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Studies on the Genotoxic Effects of Anticancer Drug Paclitaxel (Taxol) in Mice

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Abstract: This study focused on testing the toxic genetic effect of an anti-cancer drug, namely "Taxol". It is a drug extracted from a tree called "Taxus brevifolia ". It is one of the latest treating drugs in the Kingdom of Saudi Arabia. The mechanism of its action lies in the polymerization of Tubulin threads, which leads to its stabilization and thus stops their work, especially as a stimulus of chromosomes. Three doses were selected to measure the genetic toxicity of this drug, i.e. a therapeutic dose (0.6mg/kg), double of the therapeutic dose (1.2mg/kg) and three limes of the therapeutic dose (1.8mg/kg). The treated mice were used for testing "Chromosomal Aberration " and the " micronuclei ", then anatomizing, 12,18,24 hours after the peritoneal injection. The results showed a high significant increase in the total number of the chromosomal aberrations; (structural and numerical). This increase may be either due to the increase in the total of structural chromosomal aberration, represented in the chromosomal abnormality increase (PCD), or due to the increase in the numerical chromosomal aberration, often represented in the chromosomal abnormality (En.m). This increase also appeared in the micro-nuclei. This study pointed to the possibility of the occurrence of chromosomal aberration and micro-nuclei in the bone marrow cells of the mice treated with the " Taxol " drug in triple therapeutic dose.

Key words: Genotoxic · Taxol · Chromosomal Aberration · Micronuclei · Mice

INTRODUCTION

Antineoplastic drugs are responsible for the survival of cancer patients around the world. However, like many other cancer therapeutics, they themselves may cause mutations and secondary malignancies [1]. Cancer induction is therefore a toxic consequence predicted by short-term tests of genotoxicty and should be weighed against the potential therapeutic benefits of several antitumor drugs [2]. It is therefore essential that effective anticancer drugs should be tested not only for their cytotoxic potential, but also for their ability to disturb genomic integrity, in order to render a deeper understanding of the potential risks related to their clinical use [3]. To study genotoxicity, some studies [4-11] have used a variety of genetic markers, including sister chromatid exchanges (SCEs), Structural chromosomal aberrations (gapes, breaks trans-location) numerical chromosomal aberrations (polyploidy). Micronuclei increases in one or more of these markers were observed increased mutations frequency has been reported [12].

Taxol is a new anticancer drug that is isolated from stem park of the pacific yew tree; Taxus brevifolia. Its antitumour activity against a variety of rodent tumors was discovered in 1967; when, its unique mechanism of action led to the development of a new class of chemotherapeutic agents called taxanes. In 1991, The National Cancer Institute hailed Taxol (also known as paclitaxel) as the most important new cancer drug in the past 15 years and it has recently been called the best new anticancer agent developed from natural agents. Although the drug was discovered forty years ago, it was not tested experimentally until 1977. Taxol, when used on its own, produced a response in up to 60% of patients with breast and ovarian cancer [13]. It has been described to have some activity in head and neck gastric cancer and hematological cancer [14]. It is perhaps one of the most successful drugs used in the treatment of a variety of cancers [15]. Paclitaxel (Taxol) alone or in combination with other antitumor agents is widely used in a variety of tumor treatments, however, can induce chromosome damage and aneuploidy, thereby enhancing the possibility of survival of damaged cells [16]. And prevents cell division by promoting the assembly of stable microtubules from α and β tubulin heterodimers and inhibiting their depolymerization [17]. Leading to the formation of nonfunctional microtubule bundles [18, 19].

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This is an opposite effect to the Vince alkaloids, which inhibit the polymerization of the microtubules [20]. Taxol has been reported to induce micronuclei [21]. That Taxol-induced meiotic delay and spindle defects contribute to aneuploid mice oocytes and zygotes [22]. Sperm samples provided immediately after the initiation of cancer therapies may contain treatment-induced genetic defects that will jeopardize the genetic health of offspring [23]. Moreover, investigations have shown that Taxol activates a checkpoint pathway that delays cell cycle progression and induces programmed cell death [24-26].

Therefore, the objective of this study was to discover the probable genotoxic effect of different doses of Taxol drug on bone marrow in mice.

MATERIALS AND METHODS

Animals: Male Swiss albino mice *Mus musculus* MFI strain,8-9 weeks old, weighed 25-30g, were obtained from the animal house of King Fahad Medical Research Centre KAU. Animals were housed in plastic cages with steel wire tops in an air-conditioned room (22±1°C,45-75% relative humidity) maintained in a controlled atmosphere of 12h light / 12h dark cycles. The mice were maintained on basal diet (20% crude protein 4% crude fat, 3.50% crude fiber and energy 2850 k. cal/kg diet) and water were provided ad libitum. Total number of 96 male mice were used for control and three doses treatments (24 mice in each treatment).

Taxol Drug: Taxol was obtained from Dr. S. fakeeha, hospital pharmacy. The used doses in the present study was calculated based on human therapeutic dose and its another double and triple doses.

Treatments and Route of Administration: The control animals (24 animals) received an equal volume of the solvent by (9% NaCl) intraperitonealy injection (i.p.) for one day. Three doses of Taxol were intra peritoneal injected for only one day [0.6 mg /kg as a medical dose (MD), 1.2 mg /kg as an intermediate dose (ID) and 1.8mg /kg as a tolenated dose (MTD) 24 mice in each treatment]. The animals were dissected after 12h,18h and 24h.

Cytogenetic Methods

Chromosomal Aberrations (CA): Bone marrow cells of male albino mice were examined for chromosomal aberrations according the method described by Alder, [27]. Mice were intraperitonealy injected 2 hours before killing with of 0.05% colchicine dissolved in distilled water, in order to arrest metaphase dividing cells. At least 50 metaphases were examined using research microscope with oil immersion lens.

Micronucleus (MN) Assay: One thousand polychromatic erythrocytes (PCEs) per animal were scored for determining the frequency of micronucleated poly-chromatid erythrocytes (MN PCEs). Micronuclei were identified according to Schmid [28], criteria. They were morphologically identified relative to the normal nuclei (1/5 the diameter/ normal).

Statistical Analysis: SPSS program version 16 was used for analyzing chromosomal aberrations and micronuclei T-test and (analysis of variance followed by Least significant differences LSD).

RESULTS

The current study showed that exposure of mice to either dose of Taxol (0.6mg/kg, 1.2mg/kg and 1.8mg/kg) significantly increase the Chromosomal Aberration " and the " micronuclei compared with normal control ones.

The second group (0.6mg/kg), third group (1.2mg/kg) and fourth group (1.8mg/kg) were dramatically affected by the toxic effect of Taxol indicated by very highly significant increase in the average in total Chromosomal Aberrations after12 hours of treatment with doses. Also, the result revealed very highly significant increase in second group and third mice compared to the first group (control), however non-significant in fourth group after18 hours. Also, the result revealed very highly significant increase in second group, fourth and highly significant increase compared to the control after 24 hours Table (1).

The results of the "chromosomal aberrations test" revealed a highly significant increase in the average of chromosomal aberrations, Table (1) and Fig (1, 2 and 4). This highly significant increase in the average number of chromosomal aberrations, after 12,18 hours of treatment was seen in the numerical chromosomal aberrations represented in En.m, whereas the increase in the structural aberrations was only seen after treatment with the maximum tolerated dose for 12 hours in the form of PCD. However, after 24 hours of treatment the highly significant increase in the average of chromosomal aberrations, was due to the higher average number of centromeric division in the structural aberrations.

On the analysis of variance reveled highly significant differences in the average number of chromosomal aberrations (structural and numerical) among treatments after different time periods in each of three doses of the "Taxol" drug. By conducting the multiple comparisons, using the LSD, the order of treatments in terms of its

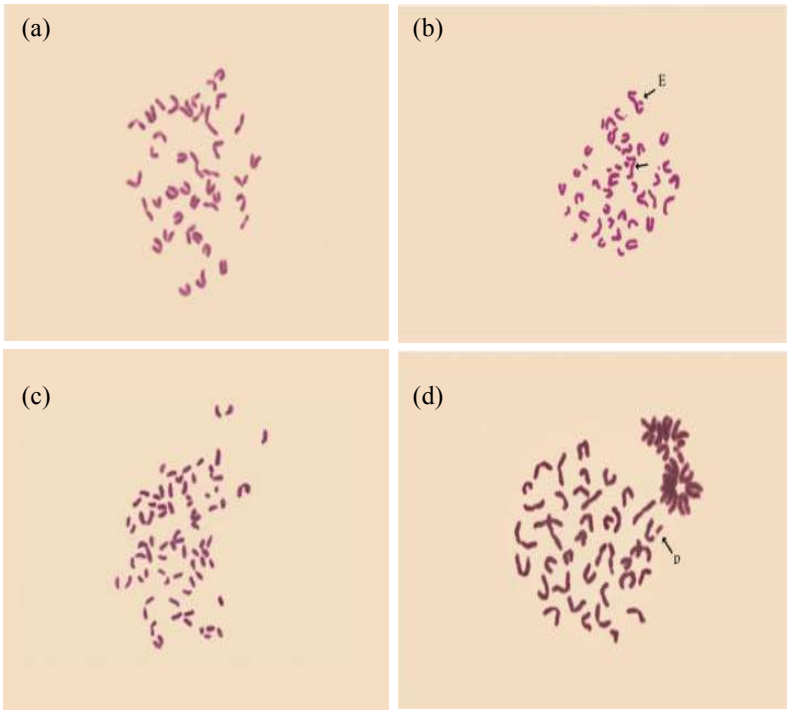

Fig. 1: Types of chromosome aberration (Structural Aberrations) from bone marrow of treated mice showing a: normal b: End to End Association (E) c: Premature (early) Centromeric Division (PCD) or Centromeric Attenuation (CA) d: Chromatid Break or Deletion (D)

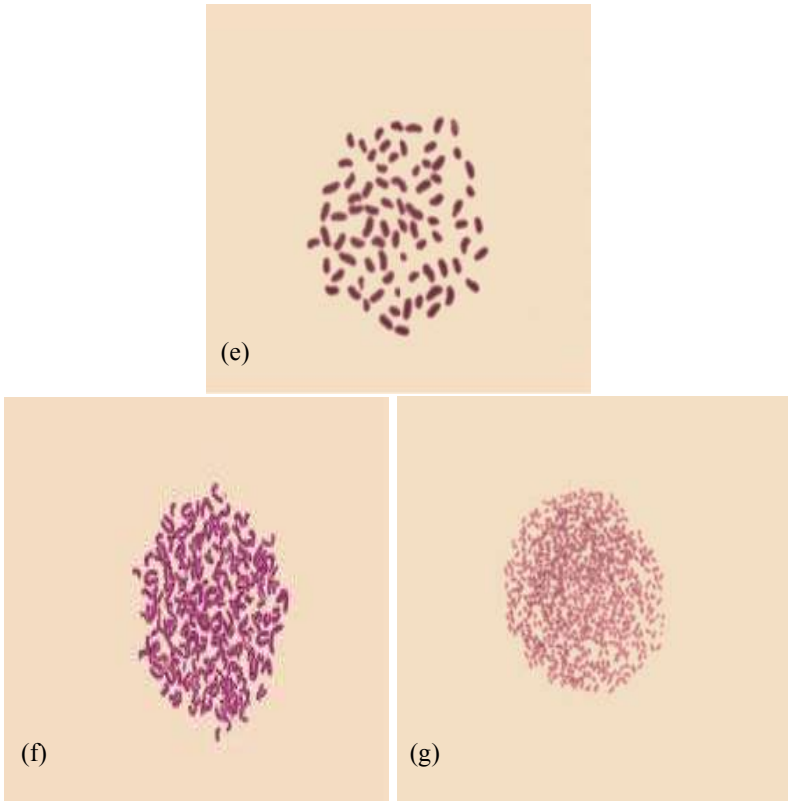

Fig. 2: Types of chromosome aberration (Numerical Aberrations)from bone marrow of treated mice showing e: Endomitosis (End.M) f: Polyploidy (Poly) g: (End. M and Poly)

Table 1: Mutagenic Effects of Drug paclitaxel (Taxol) on male mice Bone-marrow chromosomes

		Chromosomal Aberrations										
		Structural Aberrations							Numerical Aberrations			Total
type of Treatment		Break	Delet and frag	cf	End to	PCD	RC	Total	En.m	poly ploid	Total	Aberrant
12h	Group [1]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.00±6.612	0.00±0.00	8.00±6.61	0.75±1.03	0.63±0.74	1.38±0.91	9.38±6.96
	Group [2]	0.00±0.00	0.00±0.00	1.00±1.41	0.00±0.00	11.00±3.703	0.13±0.35	12.13±3.9	6.75±2.86	0.63±0.74	7.38±2.82	19.50±6.25***
	Group [3]	0.00±0.00	0.00±0.00	0.38±0.74	0.38±0.51	7.00±2.268	0.25±0.46	7.62±3.10	14.25±2.7	0.38±0.74	14.50±2.33	22.63±4.30***
	Group [4]	0.00±0.00	0.13±0.35	0.13±0.35	0.00±0.00	15.25±6.497	0.13±0.35	15.63±6.6	7.13±3.56	0.25±0. 46	7.38±3.58	23.00±7.15***
18h	Group [1]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	6.13±3.044	0.00±0.00	6.13±3.04	2.25±1.28	0.38±0.518	2.63±1.18	8.75±3.28
	Group [2]	0.00±0.00	0.13±0.35	0.00±0.00	0.00±0.00	5.13±2.850	0.00±0.00	5.38±2.87	1.88±2.03	22.50±9.54	24.38±8.31	29.63±7.24***
	Group [3]	0.38± 0.74	0.00±0.00	0.88±1.12	0.00±0.00	3.25±1.909	0.00±0.00	4.50±1.19	25.13±5.30	7.38±2.87	32.50±6.00	36.88±6.01***
	Group [4]	0.00±0.00	0.00±0.00	0.13±0.35	0.00±0.00	3.88±1.246	0.38± 0.74	4.38±1.18	17.50±2.72	2.13±1.64	19.62±3.37	7.151±3.89
24h	Group [1]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.88±0.835	0.00±0.00	0.88±0.83	0.38±.744	1.50±0.92	1.88±0.99	2.75±1.16
	Group [2]	0.13± 0.35	0.00±0.00	0.25±0.463	0.00±0.00	11.88±5.76	0.00±0.00	12.25±5.5	3.75±2.188	1.38±0.91	5.13±1.95	17.37±7.19***
	Group [3]	0.00±0.00	0.50±1.06	0.00±0.00	0.00±0.00	0.13±.35	0.00±0.00	0.63±1.06	0.50±0.535	6.25±3.91	6.75±3.91	7.38±4.24 **
	Group [4]	0.00±0.00	0.00±0.00	0.13±0.35	0.00±0.00	3.38±0.91	0.25±0.46	4.00±0.92	3.00±1.690	3.75±1.98	6.75±2.81	10.75±2.49***
LSD	*Dose*	1.289	.808	1.389	4.200	14.464	2.130	13.360	63.772	14.928	17.696	5.328
	Time	1.474	1.077	3.202	4.200	18.255	1.184	19.387	148.603	51.617	175.451	88.312
	Dose Time *	2.026	1.885	2.749	4.200	8.661	1.184	8.174	44.331	25.237	5.299	5.977

Each value represents mean ± SD; n = 8 mice PCD = Premature Centromeric Division, Polyploid = 3n.4n and 5n, Delet. and Frag. = Deletion and fragment

Statistically * Significant (P ≤ 0.05), **: highly significant (P < 0.01), ***: very highly significant (P < 0.001) from the control

Table 2: Mutagenic Effects of Drug paclitaxel (Taxol) on the Formation of Micronuclei "MN" in poly chromatic Erylrocyt5e PCEs in mice bone-marrow

		MN		LSD		
Type of treatment		Total No. of MN	Mean ± SD	Dose	Time	Dose * Time
12h	Group [1]	32	4.00 ± 1.069	11.123	223.492	9.408
	Group [2]	63	7.88±2.416 **			
	Group [3]	51	6.38±2.669 *			
	Group [4]	72	9.00±2.000 ***			
18h	Group [1]	30	3.75±1.282			
	Group [2]	130	16.25±4.950 ***			
	Group [3]	56	7.00±2.390 **			
	Group [4]	336	42.00±8.229 ***			
24h	Group [1]	29	3.63±1.847			
	Group [2]	451	56.38±11.401 ***			
	Group [3]	489	61.13±16.660 ***			
	Group [4]	481	60.13±17.133 ***			

MN = Micronuclei. No. individuals Examined = 8mice No. cells examined = 1000 / mice

Statistically * Significant (P ≤ 0.05), **: highly significant (P < 0.01), ***: very highly significant (P < 0.001) from the control.

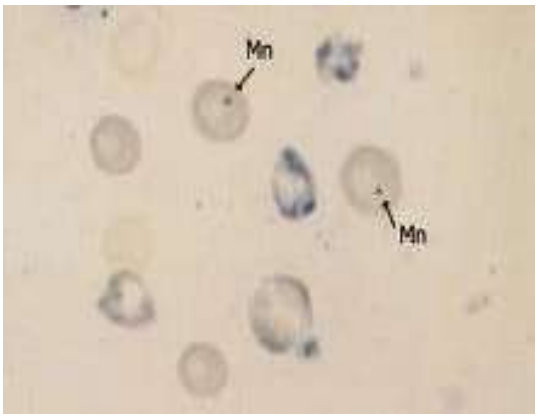

Fig. 3: Poly chromatic Erythrocytes (PCEs) with Micronucleus (Mn).

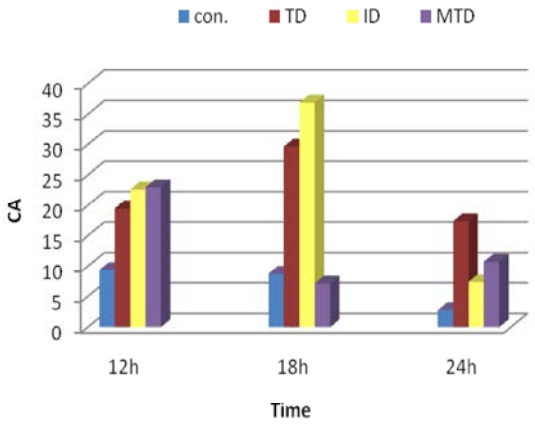

Fig. 4: Effect of Taxol drug on Chromosomal Aberrations in bone marrow cells of mice

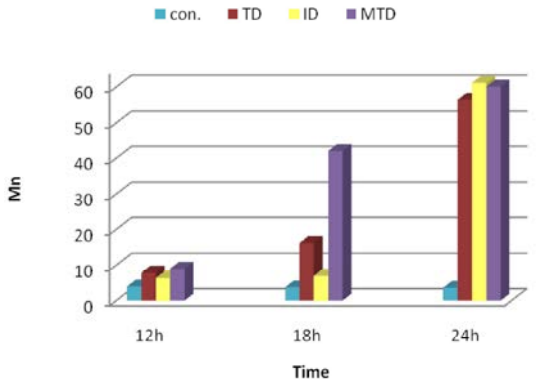

Fig. 5: Effect of Taxol drug on *Micronuclei* in bone marrow cells of mice

increase in stimulating the emergence of chromosomal aberrations was as follows: treatment after 18 hours > treatment after 12 hours > treatment 24 hours.

However, LSD, for different doses in treatments 12 hours after injection, The order chromosomal aberration was found as follows: maximum tolerated dose > intermediate dose > therapeutic dose. While after 18 hours The order was as follows: intermediate dose > therapeutic dose > maximum tolerated dose..After 24 hours of this order injection, therapeutic dose > maximum tolerated dose > intermediate dose.

Micronuclei test also recorded a significant increase in the average numbers of nuclei, after 12.18, 24 hours of treatments with therapeutic, intermediate and maximum tolerated dose, Table (2) and Fig (3 and 5).

Group 4 was dramatically affected by the toxic effect of Taxol indicated by very highly significant increase in the average in numbers of micronuclei and group 2 showed highly significant increase and significant increase in group 3 compared to the control group, after 18 hours. However the result revealed very highly significant increase in third groups 2, 4 and highly significant increase in group 3 compared to the first group, after 18 hours. Also, the result revealed very highly significant increase in the three groups compared to the control after 24 hours Table (2).

There was a highly significant increase in treatment with the therapeutic and maximum tolerated doses after 18 hours injection and the therapeutic, intermediate and maximum tolerated dose after 24 hours injection. The analysis of variance showed a highly significant differences in the average numbers of nuclei among treatments after different time periods in each of the three doses LSD multiple comparisons revealed an order increased stimulating emergence of micronuclei as follows: after 24 hours > after 18 hours > after12 hours of treatments. While for different doses this order: maximum tolerated dose > therapeutic dose > intermediate dose for both 12 or 18 hours after injection. in 24 hours of injection the increase in micronuclei was as follows: intermediate dose > maximum tolerated dose > therapeutic dose.

DISCUSSION

Genotoxicity tests able to detect drugs that cause genetic damage by interaction with other cellular targets, such as enzymes and microtubules, are particularly interesting because they play a significant role in DNA replication or in segregation of chromosomes during cell division [29].

In the present study, the induction of chromosomal aberrations in bone marrow cells *in vivo* are in agreement with the observation of [30-32] and *in vitro* [33, 34]. who reported the inductions of aneuploidy in mouse oocytes and Ozkan *et al*. [35] who observed the increase in chromosomal aberrations, in mouse bone marrow cells following Taxol administration Also, the present results are in agreement with investigations on A 549 cells [36], human T. lymphocytes [37] and peripheral blood lymphocytes [21]. As MNs are formed out of whole chromosome and Taxol found to increase significantly the micro-nucleated lymphocyte rates and over 85% of those micronuclei contained one or more whole chromosomes, Taxol is said to be aneugenic [20].

Microtubules play an important role in cell proliferation and inhibition of microtubule dynamics appears to be the mechanistic basis underlying the antitumor effects of most antimitotic compounds. Coupled with their chemical efficacy in cancer chemotherapy, spindle poisons seem to disturb the integrity of the genome, mainly inducing loss of whole chromosomes [29]. Taxanes affect microtubules, it might be expected that they would induce numerical chromosome aberrations (aneuploidy) [29]. Mitotic aneuploidy may contribute to tumorigenesis by facilitating loss of a chromosome involving tumor suppressor genes that harbor oncogenes[38 and 39]. Chemicals that can interact with the spindle apparatus or interfere with spindle function, preventing normal segregation of chromosomes or chromatids[40], are proven carcinogens [41, 42].

Treating with Taxol drug led to a significant increase in most of structural chromosomal aberrations, but the highly significant aberrations increase that were noticed in the aggregate total of chromosomal aberrations are primarily due to the increased numbers of early separation of the centromere PCD, (structural aberrations) which was accompanied by significant increase in the numerical aberrations in Endomitosis and Polyploidy.

According to [43] they reported that the PCD may be at an early stage of Endomitosis' Which in turn leads to an increased Polyploidy. This explains the significant increase in the numbers of cells containing PCD accompanied by the significant increase in Endomitosis and Polyploidy.

The pre centromeric division "PCD" data obtained in this study indicate relatively low mean PCD frequencies in control group in comparison with those in triple dose treatment. This is in agreement with an investigation on human peripheral lymphocytes PBL carried by Bajic *et al*. [21], who found that Taxol induced PCD in a dose dependent. They postulated that the properties of Taxol induced PCD in PBL and therefore, aneuploidy and genomic instability, is based on the nature of the alteration of centromere function. Also, segregation of chromosomes in anaphase is preceded by a sequential order of centromere separation and alteration of the sequence of centromere separation or PCD which has been found higher in population exposed to various xenobiotics [21,31 and 44-51]. In the present study, the statistically significant increase of polyploidy "poly" is in agreement with earlier reports [25 and 52,53]. The anticancer activity of Taxol was related to effect of inhibiting the normal cell cycle. Guo *et al*. [25] found that abnormal nuclear division arisen in the majority of cells in which cytokinesis could not proceed, resulting in the formation of multiple micro nuclear cells with polyploidy after treatment of osteosarcoma cells (Line U-2-o5) with Taxol.

In the present study, the statistically significant increase of MN per thousand of polychromatic erythrocytes "PCEs") is in agreement with earlier reports [32, 35, 54-58].

It is known that the micronuclei originates either from fragment or lagging chromosomes during the cell fission [27]. This significant increase in MN was not accompanied by significant increase in breaks or fragments. Therefore, the formation of MNs was not attributed to chromosome fragments but to lagging chromosomes [37]. Interaction between Taxol and centromere could explain a considerable amount of the centromere positive micronuclei due to multipolar mitosis. As MNs are formed out of whole chromosome and Taxol found to increase significantly the micro-nucleated lymphocyte rates and over 85% of those micronuclei contained one or more whole chromosomes, Taxol is said to be aneugenic [20].

The induction of micronuclei in bone marrow cells *in vivo* are in agreement with the observation of Kopjar *et al*. [32] who reported the induction of micronuclei in mouse bone marrow cells following Taxol administration.

Taxol was known to affect microtubule assembly and stability [59]. It enhances stability of microtubules, preventing the separation of chromosomes during anaphase. Vinblastine and paclitaxel (Taxol) are widely used as chemotherapeutic drugs that inhibit the normal function of microtubules causing mitotic arrest and cell death [60]. Despite these similarities, the signaling pathways that mediate and regulate cell death seemed to be different[61 and 62].

Spindle poisons like Taxol represent an important class of anticancer drugs that act by interfering with microtubule polymerization [24]. It seen to interfere with cell division by binding to the protein tubulin, which is a key factor in mitosis, the process of cell division and growth[63].

In an attempt to explain the mechanism by which Taxol induces cytotoxicity, micronucleus test was done in the present study using bone marrow cells. It was observed that the frequencies of micro-nucleated cells in bone marrow were significantly higher in Taxol treated groups in all doses. Micronucleus formation was taken as a sign of cytotoxicity of many drugs including Taxol [64].

In conclusion, this study pointed to the possibility of the occurrence of chromosomal aberration and micro-nuclei in the bone marrow cells of the mice treated with the " Taxol " drug in triple therapeutic dose. Further genotoxicity studies dealing with paclitaxel and related compounds are required in order to provide a deeper understanding of the possible risks that could be associated with the clinical use of taxanes.

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