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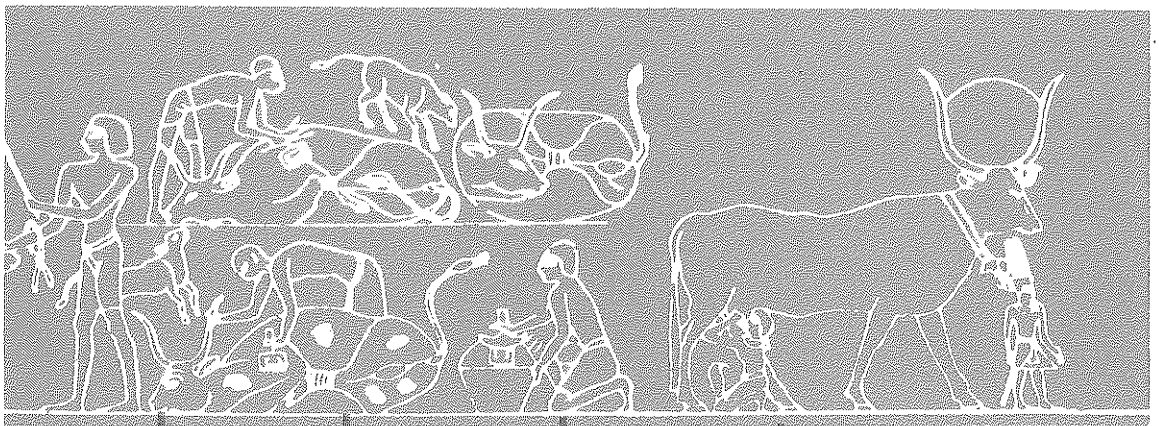

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PROTECTIVE ROLE OF ROYAL JELLY ON YELLOWISH BROWN INDUCED GENOTOXIC EFFECTS ON MALE MICE

By

NADA, H.ALTWATY

Department of biology, King Abdelaziz University

SUMMARY

Yellowish brown a food color additive was tested for its clastogenic effects on mammalian system.

Chromosomal aberrations were used for evaluation the mutagenicity of the food colors in yellowish brown.

Male mice Mus musculus, were orally administrated with yellowish brown concentrations, 4 mg/kg b.w. Bone marrow and testicles samples were taken 5, 10 and 15 days.

Yellowish brown significantly increased chromosomal aberrations, Royal jelly decreased the percentage of the induced chromosomal aberrations in bone marrow and germ cells after oral treatment.

Key words: Food colorants, yellowish brown, Royal jelly, Mutagenicity, Antimutagenicity, Chromosomal aberrations, Bone marrow.

INTRODUCTION

Yellowish brown is one of many food colorants used in food industry, especially foods. Many of these agents has side effects such as urticaria **(Chaffe and Settipane 1967),** behavioral disorders in children, **(Pollock and Warner 1990)**, endocrinal disturbance **(Jennings et. al. 1990)**, renal and hepatological changes and genotoxicity, clastogenic and carcinogenic effects **(Combes and Haveland-Smith (1982).**

The importance of the assessment of the effect of colorants on metabolism and upon the genetic make up of mankind is obviously a critical issue in the use of these chemicals. An increasing number of synthetic dyes, which are used as food and drug colorants over long periods, are shown to exhibit genotoxic effects. The human exposures to such agents are very wide, and feeding over long period is likely. Therefore, they possessed potential hazards to the human health. **Haveland and Combes (1980 and 1982),** studied the genetic effects of six major classes of synthetic food colors (anthraquinone, azo dyes, pyrollozone, indigoid, triphenyl methan and xanthane). **Abdel Aziz et al., (1989)**

studied the cytogenetic effects of sun set yellow (FCF) on the oogenesis of mice. **Agrawal et al. (1994)** studied the effect of safflower yellow and kokum red (two natural food colorants) on male mice after interpertoneally injection. In an investigation of the ***in vivo*** clastogenic potential of the food coloring erythrosine (ER), male mice were treated interperitoneally and three cytogenetic endpoints (SCEs, peripheral blood lymphocytes and micro nuclei of bone marrow) were analyzed by **Roychoudhury and Giri (1989)** they tested the effect of 4 permitted food days (Indigo Carmine, Orange G, fast green and Tartrazine) on chromosomes of Allium cepa. **Giri et al. (1988)** evaluated with ***in vivo*** cytogenetic assays in male mice the genotoxicity of the food colorant Grange G. SCEs induced by the food colorant Metanil yellow in mice were studied by **Giri et al. (1986).** All those studies showed changes in cytogentic assays relative to control animals.

Royal jelly is a thick food secreated by young nursing workers of bee. Its organic acid has bacteriostatic activity. **Bonvehi and Jorda(1991)**, reported that royal jelly is a potential antiatherosclerotic agent capable of improving the nicotine induced atherogenic lipoprotein profile. Royal jelly at about 50 - 100 mg /day decreased total serum lipids by about 10 % in a group of patients **(Vittek 1995). Sver et al. (1996)** reported the immune modulator potential of Royal jelly in rats and mice.

The aim of the present study is to study the effect of yellowish brown on bone marrow and germ cells of male mice as well as the protective effect of Royal jelly.

Materials and methods

Animals

Male mice ***Mus musculus***, weighting 20 - 25 gm were used in these studies.

Food color

The synthetic color additives that gave the desired shade of chocolate brown from the commercial Egyptian market were employed, named yellowish brown.

Class: Monoazo and triarylmethane compound

Shade: yellowish brown

Composition: composed of 4 compounds:

1- Sunset yellow (organ red) (color index No. 15985)

2- Tartrazine (yellow) (color index No. 19140)

3- Carmoisine (pink) (color index No. 14720)

4- Brilliant blue (blue) (color index No. 42900)

Treatment and methodology

The method of **Yosida and Amano (1975)** for chromosomal preparations was used.
For cytogenetic studies, animals were divided into 8 groups (each of 5 mice). The first group was kept as a control and the other groups were orally ingested subacute (Yellowish brown) for 5, 10 and 15 days yellowish brown (4 mg/kg b.w.) and Royal jelly (1 mg/kg b.w.) and their mixture. The animals were sacrificed after the last dose.
The protective Royal jelly was obtained from a beekeeper. The tested concentration (1 mg/kg b.w.) was dissolved in distilled water and given orally to animals.

RESULT AND DISCUSSION

The result of cytological examination of bone marrow and spermatocyte cells of mice treated orally with yellowish brown (4mg/kg b.w), (Royal jelly) and their mixture are listed in tables (1 and 2).

The structural aberrations induced in both types of cells were highly significant increase ($P< 0.05$) in the case of the synthetic food colorant (yellowish brown) and highly significant decrease in the mixture of the (yellowish brown and Royal jelly). They were represented by deletions, centromeric attenuations, gabs and fragments.

The frequencies of total aberrations were represented in table (1), and expressed as Mean ± S. E. All mice treated with yellowish brown doses for 15 days showed highly significant increase ($P<0.05$) in total aberrations while the mixture of the yellowish brown and Royal Jelly decrease ($P<0.05$) in the total aberrations which still in the significant level in treated animal for 15 days .Studies of numerical aberrations showed no significant level (Table 1,2) .Figures, (1, 2, 3, 4) are showing chromosomal aberrations of bone marrow cells.

As the results showed, synthetic food colorant yellowish brown caused a significant increase in chromosome aberrations in both bone marrow and spermatocyte cells.
Subacute treatment caused high a percentage of cells with aberrations in yellowish brown treated animals ($P<0.05$) at dose (4mg/kg b.w) used for 15 days. There is a highly significant decrease in those treated with the mixture (yellowish brown and Royal Jelly). The aberrations were presented by autosomal, X-Y univalent, chain and

polyploidy. Figures. (5, 6, 7, 8) are showing chromosomal aberration of spermatocyte type

Other similar results were obtained when the synthetic dyes were tested as a mutagenic agent. **El Ashmawy and Abd el Aziz (1989)** found that carmosine and amaranth induced chromosomal changes in bone marrow and spermatocyte cells of mice.

Also tartrazine (a synthetic azo-dye) was found to give high percentage of abnormal bone marrow cells, increase in the percentage of polychromatic erythrocytes indicating bone marrow toxicity **(Abdel-Aziz and El-Ashmawy 1993; Patterson and Butler 1982; Menoret 1982 and Meyer and Hansen, 1975)**. Brillrant black, brilliant blue, erythrosine and indigo carmine are four synthetic colors showed highly mutagenic effect on mice **(Osman et al., 1995).**

Abdel-Aziz and El-Ashmawy (1993) studied the effect of tartrazine on mitotic activity of cells and observed depression of the number of dividing cells which was significant after 14 days and highly significant after 21 days of treatment.

Giri et al., (1990), reported that tartrazine showed a significant increase in chromosomal aberrations at some of the higher concentrations of the dye.

It is obvious from the presented results that the percentage of chromosome abnormalities in somatic cells is higher than that in germ cells. Similar results were reported earlier **(Russell, 1978; and Abdel Aziz 1993).**

From the present results, it is concluded that yellowish brown has a mutagenic effects on mammalian somatic and germ cells.

Royal jelly decreased the percentage of total chromosomal aberrations induced by yellowish brown.

Royal jelly has been analyzed into fractions by **Young and Cho, (1977).**The first fraction consists of organic acid (phenolic material and beeswax) sterol, phospholipids and saponifiable substance. The second fraction is rich with sugar, unidentified acid, inorganic salt and nitrogen compound. The third fraction is protein in nature, including amino acid aspartic acid,arginine, thyrosine, tryptophan and histidine.The first fraction contains the physiologically active material responsible for sexual development of the queenbees. It contains phenolic,materials that may be responsible for the antimutagenic potential of royal jelly observed in the present studies .It may be mentioned in this connection that ,naturally occurring plant phenols has substantial antimutagenic and anticarcinoogenic effects against a variety of polycyclic aromatic hydrocarbons.

Table 1: Chromosomal aberrations in bone marrow cells of mice treated with the yellowish brown (Synthetic dyes) and Royal Jelly (Protective).

Treatment dose/time/day	No. of animals	No. of examined	Structural aberrations						Normal cells m±S.E	Numerical aberration		Total aberration numerica m±S.E
			Gap m±S.E	Deletions m±S.E	Fragment m±S.E	Centromeric attrenuation m±S.E	Poly ploidy m±S.E	Total aberration m±S.E		Trisomic 2n+1 m±S.E	Monosomic 2n-1 m±S.E	
Control	5	250	0.4 ± 0.24^{B}	0.60 ± 0.24^{E}	0.40 ± 0.24^{C}	5.60 ± 0.40^{C}	0.00 ± 0.00^{B}	2.00 ± 0.70^{D}	38.00±9.51	0.20 ± 0.20^{C}	0.00 ± 0.00^{B}	0.20 ± 0.20^{B}
Yellowish brown 4mg/kgb.w 5days	5	250	1.00 ± 0.44^{B}	3.00 ± 0.70^{CD}	1.80 ± 0.48^{BC}	2.40 ± 0.60^{BC}	0.60 ± 0.24^{B}	8.80 ± 1.95^{BC}	41.20±1.95	0.60 ± 0.24^{BC}	0.00 ± 0.00^{B}	0.60 ± 0.24^{B}
Yellowish brown 4mg/kgb.w 10days	5	250	1.40 ± 0.74^{AB}	5.60 ± 0.40^{B}	3.00 ± 1.22^{AB}	3.60 ± 0.74^{B}	1.80 ± 0.37^{B}	15.40 ± 3.07^{B}	43.60±3.07	1.00 ± 0.31^{AB}	0.20 ± 0.20^{AB}	1.20 ± 0.48^{A}
Yellowish brown 4mg/kgb.w 15days	5	250	2.60 ± 0.50^{A}	8.80 ± 0.58^{A}	4.60 ± 1.20^{A}	5.80 ± 0.73^{A}	3.80 ± 0.20^{A}	25.60 ± 2.50^{A}	24.40±2.50	1.40 ± 0.24^{A}	0.60 ± 0.24^{A}	2.00 ± 0.44^{A}
Yellowish brown+Royal jelly 4mg/kgb.w 5days	5	250	0.40 ± 0.24^{B}	1.00 ± 0.63^{DE}	0.80 ± 0.58^{BC}	1.20 ± 0.58^{C}	0.00 ± 0.00^{B}	3.40 ± 0.97^{CD}	36.60±9.16	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}
Yellowish brown+Royal jelly 4mg/kgb.w 10days	5	250	0.60 ± 0.24^{B}	2.20 ± 0.66^{CDE}	1.00 ± 0.63^{BC}	1.40 ± 0.60^{BC}	0.80 ± 0.48^{B}	6.00 ± 2.28^{CD}	34.00±8.67	0.40 ± 0.24^{BC}	0.00 ± 0.00^{B}	0.40 ± 0.24^{B}
Yellowish brown+Royal jelly 4mg/kgb.w 15days	5	250	1.20 ± 0.58^{AB}	3.20 ± 1.15^{C}	1.80 ± 0.48^{BC}	$2.40\pm1.12m^{BC}$	1.60 ± 0.67^{B}	10.20 ± 3.63^{BC}	29.80±7.88	0.60 ± 0.24^{BC}	0.20 ± 0.20^{AB}	0.80 ± 0.37^{B}

Mean the different letters (A, B, C, D, E) in the same column are significantly different at level ($P<0.05$).

Table: 2 Chromosomal aberrations in spermatocyte cells of mice treated with the yellowish brown (Synthetic dyes) and Royal Jelly (Protective).

Treatment dose/time/day	No. of animals	No. of examined	Structural aberrations					Normal cells m±S.E	Numerical aberration		Total aberrations numerical m±S.E
			Chain m±S.E	Autosomal m±S.E	X-Y m±S.E	Poly ploidy m±S.E	Total aberration m±S.E		Trisomic 2n+1 m±S.E	Monosomic 2n-1 m±S.E	
Control	5	250	0.00±0.00^{C}	0.40±0.24^{D}	0.60±0.24^{D}	0.00±0.00^{C}	1.00±0.44^{D}	29.00±11.84	0.00±0.00^{B}	0.00±0.00^{B}	0.00±0.00^{B}
Yellowish brown 4mg/kgb.w 5days	5	250	1.60±0.50^{BC}	3.40±0.92^{BC}	3.80±0.58^{BC}	1.40±0.40^{BC}	10.20±2.05^{BC}	39.80±2.05	0.20±0.20^{B}	0.00±0.00^{B}	0.20±0.20^{B}
Yellowish brown 4mg/kgb.w 10days	5	250	2.00±0.70^{AB}	4.60±0.40^{AB}	5.20±1.15^{AB}	2.40±0.67^{B}	14.20±2.39^{B}	35.80±2.39	0.60±0.24^{AB}	0.00±0.00^{B}	0.60±0.24^{B}
Yellowish brown 4mg/kgb.w 15days	5	250	3.40±0.67^{A}	6.40±0.67^{A}	7.60±1.12^{A}	4.00±0.83^{A}	21.40±1.72^{A}	28.60±1.72	1.00±0.44^{A}	0.40±0.24^{A}	1.40±0.60^{A}
Yellowish brown+Royal jelly 4mg/kgb.w 5days	5	250	0.60±0.40^{BC}	1.40±0.60^{CD}	1.80±0.37^{CD}	0.40±0.58^{C}	4.20±1.28^{CD}	54.80±1.28	0.00±0.00^{B}	0.00±0.00^{B}	0.00±0.00^{B}
Yellowish brown+Royal jelly 4mg/kgb.w 10days	5	250	0.80±0.37^{BC}	1.80±0.80^{CD}	2.40±0.81^{CD}	0.80±0.58^{BC}	5.80±2.28^{CD}	34.20±8.73	0.00±0.00^{B}	0.00±0.00^{B}	0.00±0.00^{B}
Yellowish brown+Royal jelly 4mg/kgb.w 15days	5	250	1.20±0.58^{BC}	2.80±0.86^{BC}	3.60±1.12^{BC}	1.40±0.60^{BC}	9.00±2.84^{BC}	31.00±7.94	0.40±0.24^{AB}	0.00±0.00^{B}	0.40±0.24^{B}

Mean the different letters (A, B, C, D, E) in the same column are significantly different at level ($P<0.05$).

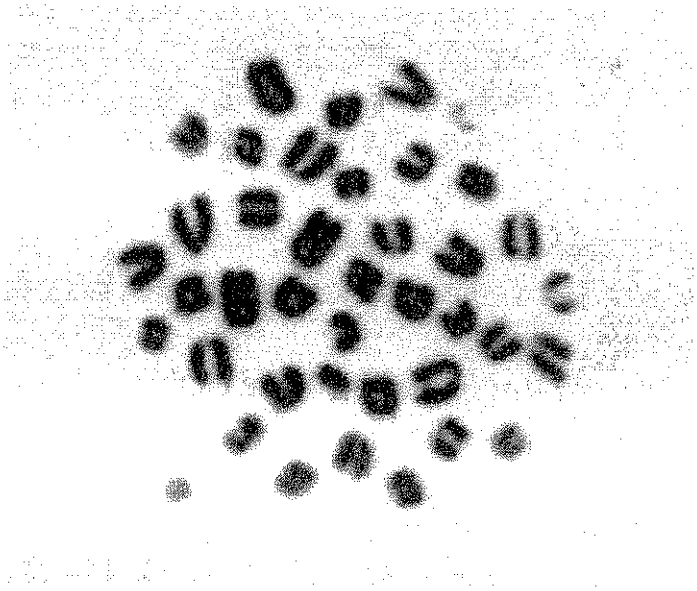

Fig. (1): A normal metaphase spread from bone marrow of control male mice photographed on (Giemsa stain, X1000).

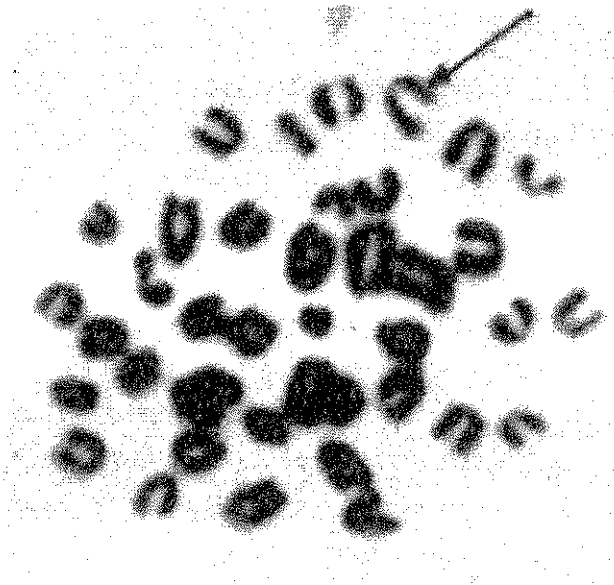

Fig. (2): Metaphase spread from bone marrow of treated male mice showing chromatid deletion type of chromosomal aberrations (Giemsa stain, X1000).

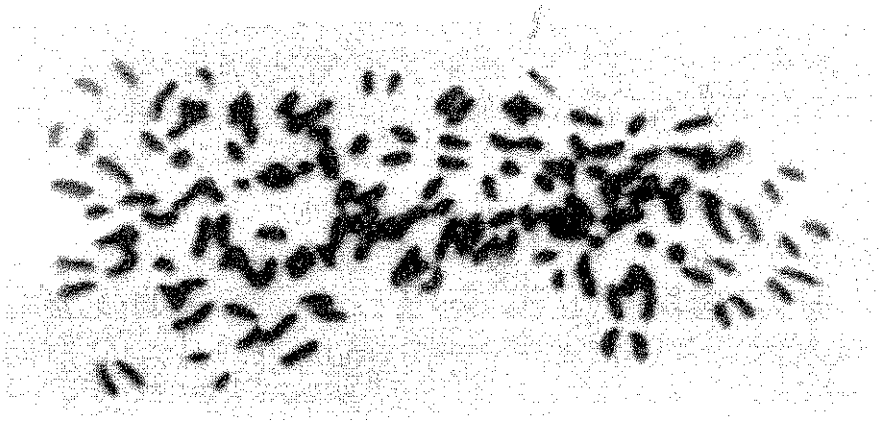

Fig. (3): Metaphase spread from bone marrow of treated male mice showing centromeric attenuation type of chromosomal aberrations (Giemsa stain, X1000).

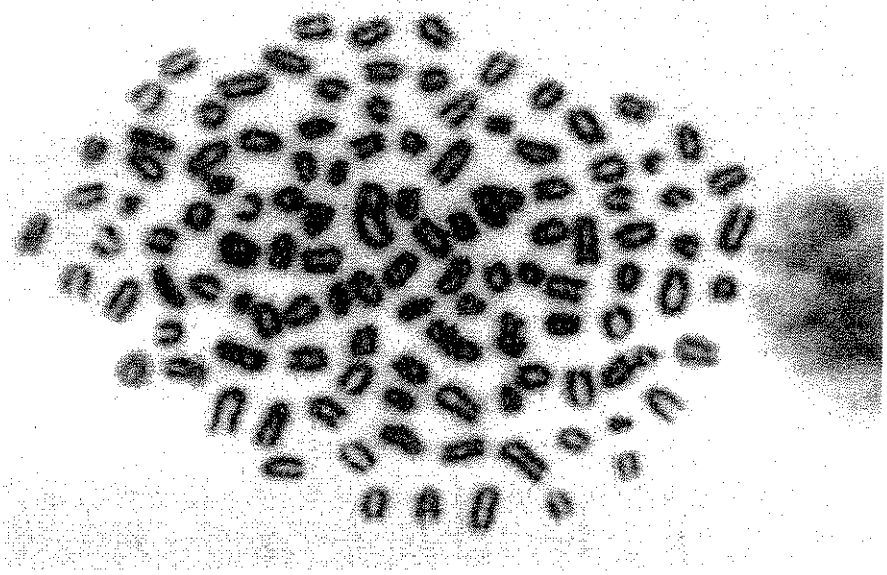

Fig. (4): Metaphase spread from bone marrow of treated male mice showing polyploid type of chromosomal aberrations (Giemsa stain, X1000).

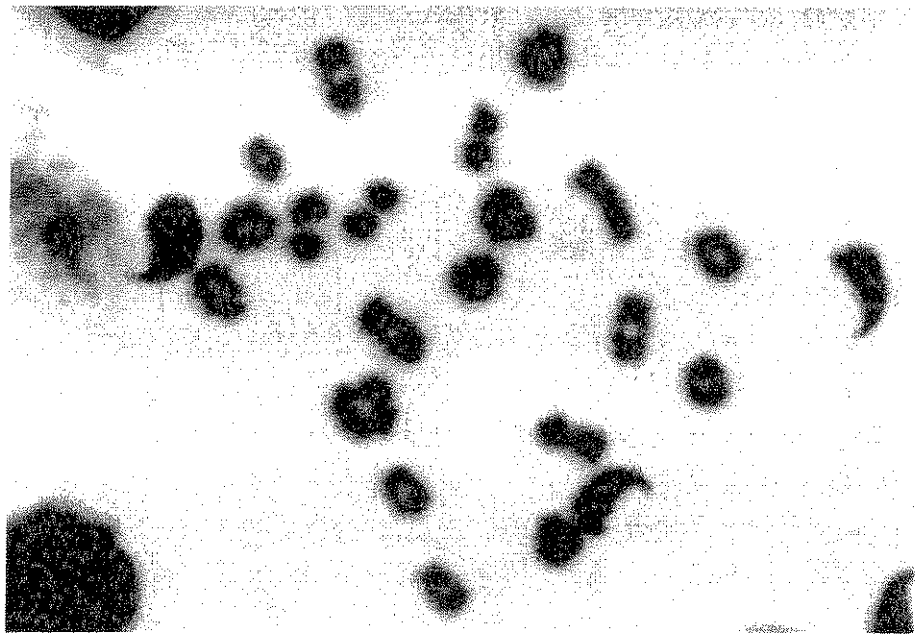

Fig. (5): A normal metaphase spread from spermatocytes of control male mice (Giemsa stain, X1000).

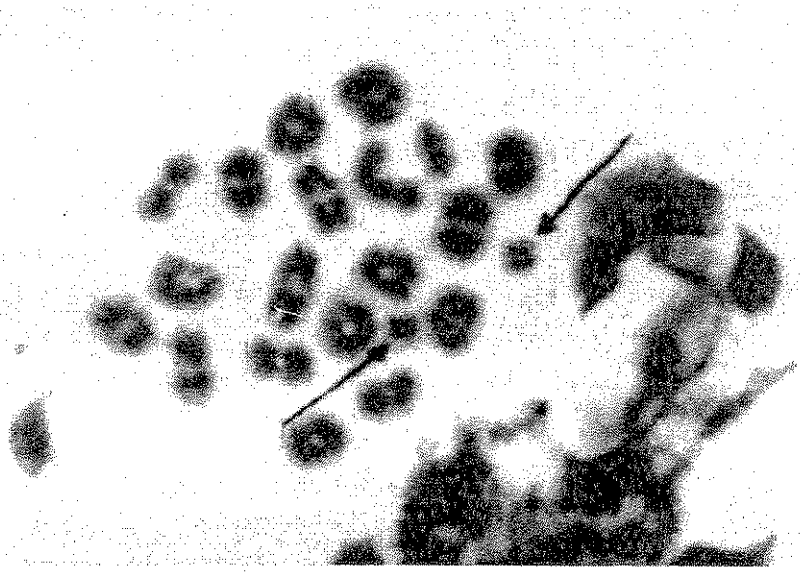

Fig. (6): Metaphase spread from spermatocytes of treated male mice showing autosomal univalent type of chromosomal aberrations (Giemsa stain, X1000).

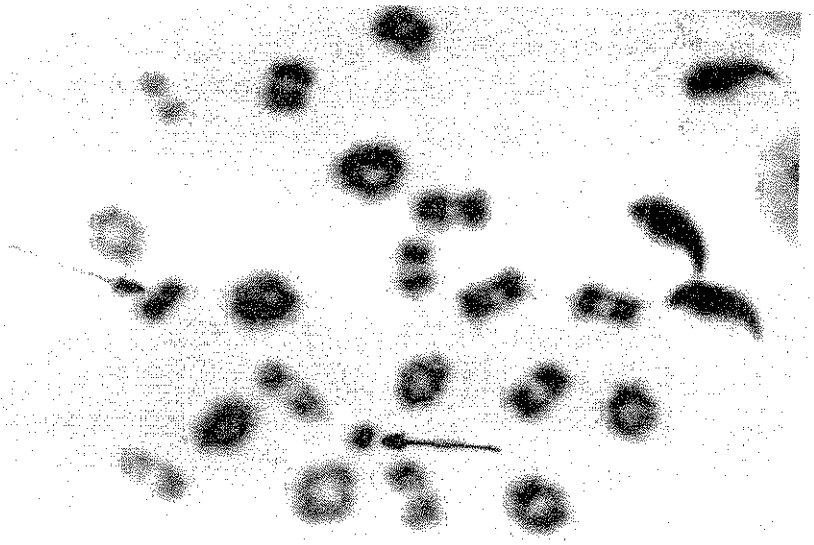

Fig. (7): Metaphase spread from spermatocytes of treated male mice showing X-Y univalent type of chromosomal aberrations (Giemsa stain, X1000)

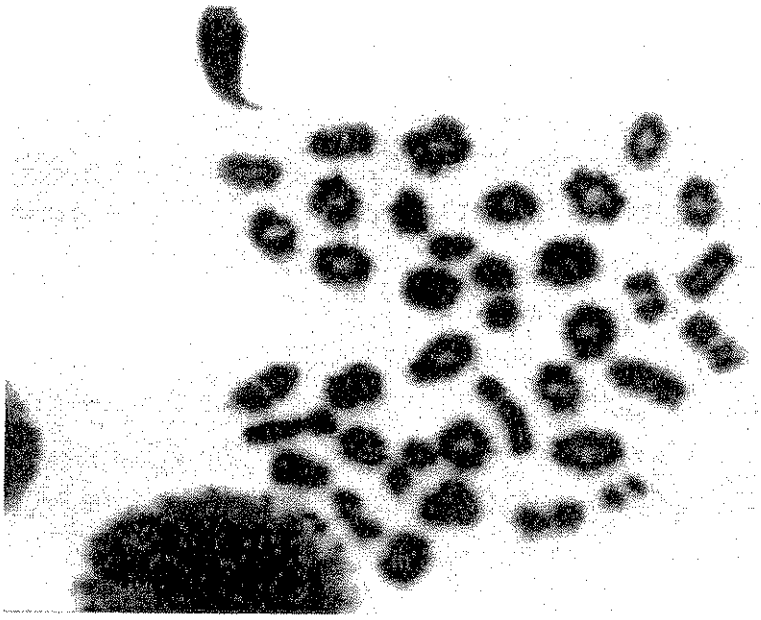

Fig. (8): Metaphase spread from spermatocytes of treated male mice showing polyploid type of chromosomal aberrations (Giemsa stain, X1000).

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المجلد ٦٥ ٢٠٠٥ العدد ٣