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Clastogenic Effect of Azadirachtin of Neemix-4.5 On SWR/J Mouse Bone Marrow Cells

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Abstract

The clastogenic effect of azadirachtin of neemix-4.5 was investigated in SWR/J mouse bone marrow cells. Males and females, 5 each per treatment time, aged 10–12 weeks and weighing 31.7–33.8 g, were orally administrated 9.0 mg/kg (1/10 LD_{50}) of azadirachtin solution. A control group (5 males and 5 females) received only sterile distilled water. The animals were sacrificed 6, 12, 24, 48 or 72 h post- treatment. The chromosome preparations were obtained from bone marrow cells. Chromatid and chromosomal aberrations were investigated in 50 metaphases per animal.

No significant differences in the frequency of chromosomal aberrations or in the percentage of mitotic index were observed between the treated male and female mice at any time intervals used. Hence, data from the two sexes were pooled when analyzed statistically. In the prersent study, the dose level 9.0 mg/kg body weight of azadirachtin of neemix-4.5 did not induce any significant ($p>0.05$) changes in the percentages of mitotic indices or in chromosomal aberrations in the bone marrow cells of treated animals at all time intervals tested compared with the control group. As the pharmacokinetics of azadirachtin is unknown, the essentially negative results in the present study may be due to a lack of genotoxic potential .

Key Words : Neemix-4.5, Azadirachtin, Clastogenic effect, Bone Marrow Cells, Mice

Introduction

Potential problems associated with continued long-term use of toxic insecticides including pest resistance and negative impact on natural enemies (Magaro and Edelson, 1990; Liang *et al.*, 2003). Moreover, increasing documentation of negative environmental and health impact of synthetic insecticides and increasing stringent government regulation of pesticides has resulted in renewed interests in the development and use of botanical pest management products (Ascher, 1993; Liang *et al.*, 2003).

Neem-based insecticides containing azadirachtin that was derived from extracts of neem tree, *Azadirachta indica*, have played an important role in crop protection (Liang *et al.*, 2003). Azadirachtin proved to be the dominating and most effective among several related limonoids (Ley *et al.*, 1993 ; Adel and Sehnal, 2000). The use of azadirachtin as an insecticide is well documented (Rembold *et al.*, 1989 ; Schmutterer, 1995 ; Kreutweiser *et al.*, 2002), and it is known to affect feeding, growth, metamorphosis and reproduction in insects. However, little is known about its toxicity, mutagenicity and clastogenicity in mammalian species (Jongen and Koeman, 1983; Stewart, 1998; Akudugu *et al.*, 2001; Awasthy, 2001; Salehzadeh *et al.*, 2003). Hence, the aim of the present study was to

investigate the possible clastogenic effect of the dose level 9.0 mg/kg (1/10 LD_{50}) of azadirachtin of neemix-4.5 on SWR/J mouce bone marrow cells .

Materials and Methods

Inbred SWR/J male and female mice , 10 – 12 weeks old and weighing 31.7 – 33.8 g , were used throughout the study . Animals were kept and bred in an environmentally controlled room with a temperature of 22±1 °C, a relative humidity of 45±5 % and a light/dark cycle of 10/14 h. Mouse Food (Commercially available in Saudi Arabia) and water were offered *ad libitum*. A total of 30 males and 30 females were used and were divided into 6 groups, each group contained 5 males and 5 females. Animals of groups 1-5 were orally treated with a single dose level 9.0 mg/kg body weight of zadirachtin of neemix-4.5 (Thermo Trilogy Corp., USA) dissolved in sterile distilled water. Animals of group 6 were similary treated with the corresponding volumes of the vehicle alone and served as control. The animals were killed by cervical dislocation 6, 12, 24, 48 or 72h following the treatment and the clastogenic effect of the insecticide on those animals using *in vivo* bone marrow cells, at the intervals mentioned , was evaluated.

The method of Preston *et al*. (1987) and Al-Hawary and Al-Saleh (1989) were used for chromosomal preparations. A minimum of 10 slides were prepared and 50 well spread and distinctly identifiable metaphases from each mouse were selected. Each selected metaphase was examined using the X100 oil immersion objective of Zeiss microscope for detecting possible chromosomal aberrations. Prior to scoring the insecticide effect on the chromosomes, the slides were covered and coded. The chromosomal aberrations scanned were : chromatid gaps (G), chromatid breaks (B), fragments (F), deletion (D) and aneuploidy ($2N^-/2N^+$). The gap was scored as a complete discontinuity narrower than the width of a chromatid according to the criterion of Matsuoka *et al.* (1979). Photomicrographs of selected metaphases were taken under bright illumination, using X100 oil immersion objective and X10 eyepiece.

Statistical analysis :

The data obtained were statistically analyzed using a 2x2 contingency table (X^2) for the actual numbers obtained (Sokal and Rohlf, 1981).

Results

In the present work, no significant differences in the percentages of mitotic indices or chromosomal aberrations were observed between azadirachtin of neemix-4.5–treated male and female SWR/J mice at any time intervals used. Accordingly, the data obtained from the two sexes were pooled together and statistically analyzed.

The dose level 9.0 mg/kg body weight (1/10 LD_{50}) of azadirachtin of neemix-4.5 did not induce any significant ($p>0.05$) changes in the percentages of mitotic indices or chromosomal aberrations in the bone marrow cells of treated animals at all time intervals used compared with the control group (Tables 1-2 , Plates 1-2).

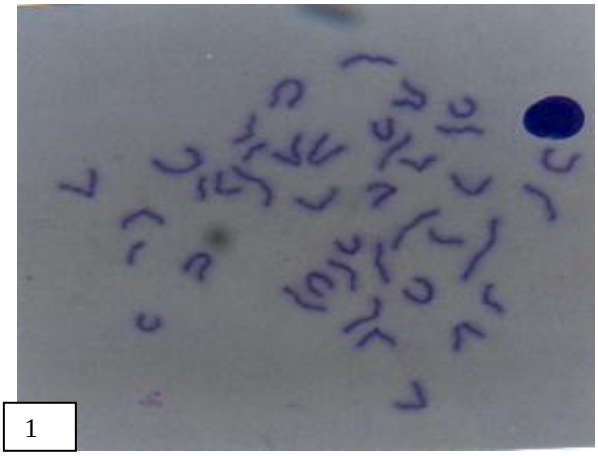
Table 1. Effect of the dose level 9.0 mg/kg (1/10 LD_{50}) of azadirachtin of neemix-4.5 on themitotic indices in bone marrow cells of SWR/J mice at various time intervals following the treatment.

Group (hour)	No. of animals used	No. of cells analyzed	No. of dividing cells	Mitotic index (%)
Control	10	10000	402	4.02
6	10	10000	393	3.93
12	10	10000	385	3.85
24	10	10000	396	3.96
48	10	10000	389	3.89
72	10	10000	392	3.92

Table 2. Effect of the dose level 9.0 mg/kg (1/10 LD_{50}) of azadirachtin of neemix-4.5 on the chromosomes of bone marrow cells of the SWR/J mice at various time intervals following the treatment.

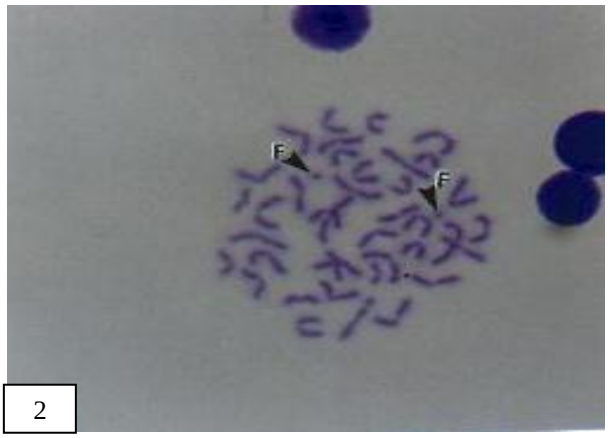
Group (hour)	No. of animals used	No. of cells analyzed	No. of abnormal cells	$2N^-/2N^+$ (%)	Aberration/500 cells*				% abnormalities	
					G	B	F	D	with Gap (G)	without gap
Control	10	500	9	4 (0.80)	5	2	2	-	1.80	0.80
6	10	500	16	3 (0.60)	9	2	5	1	3.20	1.40
12	10	500	19	4 (0.80)	11	3	4	2	3.80	1.60
24	10	500	15	5 (1.00)	7	2	4	2	3.00	1.60
48	10	500	14	2 (0.40)	6	4	3	2	2.80	1.60
72	10	500	12	3 (0.60)	7	2	4	-	2.40	1.00

* G = Chromatid gap , B = Chromatid break , F = Fragment , D = Deletion



1

Plate 1:- Photomicrograph of a normal metaphase stage of bone marrow cell of an adult mouse (control). Geimsa stain. X 4500



2

Plate 2:- Photomicrograph of abnormal metaphase stage of bone marrow cell of an adult mouse treated with 9.0 mg/kg of azadirachtin for 24 h showing fragments (F). Geimsa stain. X 4500 .

Discussion

The present results have clearly demonstrated that the oral administration of the dose level 9.0 mg/kg body weight of azadirachtin of neemix-4.5 into male and female SWR/J mice did not show any significant mitotic depression, as evaluated by the percentages of mitotic indices, or clastogenic effect, as evaluated by the percentages of chromosomal aberrations, in the bone marrow cells of treated mice compared with their control group.

Neem seed oil showed no mutagenic effect in the *Salmonella typhimurium* stains TA98 and TA100 (Jongen and Koeman, 1983). Likewise, Nimbolide and nimbic acid, two components of neem oil, also failed to cause mutagenicity by Ames test using two *S. typhimurium* tester stains (Uwaifo, 1984). Moreover, Stewart (1998) reported that NeemAzal Technical and NeemAzal 1% showed no mutagenic activity in rats using *in vitro* and *in vivo* techniques. However, a crude ethanolic extract of neem leaves was reported to cause clastogenic and aneugenic effect *in vivo*. This was evidenced by a dose-related increase in long-lasting structural chromosomal aberrations and disruption of mitosis in bone marrow cells of mice which had been administered the test material at high dose levels of 500, 1000 and 2000 mg/kg body weight/day for 7 days by oral route (Awasthy *et al.*, 1999). Furthermore, the oral administration of soxhlated crude ethanolic extract of leaves of neem to adult male mice for 6 weeks at dose rates 0.5,

1.0 and 2g/kg body weight/day increased the incidences of structural changes and synaptic-disturbances in meiotic chromosomes and also caused more disruptions of meiosis (Awasthy, 2001) .

Al ot of histological, histochemical and embryonic malformations studies are needed to detected side effects of Azadirachtin of neemix .The discrepancy between the results of the present study and those of Awasthy *et al.*, (1999; 2001) may be due to the differences in the tested doses and in the duration of the treatment periods. As the pharmacokinetics of azadirachtin is unknown, the essentially negative results in the present study may be due to the lack of genotoxic potential of azadirachtin (Srivastava and Raizada, 2001).

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التأثير المحث لتكسر الكرموسومات لأزاديراكتين النيمكس - 4.5 على خلايا نخاع عظام فئران السلالة SWR/J

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تم في هذا البحث دراسة التأثير المحث لتكسر الكرموسومات لأزاديراكتين النيمكس - 4.5 وذلك في خلايا نخاع عظام السلالة النقية SWR/J من الفئران المختبرية . استخدمت في هذه الدراسة ست مجموعات اشتمل كل منها على خمسة ذكور وخمس إناث بلغت أعمارها ما بين 12-10 أسبوعاً وأوزانها ما بين 31.7-33.8 جم . عوملت الحيوانات بالجرعة 9.0 مجم/كجم من وزن الجسم (وتمثل 1/10 LD_{50}) من محلول أزاديراكتين النيمكس - 4.5 عن طريق الفم ، كما عوملت المجموعة الضابطة بنفس الطريقة وبأحجام مماثلة من الماء المقطر فقط . تم قتل الحيوانات عن طريق فصل العنق عن بقية الجسم بعد 6 ، 12 ، 24 ، 48 أو 72 ساعة من المعاملة . ولقد تم الحصول على تحضيرات الكرموسومات من خلايا نخاع العظام . كما تميت دراسة العيوب الكرموسومية في 50 مرحلة استوائية جيدة الفرد وواضحة لكل حيوان .

أوضحت نتائج هذه الدراسة عدم وجود فروق ذات دلالة معنوية في النسب المئوية لكل من المؤشرات الميتوزية والعيوب الكرموسومية بين الذكور والإناث ، ولذا فقد تم ضم نتائج الجنسين معاً عند تحليلها إحصائياً . ولقد أظهرت نتائج هذه الدراسة ، أيضاً ، أن الجرعة 9.0 مجم/كجم من أزاديراكتين النيمكس - 4.5 لم تستحث أية تغيرات معنوية في النسب المئوية للمؤشرات الميتوزية أو العيوب الكرموسومية لخلايا نخاع عظام الحيوانات المعاملة مقارنة بالمجموعة الضابطة عند كل الفترات التي تم فحصها في هذه الدراسة .

وحيث إن الحركة الدوائية للأزاديراكتين غير معروفة ، فإن النتائج السلبية المتحصل عليها في هذه الدراسة قد تعود إلى فقد هذا المركب للقدرة السمية الوراثية .
ويوصى الباحثون بإجراء المزيد من الدراسات الهستولوجية والهستوكيميائية لتوضيح أثر المبيد لمعرفة مدى استخدامة على نطاق واسع خاصة انة بداخل النبات.