Evaluation of Some Hematological and Cytogenetic Effects of *Ammi majus* Seed Aqueous Extract in Albino Male Mice

تقييم بعض التاثيرات الدمية والوراثية الخلوية للمستخلص المائي لبذور نبات اظافر الجن (*Ammi majus*) في ذكور الفئران البيض

Rakad M. Kh. Al-Jumaily Ali H. Ad'hiah[*] Mohammed M. F. Al. Halbosiy[**] Baraa A. Abdul Hameed[**]

Department of Biology, College of Science, University of Baghdad
[*]Tropical-Biological Research Unit, College of Science, University of Baghdad.
[**]Biorechnology Research Center, Al-Nahrin University

ركاد محمد خماس الجميلي علي حسين أدحية[*] محمد محمود فرحان[**] براء عبد الهادي عبدالحميد[**]

قسم علوم الحياة/ كلية العلوم/ جامعة بغداد
[*]وحدة الأبحاث البايولوجية للمناطق الحارة/ كلية العلوم/جامعة بغداد
[**]مركز بحوث التقنيات الاحيائية/جامعة النهرين

Abstract

The present study was carried out with the aim to evaluate the hematological and cytogenetic effects of seed aqueous extract of the plant *Ammi majus* (0.5, 1.0, 1.5) mg/kg in albino male mice. The investigated parameters were total count of leucocytes (TLC), mitotic index (MI), micronucleus (MN) formation and chromosomal aberrations. The mitomycin C (MMC) was used as a mutagen in the interaction with the plant extract (pre- and post-treatment), with the aim to determine the antimutagenic efficiency of the plant extracts, and in all cases, the materials were given orally. In the first treatment, the results indicated that the dose 1.5 mg/kg of the extract enhanced the parameters investigated and a significant increase was observed in TLC (10070 cells/cu.mm.blood) as compared to negative (7290 cells/cu.mm.blood) or positive (4910 cells/cu.mm.blood) controls, and such observation was positively correlated with the mitotic index. In contrast, the spontaneous formation of MN was significantly decreased in the three investigated doses of the extract. In pre- and post-treatment experiments, a similar picture was drawn, and the plant extract was able to modulate the mutagenic effects of MMC.

المستخلص

اجريت الدراسة الحالية بهدف تقييم بعض التاثيرات الدمية والوراثية الخلوية للمستخلص المائي لنبات اظافر الجان (0.5 ، 1.0 ، 1.5) ملغم /كغم في ذكور الفار الابيض ومن خلال العد الكلي لخلايا الدم البيض ومعامل الانقسام وتكون النوى الصغيرة والزيغ الكروموسومي . لقد اختبرت فعالية المستخلص النباتي في منع او تعديل الفعل المطفر للمايتوسين سي ومن خلال التداخل مابين المستخلص النباتي والمطفر ومن خلال معاملتين (قبل وبعد المطفر) ، وفي جميع المراحل , كانت أعطاء الجرع عن طريق الفم . في المعاملة الاولى ، اظهرت الجرعة 1.5 ملغم/كغم من المستخلص النباتي تعزيز معنوي واضح لقيم المعاملات المدروسة وان العد الكلي لخلايا الدم البيض اظهر زيـادة معنوية 10070 خلية/ملم^3 دم مقارنة بالسيطرة السالبـة 7290 خلية/ ملم^3 دم او السيطرة الموجبة 4910خلية /ملم^3 دم . كذلك اظهرت الدراسة بأن المستخلص كان فعالا في رفع قيم معامل الانقسام ، وبالمقابل فان

الدراسة سجلت انخفاض في التردد التلقائي لمعدل تكون النوى الصغيرة وللجرع الثلاث. كما أكدت نتائج المعاملتين الثانية والثالثة (قبل وبعد المطفر) بأن المستخلص اظهر قدرة في تعديل التاثيرات المطفرة لعقار المايتومايسين سي .

Introduction

Plant extracts are always evoked the interest as sources of natural products, and this is reasoned by the fact they have different chemical compounds that make them of a medical importance [1]. These compounds are divided into alkaloids, tannins, carbohydrates, volatile oils, saponines, steroids and flavonoids, which are dependent on their chemical and physical characteristics [2]. Such chemical constituents have important biological potentials; for instance, antibacterial, antifungal, antiviral, antioxidant and antimutagenic properties [3]. With respect to the latter potential, it is important to note that carcinogenesis is preceded by mutagenesis, and therefore medicinal plants and/or plant products with antimutagenic properties can be considered as important anticarcinogens [4], especially if we consider that the etiology of cancer is multifactorial and 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 [5, 6]. Therefore, medicinal plants with these potentials may be able to prevent or modulate the effect of mutation, or to terminate the carcinogenesis of mutated cells, and consequently their target is mutagenesis, carcinogenesis or both [7].

One of these plants is *Ammi majus* (Umbelliferae family), which is also known as Bishop's weed and Blister weed [8]. Chemically, the plant is rich in furochromones (particularly khellin, visnagin, khellol and khellol glucoside), pyranocoumarins (particularly visnadin and samidin) and flavonoids (including quercetin and isohamnetin and their 3-sulfates) [9]. Such constituents may justify the folkloric medicinal applications of the plant in treating leprosy, kidney stones and urinary tract infections, furthermore, the plant has also been effective against some skin disorders such as psoriasis and vitiligo (acquired leukoderma) [10, 11].

Based on such findings, the present study was designed to evaluate the role of *A. majus* seed aqueous extract in modulating the genotoxic effects of mitomycin C (MMC) in albino male mice.

Materials and Methods

The dried seeds of *Ammi majus* were purchased from a local medicinal plant store in Baghdad. The seeds were powdered using a coffee grinder, and 50 grams of the seed powder was extracted with 100 ml of distilled water by the Soxhlet apparatus for three hours at 50ºC. The extract solution was centrifuged (1000 rpm for 15 minutes), and then the supernatant was collected and evaporated at 50ºC using a rotary evaporator 50 %. The resulted deposit was dissolved in distilled water to prepare three doses (0.5, 1.0 and 1.5 mg/kg), which were tested in albino male mice (*Mus musculus*) at age 8-9 weeks, and they had free access to food (standard pellets) and drinking water (*ad libitium*) during all experiments.

Three types of treatments were carried out. First, 0.25 of each dose was given to the animals for seven days (single dose/day) and in day 8, they were investigated. Such treatment was paralleled by negative (dosed with distilled water) and positive (dosed with MMC: 5 mg/kg) controls. Second, the extract was given for six successive days (single dose/day) and in day 7, MMC was given (pre-treatment). Third treatment, MMC was given in day 1, while the extract was given in the next six days (single dose/day) (post-treatment). The latter two experiments were paralleled with two controls, in which the plant extract was replaced by distilled water. In all cases, the materials were given orally, and in the first treatment, the number of mice was 25 (5 mice for each dose of plant extract and negative and positive controls), while in pre- or post-treatments**,** the number of animals was 20.

The animals were investigated for four parameters; total leucocyte count (TLC), mitotic index (MI), micronucleus (MN) formation and chromosomal aberrations (CAs).The TLC was carried out on peripheral blood that was obtained from the tail by the conventional method of blood cell counting [12]. For MI, the cells were obtained from the bone marrow of the animals, and at the same time the CAs were determined in 25 well-spread metaphases according to a previously established method [13]. For MN formation assay, the polychromatic erythrocytes of bone marrow were employed for such determination [14].

Data were presented in terms of means ± standard errors (S.E.), and significant differences between means were assessed by Duncan's test at a probability level of equal or less than 0.05, using the computer statistic programme SPSS (version 7.5).

Results

First treatment, the first two doses (0.5 and 1.0 mg/kg) of plant extract elevated the TLC (7220 and 8240 cells/cu.mm.blood) as compared to the negative (7290 cells/cu.mm.blood) or positive (4910 cells/cu.mm.blood) controls. However, the difference was significant ($p \leq 0.05$) when the comparison was made with the positive controls. The third dose (1.5 mg/kg) was more effective in increasing the TLC (10070 cells/cu.mm.blood), and the difference was significant as compared to either controls. A similar significant increase in the MI was observed in the dose 1.5 mg/kg (15.82%), in addition to the dose 1.0 mg/kg (14.02%), as compared to the negative and positive controls (11.6 and 8.04%, respectively). With respect to CAs, the negative control, as well as, the three doses of the plant extract shared similar means (1.3, 1.2, 1.4 and 1.3%, respectively), but they were significantly lower than the corresponding mean in the positive control (5.9%). In contrast, there was a significant reduction in the spontaneous formation of MN in mice treated with first two doses (0.32 and 0.34%, respectively) as compared to negative (0.50%) or positive (12.84%) controls Table (1)

Table (1): Total count of leucocytes, mitotic index, chromosomal aberrations and micronucleus formation in albino male mice treated with *Ammi majus* aqueous extract.

Groups		Mean ± Standard Error *			
		TLC x10^3 (cell/cu.mm. blood)	Mitotic Index (%)	Chromosomal Aberrations (%)	Micronucleus Index (%)
Negative Control		7290 ± 138 a	11.60 ± 0.26 a	1.30 ± 0.22 a	0.50 ± 0.07a
Positive Control		4910 ± 136 b	8.04 ± 0.07 b	5.90 ± 0.23 b	12.84 ± 0.49 b
Ammi	0.5 mg/kg	7220 ± 806 a	11.7 ± 0.68 a	1.20 ± 0.33 a	0.32 ± 0.01 c
majus	1.0 mg/kg	8240 ± 163 a	14.02 ± 0.53 c	1.40 ± 0.41 a	0.34 ± 0.04 c
Extract	1.5 mg/kg	10070 ± 472 c	15.82 ± 0.46 c	1.30 ± 0.71 a	0.71 ± 0.06 c

*** Different letters: Significant difference (P ≤ 0.05) between means of the same column.**

The results of second and third treatments (pre- and post-treatments) confirmed the forthcoming findings, and the extract was able to modulate the immunosuppressive and mutagenic effects of MMC, especially at 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).

Table (2): Total count of leucocytes, mitotic index, chromosomal aberrations and micronucleus formation in albino male mice after interaction between *Ammi majus* aqueous extract and mitomycin C.

Groups		Mean ± Standard Error *			
		TLC x10^3 (cell/ cu.mm. blood)	Mitotic Index (%)	Chromosomal Aberrations (%)	Micronucleus Index (%)
H_2O + MMC		4740 ± 128 a	5.60 ± 0.66 a	5.50 ± 0.24 a	12.38 ± 0.28 a
Extract	0.5 mg/kg	5490 ± 324 a	10.60 ± 0.28 b	3.80 ± 0.51 b	8.68 ± 0.22 b
+	1.0 mg/kg	5550 ± 317 a	9.56 ± 0.25 b	3.04 ± 0.45 b	5.64 ± 0.21 c
MMC	1.5 mg/kg	8030 ± 115 b	11.46 ± 0.67 b	2.60 ± 0.25 b	4.42 ± 0.28 c
MMC + H_2O		4580 ± 81 a	5.02 ± 0.24 a	5.80 ± 0.46 a	12.08 ± 0.47 a
MMC	0.5 mg/kg	5440 ± 269 a	8.44 ± 0.25 b	4.50 ± 0.24 a	8.80 ± 0.71 b
+	1.0 mg/kg	7660 ± 231 b	10.58 ± 0.28 b	3.20 ± 0.38 b	6.40 ± 0.23 c
Extract	1.5 mg/kg	8920 ± 159 b	11.68 ± 0.22 b	3.30 ± 0.19 b	4.60 ± 0.26 c

*** Different letters: Significant difference (P ≤ 0.05) between means of the same column.**

Discussion

The results of the first treatment showed that the TLC was increased in all groups of *A. majus* treated mice and such observation was positively correlated with the MI. The increase in TLC may be interpreted in term of a stimulated immune response. Such finding may be explained in the ground of some plant natural products that were absorbed in the intestine and entered the blood circulation, where they acted as hydrophilic anti-oxidants and increased tissue concentration of enzymes involved in free radical scavenging activity. Both consequences have the potential to enhance the immune functions [15], especially if we consider that the *A. majus* has been contraindicated in human Immune deficiency virus (HIV) infection and other autoimmune diseases [16].

The results also indicated that the plant extract has no cytotoxic or mutagenic effects, as judged by the findings of CA and MN assays in the three types of treatments. A support of such results has been presented by [17], who demonstrated that the plant is safe and has no genotoxic effects. Also, the results of pre- and post-treatments may qualify the present extract as a desmutagen and a bioantimutagen. As a desmutagen, the chemical constituents of the extract may be able to react with the mutagen or its metabolites, while as a bioantimutagen; the plant extract or its constituents may increase the error free repair fidelity in the cell or activate the promoters of DNA repair mechanisms [18, 19].

The chemistry of the plant natural products and their biological effects has been the potential of an intensive research, and therefore the observed effects can be explained in terms of the chemical constituents. In this regard, the coumarins are generally considered to be the most important element of *A. majus*, and recently it has been demonstrated that coumarins have antioxidant properties and exerted a reduced proliferative activity of cancer cell lines [20]. Additionally, coumarins have been shown to block tumor promotion [21]. Coumarins have also been found to induce glutathione-S-transferase (GST) activity in the fore stomach, liver and intestine of mice [22]. *A.majus* contains imperatorin, isoimperatorin and bergapten as a important component having the ability of antioxidant and prevention of carcinogenesis [10, 23]. Numerous other active compounds in *A.majus* have been and identified include xanthotoxin (methoxsalen, 8-methoxypsoralen(8-MOP) ammoidin), marmesin and heraclenin,all of them have antioxidant properties and also anti- carcinogenic [24,25]. Other constituents also exist, and may act in different pathways to protect the genetic make-up; for instance flavonoids [26].

In conclusion, it is possible to consider that the present plant is rich in constituents, which may have immunostimulant and antimutagenic activities. But it is too early to reach a final conclusion, because other biological properties must be studied to evaluate the pharmacological potentials and to understand the mechanisms by which this extract is acting.

References

1. Al-dhaher, Z.A. (2008). The antibacterial activity of aqueous extract of cinnamon and clove against *Staphylococcus aureus*. J.Al- Nahrain Uni. Sci., 11: 131-135.
2. Hussein, F.T.K. (1981). Agriculture and compostion of medicinal plants. Marse House for Publication Al-Reya.
3. Kujumgiev, A., Tsvetkova, I., Serkedjieva, Yu. Bankova, V., Christor, R. and Papov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin .J. Ethanopharmacol., 64: 235-240.
4. Kordali, S., kotan, R., Mavi, A., Cakir, A., Ala, A. and Yildirium, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oils of *Artemisia absinthium, Artemisia dracunculus , Artimisia santonium , and Artemisia spicigera*. J. Agric. Food chem., 53: 9452-9458.

5. Murakami, A., Ohigashi H. and Koshimizu, K. (1996). Anti-tumor promotion chemoprevention. Biosc. Biochemi., 60:1-8.

6. Ramel, C., Alkperov, U.K., Ames, B.N., Kado, T. and Wallenberg, L.W. (1986). Inhibitor of mutagenesis and their relevence to carcinogenesis.Mutation,Res.,168:47-65.

7. Mukherjes, A.K., Basu , S., Sarkar, N. and Ghosh, A.C. (2001). Advances in cancer therapy with plant based natural product. Current Medicinal Chemistry, 8:1467-1486.

8. Central Council for Research in Unani Medicine. (1987). Standardization of single drugs of Unani Medicine – Part I. New Delhi, Ministry of Health and family walfare.

9. Singab, A.N.B. (1998). Acetylated flavonol triglycosides from *Ammi majus* L. phytochemistry, 49: 2177-2180.

10. Ekiert, H. and Gomolka , E.(2000). Coumarin compounds in *Ammi majus* L. cellus cultures. Pharmazie, 55: 684-687.

11. Egyptian pharmacopoeia (1972). General Organization for Government Printing, Cairo, vol.2, ^{3d}Ed.

12. Sood , R. (1985) . Heamatology for student and practitioners. Laypee Brothers, India.

13. Stich, M. and San. C. (1981). Topics in environmental physiology and medicine in short-term tests and chemical carcinogens.Springer Vuglgare.New York.

14. Schmid, W. (1976). The cell micronucleus test for cytogenetic analysis. In: Hollaender, A. (Ed). Chemical Mutagens: Principles and Methods for their Detection, vol.4. Plenum Press, New York and London, pp.31:53.

15. Sun, F., Hayami, S., Haruna, S., Ogiri, Y., Tanaka, K. and Yamada, Y. (2000). In vivo antioxidative activety of propolis evaluated by the interaction with vitamins C and E and the level of lipid hydroperoxidase in rats. J. Agric. Food chem., 48: 1462-1465.

16. Wagner, H. and Wisenauer, M. (1995). Phytotherapie. Stuttgart, Gustar Fisher.

17. Mahmoud, I., Alkofahi, and Abelaziz, A. (1992). Mutagenic and toxic activities of several spices and some Jordanian medicinal plants. International J. pharmcognosy, 30: 81-85.

18. Bronzetti, G.(1997). The role of antimutagenesis and carcinogenesis. J. Environ. Patho. Toxical. Oncol. 16: 259-269.

19. Kuroda, Y. and Hara, Y. (1999). Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat. Res., 436: 69-97.

20. Lee, K. T., Sohn, H, C., Park, H.J., Kim, D.W., Jung, G.O. and Park, K.Y. (2000). Essential moiety for antimutagenic and cytotoxic activity of hederqenin monodemosides and bidesmosides isolated from the stem bark of *Kalapanox pictus*. Planta Med., 66: 329-332.

21. Sparnins, V.L., Venegas, P.L. and Wattenberg,L.W. (1982)Glutathione- S- transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. J. Natl. Cancer Inst., 68: 493-496.

22. Okuyama,T., Takata, M., Nishino, A., Takayasu, J. and Iwashima, A. (1990). Studies on the antitumor-promoting activity of naturally occurring substances. II. Inhibition of

tumor-enhanced phospholipid metabolism by umbelliferous materials. Chem. Pharm. Bull., 38: 1084-1086.

23. Kally,V.P.,Ellis, E.M., Manson,M.M., Chanas,S.A., Moffat, G.J., Mcleod, R., JUdah, D.J., Neal, G.E. and Hayes, J.D. (2000). Chemoprevention of aflatoxin B1 hepatocarcinogenesis by coumarin, a natural benzopyrone that is apotent inducer of aflatoxin B1-aldehyde reductase,the glulathione S-transferase A5 and P1 subunits and NAD(P)H:quinine oxidoreducatase in rat liver.Cancer Res.,60,957-969.

24. FouinFortunet,H,.Tinel,M.,Descatoire,V.,Latteron, P.,Larrey, D.,Geneve, J. and Pessayer,D. (1986). Iactivation of cytochrome P450 by the drug methoxsalen .J.Pharmacol.Exp.Ther.,236 ,237-247.

25. Larbat,R.,Kellner,S.,Specker,S.,Hehn,A Gontier, E.,Hans, J., Bonrgand, F.and Matern, U. (2007). Molecular cloning and functional characterization of psoralen synthase, the first committed monooxygenase of furanocoumarin biosynthesis. J.Biol. Chem., Vol.282 (1),542-554.

26. Eaton, E.A. (1996). Flavonoids, potent inhibitors of the human from phenol sulfotransferase: potential role in drug metabolism and chemoprevention. Drug Metab. Dispose., 24: 232-237.