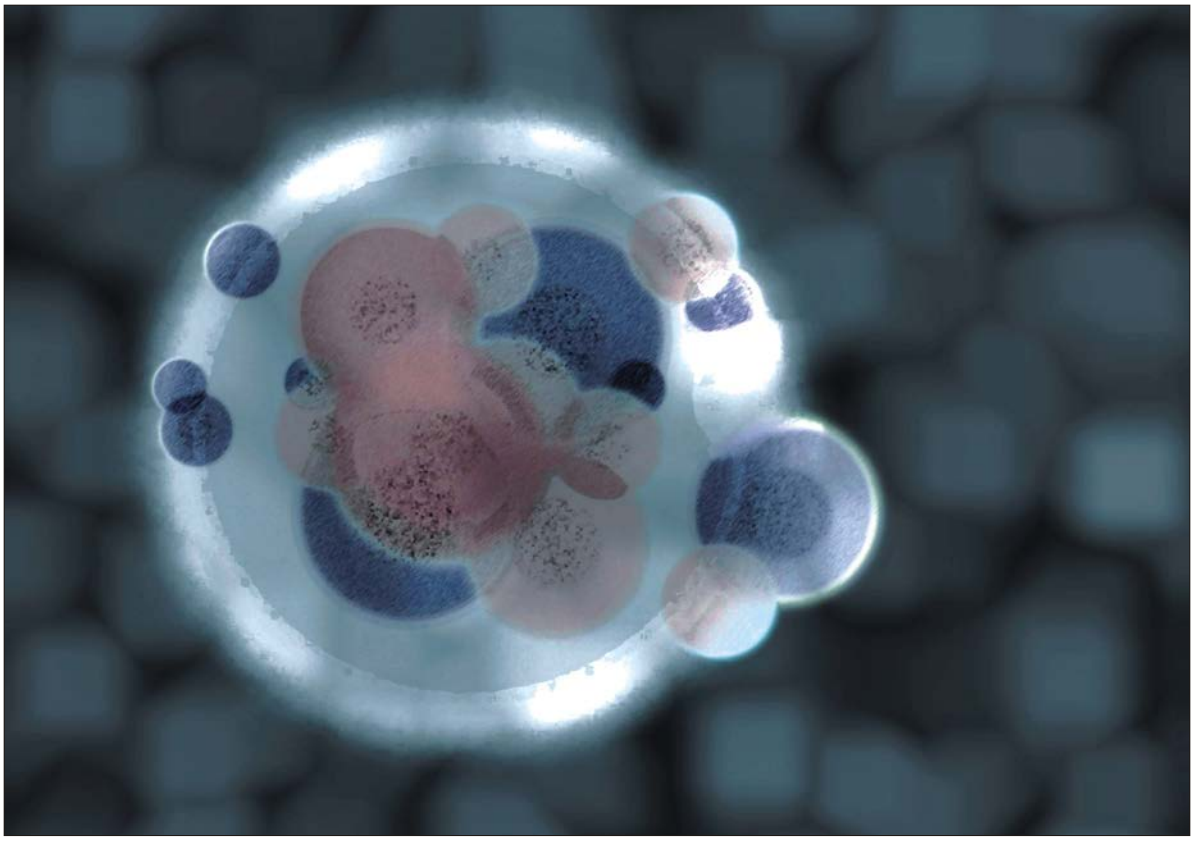

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Research Article
Protective Effects of *Echinacea purpurea* against the Genotoxic and Biochemical Damage Induced by Benzo[a]pyrene in Mice

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Abstract

Background and Objective: All living organisms could be exposed to Benzo[a]pyrene (BaP) that resulted mainly from burning of industrial carbon compounds, volcanic eruptions and forest fires causing genotoxic and carcinogenic effects. The current study aimed to examine the protective role of *Echinacea purpurea* (*E. purpurea*) against BaP-induced genotoxicity and biochemical damage in mice. **Materials and Methods:** Eight experimental groups were designed. Group 1: Negative control, group 2 received single dose of BaP (200 mg kg^{-1} b.wt.), groups 3-5 received the same dose of BaP but pretreated orally with *E. purpurea* (100 mg kg^{-1} b.wt.) for 1, 7 and 14 days respectively, groups 6-8 received oral dose of *E. purpurea* for 1, 7 and 14 days, respectively. Chromosomal abnormalities, DNA fragmentation and gene expression of *CYP1A2*, *CYP3A4* and GR genes were assessed in the liver. Lipid peroxidation and antioxidant enzyme activities were evaluated in mice. **Results:** Significant reduction ($p<0.01$) in chromosomal abnormalities induced by BaP was observed in animals pretreated with *E. purpurea* for 7 and 14 days. In liver cells, the inhibition percent of DNA fragmentation reached 37.6 and 60.4% respectively. In bone marrow, the inhibition percent of cells with comet tail reached 32.7 and 54.8%, respectively. Mice treated with *E. purpurea* combined with BaP showed significant increase ($p<0.05$) in gene expression of *CYP1A2*, *CYP3A4* and *GR* in liver cells through the experimental periods. Significant stimulation of antioxidants (GST, catalase and reduced glutathione) was observed in *E. purpurea* treated animals. Pretreatment with *E. purpurea* significantly reduced lipid peroxidation induced by BaP in liver and kidney. **Conclusion:** The *E. purpurea* might help in relief the deleterious consequence of carcinogens as BaP.

Key words: *Echinacea purpurea*, BaP, DNA fragmentation, chromosomal aberrations, gene expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Up till now, Hippocrates tenet "let food is thy medicine and medicine is thy food" has a great treasure. *Echinacea* (Asteraceae family) is a popular medicinal herb that was used early by native Indians of North America. It has a long history for the treatment of common cold, upper respiratory infections, urinary tract infections, burns and disorders such as viral infections and a lot of other disease conditions due to a deficiency of immunological responses[1,2]. Today, Commission E (Task Force E of the Federal Bureau of Health of Germany) approved it as medicine and its products are among the best-selling herbal preparations in the first world. *Echinacea purpurea* (*E. purpurea*) extract contain many active compounds as flavonoids, phenols, carbohydrates, fatty acids, polysaccharides and isobutyl amides[3] that exert wide spectrum of medical effects include anti-inflammatory, immune stimulating, anticancer and antiviral effects. Extract also shows anti-oxidative and free radical scavenging properties[4,5] and prescribed to supplement cancer chemotherapy for their immune-supportive effects, however, the extract may also influence tumorigensis[6]. The *E. purpurea* also has an effect as Interferon (IFN), enhances the production of Interleukin (IL)-1, IFN and macrophage activation[7].

Benzo(a)pyrene (BaP) is a widespread polycyclic aromatic hydrocarbon compound in the environment. It is formed during insufficient combustion of organic matters and high temperature processing of crude oil, coke, coal or other industrial carbon compounds. The main anthropogenic sources of BaP include burning of fuels and vehicle exhausts, while the major natural sources are volcanic eruptions and forest fires. BaP is also found in cigarette smoke, smoked and grilled food[8]. Therefore, all living organisms could be exposed to BaP.

Animal studies have recorded that BaP can induce carcinogenic effects in mice, rats, hamsters, rabbits, ducks and monkeys. Exposure to BaP was found to produce tumors in lymphoid organs, bone marrow and testes. Moreover, human occupational exposure to BaP elevates the risk of lung, stomach, bladder and skin cancers[9]. Genotoxic effects of BaP have been documented in both *in vivo* and *in vitro* studies[10-12]. The BaP is considered as a human carcinogen group (1) by the International Agency of Research on Cancer[9].

The present study aimed to evaluate the protective role of *E. purpurea* against BaP toxicity in mice using chromosomal aberrations in bone marrow and mouse spermatocytes, DNA fragmentation in liver and comet assay in bone marrow cells as markers of genotoxicity. Also gene expression of *CYP1A2*, *CYP3A4* and *GR* in liver cells and antioxidant enzymes in liver and kidney were examined.

MATERIALS AND METHODS

The study was started at the beginning of year 2016 because it takes 3 weeks (1) week for acclimatization period and 2 weeks for the study and then authors started to perform the laboratory work at National Research Centre, Dokki, Cairo, Egypt.

Chemicals and doses: *Echinacea* extract was purchased under the trade name Immulant® produced by MEPACO-EGYPT. The extract was dissolved in distilled water and was given via oral gavage in a dose equivalent to therapeutic human dose after modification by relative surface area between species according to the Paget and Barnes[13] formula (100 mg kg^{-1} b.wt.). Benzo(a)pyrene (BaP) (97% purity, CAS No. 50-32-8. Sigma, USA) was dissolved in Dimethyl Sulfoxide (DMSO) and was given by intraperitoneal injection in a single dose 200 mg kg^{-1} b.wt.

Experimental animals: Eight weeks old Swiss albino male mice (25±3 g) were obtained from the Animal House Colony, Giza, Egypt and were maintained *ad libitum* on standard lab diet (protein: 160.4, fat: 36.3, fiber: 41 g kg^{-1} and metabolizable energy 12.08 MJ) purchased from Meladco Feed Co. (Aubor City, Cairo, Egypt). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., National Research Centre, Dokki, Cairo, Egypt. After an acclimatization period of 1 week, the animals were divided into eight groups (10 mice/group) and housed in filter-top polycarbonate cages.

Experimental design: Eighty adult male mice were used in this study. Mice were divided into eight experimental groups as follows: Group 1 received single dose injection of DMSO and served as a negative control. Group 2 received single dose injection of BaP and served as a positive control. Groups 3, 4 and 5 received the same dose of BaP but pretreated orally with *E. purpurea* (100 mg kg^{-1} b.wt.) for 1, 7 and 14 days, respectively. Groups 6, 7 and 8 received oral dose of *E. purpurea* extract for 1, 7 and 14 days, respectively.

Experimental procedure

Chromosome abnormalities in somatic and germ cells: For somatic and germ cells preparations, animals from the different groups were injected with colchicine, 2 h before sacrifice. Chromosome preparations from bone marrow cells carried out according to the method of Yosida and Amano[14]. Chromosomal preparations from spermatocytes were made according to the technique developed by Evans *et al.*[15]. The 100 well spread metaphases were analyzed per mouse. Metaphases with gaps, chromosome or chromatid breakage and fragments were recorded in somatic cells and diakinesis metaphases-I with univalent, fragments, breaks and triploidy were recorded in germ cells.

DNA fragmentation: The colorimetric estimation of DNA content was detected according to Perandones *et al.*[16] with some modifications from Burton[17]. Tissues were dissociated in hypotonic lysis buffer (10 mM tris, 1 mM EDTA, 0.2% triton X-100, pH 8.0), incubated for 30 min at 48°C, the intact chromatin (pellet) was separated from DNA fragments (supernatant) by centrifugation for 15 min at 12.000 rpm. Pellet was resuspended in lysis buffer. Samples were precipitated by 10% trichloroacetic acid at 48°C. Samples were pelleted at 4000 rpm for 10 min, mixed with 5% trichloroacetic acid and then boiled for 15 min. DNA content quantified using diphenylamine reagent. The percentage of DNA fragmentation was expressed by the equation:

$$\text{DNA fragmentation (\%)} = \frac{\text{Optical density of supernatant}}{\text{Optical density of supernatant+ Optical density of pellet}} \times 100$$

Comet assay: The comet assay was performed as described by Singh *et al.*[18] with some modifications. Cell suspensions of bone marrow (25 µL) were mixed 1:10 with 250 µL molten low melting point (LMP) agarose and samples of 75 µL of the mixture were rapidly spread on comet slides. After gelling for 10 min at 4°C in the dark, 70 µL of 0.5% LMP agarose were added to cover the cell layer. Slides were put in a lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM tris base, 1% sodium lauroyl sarcosinate and 1% triton X-100) for 1 h at 4°C in the dark. Slides were then washed three times with neutralization buffer (0.4 M tris, pH 7.5) for 5 min and incubated in fresh alkaline buffer (0.3 M NaOH and 1 mM EDTA, pH>13) for 20 min at room temperature to allow unwinding of DNA. Electrophoresis was then carried out at room temperature in fresh ice-cold alkaline electrophoresis buffer for 30 min (at 25 V and 300 mA), then slides were gently washed three times for 5 min in fresh neutralization buffer, stained with 25 µL of ethidium bromide solution (20 µg mL^{-1}) and covered with cover slip. Comets were examined using a fluorescence microscope.

Gene expression

RNA isolation, reverse transcription and PCR amplification: Total RNA was extracted from liver tissues of mice by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). The RNA was dissolved in diethylpyro-carbonate (DEPC)-treated water by passing solution a few times through a pipette tip. Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water according to the manufacturer's instructions. The quantification of RNA was performed spectrophotometrically by measuring absorbance (Abs) at 260 nm. The purity of RNA was assessed by calculating ratios of absorbance of 260/280. The RNA used immediately for reverse transcription or kept under -80°C until use.

To synthesis cDNA of mice tissues, total RNA was reverse transcribed into cDNA in a total volume of 20 µL using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific™, Germany). An amount of total RNA (5 µg) was used with 50 µL oligo-dT primer,10 mM of dNTP mix,5x reaction buffer (250 mM tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM Dithiothreitol), 20 U Ribolock RNase inhibitor and 200 U μL^{-1} RevetAid RT.

Polymerase chain reaction was performed using the first-strand cDNA from different samples of mice as the template for amplification with the following specific primers CYP1A2, CYP3A4, Glucocorticoid receptor (GR),and β-actin, a housekeeping gene that used to normalize mRNA of studied genes (Table 1). Primers were obtained from HVD, Germany. A total reaction volume of 15 µL contained 5 ng genomic DNA, 0.2 µM of each primer, 1x of *Taq* polymerase buffer, 2 units of

Table 1: Sequences of PCR primers

	Primer sequence	
Gene primer	Forward primer	Reverse primer
CYP1A2	AGCTTCTCCTGGCCTCTGC	GGACTTTTCAGGCCTTTGGG
CYP3A4	CAGGAGGAAATTGATGCAGTTTT	GTCAAGATACTCCATCTGTAGCACAGT
GR	GGAGAATTATGACCACACTCAAC	GCAGTAGGTAAGGAGATTCTCAA
β-actin	CTGGCACCCAGCACAATG	GCCGATCCACACGGAGTACT

Taq polymerase (Promega).The PCR reaction was carried out under the cycling conditions of 94°C for 3 min followed by 35 cycles of 94°C for 15 sec, 55°C for 45 sec, 70°C for 45 sec terminated with 72°C for 5 min[19]. The PCR products were then loaded on 2% agarose gel.

Biochemical analysis

Sample collection: Livers and kidneys were removed from the dissected mice, rinsed in ice cold saline solution and frozen at -80°C. Livers and kidneys were cut into small pieces and then homogenized at 4°C in adequate volume of 0.025 mM tris-HCl buffer, pH 8, with glass homogenizer, resulting homogenate fitted with a teflon pestle. The homogenates were centrifuged at 3400 rpm for 15 min using Beckman L5-50B ultracentrifuge with 220.78VD2 rotor at 4°C. The supernatants were filtered through a plug of glass wool to remove floating lipids, the cytosolic fractions were termed as crude homogenates and stored at -20°C for further analyses. In homogenate catalase (CAT), GST activities, amount of reduced glutathione (GSH) and lipid peroxidation were measured for each group.

Glutathione S-transferase and Catalase (CAT) activity: Glutathione S-transferase activity was determined according to the method described by Habig *et al.*[20], by measuring the increase in the concentration of the conjugation product of GSH and 1-Chloro-2,4-dinitrobenzene (CDNB) at 340 nm over 3 min at 25°C. Unless stated, the assay mixture contained in a total volume of 1 mL, 0.1 M potassium phosphate buffer, pH 6.5, 1 mM CDNB in ethanol (final concentration of ethanol less than 4%), 1 mM GSH and the enzyme solution. One unit is equivalent to the amount of enzyme conjugating 1 μM of CDNB in 1 min at 25°C. The extinction coefficient of the product was taken to be 9.6 mM cm^{-1}. Protein concentration was estimated by the method of Bradford[21] using bovine serum albumin as standard.

Catalase activity was determined spectrophotometrically at 37°C. The decomposition of H_2O_2 was followed as a decline in absorbance at 240 nm for 5 min[22]. The assay reaction mixture contained in a total volume of 3 mL the substrate buffer and a suitable volume of the enzyme solution. The substrate buffer contained 30% H_2O_2 in 100 mL of 50 mM potassium phosphate buffer and pH 7. The final concentration of H_2O_2 is 0.042 M. The activity of enzyme was expressed as units of decomposed min^{-1} mg^{-1} proteins by the decomposition of H_2O_2 of the assay followed at 240 nm. The molar absorptivity of H_2O_2 = 43.6 L mol^{-1} cm^{-1}.

Estimation of non-enzymatic antioxidants: The GSH were measured in hemolysate using the Saville method[23].

Estimation of macromolecular damages-lipid peroxidation (LPO): The level of lipid peroxides was assayed by the method of Ohkawa *et al.*[24]. About 1.5 mL of thioparpturic acid, 0.2 mL of sodium dodecyl sulphate and 1.5 mL of glacial acetic acid were added to the test tubes containing 0.1 mL of samples. The test tubes were heated in the water bath for 1 h and then cooled and 1 mL of distilled water was added. The optical density was determined at 532 nm using a reagent blank. Standard malondialdehyde was also processed in a similar fashion. The results were expressed as nmol of malondialdehyde liberated mg^{-1} protein.

Determination of total phenolic content (TPC): Total concentration of phenolic compounds in the extract was determined using a series of gallic acid standard solutions (2.5-20 μg mL^{-1}) as described by Singleton and Rossi[25] but with some modifications. Each extract solution (0.1 mL) was mixed with 2 mL of a 2% (w/v) sodium carbonate solution and vortexes vigorously. The same procedure was also applied to the standard solutions of gallic acid. After 3 min, 0.1 mL of folin-ciocalteu's phenol reagent was added and each mixture was vortexes again. The absorbance at 750 nm of each mixture was measured, after incubation for 30 min at room temperature.

Free radical scavenging activity by DPPH method: The free radical scavenging activities were determined by DPPH method with some modifications of the method proposed by Blois[26]. Briefly, a 0.1 mM solution of DPPH in absolute ethanol was prepared. The initial absorbance of the DPPH in ethanol was measured at 517 nm (absorbance 1.3) and did not change throughout the period of assay. A series of extract solutions with varying concentrations were prepared by dissolving the dried extract in ethanol and 0.1 mL of solutions from each extract was added to 1.4 mL of DPPH solution. The absorbance at 517 nm was recorded after 30 min of incubation at room temperature.

Statistical analysis: For the genotoxicity analysis, the significance of each treatment was evaluated by t-test. All statements of significance were based on probability of $p<0.01$. The significance of differences between different treatments was evaluated by t-test in chromosomal aberration, DNA fragmentation and comet assays.

The signal intensities of *GR*, *CYP1A2* and *CYP3A4* genes and the reference primer β-actin were quantified by the computerized Gel-Pro program. Data for gene expression analyses were analyzed using the General Linear Models (GLM) procedure of Statistical Analysis System (SAS, Version 9.1, Stat soft Inc., Tulsa, USA) to assess significant differences between groups. The values are expressed as Mean±SE. All statements of significance were based on probability of $p<0.05$.

RESULTS

Chromosomal aberrations in somatic and germ cells: The number and percentage of the chromosomal aberrations induced in control and treated animals in bone marrow (somatic cells) and spermatocytes (germ cells) are shown in Table 2 and 3, respectively. The percentages of aberrant somatic cells in animals treated with *E. purpurea* for 1 day was not statistically different compared to control group. Treatment with *E. purpurea* for 7 and 14 days caused a highly significant ($p<0.01$) reduction in the percentage of chromosomal abnormalities induced by BaP in both types of cells. In somatic cells, the percentage of reduction reached 35.5 and 54.64% after treatment with *E. purpurea* for 7 and 14 days, respectively (Table 2). In germ cells, the percentage of reduction reached 43.5 and 63.88% after treatment with *E. purpurea* for 7 and 14 days in germ cells, respectively (Table 3).

DNA fragmentation: Administration of *E. purpurea* decreased the percentage of DNA fragmentation induced by BaP in liver cells (Table 4). The percentage of DNA fragmentation was decreased to 12.96 and 8.22% ($p<0.01$) after treatment with *E. purpurea* for 7 and 14 days, respectively compared to 20.77% for the groups treated only with BaP in liver.

Table 2: Number and mean percentage of the different types of chromosomal aberrations in bone marrow cells (somatic cells) of mice after treatment with *E. purpurea* (*E. purpurea*) for 1, 7 and 14 days alone or in combination with BaP

		Number of metaphases with							Total chromosomal aberrations		
Treatments	*E. purpurea* treatment day(s)	Gap	Frag. and/or break	Del.	Rt.	Gap+ (Frag. or break)	End.	Poly.	Excluding gaps (Mean±SE)	Including gaps (Mean±SE)	Inhibition (%)
Control	1	9	11	-	-	2	-	-	2.60±0.4	4.4±0.24	
BaP		77	120	7	4	35	10	7	36.6±0.83**	52.0±0.66**	
E. purpurea		7	10	-	-	2	-	-	2.40±0.2	3.8±0.5	
BaP+*E. purpurea*		89	109	6	3	37	12	5	34.4±0.74	52.2±0.73	6.00
Control	7	10	10	-	-	1	-	-	2.20±0.2	4.2±0.31	
E. purpurea		9	8	-	-	2	-	-	2.00±0.2	3.8±0.22	
BaP+*E. Purpurea*		68	71	4	-	31	8	4	23.6±0.46*	37.2±0.8*	35.50
Control	14	11	9	-	-	3	-	-	2.40±0.2	4.6±0.3	
E. purpurea		7	8	-	-	3	-	-	2.20±0.2	3.6±0.2	
BaP+*E. purpurea.*		44	52	-	-	29	2	-	16.6±0.24*	25.4±0.37*	54.64

The total number of scored metaphases is 500 (5 animals/group). Frag.: Fragment, Del: Deletion, Rt.: Robertsonian translocation, End.: Endomitosis, Poly.: Polyploidy. **Significant at 0.01 level (t-test) comparing to control (non-treated). *Significant at 0.01 level (t-test) comparing to treatment

Table 3: Number and mean percentage of metaphases with chromosomal aberrations in spermatocytes (germ cells) of mice after treatment with *E. purpurea* for 1, 7 and 14 days alone or in combination with BaP

		Number of different types of chromosomal aberrations						Total aberrations		
Treatments	*E. purpurea* retreatment day(s)	XY univalent	Auto univalent	XY+Auto univalent	Fragment or break	Fragment or break+XY	Triploidy	Number	Mean %±SE	Inhibition (%)
Control	1	13	6	-	-	-	-	19	3.8±0.2	
BaP		52	30	12	7	4	3	108	21.6±1.1**	
E. purpurea		14	4	-	-	-	-	18	3.6±0.2	
BaP+*E. purpurea*		49	31	12	6	3	3	104	20.8±0.7	3.70
Control	7	13	3	-	-	-	-	16	3.2±0.2	
E. purpurea		12	5	-	-	-	-	17	3.4±0.3	
BaP+*E. purpurea*		30	19	7	3	2	-	61	12.2±0.5*	43.50
Control	14	12	4	-	-	-	-	16	3.2±0.22	
E. purpurea		10	5	-	-	-	-	15	3.0±0.2	
BaP+*E. purpurea*		23	12	3	1	-	-	39	7.8±0.24*	63.88

The total number of scored metaphases is 500 (5 animals/group). **Significant at 0.01 level (t-test) comparing to control (non-treated). *Significant at 0.01 level (t-test) comparing to treatment

Table 4: DNA fragmentation in mouse liver cells after treatment with *E. purpurea* for 1, 7 and 14 days alone or in combination with BaP

Treatments	*E. purpurea* treatment day(s)	DNA fragmentation (%) Mean±SE	DNA fragmentation Inhibition (%)
Control	1	3.12±0.21	
BaP		20.77±1.06**	
E. purpurea		3.13±0.28	
BaP+*E. purpurea*		18.64±0.87	10.25
Control	7	3.23±0.26	
E. purpurea		2.96±0.20	
BaP+*E. purpurea*		12.96±0.32*	37.6
Control	14	3.22±0.27	
E. purpurea		3.12±0.20	
BaP+*E. purpurea*		8.22±0.33*	60.4

**Significant at 0.01 level (t-test) comparing to control (non-treated), *Significant at 0.01 level (t-test) comparing to treatment

Table 5: Comet assay in mouse bone marrow cells after treatment with *E. purpurea* for 1, 7 and 14 days alone or in combination with BaP

Treatments	*E. purpurea* treatment day(s)	Comet tail length (μm)	Percentage of cells show comet tail	Inhibition (%) of cells show comet tail
Control	1	0.84±0.12	1.2	
BaP		9.32±0.52**	35.4	
E. purpurea		0.83±0.10	1.6	
BaP+*E. purpurea*		9.22±0.63	34.2	
Control	7	0.86±0.14	1.6	
E. purpurea		0.82±0.12	1.4	
BaP+*E. purpurea*		6.20±0.50*	23.8	32.7
Control	14	0.87±0.14	1.6	
E. purpurea		0.82±0.12	1.2	
BaP+*E. purpurea*		4.15±0.23*	16.0	54.8

**Significant at 0.01 level (t-test) comparing to control (non-treated) *Significant at 0.01 level (t-test) comparing to treatment

Table 6: Phyto-chemical analysis of *E. purpurea* (100 mg/capsule)

Plant	Total phenolic content (mg gallic acid/capsule)	DPPH *IC_{50} (μg dry tissue mL^{-1})
E. purpurea	1.0857	39.32

DPPH IC_{50} of ascorbic acid as stranded as scavenger for free radical (27.25 μg dry tissue mL^{-1}). *IC_{50} (amount of extract which cause 50% inhibition of DPPH free radical)

Comet assay: The percentages of cells showing comet tails for bone marrow cells are presented in Table 5. Treatment with BaP increased the percentage of cells with comet tail to 35.4% and the mean tail length to 9.32±0.52 μm ($p<0.01$). Administration of *E. purpurea* for 7 and 14 days reduce the BaP-induced percentage of tailing cells and mean tail length in a significant and time dependent manner. The comet tail length reached 6.2±0.5 and 4.15±0.23 μm ($p<0.01$) after pretreatment with *E. purpurea* for 7 and 14 days in bone marrow cells, respectively.

Gene expression: Relative gene expression of liver related tumor genes Cytochrome P450 genes (*CYP1A2* and *CYP3A4*) and *GR* gene were assessed corresponding to the housekeeping gene β-actin by isolating total RNA from mice liver tissues followed by cDNA synthesis. The PCR amplification of cDNA was performed on four genes using specific primer for each gene to evaluate the induction of gene expression. Mice injected with BaP showed highest significant increase in gene expression ($p<0.05$) of liver cells compared to group 1 (negative control) and other groups in three studied genes (Fig. 1-3). Mice received oral dose of *E. purpurea* combined with BaP exhibited decrease in gene expression than injected mice with BaP for 1, 7 and 14 days at the three studied genes through experimental periods except in 1 day mice treated with *E. purpurea* and BaP of *CYP3A4* gene, which possessed the same gene expression of mice injected with BaP.

Phytochemical analysis of *E. purpurea* (100 mg/capsule): The total phenolic content was determined by using folin-ciocalteu method. Obtained results indicated that each capsule of *E. purpurea* contain TPC (1.0857 mg gallic acid/capsule), also antioxidant activities of *E. purpurea* capsule was investigated by (DPPH) scavenging inhibition method (Table 6). The IC_{50} values for DPPH (%) scavenging activity were determined from the percentage inhibition versus log plant extract concentration curve using vitamin C as a standard. These results showed low IC_{50} DPPH radical scavenging activity of 39.32 μg dry tissue mL^{-1}, which point to high antioxidant capacity when compared by DPPH IC_{50} of ascorbic acid as stranded as scavenger for free radical (27.25 μg dry tissue mL^{-1}) (Fig. 4).

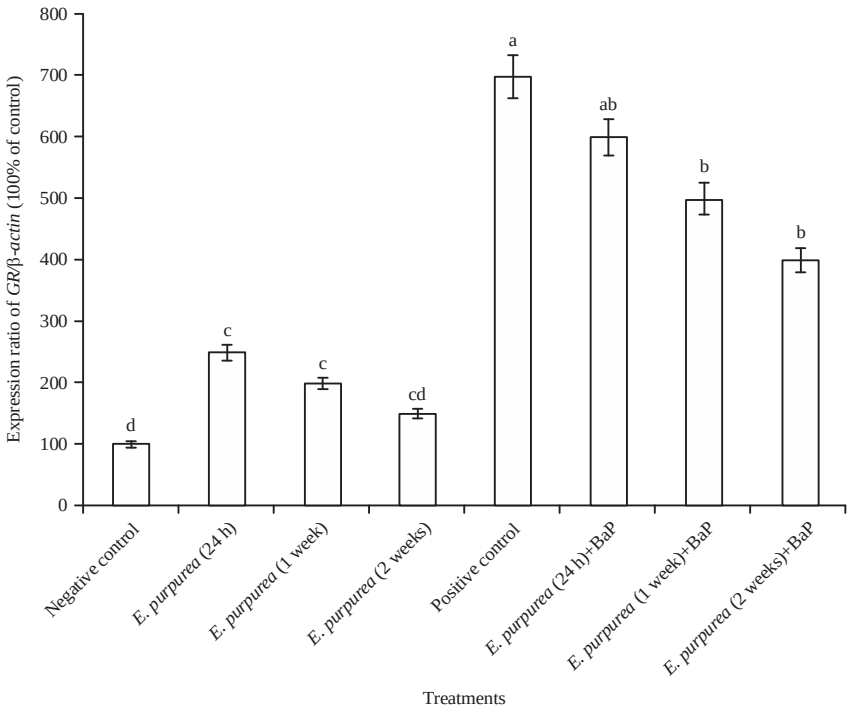

Fig. 1: PCR amplification of *GR* and *β-actin* genes expressed in liver tissues of rats

In each gene, lane 1 represents negative control, lane 2-4 represent low, medium and high doses of *E. purpurea*, respectively. Lane 5 represents BaP (positive control) only, lanes 6-8 represent low, medium and high doses of *E. purpurea* and BaP, respectively

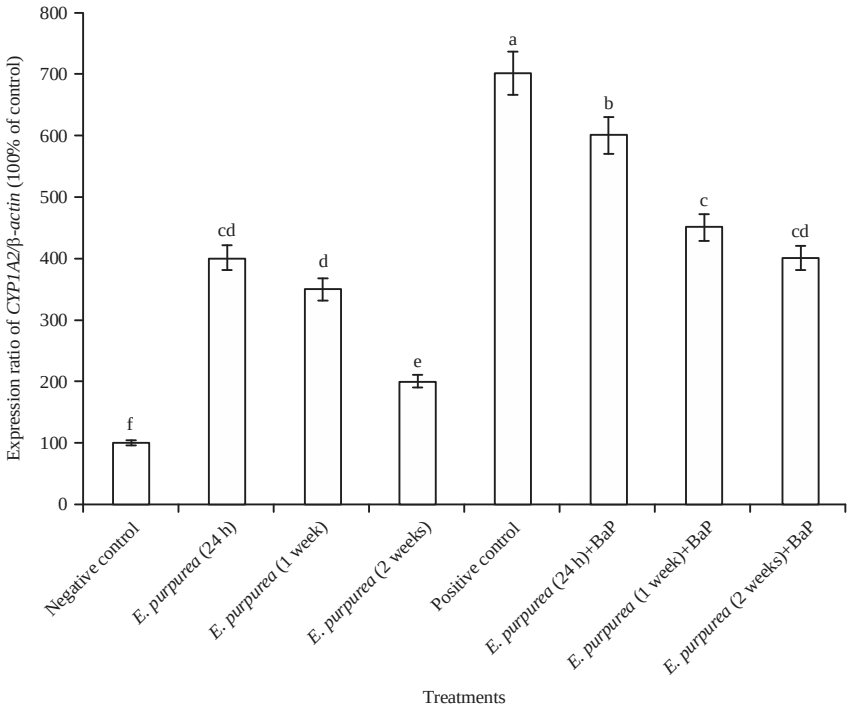

Fig. 2: PCR amplification of *CYP1A2* and *β-actin* genes expressed in liver tissues of rats

In each gene, lane 1 represents negative control, lane 2-4 represent low, medium and high doses of *E. purpurea*, respectively. Lane 5 represents BaP (positive control) only, lanes 6-8 represent low, medium and high doses of *E. purpurea* and BaP, respectively

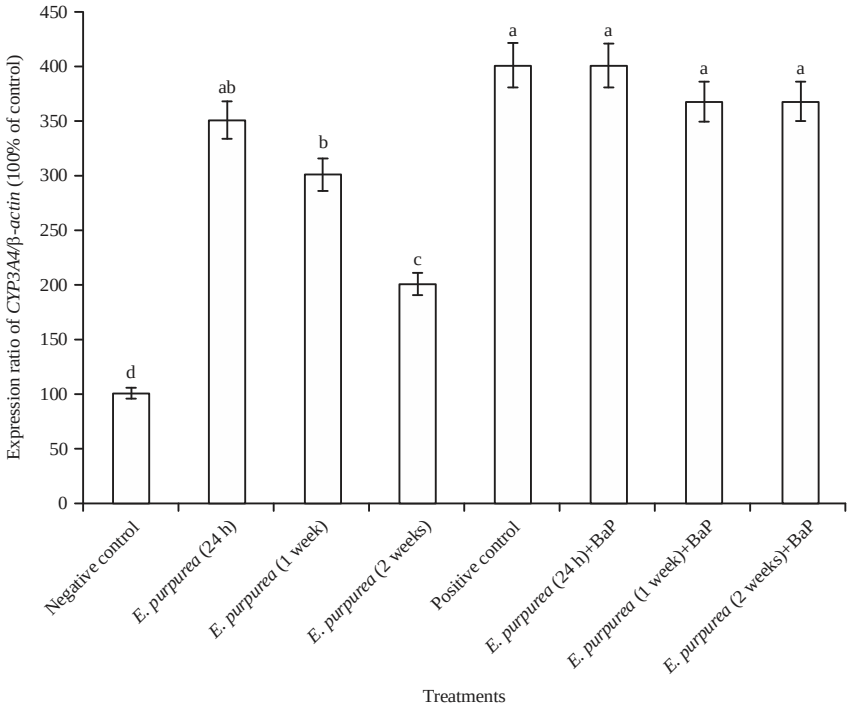

Fig. 3: PCR amplification of *CYP3A4* and *β-actin* genes expressed in liver tissues of rats

In each gene, lane 1 represents negative control, lane 2-4 represent low, medium and high doses of *E. purpurea*, respectively. Lane 5 represents BaP (positive control) only, lanes 6-8 represent low, medium and high doses of *E. purpurea* and BaP, respectively

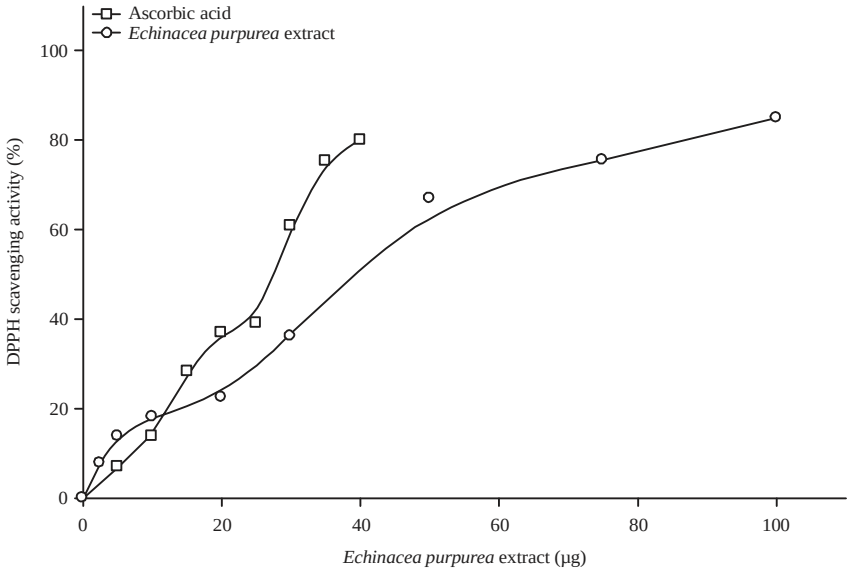

Fig. 4: Antioxidant activity of *E. purpurea* water extract compared by ascorbic acid as control (standard)

Enzymatic and non-enzymatic antioxidants: Table 7 and 8 represent the changes of enzymatic and non-enzymatic antioxidants of liver and kidney tissues, respectively in control and experimental animals. The enzymatic and non-enzymatic antioxidants such as GST, catalase and reduced glutathione levels were significantly reduced in carcinogenic mice group compared to control group ($p<0.05$). These levels did no change after oral administration of *E. purpurea* carcinogenic extraction mice for 1 day. However, same treatment with extended time for 7 and 14 days resulted in significant

Table 7: Levels of glutathione-S-transferase (GST), Catalase (CAT), Glutathione (GSH) and Malondialdehyde (MDA) in liver tissues of mice treated with *E. purpurea* extract alone or with BaP injection (Mean±SE)

Treatments	*E. purpurea* treatment days	GST (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)	GSH (μM mg^{-1} protein)	MDA (LP) (μM mg^{-1} protein)
Control	-	5.284±0.530[a]	623.40±33.73[a]	0.0560±0.0080[a]	0.0394±0.0057[a]
BaP	-	2.073±0.065*	59.27±5.12*	0.0342±0.0045*	0.2640±0.0330*
BaP+*E. purpurea*.	1	2.490±0.167*	76.11±12.1*	0.0392±0.0074*	0.2560±0.0202*
BaP+*E. purpurea*	7	3.090±0.295*	289.56±9.50*	0.0493±0.0039[a]	0.1891±0.0307*
BaP+*E. purpurea*	14	4.460±0.320[a]	544.01±33.5[a]	0.0425±0.00451[a]	0.0930±0.0162*
E. purpurea	1	4.130±0.306[a]	596.50±18.2[a]	0.0450±0.0064[a]	0.0540±0.0052[a]
E. purpurea	7	5.300±0.462[a]	543.80±15.13[a]	0.0511±0.0092[a]	0.0557±0.0036[a]
E. purpurea	14	6.6754±0.621[a]	833.60±48.3[a]	0.0695±0.0078[a]	0.0456±0.0049[a]

*Significantly different from control negative (Normal) at p<0.05, [a]significantly different from control positive (BaP) at p<0.05

Table 8: Levels of glutathione-S-transferase (GST), Catalase (CAT), Glutathione (GSH) and Malondialdehyde (MDA) in kidney tissues of mice treated with *E. purpurea* extract alone or with BaP injection (Mean±SE)

Treatments	*E. purpurea* treatment days	GST (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)	GSH (μM mg^{-1} protein)	MDA (LP) (μM mg^{-1} protein)
Control		1.084±0.0687[a]	139.30±16.455	0.0251±0.003	0.111±0.0283
BaP		0.270±0.01732*	15.77±3.54	0.0116±0.003	0.554±.0508
BaP+*E. purpurea*	1	0.290±0.012*	40.80±3.94*	0.0148±0.003*	0.4944±0.039*
BaP+*E. purpurea*	7	0.403±0.048*	88.69±3.932*	0.033843±0.003[a]	0.08216±0.0086[a]
BaP+*E. purpurea*	14	0.7864±0.0425*	151.95±10.422[a]	0.040±0.006[a]	0.09533±0.0068[a]
E. purpurea	1	0.962±0.06235[a]	120.62±11.19[a]	0.032±0.0017[a]	0.16±0.0231[a]
E. purpurea	7	1.230±0.09815[a]	150.60±11.21[a]	0.0296±0.003[a]	0.12±0.0177[a]
E. purpurea	14	1.504±0.30946[a]	174.20±10.51[a]	0.0281±0.0023[a]	0.0982±0.0065[a]

*Significantly different from control negative (Normal) at p<0.05, a significantly different from control positive (BaP) at p<0.05

(p<0.05) enhancement of enzymatic and non-enzymatic antioxidant activities. On the other hand, oral treatment of *E. purpurea* extract stimulates the antioxidant activities of both liver and kidney tissues in mice received zero dose of BaP compared to control mice.

Macromolecular damage: Levels of lipid peroxidation (LPO) in liver and kidney tissues of control and experimental animals are presented in Table 7 and 8, respectively. There was a noticeable increase in LPO in cancer bearing animals when compared to control animals (p<0.05). Significant decrease in the levels of LPO was observed in carcinogenic mice when treated with *E. purpurea* extract for 7 and 14 days. No alterations in levels of LPO were recorded for mice received oral *E. purpurea* extract alone for different period of times compared to control.

DISCUSSION

In the present study, mice remediated with BaP showed a high frequency of chromosomal abnormalities in bone marrow and spermatocyte cells when compared to control non-treated mice. In somatic cells, the most persistent type of aberrations were chromatid gaps, breaks and fragments while X-Y and autosomal univalent were the most dominant type in Diakinesis-metaphase I germ cells. There is a reliable concept that chromosomal aberration induction plays an important role in tumor development[27]. Thus, chromosomal aberrations in mouse bone marrow and spermatocyte cells were used in the present study as cytogenetic end point in genetic risk assessment. Similar to the current observations, exposure to BaP was declared to induce different types of chromosomal aberrations such as breaks, gaps, rings and dicentrics chromosomal aberrations *in vitro*[28] and chromatid breaks *in vivo*[29,30]. The BaP was also proved to induce a significant genotoxicity and oxidative stress in epididymal sperm and testis[31]. Moreover, the present results demonstrated that animals treated with BaP showed a significant elevation in DNA fragmentation in cells of liver and a significant DNA damage, determined by comet assay, in bone marrow cells. Comet assay is a sensitive and reliable method for detection of DNA damage[32]. Metabolic activation of BaP mainly produce mutagenic and DNA-reactive metabolite BaP-7,8-dihydrodiol-9,10-epoxide which could form stable and depurinating adducts with DNA through electrophilic carbonium ions lead to the formation of DNA strand breaks[33]. It was observed that treatment with BaP elevates the expression of the cytochrome P450 genes (*CYP1A1*, *CYP1A2* and *CYP3A4*) and the glucocorticoid hormone receptor gene (*GR*) in comparison with control group of mice, which stimulate the rate of DNA damage in male mice that considered as high anticipation for tumor series[34]. Cytochrome

P450 (CYP450) superfamily is a very important system involved in biotransformation of toxins, drugs and carcinogens. The CYP1A2 and CYP3A4 are involved in the biotransformation of chemicals especially in pre-carcinogen metabolic activation, whereas CYP3A4 is responsible for drug metabolism[35]. The CYPs Genetic polymorphism may produce an increase in individual's susceptibility to specific chemicals to motivate cancers[36] and intense toxicity or therapeutic defeat of drugs[37]. The GR is steroid hormone receptors that mediated biological processes such as development, growth, metabolism, apoptosis, behavior, electrolyte homeostasis, reproduction, stress response and immune response individually or in combination with other receptors by mediated the glucocorticoid hormones[38,39]. The CYP enzymes are also involved in steroid synthesis and metabolism[40]. The mutated GR implicated in severe complications[41,42]. The BaP metabolism, via cytochrome P4501A1 (CYP1A1), is also correlated with the formation of reactive oxygen species and induction of oxidative stress that could be involved in BaP-induced oxidative damage to DNA, its genotoxicity and carcinogenicity[43,44]. This may explain the present results on liver cancer related genes *CYP1A2*, *CYP3A4* and mediated biological processes *GR* gene. These results were also supported by the current biochemical investigation. Potential and oxidative effect indicated by increase of GST, CAT and GSH activities in both liver and kidney of mice exposed to BaP is demonstrated. It is known that ROS are created during normal mitochondrial respiration process and through oxidative metabolites of foreign chemicals. Oxidative metabolites of BaP can produce many different chemicals including free radicals via interaction with membrane lipids, peroxides, quinones or other reactive intermediates[45,46], where free radicals could interact with lipids of cell membranes and other lipid-containing structures in condition of oxidative stress[47]. The action of lipid peroxidation was increased by BaP due to induction of malondialdehyde (MDA) producing a genotoxic agent that may participate in the progression of human cancer[46] which may induce impairment of cell's natural protective system and could be, mainly, related to GSH depletion[48].

The current results reported that the treatment with *E. purpurea* for one day interval did not induce any significant changes in cytogenetic, gene expression and biochemical parameters, while the treatment with *E. purpurea* for 7 and 14 days exhibited significant changes in the previous parameters. Likewise, Stanisavljevic *et al.*[49] showed that extracts of *E. purpurea* were a potential source of active natural and non-toxic substances, which had functions as antioxidants, antimicrobials and antibiotics. In addition, the present investigation proved that treatment with *E. purpurea* was not only safe but also had a powerful and improvement effect against BaP induced genotoxic and biochemical damage. It significantly decreased chromosomal abnormalities in somatic cells, DNA fragmentation in liver and DNA damage in bone marrow cells and regulates the gene expression. After 14 days of treatment with *E. purpurea*, the inhibition percent reached to 60.4 and 54.8% in DNA fragmentation and the percent of cells showing comet tail, respectively. These results were compatible with those performed by Joksi *et al.*[50]. Barrett[1] was demonstrated that the treatment of ionizing radiation workers with *E. purpurea* for 2 weeks significantly reduces chromosomal aberrations and micronuclei in blood cells. Sayed[51] reported that *E. purpurea* exerts a preventive effect against cisplatin-induced genotoxicity in mice. Only few data was reported about protective effect of *E. purpurea* on germ cells. Treatment with *E. purpurea* for 7 and 14 days decreased significantly ($p<0.01$) the percentage of chromosomal aberrations in diakinesis metaphases-I of spermatocytes. The percentage of reduction reached 43.5 and 63.88% after treatment, respectively. This effect could likely be ascribed to antioxidants in *E. purpurea* such as flavonoids, phenolic acids or phenolic diterpenes[49-52]. Echinacoside, caffeic acid, chlorogenic acid, cynarine and caftaric acid in *E. purpurea* were observed to be potent scavengers of free radicals such as hydroxyl radicals (OH·) and superoxide (O_2^-) and were able to inhibit lipid peroxidation[53-56].

Echinacea purpurea extract has an influence on mRNA expression of major CYP450 enzymes[57], whereas, its influence on mRNA expression of GR still obscure. In the present study, treatment with *E. purpurea* exhibited reduction in BaP-induced alterations in gene expression of tumor initiation genes (*CYP1A2* and *CYP3A4*) and *GR* gene, these findings are in accordance with current cytogenetic analysis. Modarai *et al.*[58] studied the inhibitory effect of *E. purpurea* extracts on CYP3A4 expression due to alkylamide content of *Echinacea* extracts, which may have the same inhibitory effect on CYP1A2 and GR that controlling genes responsible for metabolism, development and immune response. *Echinacea purpurea* regulates the expression of CYP1A2 and CYP3A4 by activating pregnane xenobiotic receptor of human carcinoma of liver[59], while Naspinski *et al.*[60] stated that the expression of CYP1A2 and CYP3A4 up-regulated by pregnane xenobiotic receptor.

In this study, low IC_{50} DPPH radical scavenging activity referred to the high antioxidant capacity of *E. purpurea* in comparison with DPPH IC_{50} of ascorbic acid as stranded, which proved that *E. purpurea* has scavenging and antioxidative activity for free-radicals[55]. In the present study, measuring of

total phenolic content in *E. purpurea* extract showed that the level of these phenolic compounds was considerable, therefore, some of its pharmacological action could be due to the presence of these valuable constituents[61]. Several enzymes have a critical role in the elimination of Reactive Oxygen Species (ROS) in living organism such as Catalase (CAT) and glutathione-S-transferase (GST), which considered the most crucial enzymes in the cellular antioxidant system[62]. Catalase degrades H_2O_2 to water and oxygen and GPx detoxifies both H_2O_2 and hydro-peroxides (ROOH) using reduced glutathione (GSH) as a cofactor. The GST catalyze GSH conjugation reaction to electrophilic metabolites of xenobiotics including carcinogens and mutagens, to the endogenous nucleophile GSH[62-63]. These results demonstrated a significant reduction in hepatic and renal concentration of GSH in BaP treated mice which may reflect increase in GSH consumption via GST or it may react directly with ROS. Treatment with *E. purpurea* may improve natural antioxidants that may intervene with ROS induced by BaP or stimulate the endogenous GST production.

Interests in herbal medicine for cancer therapy have dramatically grown over the preceding years. Exogenous intake of antioxidants can help the body scavenge free radicals and have great potential in ameliorating the processes of free radicals diseases[64]. From this view point, the crucial target of this study was to study the ability of *E. purpurea* extract, at its human therapeutic dose and to counteract genotoxic and biochemical consequences induced by BaP.

CONCLUSION

The treatment with *E. purpurea* as therapeutic medical plant against environmental chemical-induced oxidative damage and genetic deleterious such as BaP is safe, powerful and could relieve the deleterious consequence of carcinogens in mice. So it is recommended to use this medical plant in the treatment of human genotoxicity and oxidative damage and make a further study for that plant.

SIGNIFICANT STATEMENT

This study discovers that the treatment with *E. purpurea* as a therapeutic medical plant is safe, powerful and promise candidate to counteract toxicity of different environmental chemical-induced oxidative damage and genetic deleterious such as BaP using genetic and biochemical analyses. This is very beneficial, where, *E. purpurea* might help in relieve the deleterious consequence of carcinogens in mice. So, it could help the researchers to use this medical plant in the treatment of human genotoxicity and oxidative damage against the toxic materials. Thus, a new theory on *E. purpurea* as therapeutic plant against several genotoxic and chemical induced oxidative damage may be arrived at.

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