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Genotoxic effects of *Peganum harmala* L. in relation to traditional use

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***Peganum harmala* L. is a well-known medicinal plant, widely distributed and used in folk medicine. Recently, numerous reports have been published regarding the pharmacological and therapeutic effects of *P. harmala* and its major alkaloids components. However, mammalian toxicity due to *P. harmala* alkaloids extract has been rarely investigated. The present research aimed to study the genotoxic effects of *harmala* plant extract in relation to traditional use. *Harmala* crude alkaloid was extracted and the toxicity of the extract was examined on albino mice by intraperitoneal injection. The genotoxic effects of the alkaloid extract was tested on bone marrow cells isolated from sacrificed mice. The total seeds alkaloids showed moderate toxic effects on male albino mice with LD_{50} 350 mg/ Kg body weight. *P. harmala* alkaloids extract showed significant reduction in the mitotic index (MI) and increased depression of mammalian cells division possibly through chromosomal aberrations. *P. harmala* alkaloids induced different types of chromosome aberrations including rings, breaks, polyploidy, sticky, laggards and bridges with the sticky form as the most abundant type. Furthermore, *P. harmala* alkaloids induced a significant increase in sister chromatid exchange (SCE) compared to untreated controls. The frequency of micronuclei was increased with increasing the concentration but was not affected by increasing the exposure time. The medicinal use of *harmala* should be under control since higher doses and/ or longer exposure is genotoxic. An amount of plant that contains ≥ 12 mg alkaloids cannot be safe for traditional use.**

Key words: *Peganum harmala*, genotoxicity, chromosome aberration, sister chromatid exchange.

INTRODUCTION

Peganum harmala L. is a perennial herbaceous and glabrous plant that belongs to the family *Zygophyllaceae* and can grow up to 30-100 cm in semi-arid condition. The plant is widely distributed in Asia, North Africa and Middle East (Moshiri et al., 2013). All parts of the plant including leaves, seeds, fruits, roots and barks have been used in

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folk medicine (Moloudizargari et al., 2013). The plant is well-known for its medicinal values including hypotensive and cardiovascular activities (Tahraoui et al., 2007), antimicrobial effects (Prashanth and John, 1999), anti-fungal (Saadabi, 2006), antitumor activities (Li et al., 2007), anti-diabetics (Waki et al., 2007), anti-inflammatory effects (Hamsa and Kuttan, 2010), anti-leishmanial effects (Mirzaie et al., 2007) and in some nervous system disorders such as Parkinson's disease, psychiatric nervousness, and in rigorous pain (Leporatti and Ghedira, 2009)

P. harmala is known as a major source of alkaloids (Rafie et al., 2017) including β-carbolines such as harmine, harmaline, harman and harmalol and the quinazoline derivatives such as vasicine and vasicinone (Di Giorgio et al., 2004). Harmala alkaloids showed a variety of biochemical, psychopharmacological, and behavioral effects on animals and humans (Farzin and Salimi, 2009). For example, harmine is considered as an important antitumor and anti-HIV agent (Begum et al., 2008). Similarly, harmaline, can cause 96.6% inhibition rate of human retinoblastoma (Rb) cell line within 72 h (Jin, 1990). On the other hand, γ -harmine is considered as the first alkaloid with relatively good radio-protective effect (Li et al., 1995). Furthermore, gastro-protective mechanism by peganine (Singh et al., 2013) and treatment of psychiatric disorders by deoxyperganine (Tursunkhodjaeva et al., 2015) were reported.

With all of the aforementioned pharmacological effects of *P. harmala* and its known folk use medicine, its cytotoxicity in particular genotoxicity on mammalian cells was rarely investigated. Here, in this study, the genotoxicity of *P. harmala* alkaloids on mammalian cells was investigated using different genotoxic parameters including mitotic index, chromosome aberration, sister chromatid exchange and micronucleus assay.

MATERIALS AND METHODS

Chemicals and reagents

Mitomycin C (MMC) was obtained from Kyowa Hakko Co. (Tokyo, Japan) and used as positive control. Fifty mg of 5-BrdU tablets were obtained from Boehringer-Mannheim (Mannheim, Germany). DMSO and Hoechst 33258 were purchased from Sigma Chemical Co. (St Louis, MO). Giemsa stain was procured from Glaxo India Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Preparation of *P. harmala* alkaloid extract

P. harmala L. seeds were collected from marginal and desert lands from various locations in Jordan including Irbid, Salt, Dead Sea, Zarqa, Mafraq and Ma'an, at the end of summer, 2015. A voucher specimen was preserved in the herbarium of the Hashemite University, Department of Biology and Biotechnology, Zarqa, Jordan.

P. harmala seeds were extracted according to Tharib and El-Migirab (1984) (Brkljača et al., 2015) with some modifications. Briefly, 500 g air dried and finely ground seeds were refluxed with ethanol in a Soxhlet extraction apparatus. The ethanol was evaporated using rotatory evaporator and the residual extract was mixed with10 % HCl and re-extracted with chloroform. The aqueous acidic layer was collected and its pH was adjusted to 9.5 by ammonium hydroxide (NH_4OH) solution. The aqueous basic solution was re-extracted 3 times with chloroform. The presence of alkaloids in the chloroform layer was confirmed by Mayer's precipitation test. The chloroform layer was evaporated under vacuum by a rotary evaporator at 45-50ºC to afford 0.5 g alkaloid residue (Memelink et al., 2001). A stock solution of the alkaloid residue was prepared by dissolving 500 mg of the alkaloidal fraction in 5 mL dimethyl sulphoxide (DMSO) and aliquoted in different concentrations and kept at 4ºC for further analysis.

Determination of the median lethal dose LD_{50}

Animal study performed in this report was approved by institutional review board (IRB) at the Hashemite University. Crude alkaloid from *P. harmala* were demonstrated using Swiss albino male mice (*Mus musculus*, 2n = 40); JVC 1, 10–12 weeks old and 20–25 g weight, were obtained from the animal house (Biology Dep., Hashemite University, Jordan) and maintained under conventional laboratory conditions at room temperature on a 12 h dark/ 12 h light cycle. LD_{50} was determined using behavioral and pharmacological studies of the extract (Benbott et al., 2013) with some modifications. The extract of *P. harmala* was administered at different doses intraperitoneally. Crude alkaloid extract with 200 mg/Kg b.w. was chosen as primary dose. The mice were divided into 8 groups each of 5 animals including a control group.

Preparation of mouse bone marrow cells

Mice were sacrificed by cervical dislocation, after a single intra-peritoneal injection with alkaloids. Femora were cleaned from adherent tissues and the tip of each bone was removed followed by harvesting the bone marrow using sterile saline. Metaphase bone marrow cells were prepared for mitotic chromosomal aberrations by classical methods (Omari et al., 1996). The prepared cells were stained with Giemsa solution and the slides were coded and scored for the presence of dividing cells. Different alkaloid concentrations including 12.5, 25, 50, 75 and 100 mg/ Kg b.w were applied to investigate their genotoxic effects on bone marrow cells at different time periods: 8, 24, 48 and 72 h. At least, 3000 cells from four male mice were used for each experimental and control group. Control groups received equivalent volumes of saline solution. Frequency of normal and aberrant chromosomes was examined, and chromosomal aberration was calculated according to the following formula:

Chromosomal aberration (CA) (%) = (Total number of chromosomal aberrations / Total number of cells examined) x 100

The mitotic index and mito-depression were calculated according to the following equations (Omari et al., 1996):

Mitotic index= (Number of divided cells/Total number of cells) x 100
And the mito-depression index was calculated using the following equation:

MI (control) - MI (treatment) x 100/ MI (control).

Sister chromatid exchange (SCE)

The SCE assay was applied as described by Goto et al. (1978)

with some modifications. The bromodeoxyuridine 5-BrdU tablets (50 mg) were prepared and implanted. The treated animals were sacrificed by cervical dislocation after 24 and 48 h treatments and the femora were dissected. The animals were treated intraperitoneally with colchicine (4 mg/ Kg) for 90 min. Different concentrations of alkaloids extract including 12.5, 25, 50, 75 and 100 mg /Kg b.w, were applied and 4 male mice were used for each injected alkaloid dose.

Bisbenzimide (Hoechst 33258) dye was applied and metaphase bone marrow cells were prepared by the classical methods. The preparations were stained in Giemsa solution and at least 200 well-spread second division metaphases were analyzed for SCE. Mitomycin C was chosen as a positive control, and DMSO was used as negative control.

Micronucleus assay (MN)

Different alkaloids concentrations (12.5, 25, 50 and 100 mg / Kg b.w) were injected intra peritoneal for 24, 47 and 72 h. Four male mice were used per each concentration. The micronucleus test was performed according to Schmid (1975) with minor modifications. The preparations were stained with Giemsa solution and a total of at least 4000 cells were scored for each animal at a magnification 1000 x using the oil immersion lens. Mitomycin C and DMSO were chosen as a positive and a negative control respectively.

Statistics

Statistical analysis was performed using SPSS version 17. Significant differences between the results of different groups using one way analysis of variance (ANOVA) was applied. The differences were considered statistically significant when $P \leq 0.05$ and highly significant at $P \leq 0.001$.

RESULTS

P. harmala is generally toxic

To establish a base line of extract cytotoxicity, LD_{50} of *P. harmala* alkaloids extract in mice was measured. The LD_{50} of mice treated intraperitoneally with *P. harmala* alkaloids were calculated using the second phase and was found to be ≥350 mg / Kg b.w.

P. harmala is genotoxic

P. harmala reduced mitotic index (MI) and increased mito-depression of mammalian cells

The cytological effect of the alkaloids was estimated on the basis of the mitotic index in bone marrow cells of Swiss albino mice following a single intraperitoneal dose of 12.5, 25, 50, and 100 mg / Kg b.w. The mitotic index was detected from 8 to 72 h following injection. A significant reduction in the mitotic index from ~4.45 to ~3.31 was evident in all treated mice cells compared to positive and negative controls (Table 1). The depression index was increased with increasing the exposure time in almost all treatments (Table 1).

P. harmala caused significant cchromosome aberrations

Cytogenetic studies showed that *P. harmala* seeds alkaloids extract induced significant increase in the % of chromosome aberrations in almost all treatments when compared to control. Chromosome aberrations including rings, breaks, polyploidy, sticky, laggards and bridges were shown. The sticky chromosomes were the most abundant, while rings, broken and poly ploidy were produced in considerable frequencies (Table 2). Other types of aberrations (gaps, dicentric and fragments) were also produced in very low frequencies (Table 2). Furthermore, *P. harmala* alkaloids induced sister chromatid exchange (SCE) at all tested concentrations (12.5, 25, 50 and 100 mg / Kg b.w) compared to untreated controls. The potency of SCEs due to alkaloid treatment was significantly lower than those produced by the positive control (MMC) by ~ 60% and the frequency of SCE was not increased by increasing exposure time (Table 3). Additionally, the frequency of micronuclei was significantly increased with increasing the alkaloid concentrations compared to control, while the increase in exposure time did not alter this frequency. However, there were no significant differences between treated groups (Table 4).

DISCUSSION

P. harmala alkaloid extract showed LD_{50} of ≥ 350 mg / Kg b.w which is consistent with that obtained previously (Benbott et al., 2013). These identical values may be attributed to similar conditions used in this respect. To assess the lethal effect of the extract, several genotoxic effects of the extract were tested including mitotic index (MI), chromosome aberration (CA), sister chromatid exchange (SCE) and micronucleus (MN) assays. The extract showed marked reduction in the mitotic index (MI) of the injected mice compared to controls; similar to results obtained with *Catha edulis* (khat) alkaloid (Kabarity and Malallah 1980) and *Rubia cordifolia* alkaloid extracts (Abderrahman 2004).

In reference to Kabarity and Malallah (1980), the marked reduction in MI suggested that the alkaloid extract could affect the onset of mitosis and the spindle formation. Thus, possible chromosomal aberrations (CA) due to the alkaloid extract were tested. Cytogenetic analyses showed significant increase in the % of chromosome aberrations due to alkaloid extract including rings, breaks, polyploidy, sticky, laggards and bridges with sticky chromosomes being the most abundant type. Other types of aberrations including gaps, dysenteric and fragments were also observed but in very low

Table 1. Mitotic index in bone marrow cells treated with different concentrations of *Peganum harmala* alkaloid extract.

Exposure Time (h)	Dose (mg/kg)	Number of cells	Mitosis	Mitotic index (MI)	Mitodepressive index
8	100	3100	122	3.93*	13.81
	75	3001	117	3.90*	14.47
	50	3675	143	3.89*	14.69
	25	3120	133	4.26	6.58
	12.5	3000	133	4.43	2.85
	Cont.	4300	196	4.56	-
24	100	3195	116	3.63*	13.37
	75	3020	112	3.71*	11.46
	50	3515	136	3.87*	7.64
	25	3110	120	3.86*	7.88
	12.5	3121	138	4.42	5.49
	Cont.	3009	126	4.19	-
48	100	3002	110	3.66 *	15.86
	75	3005	112	3.73*	14.25
	50	3111	117	3.76*	13.56
	25	3035	125	4.12	5.29
	12.5	3105	132	4.25	2.30
	Cont.	3440	150	4.35	-
72	100	4125	-	-	-
	75	4012	-	-	-
	50	3177	105	3.31*	24.77
	25	3940	161	4.09	7.05
	12.5	3935	175	4.45	1.14
	Cont.	3126	138	4.40	-

*Significant value.

frequencies. The results are consistent with previously reported results of extract using *Vicia faba* plants (Mekki et al., 2015). On the other hand, chromosomal aberration (CA) is considered to be very sensitive end points recognizing the genotoxicity induced by chemicals (Majak et al., 2010). It is evident that sticky chromosomes caused spindle deformation (Tawab et al., 2004) and it is attributed to an increase in the viscosity of cytoplasm (Abderrahman, 1997). *P. harmala* alkaloids showed significant genetic toxicity measured by sister chromatid exchange (SCE) test. SCE is considered as sensitive indicator of genetic toxicity and biomarker of genotoxic substances (Jeyapradha et al., 2011). *P. harmala* alkaloids showed significant increase in the frequency of SCEs and the increase was dose-dependent, but not time-dependent, consistence with results obtained on other genotoxic agents (Das et al., 2004; Abderrahman and Modallal, 2008).

Furthermore, assay of micronuclei (MN) serves as an indicator of genetic damage (Jois et al., 2010). MN are very small extra-nuclear bodies which arise from acentric chromatid / chromosome fragments caused by unrepaired or misrepaired DNA breaks during anaphase (Fenech et al., 2011). Thus, the quantitative estimation of MN may serve as an indicator of genetic damage that has taken place (Jois et al., 2010). MN assay was chosen to assess alkaloids possible genotoxic and cytotoxic effects on mice cells. MN assay has become one of the most commonly used methods for assessing chromosome breakage and loss in mammalian lymphocytes (Jiménez et al., 2008). The increase in the frequency of MN in exfoliated cells revealed a statistically significant effect on mice cells treated with *P. harmala* alkaloids when compared to control group. This result is similar to data reported on the genotoxic induction by the Brazilian medicinal plant, *Strychnos pseudoquina* (Santos et al., 2006).

Conclusion

In summary, our results showed clearly that alkaloids

Table 2. Frequency of chromosome aberrations in mice cells treated with different concentrations of alkaloids from *Peganum harmala.*

Exposure Time (h)	Conc. (mg/kg)	Cells Examined	Types of chromosome aberrations						% of CA
			Rings	Breaks	Polyploidy	Sticky	Lagg.	Bridges	
8	100	3820	25	26	15	49	2	1	3.09**
	75	3200	20	20	12	40	1	2	2.97**
	50	3711	22	23	14	40	-	1	2.69**
	25	3686	14	14	8	26	1	-	1.71**
	12.5	3502	9	8	4	18	-	1	1.14*
	Cont.	3005	2	2	2	4	-	-	0.33
24	100	3522	28	28	18	61	2	1	3.92**
	75	3010	22	22	12	50	1	2	3.62**
	50	3711	25	26	17	56	2	2	3.45**
	25	3900	18	21	14	50	1	1	2.69**
	12.5	3615	14	12	8	30	-	1	1.80*
	Cont.	3623	2	2	1	3	1	1	0.28
48	100	3520	36	34	24	68	4	3	4.80**
	75	3800	29	30	20	54	4	4	3.71**
	50	3908	27	28	18	48	3	2	3.22**
	25	3912	18	20	10	30	2	-	2.06**
	12.5	3844	12	13	9	21	1	1	1.48*
	Cont.	3998	4	111	1	3	1	1	0.25
72	100	3622	38	37	23	72	5	3	4.91**
	75	3400	30	30	20	45	5	4	3.94**
	50	3952	31	30	18	60	4	3	3.69**
	25	3945	30	30	20	55	3	2	3.55**
	12.5	3800	18	20	11	24	1	1	1.97*
	Cont.	3678	3	3	23	33	1	1	0.27

*Significant value, ** The result shows exceptionally a significant value.

Table 3. Frequency of sister chromatid exchange in mice cells treated with different concentrations of alkaloids from *Peganum harmala.*

Parameter	Dose (mg/ Kg b.w)				
	12.5	25	50	75	100
24 h					
MMC[a]2.0	12.85±0.20				
DMSO[b]	3.85±0.28				
SCE/cell (mean±S.D)	4.35±0.32*	5.44±0.34*	6.68±0.22**	7.85±0.28**	8.25±0.42**
48 h					
MMC[a]2.0	12.54±0.36				
DMSO[b]	3.52±0.26				
SCE/cell (mean±S.D)	4.48±0.38*	5.58±0.40*	6.74±0.42*	8.02±0.24**	8.44±0.38**

[a]MCC, mitomycin C (positive control); [b]DMSO, dimethyl sulfoxide (negative control). *Significant value; **The result shows an exceptionally significant value.

isolated from *P. harmala* exhibited genotoxic effects by inducing significant mitodepression, chromosomal aberration, sister chromatid exchange and micronuclei. These effects were more prominent at higher doses, and

Table 4. Effect of alkaloids extracted from *Peganum harmala* on micronucleus (MN) formation in mouse bone marrow cells.

Exposure Time (h)	Dose (mg/kg b.w)	Number of cells examined	Number of micronuclei	Percentage of micronuclei
24 h	**12.5**	**4005**	**42**	**1.04**
	25	4100	59	1.44*
	50	4002	61	1. 52*
	100	4021	95	2.36**
	DMSO	4000	25	0.86
	Mytomycin C 2.0	4012	174	4.34
48 h	12.5	4001	43	1.07
	25	4010	57	1.42*
	50	4015	61	1.52*
	100	4116	95	2.31**
	DMSO	4008	29	0.72
	Mytomycin C 2.0	4015	179	4.46
72 h	12.5	4105	44	1.09
	25	4211	56	1.33*
	50	4005	58	1.45*
	100	4211	97	2.30**
	DMSO	4019	29	0.90
	Mytomycin C 2.0	4113	185	4.50

*Significant value; ** the result shows an exceptionally significant value.

hence it is important to direct the traditional use of the medicinal plant to avoid any genotoxic effects. However, further investigation is still needed to identify the active compounds from the crude alkaloid extracted that is responsible for these activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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