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GC-MS Analysis and Evaluation of Mutagenic and Antimutagenic Activity of Ethyl Acetate Extract of *Ajuga bracteosa* Wall ex. Benth: An Endemic Medicinal Plant of Kashmir Himalaya, India

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Abstract

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The natural products remain an important source of new drugs, new drug leads and new chemical entities. The ethyl acetate extract of *Ajuga bracteosa* was evaluated for mutagenic and antimutagenic assay against mice pre-treated with 1/4th LD50 (117.5 mg/kg bw) of ethyl methane sulphonate by micronucleus and chromosomal aberration assay. Mice were treated with ethyl acetate extract of *Ajuga bracteosa* (Ab-EAE) (100, 200 300 & 400 mg/kg bw) for 30 days. Without the doses of EMS, no mutagenic effects were observed in blood and bone marrow samples of the mice. But Ab-EAE showed antimutagenic effects on EMS induced mutagenicity in mice. It was observed that high doses of Ab-EAE showed protective effects. The reduction profiles in the EMS induction MN at concentration of ethyl acetate extract of *Ajuga bracteosa* (100, 200, 300 and 400 mg/kg bw) were estimated as 2%, 4.9%, 16.4% and 20.7% respectively. It can be concluded from the study that ethyl acetate extract of *Ajuga bracteosa* exhibited no mutagenic effects but only possessing antimutagenic effects. This antimutagenic activity is an induction of medicinal relevance.

Keywords: Ajuga; Antimutagenicity; EMS; Micronucleus; GC-MS

Introduction

Traditional herbal medicine practitioners have described the therapeutic effectiveness of many indigenous plants [1]. The plants are the source of synthetic and traditional herbal medicine and hence are useful for healing and curing of human diseases because of the presence of phytochemical constituents [2-4]. These phytochemicals are naturally present in all parts of medicinal plants viz., leaves, vegetables and roots. The Phytochemicals are synthesized by a plant itself as primary and secondary metabolites. Chlorophyll, proteins and common sugars are included in primary constituents while as terpenoids, alkaloids and phenolic compounds come under secondary compounds [5]. Terpenoids and phenols exhibit various important pharmacological activities viz., anti-inflammatory, anticancer, anti-malarial, anti-viral, anti-bacterial activities and inhibition of cholesterol synthesis [6]. Alkaloids are known to possess anesthetic properties [7,8].

The pharmacological and therapeutic properties of traditionally used medicinal plants are attributed to various chemical constituents isolated from their crude extracts [9-11]. It is very common among the people who live in upper reaches of Kashmir Himalaya to use herbs for curing of various diseases [12]. Although the diversity of plant species in Kashmiri Himalayas is a potential source of biologically active compounds, the effects on human health and genetic material are often unknown. Interest in such popular usage has recently gained strength, through recent knowledge that chemicals, such as proteases and antioxidants may prevent or reduce the development of cancer by blocking genetic damage [13-15].

Ajuga bracteosa Wall ex. Benth of family Lamiaceae is commonly known as 'Bungle' in English and 'Jan-i-adam' in Kashmiri. It is a perennial erect, ascending hairy herb, often prostrates with oblanceolate or sub-spathulate leaves and grows up to 5-50 cm tall. It is found along roadsides, open slopes, and rock services [16,17]. Its distribution extends from temperate regions of Western Himalayas viz., Kashmir, Pakistan, Afghanistan and China to Bhutan in Eastern Himalayas; Indian subtropical regions [18] viz., plains of Punjab and upper Gangetic plains at an altitude of 1300 m [19] and in tropical regions of Malaysia. In Pakistan, it is found in northern hilly areas, where in local Hindi/Punjabi language it is called kori booti (means bitter herb) owing to its bitter taste. The plant is effectively used for the treatment of gout, rheumatism, palsy, jaundice, hypertension, sore throat and as a blood purifier. Locally, the leaves are used to cure headache, pimples, measles, stomach acidity, burns and boils.

Materials and Methods

Collection and air drying of plant material

Aerial parts of *Ajuga bracteosa* were collected from Sinthan Top area of District Anantnag (Kashmir) in the month July, 2013. The plant was identified at the Centre of Biodiversity and Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar, J & K and a voucher specimen (JKASH/CBT/226 Dated 08. 08. 2014) was deposited there. The parts were allowed to dry under shade (30°C) for 8-10 days.

Preparation of extracts

After shade drying, the aerial parts were macerated to fine powder, 1 kg of leaves were extracted successively with hexane for defatenning and methanol for 16 h using Soxhlet apparatus. The extracts were filtered through a Buchner funnel using Whatman No.1 filter paper, and all the extracts were concentrated to dryness under vacuum using a Heidolph rotary evaporator, yielding hexane, ethyl acetate, methanol and aqueous crude extracts of 65, 52, 46 and 36 g respectively. All the extracts were stored at 4°C in air tight glass bottles before use.

Phytochemical screening

Chemical tests were carried out on the extracts using standard procedures to identify various constituents like Tannin, saponin, flavonoids, steroids, terpenoids, glucosides, alkaloids, carbohydrates, phytosterols, phenol, proteins and amino acids [20-23].

GC-MS analysis

GC-MS analysis was carried out with GCMS-QP2010 Plus, Shimadzu, Japan fitted with programmable head space auto sampler and auto injector. The capillary column used was DB-1/RTX-MS (30 metre) with helium as a carrier gas, at a flow rate of 3 mL/min with 1 μL injection volume. Samples were analysed with the column held initially at 100°C for 2 min after injection, then increased to 170°C with 10°C/min heating ramp without hold and increased to 215°C with 5°C/min heating ramp for 8 min. Then the final temperature was increased to 240°C with 10°C/min heating ramp for 15 min. The injections were performed in split mode (30:1) at 250°C. Detector and injector temperatures were 260°C and 250°C, respectively. Pressure was established as 76.2 kPa and the sample was run for 70 min. Temperature and nominal initial flow for flameionization detector (FID) were set as 230°C and 3.1 mL/min, correspondingly. MS parameters were as follows: scan range (m/z): 40-650 atomic mass units (AMU) under the electron impact (EI) ionization (70 eV). The constituent compounds were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC and as well as the mass spectra from the Wiley libraries and National Institute of Standards and Technology (NIST) database.

Animals and treatments

Both sex of albino mice, Balb/c strain useful for research in cancer and immunology, weighing 25-35 g were obtained from the Indian Institute of Integrative Medicine (IIM), Canal Road Jammu, kept in plastic cages in an experimental room under controlled conditions of temperature (22 ± 2°C), humidity (55 ± 10%), 12 h light/dark cycles and access to food and water. They were randomized at the beginning of the experiment. The study design was approved by the Institutional Animal Ethical Committee, and the experiments undertaken in accordance with the ethical principles of the CPCSEA norms. The mice were divided into 8 groups, with 5 animals per group (Table 1). Ethyl methane sulfonate (EMS, Sigma Aldrich) was used to induce mutations and chromosomal aberrations for antimutagenic evaluation of ethyl acetate extract of *Ajuga bracteosa*.

Group	Dose	Purpose of group	Duration
Group 1	Distilled water	Negative control	15 days
Group 2	1/4th LD50 EMS	Positive control EMS	24 h
Group 3	Ab-EAE 100 mg/kg bw	Positive control *Ajuga bracteosa*	24 h
Group 4	Ab-EAE 400 mg/kg bw	Positive control *Ajuga bracteosa*	24 h
Group 5	Ab-EAE 100 mg/kg bw + EMS	Treated Group	30 days
Group 6	Ab-EAE 200 mg/kg bw + EMS	Treated Group	30 days
Group 7	Ab-EAE 300 mg/kg bw + EMS	Treated Group	30 days
Group 8	Ab-EAE 400 mg/kg bw + EMS	Treated Group	30 days

Table 1: Grouping, dose (distilled water, EMS and Ab-ME in concentrations of 100, 200, 300 and 400 mg/kg bw) and duration of experiment. Ab-EAE=Ethyl acetate extract of *Ajuga bracteosa*.

The micronucleus test

The method of MacGregor et al. was used for micronucleus test. Mice were injected intraperitoneal with 0. 5 ml of 0.06% colchicine and two hours later, mice were sacrificed by cervical dislocation. Slides were prepared with blood collected from the jugular vein. The slides were air-dried, fixed in absolute methanol, stained in 10% Giemsa and then coded for blind analysis. One thousand polychromatic erythrocytes (PCE) were analysed per mouse. The proportion of PCE and normochromatic erythrocytes (NCE) in 200 erythrocytes/animal was calculated, to detect possible cytotoxic effects. The slides were scored blindly, using a light microscope with a 65x objectives.

Chromosomal aberration

Both the femurs were fleshed out from the muscles and kept in HBSS (Hank's balanced salt solution). The femurs were then rinsed with 3 ml 0.056% KCl solution in a centrifuge tube. The tube was then incubated at 37°C for 20 minutes. After incubation, centrifugation at 800 rpm for 4 minutes was carried out. Supernatant was discarded and fresh Carnoy's fixative was added (3:1methanol: acetic acid). The process of centrifugation was repeated three times. Then slides were prepared, stained with 4% Giemsa, air dried and studied under compound microscope.

Results

Phytochemical screening

Therapeutic values of medicinal and aromatic plants (MAPs) are due to the presence of major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, saponins, steroids etc. The phytochemical investigation of *Ajuga bracteosa* extracts in the present study revealed presence of different active ingredients (secondary plant metabolites) like flavonoids, phenolics, alkaloids, tannins, cardiac glycosides, terpenes, saponins, steroids, carbohydrates, amino acids and proteins as shown in Table 2. It supports the resourcefulness of the plant extract.

Phytoconstituents	Test	Result
Alkaloids	Wagner's test	+ +
Phenolics	phenol test	+ +
Tannins	Ferric chloride test	+ +
Cardiac glycosides	Keller-Killani test	+ +
Terpenes	Salkwaski's test	+
Flavonoids	Shinoda's test	+ +
Saponins	Frothing test	+
Steroids	Libermann-Buchard's test	+
Carbohydrates	Molish test	+ +
Proteins	Biuret test	+
Polysterols	Salkowski's Test	+
Amino acids	Ninhydrin Test	+

Table 2: Qualitative phytochemical screening of *Ajuga bracteosa*. (++) = strong presence, (+) = moderate presence

GC-MS analysis

In order to find out the phytocomponents responsible for antimutagenic activity, ethyl acetate fraction of *Ajuga bracteosa* was subjected to GC-MS analysis. The active principals present in the ethyl acetate fraction of *Ajuga bracteosa* along with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) are presented in Table 3. The chromatogram of Ab-ME showed six major peaks (Figure 1): 1, 2, 3-Propanetriol (18.15%), 1, 2, 3-Propanetriol, 1-acetate (11.35%), Stigmast-5-en-3-ol (11.35%), 2, 6, 10-Trimethyl, 14-ethylene-14-pentadecane (8.51%), 2-Hexadecene-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R (5.76%) and l-(+)-Ascorbic acid 2, 6-dihexadecanoate (4.75%) comprising 59.87% of total peak area.

S. No.	Compound	Retention time	% Area	Molecular formula	Molecular weight
1	1, 2, 3-propanetriol	5.7	18.15	$C_3H_8O_3$	92
2	1,2,3-Propanetriol, 1-acetate	7.47	11.35	$C_5H_{10}O_4$	134
3	1,2,3-Propanetriol, 1-acetate	8.5	0.77	$C_5H_{10}O_4$	134
4	1,2,3-Propanetriol, 1-acetate	11.12	0.94	$C_5H_{10}O_4$	144
5	2,3-Dihydroxypropyl acetate	11.23	0.45	$C_5H_{10}O_4$	134
6	1,2,3-Propanetriol, 1-acetate	14.42	0.59	$C_5H_{10}O_4$	134
7	2(4H)-Benzofuranone, 5,6,7,7A-tetrahydro-6-H	24.04	0.83	$C_{11}H_{16}O_3$	196
8	(2E)-3, 7, 11, 15- Tetramethyl-2-Hexadecane	25. 08	0.24	$C_{20}H_{40}$	280
9	2, 6, 10- Trimethyl, 14- Ethylene-14-Pentadecne	25.19	8.51	$C_{20}H_{38}$	278
10	Acetic acid, 3, 7, 11, 15- Tetramethyl-hexadecyl ester	25.32	0.8	$C_{22}H_{44}O_2$	340
11	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R	25.7	2.82	$C_{20}H_{40}O$	296

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12	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R	26.07	4.37	$C_{20}H_{40}O$	296
13	Hexadecanoic acid, methyl ester	26.89	0.42	$C_{17}H_{34}O_2$	270
14	1-Hexadecne-3-ol, 3, 5, 11, 15-Tetramethyl	27.29	0.24	$C_{20}H_{40}O$	296
15	I- (+)- Ascorbic acid 2, 6-dihexadecanoate	27.69	4.75	$C_{38}H_{68}O_8$	652
16	Hexadecanoic acid, ethyl ester	28.01	0.78	$C_{18}H_{36}O_2$	284
17	9, 12-Octadecenoic acid (Z, Z)-, methyl ester	29.44	0.23	$C_{19}H_{34}O_2$	294
18	9, 12, 15-Octadecenoic acid, methyl ester, (Z, Z, Z)-,	29.53	0.78	$C_{19}H_{32}O_2$	292
19	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R	29.7	5.76	$C_{20}H_{40}O$	296
20	9,12, 15-Octadecatrienoic acid, (Z,Z,Z)-	30.19	1.55	$C_{18}H_{30}O_2$	278
21	Ethyl (9Z, 12Z)-9, 12-Octadecadienoate	30.35	1.05	$C_{20}H_{36}O_2$	308
22	Octadecanamide	30.6	0.15	$C_{18}H_{37}NO$	283
23	Phytol, acetate	30.9	0.88	$C_{22}H_{42}O_2$	338
24	1-Chloroheptacosane	31.72	0.48	$C_{27}H_{55}Cl$	414
25	9-Hexadecanoic acid, 9-Octadecenyl Ester, (Z)	31.88	0.19	$C_{34}H_{64}O_2$	504
26	13-Octadecenal, (Z)	31.97	0.73	$C_{18}H_{34}O$	266
27	Cyclohexane, Decyl-	32.19	0.43	$C_{16}H_{32}$	224
28	1-Heptacosanol	32.43	0.27	$C_{27}H_{56}O$	396
29	1-Henicosanol	32.64	0.36	$C_{21}H_{44}O$	312
30	1, 3, 5- Trisilacyclohexane	33.42	0.26	$C_3H_{12}Si_3$	132
31	Octadecanal	33.87	0.5	$C_{18}H_{36}O$	268
32	1, 2-Benzenedicarboxylic acid	34.06	0.62	$C_{24}H_{38}O_4$	390
33	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	34.6	0.9	$C_{27}H_{50}O_6$	470
34	1,3-Dioxolane, 4-[(2-Methoxy-4-Hexadecenyl)	34.95	0.14	$C_{23}H_{44}O_4$	384
35	Heneicosane	35.31	0.16	$C_{21}H_{44}$	296
36	14-Beta-H-Pregna	35.74	0.19	$C_{21}H_{36}$	288
37	1-(3,4-dimethoxybenzyl)-6,7-Dimethoxy-2-Meth	35.95	0.44	$C_{21}H_{27}NO_4$	357
38	E, Z-1, 3, 12-Nonadecatriene	36.39	0.62	$C_{19}H_{34}$	262
39	Methyl (Z)-5, 11, 14, 17-Eicosatetraenoate	36.51	0.62	$C_{21}H_{34}O_2$	318
40	Tetracontane	37.61	2.12	$C_{40}H_{82}$	562
41	2-Pentatriacontanone	39.66	1.3	$C_{35}H_{70}O$	506
42	Protopine	40.29	0.59	$C_{20}H_{19}NO_5$	353
43	1, 54- Dibromo tetrapentacontane	41.1	1.35	$C_{54}H_{108}Br_2$	914
44	Stigmast-5-en-3-ol, (3.beta.)-	41.48	0.57	$C_{29}H_{50}O$	414
45	Vitamin E	42.05	0.61	$C_{29}H_{50}O_2$	430
46	Octacosyl acetate	43.54	0.48	$C_{30}H_{60}O_2$	452
47	Ergosta-5, 24-dien-3-ol, (3 beta)	44.39	0.22	$C_{28}H_{46}O$	398

48	Ergost-5-en-3-ol, (3 beta)	44.59	1.32	$C_{28}H_{46}O$	400
49	Octadecanoic acid, octadecyl ester	45.88	0.31	$C_{36}H_{72}O_2$	536
50	Stigmast-5-en-3-ol, (3.beta.)-	47.18	11.35	$C_{29}H_{50}O$	414
51	Cholest-5-en-3-ol, 24-propylidene-, (3 beta)	47.69	1.27	$C_{30}H_{50}O$	426
52	9, 19-Cyclocholeatan-3-ol, 14-methyl- (3 beta)	48.34	0.91	$C_{28}H_{48}O$	400
53	9, 19-Cyclolanost-24-en-3-ol (3 beta)	49.6	0.52	$C_{30}H_{50}O$	426
54	Stigmast-5-en-3-ol, oleate	51.71	3.01	$C_{47}H_{82}O_2$	678
55	2, 6, 10- Trimethyl, 14-Ethylene-14-Pentadecne	56.47	0.74	$C_{20}H_{38}$	278

Table 3: Phytocomponents identified in the ethyl acetate fraction of *Ajuga bracteosa* (Ab-EAE) by GC-MS.

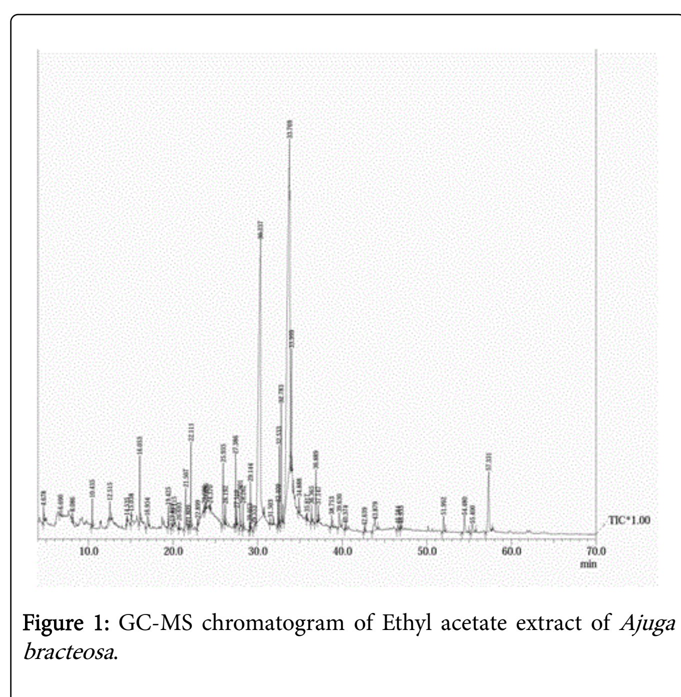

Figure 1: GC-MS chromatogram of Ethyl acetate extract of *Ajuga bracteosa*.

Micronucleus test

According to MN testing of mouse blood cells the low frequencies of micronucleated cells presumes the meagre effects of ethyl acetate extract of *Ajuga bracteosa* (Ab-EAE) 100 and 400 mg/kg (Table 4), there by indicating the virtual absence of mutagenic effect. In other words, nonstatistically significant difference in the frequency of MN polychromatic erythrocytes (PCE) or the ratio of PCE to normochromatic erythrocytes (NCE), between the negative control and thegroups that ingested extracts could be detected.

	Treatment	Total No. of cells analysed per mice	No. of cells with micronuclei	% age of MN	% Reduction	P value
Group 1	Negative Control (Distilled water)	1000	2.35 ± 0.12			
Group 2	Positive control (EMS)	1000	7.23 ± 0.89			0.05*
Group 3	Ab- EAE 100 mg/kg bw	1000	2.32 ± 0.08			
Group 4	Ab- EAE 400 mg/kg bw	1020	2.30 ± 0.05			
Group 5	Ab- EAE 100 mg/kg + EMS	1000	7.09 ± 0.76	98	2	
Group 6	Ab-EAE 200 mg/kg + EMS	1000	6.88 ± 0.54	95.1	4.9	0.05*
Group 7	Ab-EAE 300 mg/kg + EMS	1000	6.05 ± 0.45	83.6	16.4	0.05*
Group 8	Ab-EAE 400 mg/kg + EMS	1000	5.74 ± 0.35	79.3	20.7	0.05*

Table 4: Effects of Ethyl Acetate Extract of *Ajuga bracteosa* on MNPCE frequencies (mean ± SD) in mice, induced with ethyl methane sulfonate (EMS) 117.5 mg/kg bw (1/4th LD50). Ab-EAE = Ethyl Acetate extract of *Ajuga bracteosa*.

When evaluating antimutagenicity in Ab-EAE, a significant decrease in the frequency of EMS-induced MN PCE was observed only in mice that had received 100, 200, 300 and 400 mg/kg of Ab-EAE (p=0.05). In the present study, the ethyl acetate extract of *A. bracteosa* showed antimutagenic activities by reducing the % age of micronuclei with increase in the dose of the extract (Table 4 and Figure 2). The number of cells with micronuclei also decreased with increase in the dose of the extract i.e., from 100 mg/kgbw to 400 mg/kgbw (Figure 3).

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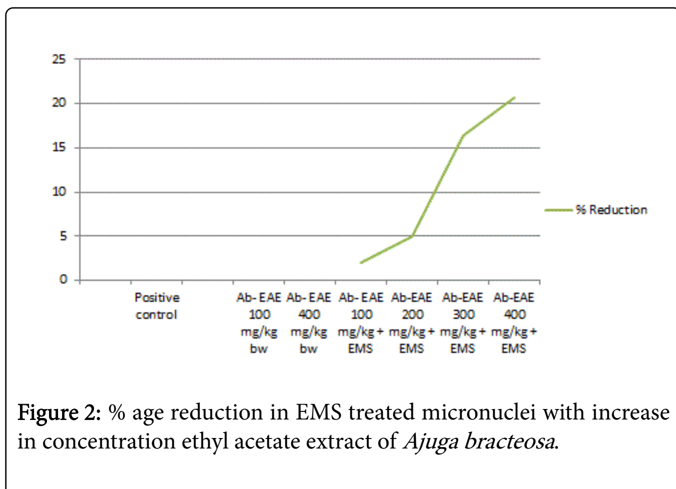

Figure 2: % age reduction in EMS treated micronuclei with increase in concentration ethyl acetate extract of *Ajuga bracteosa*.

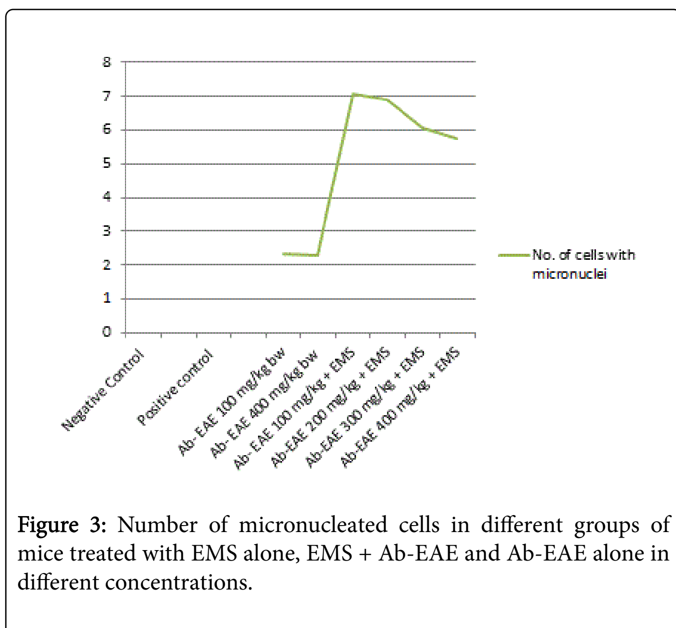

Figure 3: Number of micronucleated cells in different groups of mice treated with EMS alone, EMS + Ab-EAE and Ab-EAE alone in different concentrations.

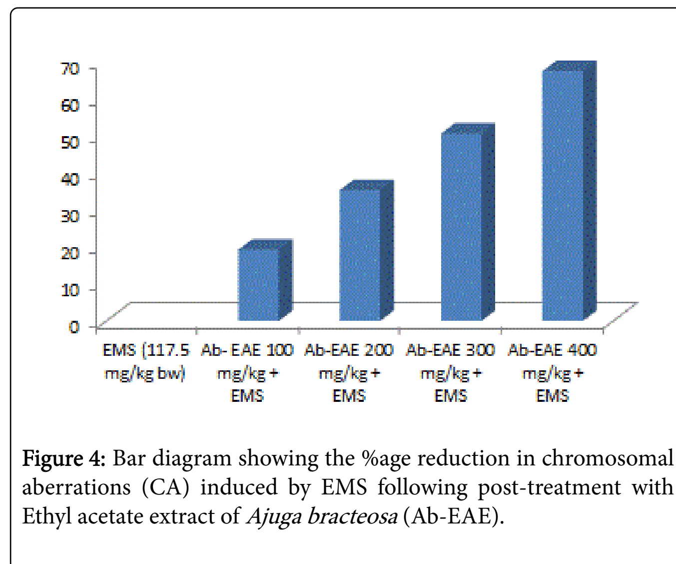

Figure 4: Bar diagram showing the %age reduction in chromosomal aberrations (CA) induced by EMS following post-treatment with Ethyl acetate extract of *Ajuga bracteosa* (Ab-EAE).

Chromosomal aberration

Chromosomal aberration frequencies observed after various treatment schedules with EMS and different doses of Ab-EAE is shown in Table 5. The chromosomal aberrations decreased from 25.09% in EMS induced cells to 20.59%, 16.50%, 12.63% and 8.27% at 100, 200, 300 and 400 mg/kg bw of the ethyl acetate extract of *Ajuga bracteosa* (Figure 4). EMS produced predominantly breaks, gaps, fragments and exchanges.

Discussion

From ancient times, medicinal plants are being used as remedies for various diseases in humans. In today's industrialized society, the use of medicinal plants has been traced to the extraction and development of several drugs as they were used traditionally in folk medicine [24]. Medicinal plants have potent phytoconstituents which are important source of antibiotic compounds and are responsible for the therapeutic properties [25-32]. These phytoconstituents bestow them with medicinal properties [33,34]. The antioxidant properties, in many plants, are attributed to the presence of phenolic compounds. These phenolic compounds are known to possess various biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [35]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [36]. They are also effective antioxidants and show strong anticancer activities [37]. Tannins bind to proline rich protein and interfere with protein synthesis. Besides, most of the phytochemicals are known to have therapeutic properties such as insecticidals [38], antibacterial, antifungal [39] and anticonstipative [40] activities etc.

Treatments		**Chromosomal Aberrations**							
Concentration (mg/kgbw)	**No. of cells**	**Rings**	**Fragments**	**Exchange**	**Breaks**	**Dicentrics**	**Gaps**	**Total Aberrations**	**%age of Aberrations**
Distilled water	1004	2	6	-	15	-	-	23	2.29
EMS 117.5 mg/kg bw	1020	8	35	28	123	-	62	256	25.09
Ab-EAE Alone 100 mg/kg bw	1000	3	4	-	14	-	-	21	2.1

Ab-EAE Alone 400 mg/kg bw	1000	2	6	-	12	-	-	20	2
Ab-EAE 100 mg/kg bw + EMS	1005	8	32	24	102	3	38	207	20.59
Ab-EAE 200 mg/kg bw + EMS	1000	6	28	20	86	-	26	166	16.5
Ab-EAE 300 mg/kg bw+ EMS	1005	4	24	18	62	1	18	127	12.63
Ab-EAE 400 mg/kg bw + EMS	1015	2	18	14	38	-	12	84	8.27

Table 5: Frequency of Chromosomal aberrations observed after post-treatment with Ethyl Acetate extract of *Ajuga bracteosa* in EMS treated mouse bone marrow cells. Ab-EAE=Ethyl Acetate extract of *Ajuga bracteosa*.

The phytochemical screening of *Ajuga bracteosa* showed that their aerial parts were rich in saponins, alkaloids, phenol, tannins and Steroids. The presence of these phytochemicals in the tested plant indicates that this plant may be a good source for production of new drugs for various ailments. Saponins are known to produce inhibitory effect on inflammation [41]. They also have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity and cholesterol binding properties [42]. Steroids have been reported to possess antibacterial properties [43] and they are very important compounds especially due to their relationship with compounds such as sex hormones [44,45]. It has been reported that alkaloids possess analgesic [46], antispasmodic and antibacterial [47,48] properties. According to many reports, glycosides are known to show blood pressure lowering property [49]. Thus from the present study, it could be suggested that the identified phytoconstituents from *Ajuga bracteosa* make the plant valuable for bioactive compounds of sustainable medicine.

Important sources of new bioactive agents are the natural products. These natural products are obtained from medicinal herbs which are not only being used world-wide for the treatment of various diseases but also have a great potential for providing novel drug leads with novel mechanism of action [50]. The majority of higher plants contain a number of agents or phytoconstituents that are capable of causing mitigating effects to a number of mutagens [51]. The phytoconstituents from Terminalia arjuna suppressed the mutagenic effect of the aromatic amine, i.e., 2-aminofluorene (2-AF). The observed activity caused the inhibition of the metabolic activation of pro-mutagens [52]. It was also found that the extracts of Acanthopanax divaricatus were able to rapidly eliminate the mutagenic compounds from the cells before they induce the DNA damage [53]. In a similar study, it was observed that the methanol extracts of the lichens have antimutagenic effects against sodium azide [54]. The antimutagenic properties of plant extracts was also demonstrated against cyclophosphamide induced mutagenicity in mice [55]. The different extracts of Dioscorea pentaphylla were found to significantly inhibit the effects of methyl methanesulphonate (MMS) induced mutagenicity [56]. An edible wild plant, Tragopogon longirostis was also evaluated for antioxidant, mutagenic and antimutagenic properties and it was found that the ethanolic extract of its leaves exhibited antimutagenic properties at 2.5, 0.25, and 0.025 mg/plate concentrations [57]. The ethanolic extract of Origanum vulgare also reduced the frequency of MN PCR from 10.52 ± 1.07 for CP to 2.17 ± 0.6 for the synergic test of CP and the ethanolic extract [58].

Conclusion

The medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants play a vital role in preventing various diseases. The presence of secondary metabolites in *Ajuga bracteosa* bestow it with different biological properties like antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, antimalarial, anti-bacterial and anti-fungal. Thus, *Ajuga bracteosa* can be used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. Thus we hope that the important phytochemical properties identified in this study in the local plant of Kashmir Himalaya will be helpful in copping different diseases of this particular region.

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