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Modulating the Mutagenic Effects of Mitomycin C by Aqueous Extract of *Alhagi graecorum*

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Abstract

The role of *Alhagi graecorum* Boiss aqueous extract (0.5, 1.0 and 1.5 mg/kg) in modulating the genotoxic effects of mitomycin C (MMC) in albino male mice was evaluated through three types of treatments. In the first, the extract was given alone to study its effect, while in the second and third treatments, interactions with MMC were carried out (Pre- and Post-treatments). The investigated parameters were total leucocyte count (TLC), mitotic index (MI), chromosomal aberrations (CA) and micronucleus (MN) formation.

In the first treatment, the MI was significantly increased in the doses 1.0 and 1.5 mg/kg (16.66 and 17.84%, respectively) as compared to either negative (13.32%) or positive (7.32%) controls, and such observation was positively correlated with the TLC. Moreover, CA and MN assays revealed that the extract has no mutagenic effects, in contrast, a reduction in the spontaneous frequency of CA and MN was observed, especially the MN formation, in which the reduction was around 50% in the three doses. The results of second and third treatments (Pre- and Post-treatments) confirmed the forthcoming findings, and the extract was able to modulate the mutagenic effect of MMC, especially the dose 1.5 mg/kg, in which a significant enhancement of TLC and MI, and a significant reduction in the induced CAs and MN formation were observed.

Introduction

The plants and their products are important sources of remedies for human diseases, and based on this fact, the studies are engaged to investigate the role of plants and their chemical compositions in treatment and prophylaxis of different diseases (1,2). In this regard, cancers have gained much concern, and this reasoned by the fact that these diseases are showing increase incidences world wide (3). The aetiology of cancer is multifactorial, but in all cases, a genetic abnormality (i.e. mutation) is a universal factor, and such abnormality is considered as a trigger for the initiation and progression of carcinogenesis (4). Accordingly, the plant extracts and their active gradients are investigated with respect to their role in protecting the genetic material, and equally important, their role in enhancing the DNA repair mechanisms (5). Such investigations have been fruitful in describing many anti-cancers medicines of plant origins (6). The *Alhagi graecorum* Boiss (Family: Papilionaceae) is a further plant of interest in researches of anti-mutagenicity and anti-carcinogenicity (7,8), and the chemical constituents of the plant may favour such interest. The chemical analysis of the plants have revealed compounds (flavonoids and flavonoid-derivatives, tannins, coumarin, vitamin C, essential oils and steroids) that are considered as anti-oxidants, anti-mutagens and anti-
carcinogens (9,10,11). In this light, the present study was designed with the aim to evaluate the role of *Alhagi graecorum* aqueous extract in modulating the genotoxic effects of mitomycin C (MMC) in albino male mice.

Materials and Methods

1.***Laboratory Animals***: Albino male mice (*Mus musculus*) were the investigated animals, which were 9-10 weeks old at the beginning of experiments. They had free excess to food and water (*ad libitum*).

2.Plant Extraction: A fresh plant was collected from open fields located 40 km north-west the capital Baghdad in April 2005. The plant was identified as *Alhagi graecorum* by Professor Ali Al-Mousawi (Department of Biology, College of Science, University of Baghdad). After washing the plant with distilled water, 50 grams of the dry plant was blended in 100 ml distilled water for 15 minutes. Then, the mixture was centrifuged (3000 rpm for 15 minutes), and the

supernatant was collected. By means of a rotary-evaporation (45°C), the deposit was collected and dissolved in distilled water to prepare the required doses (12).

3.Experimental Design: Three oral doses (0.5, 1.0 and 1.5 mg/kg) of the extract were tested through three types of treatments. In the first, the animals were given seven successive doses (single dose/day) of the extract, and then dissected in day 8. Such treatment was paralleled by negative (dosed with distilled water) and positive (dosed with MMC; 5 mg/kg) controls. In the second, the extract was given for six successive days, and in day 7, MMC was given (Pre-treatment). In the third, MMC was given in day 1, while in the next six days, the plant extract was given (Post-treatment). In both interactions, the animal dissection was carried in day 8. Also, the latter two treatments were paralleled by two corresponding controls, in which the plant extract was replaced by distilled water.

4.Laboratory Methods: Four parameters were evaluated; they were total leucocyte count (TLC), mitotic index (MI), chromosomal aberrations (CA) and micronucleus formation (MN). The TLC was carried out on a blood obtained from the tail by the conventional method of blood cell counting (13). The MI and CA were assessed in the bone marrow cells after injecting the animals with □olchicines (14). For MN formation assay, the bone marrow cells were also employed, but without injecting the animal with MMC (15).

5.Statistical Methods: The data were given in terms of means ± standard errors, and significant differences between means were assessed by Duncan test using the computer programme SAS.

Results and Discussion

In the first treatment, the MI was significantly increased in the doses 1.0 and 1.5 mg/kg (16.66 and 17.84%, respectively) as compared to either negative (13.32%) or positive (7.32%) controls, and such observation was positively correlated with the TLC. Moreover, CA and MN assays revealed that the extract has no mutagenic effects, in contrast, a reduction in the spontaneous frequency of CA and MN was observed, especially the MN formation, in which the reduction was around 50% in the three doses (Table 1). The results of second and third treatments (Pre- and Post-treatments) confirmed the forthcoming findings, and the extract was able to modulate the mutagenic effect of MMC, especially the dose 1.5

mg/kg, in which a significant enhancement of TLC and MI, and a significant reduction in the induced CAs and MN formation were observed Table (2). Both types of interactions with MMC rendered similar results, and no significant differences were recorded between Pre-treatment and Post-treatment with respect to the investigated parameters. Therefore, the present extract can be considered as desmutagen and bioantimutagen, and its antimutagenic effect is acting through two pathways. In the first, and as a desmutagen, the chemical compounds of the extract react with the mutagen or its metabolites, a mechanism that can modify the mutagenic properties of the mutagen. Otherwise, the extract can act on the body enzymes of detoxification. As bioantimutagen (the second pathway), the action is on DNA replication and repair mechanisms (16,17).

Such effects can be explained in the ground of chemical constituents of the plant. One of the important compounds are flavonoids, which are phenolic compounds that are easily dissolved in water, and are powerful anti-oxidants acting on the enzyme glutathione-S transferase. The later enzyme is a cellular factor that can counteract the effects of mutagenic and carcinogenic agents (11,18). Tanic acid (hydrolytic product of tannin) is a further compound that enhances the error-prone DNA repair mechanism (19). Other constituents also exist, and act in different pathways to protect the genetic make-up (20,21). Therefore, it is possible to add the present plant to the list of medicinal plants that have anti-mutagenic effects, but before reaching such substantial conclusion, further researches are certainly required, and other more advanced mutagenic assay must be applied.

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Table (1): Total leucocyte count, mitotic index, chromosomal aberrations and micronucleus formation in albino male mice treated with an aqueous extract of *Alhagi graecorum*, and two controls.

Groups		Mean ± Standard Error			
		TLC x 10^3 (cell/cu.mm. blood)	Mitotic Index (%)	Chromosomal Aberrations (%)	Micronucleus (%)
Negative controls		6.7 ± 0.3 a	13.2 ± 0.3 a	1.3 ± 0.3 a	0.62 ± 0.07 a
Positive controls		4.5 ± 0.3 b	7.3 ± 0.2 b	6.6 ± 0.2 b	13.4 ± 0.2 b
Alhagi graecorum extract	0.5 mg/kg	5.4 ± 0.2 a	13.6 ± 0.3 a	1.2 ± 0.1 a	0.38 ± 0.01 c
	1.0 mg/kg	6.7 ± 0.2 a	16.7 ± 0.1 c	1.4 ± 0.7 a	0.42 ± 0.07 c
	1.5 mg/kg	7.7 ± 0.1 c	17.8 ± 0.8 c	1.3 ± 0.5 a	0.39 ± 0.05 c

Different letters in the same column: significant difference ($P \leq 0.05$).

Table (2): The effect of *Alhagi graecorum* aqueous extract-mitomycin C interaction (pre- and post-treatments) on total leucocyte count, mitotic index, chromosomal aberrations and micronucleus formation in albino male mice.

Groups		Mean ± Standard Error			
		TLC x 10^3 (cell/cu.mm. blood)	Mitotic Index (%)	Chromosoml Aberrations (%)	Micronucleus (%)
H2O + MMC		4.4 ± 0.1 a	5.9 ± 0.4 a	6.1 ± 0.7 a	12.8 ± 0.7 a
Extract + MMC	0.5 mg/kg	5.0 ± 0.1 a	6.7 ± 0.3 a	2.6 ± 0.4 b	7.1 ± 0.5 b
	1.0 mg/kg	5.6 ± 0.1 ab	8.5 ± 0.4 b	2.5 ± 0.2 b	6.0 ± 0.4 b
	1.5 mg/kg	9.1 ± 0.1 c	10.5 ± 0.4 c	1.7 ± 0.2 c	4.5 ± 0.6 c
MMC + H2O		4.5 ± 0.2 a	5.8 ± 0.2 a	5.7 ± 0.2 a	11.8 ± 0.3 a
MMC + Extract	0.5 mg/kg	5.2 ± 0.2 a	7.9 ± 0.4 b	3.4 ± 0.2 d	6.1 ± 0.5 b
	1.0 mg/kg	7.6 ± 0.2 d	9.9 ± 0.3 c	2.5 ± 0.3 b	5.1 ± 0.6 b
	1.5 mg/kg	8.8 ± 0.1 cd	13.4 ± 0.2 d	1.5 ± 0.2 c	3.9 ± 0.4 c

Different letters in the same column: significant difference ($P \leq 0.05$).

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تعديل التأثيرات الوراثية لعقار المايتوسين سي باستعمال المستخلص المائي لنبات العاقول

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الخلاصة

قيم دور المستخلص المائي لنبات العاقول (0.5 ، 1.0 ، 1.5 ملغم / كغم) في تعديل التأثيرات الوراثية لعقار مايتومايسين سي في ذكور الفئران البيض من خلال ثلاثة انواع من المعاملات. ففي المعاملة الاولى اعطي المستخلص لوحده لدراسة تأثيره ، بينما في المعاملتين الثانية والثالثة ، فقد قيم التداخل ما بين المستخلص والعقار (اعطاء المستخلص قبل العقاروبعده) ومن خلال العد الكلي لخلايا الدم البيض ومعامل الانقسام والزيغ الكروموسومي وتكون النوي الصغيرة في المعامل الاولى ، اظهر معامل الانقسام زيادة معنوية في الجرعتين 1.0 و 1.5 ملغم / كغم (16.66 و 17.84 % على التوالي) مقارنة بالسيطرة السالبة (13.32 %) او السيطرة الموجبة (7.32 %). كذلك اظهرت النتائج أن المستخلص كان فعالاً في رفع قيم معدل عدد خلايا الدم البيض. وبما يتعلق بالزيغ الكروموسومي وتكون النوى الصغيرة فقد اظهرت النتائج عدم امتلاك المستخلص تأثيرات تطفيرية ، وعلى العكس من ذلك فقد سجلت النتائج انخفاضا" في التردد التلقائي لمعدل الزيغ الكروموسومي وتكون النوى الصغيرة ، لاسيما ان معدل تكون النوى الصغيرة والذي كان نسبة الانخفاض فية حوالي 50 % وللجرع الثلاث المستعلمة. كما اكدت نتائج المعاملتين الثانية والثالثة (قبل المطفر وبعد) خلو المستخلص من التأثيرات السمية الوراثية ، وان المستخلص اظهر قدرة في تعديل التأثيرات التطفيرية الوراثية لعقار المايتومايسين سي ، لاسيماعند الجرعة 1.5 ملغم / كغم ، إذ كانت هناك زيادة معنوية في معدل عدد خلايا الدم البيض ومعامل الانقسام وانخفاض معنوي في معدل الزيغ الكروموسومي وتكون النوى الصغيرة.