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Aneuploidy of Urethane in Mouse Bone Marrow Cells and Potential Recovery with Lupin Water Extract

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Abstract: The incidence of in vivo urethane-induced chromosomal aberrations, sister chromatid exchanges (SCEs) and aneuploidy was examined in male mice. Single oral administration by gavage with urethane (0.5 and 1 g/kg) caused a significant increase in chromosomal aberrations in bone marrow and spermatocyte cells, and statistical significant in SCE induction. The clastogenic effect observed was dose- and time- dependent. Aneuploidy was observed clearly with the high dose recording a significant value. Administration of lupin water extract at 5000 ppm/mice/day (added with the drinking water) reduced the frequency of chromosomal aberrations, but still at the significant values (P, 0.001) while that administration of lupin extract elevated the aneuploidy induced with urethane. It can be concluded that urethane is a strong clastogenic and weak aneugenic agent when administered orally and administration of lupin water extract can be elevateing the aneugenic property of urethane.

Keywords: Urethane; Mice; lupin-extract; Chromosomal aberrations; aneuploidy; spermatocytes.

1. Introduction

Aneuploidy is a condition in which the chromosome number of a cell or individual differs from a multiple of the haploid state. Numerous pathophysiological conditions such as spontaneous abortions, birth defects and cancer are associated with the induction of aneuploidy in humans [1, 2]. Nonrandom losses or gain of specific chromosomes are commonly observed in human and animal tumors [3-5]. In addition, certain important human carcinogens such as benzene, diethylstilbestrol and asbestos, which are reportedly non- mutagenic in standard gene mutation assay, are thought to induced neoplasia through mechanisms involving mitotic disruption and aneuploidy induction [6, 7].

Aneuploidy in humans is associated with approximately 60-70% of early spontaneous abortion [8] and represents a major part of the genetic disorders among newborns [2]. Also, a growing body of evidence suggests that aneuploidy is a direct causal event in the induction of neoplastic transformation [6, 9] . Although various compounds can induce aneuploidy in mammalian cells [10, 11].

Urethane as used as antineoplastic and anesthetic drug, it has a presence in some drugs, pesticides, beverages as well as in tobacco smoke increase the possibility of industrial or environmental exposure of humans to urethane [12, 13].

Yeowool and Cho [14] demonstrated that urethane significantly depresses both tonic and burst firing activities of thalamic neurons, and the response of thalamic neurons to formalin-induced nociception under urethane is different from that of the awake state.

Some contradictory data concerning the clastogenic effects of urethane studied intraperitoneally (i.p.) *in vivo* [15-17] and *in vitro* [18, 19].

Regarding to lupin, seed alcoholic extracts obtained from *Lupinus albus* and *Lupinus angustifolius* were non-toxic. There acute toxicity (LD_{50}) in the mouse is > 4000 mg kg^{-1} body wt. After fractionation, the extract from *Lupinus angustifolius* seeds afforded several fractions with differing toxicities (LD_{50} 750-4000 mg kg-1 body wt.). None of the fractions tested in vitro were toxic [20]. Gulewicz, *et al.* [21] demonstrated that alpha-galactosides from lupin and pea seeds were essentially nontoxic, alpha-galactoside preparations were not cytotoxic for mouse cymocytes *in vivo*. They added that the *in vivo* test that oligosaccharides from lupin and pea are utilized by selected beneficial colon bacterium strains. The chemical composition of the tested preparations had no significant effect on their biological activity. On the contrary, Pothier, *et al.* [22] have an opposite results, they concluded that Lupin is toxic due its alkaloid contents, spartine and lupanine in particular. Their results indicate that lupanine and lupin extract

are less toxic than spartine and that are the doses studied the three products have a week sedative effect on the CNS.

Our primary objective was to elevate the ability of urethane to induce the clastogenecity and aneuploidy in somatic and germ cells. Mice were treated orally by gavage with two doses (0.5 and 1 gm uretnae kg^{-1}), Urethane as selected based upon its reported ability to induce aneuploidy in male or female rodent germ or somatic cells. The main target of our objective was to obtain the potential recovery of the aneuploidy induction after administration with lupin water-extracts in drinking water to mice.

2. Material and Methods

2.1. Animals

White male Swiss mice 7-8 weeks old, obtained from the Animal Housing, National Research center, Cairo, Egypt, were used in our investigations. They were housed five to a cage and acclimated for 7 days before dosing. Food and water was provided *ad libitum*.

2.2. Chemicals

Urethane (ethyl carbamate), and 5-Bromodeoxyuridine were purchased from Sigma Co. Methanol, glacial acetic acid (Merk), Colchicine and Giemsa stain Gurr R66 (BDH).

Urethane was dissolve with distilled water and orally by gavage administered to mice at a dose 0.5 and 1 g/kg body weight as a single treatment. Samples were taken 24 and 48 hours post urethane treatment. Group of mice which treated with high dose (1 g/kg b wt) treated with lupin extract (5000 ppm/mouse/day) for one week. Samples of negative (untreated) control and lupin extract treatment were analyzed 7, 14 and 30 days along lupin administration.

2.3. Chromosomal Aberration Assay in Mouse Bone Marrow Cells

Somatic chromosomal aberrations in mouse bone marrow cells were scored after preparing metaphases according to Yosida and Amano [23] with some modifications. Metaphase were stained with Giemsa in phosphate buffer, five mice were taken for each treatment. About 100 well-spread metaphases were analyzed per animal.

For karyotyping, an Olympus computerized photomicroscope, attached with a high resolution CCD camera was used. An objective 100x and eyepiece 25x magnification performed the analysis of metaphases. The software Leica Chantal (Leica Q550CW Cytogenetic Workstation) provided by (Leica, London) was used for chromosome measurement and karyotyping. Total chromosome length, short and long arms were measured foe each processed chromosome pair, the short/long arm ratio and the relative length were calculated on Microsoft Excel computer program.

2.4. Chromosomal Aberration Assay in Mouse Spermatocyte Cells

Meiotic chromosomal preparation was made according to Evans, *et al*. [24] and stained with Giemsa in phosphate buffer (pH 6.8). 50 well spread primary spermatocytes/mouse at diakinasis-metaphase I were analysed.

According to Allen [25] SCE was prepared with some modifications. Tablet of 5- Bromodeoxyuridine (BrdU) was implanted into the mouse fur, 22 hours later, colchicine (14 ug/kg b.wt.) was injected intraperitoneally (i.p.). Two hours later, animal was cervical dislocated. Both femora were treated as chromosomal preparation assay. Staining was performed as Perry and Wolff [26] using the fluorescence plus Giemsa (FbG) technique. About 25 well spread metaphases were scored and analyzed/mouse.

2.5. Statistical Analysis

Possible differences between control and treated animals in the mean percentage of bone marrow, spermatocyte and SCE's were analyzed by Student t-test.

3. Results

The cytotoxic effects of urethane in mouse bone marrow cells exposed to 0.5 and 1 g/kg b.wt. were shown in table 1. The two doses have a highly significant effect comparing to the control, a significant increase in the percentage of chromosomal aberration as observed after 24 and 48 hours of treatment. The effect was also dose-related with a maximum of 63.5% after 48 hours with a g/kg. The main types of chromosomal aberrations were centromeric attenuation, deletion and break as structural type (Fig. 1). Polyploidy was noticed only with the high dose treatment, while aneuploidy (Fig. 2) was observed with

the two doses in a dose-dependent relationship recorded 3.5% and 6.5% after 24 and 48 hours of oral treatment with the high concentration.

Table 2 shows the cytogenetic effect of urethane on the spermatocyte cells as germ cells of mice. It can be noticed that a high percentage of chromosomal aberration was recorded with the two tested doses. There is a dose-dependent increased with time. Autosomal univalent and x-y univalent were the dominant types of germ aberrations beside the presence of translocation as chain VI and polyploidy, whereas aneuploidy cells were represented as numerical aberrations.

Induction of sister chromatid exchanges (SCE's) as another end point induced with urethane in our objective was illustrated in table 3. It is clearly noticed that urethane induced a significant increase in the induction of SCEs (Fig. 3) with the two tested doses comparing to the control value.

After administration of lupin water-extract (1 g/kg b. wt.) added in the drinking water to mice, the percentage of chromosomal aberrations in bone marrow cells was recorded in table 1. Seven days post extract administration period, the frequency of chromosomal aberration still highly significant. After14 days of administration, the frequency was declined but still significant. It was noticed that the aneuploidy and polyploidy were reduced. Samples taken after 30 days of extract-administration showed a significant increase in the structural aberrations, but the numerical type (aneuploidy and polyploidy) were didn't observed (Table 1).

The frequencies of chromosomal aberrations in mice spermatocyte cells after administration of lupin water-extract were recorded in table (2). The frequencies were slightly decreased after 7 days but still significant. After 14 and 30 days of administration, the frequency of chromosomal aberrations recorded a significant effect, comparing to control. The common types of aberration in germ cells were autosomal univalent and x-y univalent, while polyploidy and aneuploidy types were reduced to zero level.

4. Discussion

Bone marrow cells have the advantages of being highly proliferative and easy to extract, and of having much correlative data for other chromosomal end-points [27]. The same authors reported that the actual numerical counting of chromosomes was the most reliable evidence of aneuploidy induction by chemical compounds in somatic cells of mammalian *in vivo*. They also added that the premature or delayed separation of centromers during anaphase could lead to increased aneuploidy if cytokinesis occurs with chromatids unequally distributed [28]. Chatterjee, *et al.* [29] in their studies on chromosomal aberrations and alterations in the mitotic index in *Rattus norvegicus*, *in vivo*, they reported that significant increase of C-mitosis, chromosome stickiness, and diplochromosome indicate disturbances of spindle function that might produce subsequent gain or loss of chromosomes.

There are modern techniques like FISH technique for aneuploidy detection in interphase cells and identification of aneuploidy-inducing agents in human cells, but the actual numerical counting still has the most reliable evidence of aneuploidy induction with aid of karyotyping of G-banded stained metaphases, which carried out in our investigations. That combination gives most sharp results of aneuploidy picture without using any probs or hyperdization.

The frequencies of chromosomal aberrations induced with urethane was highly significant indicating a heavy damaged cells, the same results were reported by Niyashita, *et al.* [30] on the chromosomal aberrations induction with urethane in H-2 congenic strains of mice with B10 and A backgrounds.

Induction of sister chromatid exchanges (SCEs) post urethane orally administrated with 1 g/kg shows a clear dose-response relationship were apparent in mouse bone marrow cells. Our results are confirmed with the finding by Cheng, *et al.* [31] who reported that there is a significant increase in SCE frequencies were observed relative to corresponding controls. They added that at concentration of urethane –inhalation with 0.1mg/L and higher induced SCE frequencies were linearly to log urethane concentration.

Data were confirmed with the published results [16, 32], where they reported that urethane is an effective clastogen in mouse bone marrow and germ cells. Also when given orally as the micronucleated normochromatic erythrocytes (MNNCE) detected in peripheral blood were induced in bone marrow[33]. Another report illustrated that Urethane is genotoxic, mutagenic and clastogenic, especially in the presence of metabolic activation [34].

Lupin water-extract from lupin seeds grown in Egypt was studied [35], who reported that the proteins were high in lysine but relatively low in sulfur-containing amino acids. Polyphenols [36] and 10% alkaloids [20]. These alkaloids containing mainly sparteine and lupanine. Results of Pothier, *et al.* [22] illustrated that lupanine and lupin extract are less toxic than sparteine at the tested doses on the central nervous system. In other words, Kennelly, *et al.* [37] reported that both spatreine and lupanine exhibited teratogenic activity in the rat embryo culture (REC), an in vitro method to detect potential teratogens.

Administration of lupin water-extract in the drinking water to mice received urethane at 1 g/kg showed a reduction in the aneuploidy after 14 and 30 days post treatment to zero percent, which deals that lupin water extract has an anti-aneugenic property. The mode of its action on the aneuoploidy is not detected, but it can be obviously mentioned that the effect of sparteine and or lupanine was performed.

On the other hand, lupin extract does not affect the clastogenicity of urethane. That the extract decreased the frequency of chromosomal aberrations in mouse bone marrow and spermatocyte cells along the experimental period, but it still around the significant levels as shown in tables 1 and 2.

From our collected data of the administration of lupin water extract (5000 ppm/day/mouse) in the drinking water received 1 g urethane/kg orally by gavage as a single dose. It was clearly noticed thaturethane has aneugenic property in somatic and germ cells of mice, beside other effect as polyploidy. It can be noticed that Lupin extract failed to recover that clastogenicity of urethane, while lupin has a potential recovery effect on the aneuploidy as reducing the aneuploidy cells to zero percent, meaning that the lupin extract can be considered as ant-aneugenic agent.

It can be concluded that urethane is strong clastogenic and weak aneugenic agent, when administered orally to mice and when followed by lupin water extract administration, it can be elevate the aneugenic property of urethane.

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Table-1. Mean percentage of chromosomal aberrations and percentage of the different types of chromosomal aberration in mouse bone marrow cells post oral treatment of urethane and lupin water-extract

Treatment	Mice killed after (h)	Scored metaph. No	% of metaphase with:								Chrom. Aberr.% Mean ± S.E.
			Centr. Atten.	Del.	Chromt. gap	Frag.	Chromt. Break	Chr. break	Aneu ploidy	Poly ploidy	
I- Control (Non-treated)	-	500	0	1.8	1.6	1.2	0.8	0	0	0	5.4 ± 0.5
II-Urethane treatment											
0.5 g kg^{-1} b. wt.	**24**	500	22	15.5	4.5	3	4.5	0	0	0	49.5 ± 0.98 **
	48	500	19.5	16.5	6.5	3.5	5.5	0	1	0	52.5 ± 0.93**
1 g kg^{-1} b. wt.	**24**	500	21.5	16	6.5	4.5	7.5	2	3.5	2.5	60.0 ± 0.63**
	48	500	17.5	16	6.5	6.5	5.5	2.5	6.5	2.5	63.5 ± 1.54 **
III-Urethane followed by lupin extract (5000ppm)											
Control	**7 days**	500	0.4	3	1.4	2.2	1.0	0	0	0	8.0 ± 0.55
Treated		500	16.2	15.6	5.4	3.4	4.8	1.4	0.8	1.2	48.8 ± 2.05**
Control	**14 days**	500	0.2	1.6	0.8	2.4	1.6	0.6	0	0	7.2 ± 0.66
Treated		500	14.6	15.2	4.6	3.0	3.8	1.0	0	0	42.2± 1.27 **
Control	**30 days**	500	0.2	1.6	1.2	1.6	1.2	0	0	0	5.8 ± 0.22
Treated		500	13.4	13.0	3.6	2.2	3.0	0.6	0	0	35.8 ± 1.98**

** Significant at ($P < 0.001$) t-test

Table-2. Mean percentage of chromosomal aberrations and percentage of the different types of chromosomal aberration in mouse spermatocyte cells post oral treatment of urethane and lupin water-extract

Treatment	Mice killed after (h)	Scored metaph. No	% of metaphase with:								Chrom. Aberr.% Mean ± S.E.
			Auto. Univ.	X-Y univ.	A.U +X-Y univ.	Chain	Frag. And/ or break	A.U + break	Aneu ploidy	Poly ploidy	
I-Control (Non-treated)	-	250	1.2	0.8	1.6	0	0.4	0	0	0	4.0 ± 0.16
II-Urethane treatment											
0.5 g kg^{-1} b. wt.	24	250	22.0	11.6	6.0	8.0	2.0	0	0	0	49.6 ± 0.15 **
	48	250	23.2	8.0	7.6	4.8	4.4	0	0.8	2.0	50.8 ± 0.96**
1 g kg^{-1} b. wt.	24	250	22.8	7.2	4.8	8.0	2.4	0	1.6	3.6	50.4 ± 1.09**
	48	250	26	8.0	4.4	7.2	5.2	0	1.6	3.2	66.6 ± 0.67 **
III-Urethane followed by lupin extract (5000ppm)											
Control	7 days	250	2.4	1.8	3.2	0.4	2.4	0.8	0	0	10.4 ± 0.63*
Treated		250	13.2	11.2	12.8	2.0	4.0	8.0	1.2	1.2	53.6 ± 0.24**
Control	14 days	250	2.0	1.6	2.8	0.4	1.2	0.4	0	0	8.4 ± 0.78*
Treated		250	11.2	10.4	11.6	1.6	4.0	6.8	0	0	45.2± 0.96 **
Control	30 days	250	1.6	0.8	2.0	0	1.6	0.4	0	0	6.4 ± 0.67
Treated		250	10.4	9.6	10.0	2.0	3.2	5.6	0	0	40.8 ± 1.53**

* Significant at ($P < 0.05$) ,
** Significant at ($P < 0.001$) t-test

Table-3. Frequency of sister chromatid exchanges (SCEs) in mouse bone marrow cells treated with two doses of urethane

Treatment	Scored metaph. No	Scored SCE			SCE s/metaphase Mean%
		Single	Double	Trible	
Control (Non-treated)	200	59	11	2	3.6
Urethane treatment					
0.5 g kg^{-1} b. wt.	75	397	95	3	6.64 *
1 g kg^{-1} b. wt.	75	582	101	16	9.32 *

* Significant at ($P < 0.001$) t-test

Fig-1. Structural chromosomal aberrations (as chromatid breaks) in mouse bone marrow cells induced with 1 g/kg b.wt. of urethane after 48 hrs.

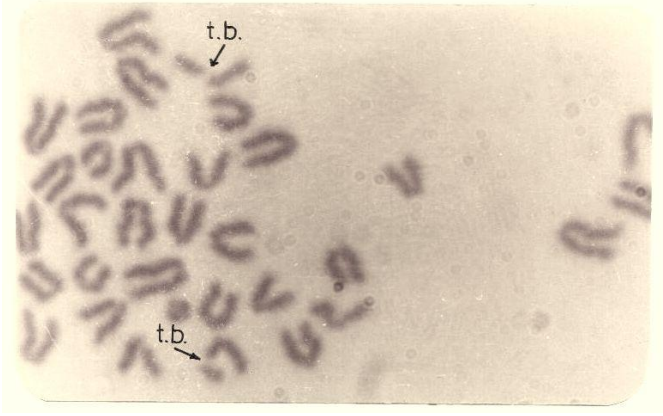

Fig-2. Aneuploidy in chromosomes No. 16 and 19, after treatment with 1 g Urethane /kg b.wt. in mouse bone marrow cells for 48 hrs.

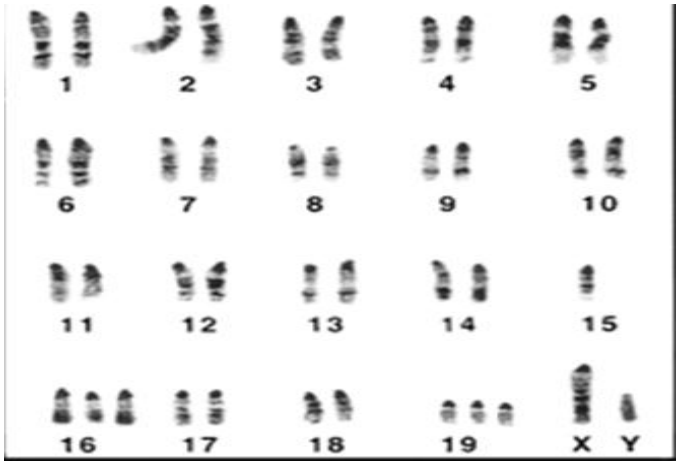

Fig-3. Sister chromatid exchanges induced with mouse bone marrow cell after treatment with 1 mg Urethane /kg b. wt. for 24 hrs.