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Cytogenetic Effect of B-sitosterol on the Frequency of Micronucleated Polychromatic Erythrocytes in Bone Marrow of the Rat

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Abstract: In the present work micronuclei development in newly formed erythrocytes in bone marrow in newly born rat was taken as an indication of genotoxic effects of B-sitosterol treatment (Phytoestrogenic compound) on treated parents. Parents were given B-sitosterol (5 and 7 mg/kg of body weight as a low and high doses, respectively) subcutaneous injections to make parents for 5 successive days, then allowed to mate with untreated females. Another case only female parents, were treated while, the final case both parents were treated before mating. Bone marrow smears were prepared from newly born rats, stained, and comparisons made of micronuclei erythrocytes between treat and untreated parents.

Through investigation the control group showed a micronuclei frequency of approximately 0.13%. Treatment at two doses levels resulted in an increase in number of micronucleated erythrocytes. The frequency was highest when both parents were treated by a high dose (0.49%) followed by gradual decline towards treatment male parents (0.33%), and both the increase and the gradual decline were dose dependent.

Introduction

The micronucleus test has become a well established *in vivo* technique for the detection of clastogenic chemicals. The assay has been developed and introduced for mutogenicity testing according to [1, 2; p. 3]. The evaluation of micronuclei has gained increased popularity as an alternative to classical chromosomal aberration analysis for detecting clastognic agents [3, 4, 5]. The scoring of micronuclei is simple and less time consuming than the analysis of chromosomal aberration, which permits a greater number of cells to be examined for the same effort than most widely used micronuclei assays that involve the enumeration of micronucleated polychromatic erythrocytes in bone marrow preparation of rodnets [3, 5]. It is assumed that micronuclei arise from fragments resulting from clastogenic action of intact whole chromosome that have lagged at anaphase due to spindle destruction [2; p. 3]. They are lost from the nucleus but retained by the cytoplasm of the daughter cells [6]. Many authors believed that there is a

correlation between micronuclei induction and chromosomal aberration in metaphases of bone marrow cells due to treatment with irradiation or chemicals [7, 8]. They were detected also in leucocytic series, unfortunately micronuclei in developing leucocytes are not always easily distinguishable from normal nuclear lobes. Therefore, mature erythrocytes are much more suitable for micronuclei detection [9, 10, 11].

The relation between frequency of micronuclei polychromatic erythrocytes in mammalian bone marrow and phytoestrogenic B-sitosterol treatment remain neglected and very rare. The present work is designed to measure the treatment effect of B-sitosterol on newly formed erythrocytes of rat bone marrow.

Material and Methods

The test animal used throughout this work was the white rat (*Rattus norvegicus*). Both adult sexes were used weighing an average 120 ± 20 gm. The test consisted of 42 virgin female rats and 21 adult male rats that were separated into 7 groups each group contained 6 females and 3 males. In each group 2 females were housed with one male for mating. Mating was ensured through the presence of the vaginal plug then the males were isolated. Micronuclei were obtained from the bone marrow of two randomly selected newly born from each mother rat. They were examined one week after delivery.

Group I

The first group served as a control and consisted of 12 newly born rats from parents treated with olive oil, the remaining 6 groups were treated subcutaneously with B-sitosterol dissolved in olive oil as follow:

Groups II & III

Male parents were injected daily with B-sitosterol (5 successive days) 5 mg/kg of body weight in group II and 7 mg/kg of body weight in group III, respectively, and two weeks postinjection then were allowed to mate with untreated virgin females.

Groups IV & V

Virgin female parents were administered a dose of B-sitosterol 5 mg/kg of body weight in group IV and 7 mg/kg of body weight in group V, daily injection for 5 successive days. On day 6 treated females were housed with untreated males until the process of mating occur.

Groups VI & VII

Both parents (male and female) were injected with the same above mentioned doses in group VI and VII, respectively. After the end of injection period of each dose, males were housed with females until the processes of mating occur.

In all groups of treatment, selected randomly two newly born rats for each mother rat (12 young rats from each group) were sacrificed at one week from delivery. Then the

bone marrow smears were prepared, smears from an extracted femur were flushed in fetal calfserum.The flushed material was mixed well and then spread on drying slides. After air drying, the slides were fixed by treating with methanol for 15 min, stained, and permanent preparation were made. Different staining methods including May Gruenwold-Giemsa stain [2; p. 2] Wrihgt-Giemsa stain by [12], haemotoxylin and eosin staining method resulted in the best, differentiation. Slides were coded and 1000-erythrocytes were scored blindly for each newly born at 1000 x magnification.

Micronucleated polychromatic erythrocytes were recorded and photomicro-graphs were taken whenever necessary. Statistical analysis for each dose level were evaluated by a dispersion test based on X2 according to Snedecor & Cochran[13].

Results

The frequency of micronuclei in non treatment conditions was determined in 12000 bone marrow polychromatic erythrocytes from the control group of 12 young rats (1.3 ± 0.4) out of each 1000 polychromatic erythrocytes had micronuclei. In groups 2 & 3, the frequency of micronuclei from 12 young rats from treated male parents with two doses of b-sitosterol were increased over the control group {Table 1 and Fig. 1}. The increase was dose dependent. Under treatment with low dose the difference was significant (0.24%) at the higher dose, the frequency of micronuclei reach (0.33%).

Table 1. Frequencies of micronuclei polychromatic erythrocytes form bone marrow of newly born rats to treated parents with B-sitosterol

Dose mg/kg	No. of parents	No. of new born rats	No. of scored cells	Positive micronuclei in newly formed erythrocytes		
				T. No.	%	M ± S.D.
Control	6 ♀ + 3 ♂	12	12000	15	0.13	1.3± 0.4
5mg/kg	C_6♀ +T_3 ♂	12	12000	29	0.24	2.4±0.4**
7mg/kg	C_6♀ +T_3 ♂	12	12000	40	0.33	3.3±0.6**
5mg/kg	T_6♀ +C_3 ♂	12	12000	31	0.26	2.6±0.5**
7mg/kg	T_6♀ C_3 ♂	12	12000	51	0.43	4.3±0.9***
5mg/kg	T_6♀ +T_3 ♂	12	12000	45	0.38	3.8±0.6**
7mg/kg	T_6♀ +T_3 ♂	12	12000	59	0.49	4.9±0.6***

N.B.: C = Untreated + = mating *p<0.05 **)<0.01 ***P<0.001
T = Treated
M = Mean/rat.
T. No. = Total number.

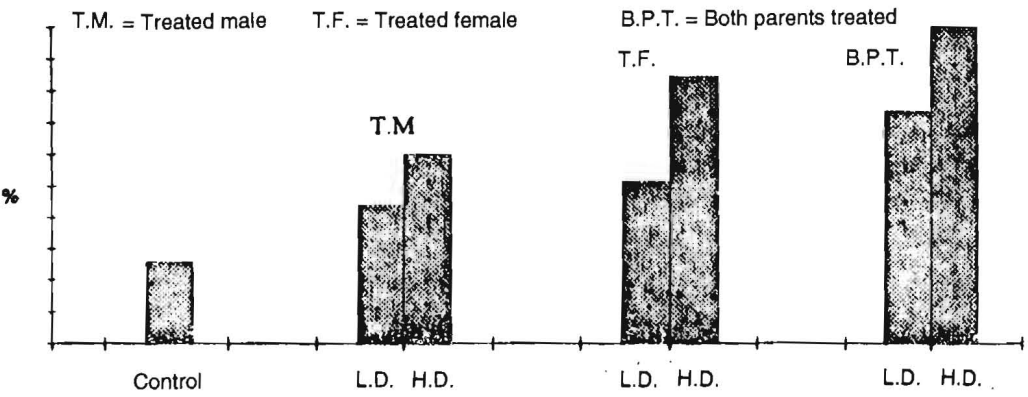

Fig. 1. Percentage of the micronuclei polychromatic erythrocytes of bone marrow smear from newly borns rat to treated parents with B-sitosterol.

In groups IV & V: The frequency of micronuclei in 12,000 polychromatic erythrocytes of 12 baby rats to treated mother rats with a low dose of B-sitosterol reached (2.6 ± 0.5). When the female parents received a large dose of B-sitosterol micronuclei were (4.3±0.9). The young rats from treated mother parents with B-sitosterol were more seriously affected than young rat from treated father parents with a large dose.

In groups VI & VII : The B-sitosterol is more damaging on newly formed erythrocytes of new born rats when both parents were treated by a low dose of B-sitosterol. The positive micronuclei in bone marrow polychromatic erythrocytes reached 45 in 12,000 cells (0.38%). At the high dose of B-sitosterol, the number of positive micronuclei increased to 59/12000 cells (0.49%) (Table 1 & Fig. 1).

Discussion

In these experiments we evaluated the micronuclei of newly formed erythrocytes in bone marrow of new born rats to treated parents with B-sitosterol, through which B-sitosterol may effect the activity of dividing cells and induction of chromosome breakage.

The micronucleus test was recommended as a rapid method of screening clastogenic effects in mammals *in vivo* [14, 15, 1.9.3]. The test depends upon the scoring of the micronuclei which arise from chromosome fragments and/or intact chromosomes that have lagged behind at anaphase. In the former case, the lagging of some chromosomes is the result of a lack of a centromere. In the latter case whole chromosomes become left behind during anaphase, most probably due to some damage affecting the spindly structure [2, p. 5]. In either case, micronuclei are distinct and easily scored structures that persist in the cytoplasm of affected cells. Furthermore, the induction of micronuclei is believed to be related to the frequency of metaphase chromosomal aberrations [16]. In

other papers [17] investigating of micronuclei induced by clastogenic compounds were considered to result from chromosomal aberrations, especially the chromosomal breakage. Clastogens and spindle poisons proved to increase the incidence of micronuclei [2; p. 5]. A further support to such correlation was submitted byHeddle & Carrano [6]. According to their view, the amount of DNA present in a centric chromosome fragments produced by random breakage of mouse genome is highly correlated with DNA content of micronuclei. Several compounds were reported to inhibit mutagenesis in bacteria after the mutagen damaged the DNA [18].

Through the present work, it becomes evident that two doses of B-sitosterol did have a clastogenic effect on mitotically dividing chromosomes of bone marrow erythroblasts. Thus micronucleus of a small size could be speculated as in Fig. 2. Moreover, B-sitosterol seemed also to have attached the spindle. Thus micronuclei of a large size could be spotted also [2; p. 6]. [15] suggested that the B-sitosterol seemed also to have genotoxic effect on germ cell and dividing mitotic cells by exerting its action on spindle disturbance and/or endoreduplication. B-sitosterol at all applied doses resulted in an increase in the frequency of micronucleated erythrocytes above the control level. The increase in frequency of micronucleated erythrocytes in bone marrow of new born rats was dose dependent and either one or both parents were treated. The new born rats from treated mother rats were affected more than those of treated father rats. This agrees with various studies previously reported for B-sitosterol [20-23] which investigated the interference of phytoestrogen and reproductive performance in ewes, female rabbit, and

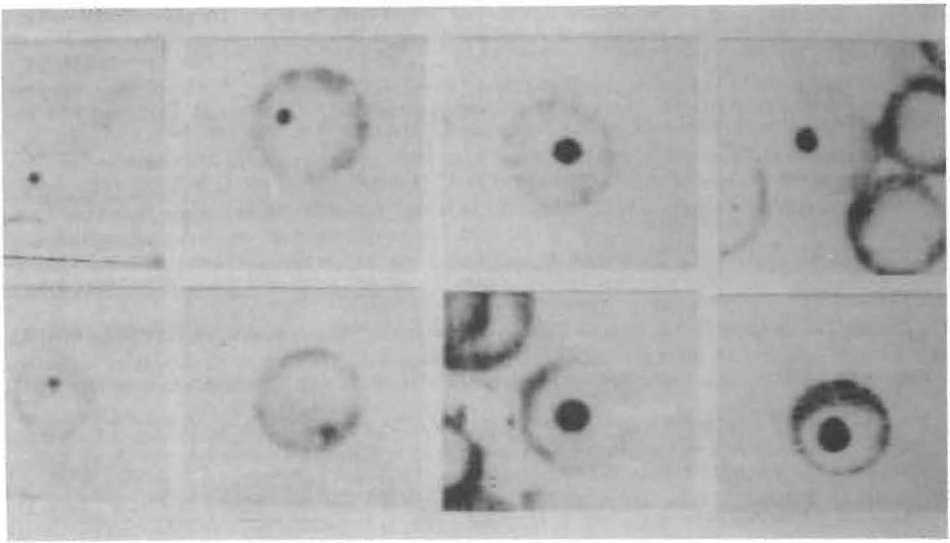

Fig. 2. Difference in size of various micronuclei from bone marrow smear of new born rats to treated parents (MN).

female rat. Whenever both parents were treated the number of micronuclei erythrocytes in bone marrow of young rats peaked (0.49%). These data accepted by various previous reports [23-26] concluded that phytoesterogen effect on number, motility and morphological shape of sperms of mice and rat animals. The increase in number of dead foetuses through action on mitotically dividing cell and genotoxic on number of morphology of chromosome with B-sitosterol treatment reported by Fawzia and Munshi *et al.* [19, 27].

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التأثير الوراثي الخلوي *للبيتاسيتوستيرول* على معدّل تكوين النوى الدقيقة في كرات الدم الحمراء من نخاع عظم الفأر الأبيض

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(سُلِّم في ٢ ذي القعدة ١٤١٤هـ؛ وقُبل للنشر في ٢٣ ذي الحجة ١٤١٥هـ)

ملخص البحث. يعتبر هذا البحث محاولةً لدراسة بعض الآثار الجانبية للإستروجين النباتي *(البيتاسيتوستيرول)* على المستوى الوراثي الخلوي من خلال دراسة معدّل ظهور النوى الدقيقة بكرات الدم الحمراء حديثة التكوين بنخاع العظم للمواليد لكل من مجموعة الذكور المعالجة، مجموعة الإناث المعالجة ومجموعة الأبوين المعالجين في حيوان التجربة (الفأر الأبيض).

وتمّت المعالجة بالحقن تحت الجلد *بالبيتاسيتوستيرول* المذاب في زيت الزيتون لأحد الأبوين أو كليهما وذلك بجرعتين من *البيتاسيتوستيرول* (٥مجم لكل كجم من وزن الجسم. و٧مجم لكل كجم من وزن الجسم).

وقد تبين من هذه الدراسة: أنّ المعالجة أدّت إلى تغيير في معدّل تكوّن النوى الدقيقة عن المجموعة الضابطة وهذا التغيير معنويًا مع المعالجة بالجرعتين والزيادة متناسبة تناسبًا طرديًا مع الجرعة المستخدمة وكذلك نوع معالجة الأبوين.

زوّجت مجموعة الذكور (الآباء المعالجة) فقط بإناث غير معالجة. تبين من فحص النوى الدقيقة لكرات الدم الحمراء من نخاع عظم المواليد وجود زيادة معنوية وطردية مع الجرعتين المستخدمتين (٢٤,٪ و٣٣,٪) على المجموعة الضابطة (١٣,٪) في مجموعة الإناث (الأمهات المعالجة) والتي زوّجت بذكور غير معالجة. تبين من فحص معدّل تكوين النوى الدقيقة في كريات الدم الحمراء بنخاع عظم المواليد أن التأثير الجيني لمركب *(البيتاسيتوستيرول)* على المواليد (٢٦,٪ و٤٣,٪) أكثر تأثير عنه عند معالجة الأباء فقط.

وبمعالجة الأبوين كان التأثير الجيني للمركب على الصغار أكثر خطورة عنه عند معالجة أحد الأبوين (٣٨,٪ و٤٩,٪) وكان التأثير معنويًا ومتناسب طرديًا مع الجرعات المستخدمة.