana* extract restored antioxidant defence.

Keywords: *Gymnosporia montana,* ames test, *Salmonella typhimurium,* antimutagenic activity

1. Introduction

Mutations are the cause of innate metabolic defects in cellular system, triggering the morbidity and mortality in living organism. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents are known to act as mutagenic, co-carcinogenic and/or carcinogenic agents [1]. Since, the mutagens are involved in the initiation and promotion of several human diseases including cancer, the significant of novel bioactive phytocompounds in counteracting the promutagenic and carcinogenic effects are gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are called as antimutagens. Antimugenic and anticarcinogenic properties of a wide variety of dietary constituents and plant secondary metabolites have been reported [2, 4]. The antimutagenic or protective effect has been attributed to many classes of phytocompounds, however, such compounds have also been reported to exhibit a wide range of other biological activities [5, 6]. Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention and have n undesirable xenobiotic effects on living organisms [7, 9]. The rich diversity of Indian medicinal plants have not yet systematically screened for antimutagenic activity [10, 13]. More than 800 plants are used in the treatment of various ailments in the traditional systems of Indian medicine (Ayurveda, Siddha and Unani). Based on the chemical diversity of known active polyantimutagens [14], many traditionally used Indian medicinal plants may exhibit such desired properties due to similarity in the major class of phytocompounds.

Gymnosporia montana belongs to the family *Celastraceae*. Much- branched, spinescent shrub or a small tree, occurring through the dried parts of India. The leaves of *G.montana* Are simple, stipules are small. The flowers are small and bisexual. The fruit is a capsule or berry. Stems are purplish brown, straight and hard spines, which are modified branches with single node from which leaf originates. In several Ayurvedic literatures like Nighantu Adarsh [15], Vanaspati Shashtra [16], Aryabhishek [17], Vasundhrani vanaspatio [18] the plant has been mentioned for the various uses. It is claimed to be use for curing jaundice, inflammation, rheumatic pain, gastrointestinal disorders, and also as a vermifuge [19, 23].

In vitro and *in vivo* antimutagenic activity of various extracts of stem and leaf of *G.montana* were studied.

2. Materials and Methods

2.1 Collection and authentification of plant material:

Plant material of *Gymnosporia montana* were collected from the Vijapur, Gandhinagar, Gujarat, India and was identified by Dr. S. K. Patel, Head of the Botany Department, Government Science College, Gandhinagar. The voucher specimen KB/O8/0011 has been deposited in K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

2.2 Preparation of different extracts

The selected plant parts i.e., stem and leaf of *G. montana* were separated, dried under sunlight and powdered. The powdered was passed through sieve of 60 mesh (#) size and was stored in airtight containers. Shade dried stem and leaf powder were extracted successively with petroleum ether (60-80), 70 % methanol and water. The extractions were carried out using soxhlet assembly, for 6-8 hours. The process was repeated for three times in the same manner. The extracts were concentrated and dried at a temp of 60^0 C on a water bath and used for the further work.

2.3 Mutagens and other analytical reagents

Glucose-6-phosphate, L-histidine, D-biotin were purchased from Sisco Research Laboratories, Mumbai. Benzo [*a*] pyrene (B [*a*] P), 4-nitro-*o*-phenylene diamine (NPD), 2 Aminoflourence (2-AF) and amberlite XAD-4 from Sigma chemicals and Sodium azide (NaN_3) purchased from Hi-Media, Mumbai, India were used. All other chemicals employed in the studies were of analytical reagent grade.

2.4 Preparation of mutagens

All of the chemical mutagens Benzo [*a*] pyrene, (B [*a*] P), 4-nitro-*o*-phenylene diamine (NPD), 2 Aminoflourence (2-AF) were dissolved in DMSO except sodium azide (NaN_3), which was dissolved in water.

2.5 Bacterial strain

Histidine requiring strains of *Salmonella typhimurium* MTCC 1551, MTCC 1552 and MTCC 1553 were obtained from MTCC (Microbial Type Culture Collection and Gene Bank), Chandigarh. They were grown in nutrient broth for 12 h and frozen permanents were prepared by freezing at -70^0 C in the presence of 9 % dimethyl sulfoxide (DMSO). Fresh cultures were prepared by inoculating 40 µl of frozen permanents in 5 ml of nutrient broth and incubated for 12 h at 37^0 C. The cultures thus obtained were used for the experiments. The cultures when tested for their spontaneous revertants and also for their revertant in the presence of different mutagen it was found that MTCC 1551 is almost identical to TA 98, MTCC 1552 is almost identical to TA 100, MTCC 1553 is almost identical to TA 102 of American type culture collection [2].

2.6 Animals

Male Wistar rats (190 ± 200 g) were housed in well ventilated cages, fed on standard pelleted diet and kept at air-controlled room. All animal experiments were conducted according to guidelines with the approval of the Institutional Animal Ethical Committee (KBIPER/08/117).

Genotype conformation of the *Salmonella* strains (Histidine requirement, *Rfa* mutation, U*vr*B mutation, R-factor) was evaluated by the method of Maron and Ames (1983).

2.7 Preparation of Rat Liver Microsomal FRACTION (S9) for *in vitro* Antimutagenic Activity

Male Wistar rat (200 g) was administrated with sodium phenobarbitone (0.1%) in drinking water for 4 days [24]. After an overnight fasting, animal was killed by decapitation; liver was removed and washed several times in chilled 0.15 M KCl. Homogenate was prepared aseptically in 0.15 M KCl (3 ml/g wet liver) [25]. The homogenate was centrifuged in a cooling centrifuge at 8,600 rpm for 10 min at 40 ^{0}C. The supernatant was used as the S9 fraction.

2.8 Preparation OF S9 Mix

5 ml of the S9 mix was prepared by adding sterile reagents in the following order, 1.675 ml sterile distilled water, 2.5 ml 0.2M sodium phosphate buffer (pH 7.4), 0.2 ml 0.1 M NADP (Nicotinamide adeninedinucleotide phosphate), 0.025 ml 1M glucose-6-phosphate, 0.1 ml $MgCl_2$-KCl solution (1.64 M KCl +0.4 M $MgCl_2$) and 0.5 ml of rat liver S9.

2.9 Determination OF *In Vitro* Antimutagenic activity of G. *Montana* Extracts

Against direct acting mutagens

Antimutagenicity of all six extracts of stem and leaf of *G. montana* against direct acting mutagens was determined according to the methods of Maron and Ames (1983). For this 2 ml of top agar containing 0.2 ml of 0.5 mM histidine–biotin was mixed with mutagens at a concentration given (NaN_3=0.0025 mg/ml, NPD=0.020 mg/ml). Different concentrations of *Gymnosporia montana* stem and leaf extracts (1, 2 and 3 mg/ml) were dissolved in DMSO and 0.1 ml freshly grown *S. typhimurium* culture (1×10^9 cells/ml approximately) were poured onto minimal agar plates and incubated at 37^0 C for 48 h. After the incubation, the revertant colonies were counted using a colony counter. Each test was carried out in triplicate (n=3).

Against mutagens requiring activation

Antimutagenic assay against mutagen that requires metabolic activation (B [*a*] P) and 2-AF was carried out as follows. Activation mixture was prepared by mixing 50 µl of the S9 fraction, and various concentration of *G.montana* extracts (1, 2 and 3 mg/ml) mixed with the mutagens at a given concentration (B [*a*] P=0.025 mg/ml and 2-AF=0.025 mg/ml) and 0.1 ml freshly grown *S. typhimirium* (TA 98, TA 100 and TA 102) (10^9 cells/ml approximately) poured onto minimal agar plates and incubated for 48 h at 37^0 C. After incubation, number of revertants was counted using a colony counter. Each test was carried out in triplicate (n=3).

Toxicity of different extracts, if any, against bacterial strains (TA 98, TA 100 and TA 102) was determined by incubating various concentrations of different extracts with cultures of different tester strains of for 48 h and checking the number of revertants and background lawn.

Percent inhibition of mutagenicity was determined by the following formula:

$$\text{Inhibition (\%) of mutagenicity} = \frac{(R1 - SR) - (R2 - SR)}{(R1 - SR)} \times 100$$

Where, $R1$ = the number of revertants without different extract, $R2$ =the number of revertants with extracts of *G.montana* and SR = the spontaneous revertant.

2.10 Determination of *In Vivo* Antimutagenic Activity Of *G. Montana* Extracts

Wister rats were divided into six groups and the following treatments were given.

Group I (Normal): Oral administration of distilled water as vehicle without any treatment

Group II (Control): Animal received B [*a*] P (10 mg/rat) by i.p injection as a Single dose, served as control.

Group III and IV: Oral administration of *G.montana* 70 % methanolic (Extract treatment) extract of leaf (200, 400 mg/kg b wt) for 30 days.

Group V and VI: Oral administration of *G.montana* aqueous extract of (Extract treatment) leaf (200, 400 mg/kg b wt) for 30 days.

On 31st day B [*a*] P (10 mg/rat i.p) were administered as a single dose to Group III, IV, V and VI.

After 24 h of administration of B [*a*] P, urine was collected from all the animals for 24 h in metabolic cages. The urine, thus collected was filtered using Whatman No.1 filter paper, and 20 ml of urine was passed through XAD-4 amberlite column (40mm x 10mm) to concentrate the mutagen [26]. The weakly anionic components absorbed were eluted from XAD colunm with 10 ml acetone. The elutes were evaporated to dryness at 60^0 C and stored at -20^0 C and reconstituted in 1.5 ml DMSO just before the antimutagenicity assay [27]. *S. typhimurium* strains TA 98 and TA 100 was used for the assay. Fresh *Salmonella* culture (10^9 cells/ml) and 0.1 ml of urine concentration were mixed with 2 ml top agar containing 0.5 mM histidine and biotin on minimal glucose agar plate. The revertants were counted after incubation for 48 h at 37^0 C. The percent inhibition of revertants was calculated against the control using the formula given above.

Statistical analysis

The results were expressed as Mean ± SEM. The significance of difference between mean values for the various treatments was tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. Data showing P values less than 0.05 ($P<0.05$) was considered as statistically significant.

3. Results

Toxicity study of *G. montana* extracts against *Salmonella typhimurium* spontaneous reverting in the presence of (+S9) or absence (-S9) of liver microsomal fraction shown in Table 1.

Table 1: effect of *g. Montana* extracts on *salmonella typhimirium* spontaneous revertant in the presence or absence of (s9) liver microsomal fraction

Extract	S9	Average No of Revertants Per Plate		
		TA 98	TA 100	TA 102
Stem Petroleum Ether (3mg)	+	50.40 ± 03.00	114.40 ± 04.10	103.40 ± 07.31
Stem Petroleum Ether (3mg)	-	48.40 ± 10.50	101.30 ± 02.30	113.40 ± 04.10
Stem 70 % Methanolic (3mg)	+	54.40 ± 04.10	113.40 ± 04.10	110.41 ± 06.20
Stem 70 % Methanolic (3mg)	-	41.40 ± 07.50	100.40 ± 03.30	135.43 ± 05.60
Stem Aqueous (3mg)	+	43.25 ± 01.30	104.40 ± 04.10	107.40 ± 05.20
Stem Aqueous (3mg)	-	47.40 ± 07.50	098.30 ± 04.10	142.42 ± 07.02
Leaf Petroleum Ether (3mg)	+	44.64 ± 09.00	098.40 ± 04.10	114.40 ± 06.13
Leaf Petroleum Ether (3mg)	-	41.40 ± 02.50	096.40 ± 03.50	153.40 ± 08.12
Leaf 70 % Methanolic (3mg)	+	51.40 ± 10.40	096.40 ± 04.10	118.41 ± 07.07
Leaf 70 % Methanolic (3mg)	-	51.40 ± 02.50	110.40 ± 02.50	117.42 ± 06.05
Leaf Aqueous (3mg)	+	48.70 ± 04.00	093.40 ± 04.10	167.43 ± 05.50
Leaf Aqueous (3mg)	-	43.40 ± 10.50	101.60 ± 04.20	175.40 ± 07.04
SR	+	48.62 ± 05.10	094.40 ± 04.10	113.40 ± 03.70
SR	-	50.40 ± 02.50	096.40 ± 03.00	113.41 ± 0 5.01

Values represent mean ± sem, n=3,

3.1 *In Vitro* Antimutagenic Activity of *G. Montana* Stem Extracts

Against direct acting mutagens

There was no significant difference in antimutagenic activity between petroleum ether extract and 70 % methanolic stem extract and aqueous extract. The maximum antimutagenic activity was shown at 3 mg/ml concentration of different extracts and between different concentrations there was no significant difference in antimutagenic activity against Sodium Azide (Table 2).

Table 2: *In vitro* antimutagenic activity of *g. montana* stem and leaf extracts against sodium azide (nan3)

Concentration of extracts mg/ml	Petroleum ether Extract				70 % Methanolic extract				Aqueous extract			
	% Inhibition											
	Stem		Leaf		Stem		Leaf		Stem		Leaf	
	TA 100	TA 102	TA 100	TA 102	TA 100	TA 102	TA 100	TA 102	TA 100	TA 102	TA 100	TA 102
1 mg/ml +0.0025 NaN_3	31.32	41.68	36.62	37.66	32.66	38.83	40.70	54.15	35.66	30.88	44.47	44.47
2 mg/ml +0.0025 NaN_3	38.05	43.86	41.28	43.29	41.07	42.15	49.32*	56.30*	38.99	39.04	51.48*	48.66
3 mg/ml +0.0025 NaN_3	41.07	45.65	46.40	44.73	38.60	43.76	58.48*	58.51*	43.30	41.32	58.75*	53.72*

Antimutagenic activity of 70 % methanolic and aqueous extracts at a concentration of 3 mg/ml was 40.31 % (TA 98), 39.49 % (TA100) and 29.17 % (TA 98) 31.05 % (TA 100) respectively against 4-nitro-*o*-phenylene diamine (Table 3).

Table 3: *In vitro* antimutagenic activity of *g. Montana* stem and leaf extracts against 4-nitro-o-phenylene diamene (npd)

Concentration of extracts mg/ml	Petroleum ether Extract				70 % Methanolic extract				Aqueous extract			
	% Inhibition											
	Stem		Leaf		Stem		Leaf		Stem		Leaf	
	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100
1 mg/ml +0.020 NPD	26.47	34.08	28.16	20.28	31.96	22.52	33.31	25.54	13.75	21.86	39.22	37.64
2 mg/ml +0.020 NPD	32.16	36.87	30.05	32.34	37.07	29.11	43.18	32.37	15.71	26.55	47.00	46.31
3 mg/ml +0.020 NPD	37.45	42.05	31.90	39.14	40.31	39.49	53.89*	42.98	29.17	31.05	54.62	58.53*

Against mutagens requiring activation

70 % methanolic extract at a concentration of 3 mg/ml showed 45.3 % inhibitory activity against B [*a*] P in the case of TA 98, 14.38 % in the case of TA 100 against Benzo [*a*] pyrene (Table 4).

Table 4: *In vitro* antimutagenic activity of *g. Montana* stem and leaf extracts against benzo [a] pyrene (b [*a*] p)

3	Petroleum ether extract				70 % Methanolic extract				Aqueous extract			
	% Inhibition											
	Stem		Leaf		Stem		Leaf		Stem		Leaf	
	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100
1 mg/ml +0.025 B [*a*] P	20.52	09.90	32.02	06.26	18.88	06.03	51.43	23.43	13.73	11.64	43.72	43.66
2 mg/ml +0.025 B [*a*] P	30.27	20.11	40.01	11.91	25.68	08.93	59.42*	40.48	20.07	16.90	46.91	47.52
3 mg/ml +0.025 B [*a*] P	36.29	25.17	45.12	16.32	45.30	14.38	64.04*	43.23	20.18	19.95	61.14*	61.25*

The 70 % methanolic and aqueous extract at a concentration of 3 mg/ml showed 37.52 % (TA 98), 44.56 % (TA 100) and 33.75 % (TA 98), 35.52 % (TA 100) respectively against 2 Aminoflourence (Table 5**).**

Table 5: *in vitro* antimutagenic activity of *g. Montana* stem and leaf extracts against 2-aminofloerene (2-af)

Concentration of extracts mg/ml	Petroleum ether extract				70 % Methanolic extract				Aqueous extract			
	% Inhibition											
	Stem		Leaf		Stem		Leaf		Stem		Leaf	
	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100
1 mg/ml +0.025 2-AF	28 .61	37.85	41.02	36.72	28.41	37.89	47.65	50.16	25 .96	33.3 3	36.77	42.33
2 mg/ml +0.025 2-AF	29.57	44.51	46.32	40.79	30.45	40.27	52.04	55.89	30.14	3 4.81	44.93	50.25
3 mg/ml +0.025 2-AF	30.65	47.84	50.27	45.46	37.52	44.56	53.26*	58.19*	33.75	35.52	48.94	55.70

3.2 In Vitro Antimutagenic Activity of *G. Montana Leaf* Extracts

Against direct acting mutagens

The 70 % methanolic extract at a concentration of 3 mg/ml inhibited NaN_3 induced mutagenicity by 46.40 % (TA 100) and 44.73 % (TA 102). 70 % methanolic and aqueous extracts showed significant ($p<0.05$) activity against Sodium azide (Table 2). 70 % methanolic and aqueous extract of leaf showed significant ($p<0.05$) antimutagenic activity against NPD. 53.89 % inhibition of mutagenicity was seen in 70 % methanolic leaf extract (TA 98) and 58.53 % in aqueous extract (TA 100), at a concentration of 3 mg/ml against 4-nitro-*o*-phenylene diamine (Table 3).

Against mutagens requiring activation

Significant antimutagenic activity ($p<0.05$) was found in 70 % methanolic leaf extract and aqueous leaf extract at a concentration of 3 mg/ml against B [*a*] P. 64.04 % (TA 98) inhibition of B [*a*] P induced mutation was found in 70 % methanolic leaf extract and 61.25 % (TA 100) was seen in aqueous leaf extract of *G. montana* at a concentration of 3 mg/ml (Table 4). 70 % methanolic leaf extract showed significant ($p<0.05$) antimutagenic activity against 2-Aminofluorence. 53.26 % (TA 98) and 58.19 % (TA 100) inhibition of mutagenic activity of 2- Aminoflourence was shown by 70 % methanolic leaf extract at a concentration of 3 mg/ml. There was no such significant difference between different concentrations (Table 5).

In Vivo Antimutagenic Activity of *G. Montana* Leaf Extracts Against B [*A*] P

49.86 % (TA 98) and 45.39 % (TA 100) *in vivo* antimutagenic activity was seen in 200 mg/ml concentration of 70 % methanolic extract of leaf. 53.10 % (TA 98) and 51.01 % (TA 100) significant ($p<0.05$) inhibiting activity was seen in 400 mg/ml concentration of 70 % methanolic extractof leaf. Between different extracts of leaf there was no significant difference in antimutagenic activity. Aqueous extract of leaf with 55.00 % (TA 98) and 57.94 % (TA 100) showed significant ($p<0.05$) antimutagenic activity at 400 mg/ml concentration (Figure: 1).

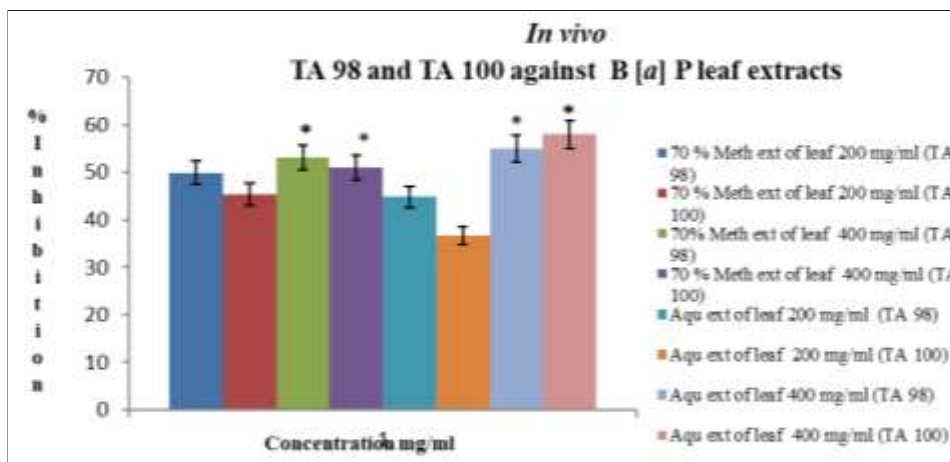

Fig 1: In *Vivo* Antimutagenic Activity of 70 % Methanolic and Aqueous Extracts of Leaf of *G.Montana*

4. Discussion

Extracts of *G.montana* showed significant inhibition of mutagenicity induced by both direct acting mutagens (NaN_3 and NPD) and mutagens require metabolic activation ((B [*a*] P) and 2-AF) in a dose dependent manner. The extracts did not show any toxicity to the tester strains at the doses tested. The conclusion was based on the number of revertants of *Salmonella typhimurium*. Hence, the activity is not the consequence of the toxic effect of extract on bacterial colony. The antimutagenic activity of the extract against direct acting (NaN_3, NPD) mutagens probably may be due to the inactivation of the mutagens. Natural substances such as alkaloids, flavonoids, tannins or their derivatives present in plant extracts [28, 29] possible antimutagenic property and their metabolites could be involved in mutagen deactivation [30].

Antimutagenic activity of the 70 % methanolic leaf extract may be due to a variety of mechanisms such as inhibition of genotoxic effects, scavenging of free radicals, induction of detoxification and inhibition of topoisomarase I or II activity. Hence, the possible mechanism of antimutagenic behavior of the extract would be due to the presence of bioactive constituents (like, flavonoids, flavonenone, flavones) which can either scavenging the free radicals or detoxify the mutagen or induce detoxifying enzymes.

Significant correlations have been observed between carcinogenicity of a series of polycyclic aromatic hydrocarbons (PAH) and their covalent binding to mouse epidermal DNA [31]. Based on extensive evidence accumulated in the last two decades, it is believed that PAH must be metabolically activated to electrophillic intermediates, which can bind to DNA and exert its carcinogenic effects [32]. B [*a*] P is metabolized by mixed function oxidase (MFO) of rat liver to active intermediate benzo [*a*] pyrene 7, 8 diol, 9, 10 epoxide (BPDB) [33]. These can attack cellular macromolecules like DNA, RNA, proteins, membranes etc and cause dysfunction and damage. Reactive oxygen species increase the lipid peroxidation, which in turn alter the integrity of membrane bound enzymes. The free radical scavenging efficiency of the extract thus might be playing, an important role in the antimutagenic activity. The activity of the *G.montana* extracts might be mediated through the inhibition of the MFO or through the inactivation of activated intermediates. The experimental results indicate that the extracts of *G.montana* were effective in preventing *in vitro* and *in vivo* mutagenic activity induced by benzo [*a*] pyrene. This indicates the possible protective effect of the extract against the attack of cellular macro molecules by PAH such as benzo [*a*] pyrene.

The present investigations showed that 70 % methanolic extracts leaf of *G.montana* are significantly effective in preventing mutagenic activity induced by both direct and indirect acting mutagen, indicating their potential in chemoprevention.

5. References

1. Mitscher LA, Drak S, Gollapuri SR, Harris JA, Shankel DM. Antimutagenesis and Anticarcinogenesis mechanisms edited by Shankel DM, Hartmen PE, Kada T, Hollaender A. (Plenum Press, New York), 1986, 153.
2. Ames BN. Dietary carcinogenesis and anticarcinogenesis. *Science*. 1983; 22:1256- 1263.
3. Kada T, Kaneko K, Matsuzaki S, Mastsuzaki T, Hara Y. Detection and chemical identification of natural bio antimutagens. Mutation Research, 1985, 150:127.
4. Stavric B. Antimutagens and anticarcinogens in food. Food and Chem Toxicology, 1994, 32:79.
5. Steinmetz KA & Potter JD, Vegetables, fruit and cancer, I. Epidemiology. Cancer Cause Control, 5, 1991, 325.
6. Shon MY, Choi SD, Kahng GG, Nam SH, Sung NJ. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate from white, yellow and red onions. Food Chem Toxicol. 2004; 42(4):59.
7. Feroson LR, Antimutagen as cancer chemoprotective agents in the diet. Mutation Research, 1994, 307:395.
8. Flora SD, Mechanism of inhibitors of mutagenesis and carcinogenesis. Mutation Research, 1998, 402:151.
9. Negi PS, Jayprakash GK & Jena BS, Antimutagenic and antioxidant activities of pomegranate peel extracts. Food Chem, 2003, 80:393.
10. Bala S, Grove IS. Antimutagenicity of some citrus fruits in Salmonella typhimurium. Mut Res, 1989, 222:141.
11. Kaur K, Arora S, Kumar S, Kumar S. Bioassay-guided isolation of antimutagenic factors from fruits of Terminallia bellerica. J. Environ Pathol Toxicol Oncol. 2003; 22(1):69.
12. Kaur K, Arora S, Kumar S & Nagpal A, Antimutagenic activities of acetone and methanol fractions of Terminallia arjuna. Food Chem Toxicol. 2002; 40(10):1475.
13. Kaur SJ, Grover IS, Kumar S, Modulatory effects of a tannin fraction isolated from Terminalia arjuna on the genotoxicity of mutagens in Salmonella typhimurium. Food Chem Toxicol, 2000, 38:1113.
14. Sangwan S, Shanker S, Sangwan RS, Kumar S. Plant derived products as antimutagens. Phytother Res, 1998, 12:389.
15. Vaidya BG. Vikankat, In: Nighantu Adarsh Part-I Chaukhambha Bharti Acadamy, Varanasi, India, 1965, 298-299.
16. Thakar J, Celastraceae: In: Vanaspati shastra 2nd Ed. Pravin Prakashan Pvt. Litd, Rajkot, Gujarat, 1998, 134-136.
17. Pade SD, Vikalo. In: Shree Aryabhishek (India's Ayurvedic Treatise), 3rd Ed. Sastu Sahitya Press, Ahmedabad, 1966, 636.
18. Ashok Sheth, Vikalo. In: Vasundhrani Vanaspatio (Ayurveday amrutanam), 2nd Ed. Navbharat sahitya mandir, Ahmedabad, 2007, 159-160.
19. Kirtikar KR, Basu BD. Celastraceae, In: Indian Medicinal Plants 2nd Ed. Vol.1. International book Distributors, Deharadun, India, 1933, 577.
20. The Wealth of India (Raw materials). Vol. V: Council for Scientific and Industrial Research. New Delhi. India, 1956, 277.
21. De Subrata, Bhavsar GC. Pharmacognostical study of Gymnosporia Montana. International journal of pharmaceutical science, 1993; 55:21-25.
22. Ambsta SP, Kamala R, Kashysps K, Ramesh Chand. Celastraceae. In: The useful plants of India. Publication and Information directorate, CSIR. 3rd Ed. New Delhi, 1994, 252.

23. Pullaih T. Celastraceae. In: Encyclopedia of world medicinal plants. Regency publications, New Delhi, 2006, 1:1316.
24. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutation Research. 1983; 113:173-215.
25. Lakshmi B, Ajith TA, Nayana Jose, Janardhanan KK. Antimutagenic activity of methanolic extract of Ganoderma lucidum and its effect on hepatic damage caused by benzo [a] pyrene. Journal of Ethnopharmacology. 2006; 107:297-303.
26. Yamasaki E, Ames BN. Concentration of mutagens from urine by absorption with the non-polar resin XAD-2; Cigarette smokers have mutagenic urine. preceding of Natural Academic Science (USA). 1997; 74:3555-3559.
27. Polosa K, Sesikeran B, Krishna TA, Krishnaswamy K, Turmeric (Curcuma longa) induced reduction in urinary matagens, Food and Chemical Toxicology, 1991; 29:25-30.
28. Dhru B, Zaveri M, Lakshmi B. Phytopharmacognostical study of stem of Gymnosporia montana: A Native of Gujarat. International Journal of Pharmaceutical Sciences Review and Research. 2010; 5(3):1-4.
29. Dhru B, Zaveri M, Lakshmi B. Pharmacognostical and phytochemical study of leaf of Gymnosporia montana (Vikalo). Journal of Global Pharma Technology. 2011; 3(1):23-27.
30. Hoel DG, Kaplan NL, Anderson MW. Implication of nonlinear kinetics on risk estimation in carcinogenesis. Science. 1993; 213:1032-1037.
31. Brookes P, Lawley PD. Evidence for the binding of polynuclear aromatic hydrocarbons to nucleic acids of mouse skin: relation between carcinogens power of hydrocarbons and their binding to DN. Nature (Lond). 1964; 202:781-784.
32. Pelkonen O, Nebert DW. Metabolism of polycyclic aromatic hydrocarbons: etiological role in carcinogenesis. Pharmacological Review. 1982; 34:189-222.
33. Smith WA, Gupta RC. Use of a microsome mediated test system to asses efficacy and mechanism of cancer chemopreventive agents. Carcinogenesis. 1996; 17(6):1285-1290.