

The Ability of Fresh Pineapple Juice in Reducing the Cytogenetic Effect of Ifosfamide on the Bone Marrow Cells of Male Albino Mice

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ABSTRACT

The main aim of this study is to evaluate the potential prophylactic and anti-mutagenic role played by a nutritional agent of natural origin i.e. fresh pineapple juice, against the cytotoxicity and genotoxicity exerted by the widely used anti-neoplastic agent, Ifosfamide, on bone marrow cells of male white mice, as an *in vivo* model, through the use of the Micronucleus test, which is one of the short term tests approved for such studies. Towards the achievement of such a goal, an experiment was conducted on male mice assigned to 4 groups, namely: The First Group or Control Group (C): This group includes male mice treated solely with a physiological solution for five (5) successive days. The Second Group II or Singly Treated Group (P): This group includes male mice treated solely with Pineapple juice at a dose of 0.40 ml/kg/day, administered orally for five successive days. The Third Group III or Singly Treated Group (T1): This group includes male mice treated with Ifosfamide at doses of 30, 60 and 150 mg/kg/day, administered by intra-peritoneal injection for five successive days. The Fourth Group IV or Doubly Treated Group (P +T1): This Group includes male mice treated with pineapple juice at a dose of 0.40 ml/kg/day, administered orally, followed by intra-peritoneal injection of the drug (Ifosfamide) at doses of 30, 60 and 150 mg/kg/day for five successive days. The results of the current study demonstrate the importance of using short term tests to gauge the prophylactic (anti-mutagenic) role played by a certain substance against the genotoxic and cytotoxic effect of chemical agents, including anti-neoplastic agents. It further demonstrated that the ingestion of fresh pineapple juice prior to treatment with ifosfamide had a positive impact against the cytotoxic and genotoxic effects on subject bone marrow cells, resulting from treatment with Ifosfamide.

KEYWORDS: Ifosfamide, Micronucleus, cytotoxicity, genotoxicity, pineapple, Mice

INTRODUCTION

The grace of God Almighty to make for every disease medicament fit him, which prompted researchers and scientists to think and find the right medicine for each disease, especially in the presence of modern civilization and caused huge changes and stunning contributed not only to human well-being and development, on the other hand, contributed to the increased lot of diseases, principally cancer so that environmental pollution and chemical products and the changing nature of food and life and work may be one of the reasons led to a dramatic increase in the numbers of people living with this disease [1].

Has been selected for the current study property Ifosfamide as one of the chemotherapy drugs used in the treatment of testicular tumors [2], bladder tumors [3], lung cancer, liver, lymph nodes [4], kidney cancer [5] and lymphoma [6].

Some studies have found that drug Ifosfamide possesses mutagenic activity [7; 8] that is one of the most chemotherapy drugs mutagenicity and events for chromosomal fractions [9], as the treatment dose of 30, 45, 60 mg/kg of the drug Ifosfamide for 10 weeks in adult rabbits have caused a shortage of sperm count and an

increase in virtual form for these abnormalities of sperm as well as a significant decrease in the vitality of the sperm [10].

In other studies, it was observed that the transaction property Ifosfamide has had a toxic impact cellular and genetically it may cause an increase in the rate of micronucleus after 24, 48 hours of treatment with a dose of 60 mg/kg rat [11], as well as nuclear imbalances, nuclei pycnosis and karyorrhexis of nuclear chromatin, pycnosis in the mucous cells of a human with a higher rate of production of micronucleus [12].

To reduce cellular and genetic effects caused by treatment with various chemical compounds, some researchers use materials known their immunosuppressive effects. In a study of Badary [13] found that treatment with a dose of 50 mg/kg of the drug Ifosfamide (IFO) for five days have caused nephrotoxicity in rats, while the prior treatment with amino acid taurine as an antioxidant for seven days in drinking water and then for five days with IFO may increase in anti tumor activity as it helped to reduce weight loss rates and decrease death rates of rats as a result of individual drug treatment Ifosfamide (IFO). Also Alvarez-Gonzalez *et al.* [11] noted that when treated mice with a dose of 60 mg/kg of drug Ifosfamide that this dose caused an increase in the rate of micronucleus formation after 24, 48 hours of treatment, while treatment with doses of Nargin (substance present in high concentration in grapefruit) dose of 50, 250, 500 mg/kg did not have any toxic effect on cell or genetically, but dual treatment with the IFO and Nargin has caused a significant decline in the rate of micronucleus in blood of rat specially after 24 and 48 hours of treatment, 48, the rate of decline reach to 54.2% after 48 hours of treatment with 500 mg/kg. Many researchers in different studies recorded that fruits and vegetables have antioxidant activities and anti tumor growth and also mango juice had properties of chemical protection [14]. The Litchi juice; which containing sufficient amounts of polyvinyl; is considered antioxidant and it is useful against fat oxidation in persons infected with breast cancer [15]. Grapefruit juice is a rich source of molecules which are known for their cancer-fighting properties [16], The hat fatty acids in Magnolia seed extract (MagS) may ameliorate cardiotoxicity of the anticancer drug [17].

So the aim of the present research is to study the influence of cytogenetic toxicity of the Ifosfamide drug, and can reduce the toxic effect by natural compound treatment as fresh pineapple juice. Some studies have indicated that pineapple fruit under research may be reduce the risk of chronic diseases such as cardiovascular diseases and cancers, especially fruits containing phenol, which is considered one of the most important antioxidants [18]. It is also an antidote to diabetes and antifungal activity of oxide [19], and it used in medical treatments as a facilitator for digestion [20 and 21], and vaccine formulation, it possesses attributes that active as antitumor and Antimetastatic activity. It also has high efficiency anthelmintic intestinal [22], and at killing intestinal nematodes which inhabit anterior sites in the small intestine [23], it is used in Brazil in folk medicine to treat gastrointestinal disorders, and found that not acute toxicity and mutagenicity, also a safe and potential new anti-ulcerogenic drug [24].

MATERIALS AND METHODS

Ifosfamide; which known commercially as Holoxan; powder white in color and are easily dissolved in distilled water distilled water or normal saline (NaCl 0.9%) and selected property packages the size of 2 g, obtained from Dr. Abd El-Rahman Taha Bakhsh hospital in Jeddah, holoxan was import from Germany (Baxter Oncology GmbH, Kantstrasse 2, D-33790, Haalle).

As used in this study fresh juice of pineapple (*Ananas comosus*) and the specific dosage depending on the Xie *et al.* [25] highlighting the preventive role of pineapple against toxic cytogenetic property ifosfamide as mutagenic.

Animals:

Forty male of the white Albino mice, aged 10-12 weeks and obtained from animal House of the King Fahd Medical Center located at King Abdul Aziz University in Jeddah, where he placed the mice in a special plastic cages under laboratory conditions suitable in a well-ventilated room, room temperature 22-25°C, relative humidity 45-75%, 12 hours daytime lighting, water is provided daily and fed dry balanced feed for experimental animals.

Methods:

Forty male mice divided into four experimental groups as follows (as shown in Table 1):

Group I: control (C) and included 5 rats treated with physiological solution through intestinal tube.

Group II: treatment (P) pineapple only included 5 rats treated with 0.4 ml/kg/day according to Xie *et al.* [25], through the mouth by intestinal tube for five consecutive days.

group III: treatment (T1) included 15 rat treated with ifosfamide 30, 60 and 150 mg according to Yamada *et al.* [26] injection in peritoneal cavity for five consecutive days [27].

Group IV: dual treatment (P + T1) included 15 rat treated with pineapple 0.4 ml/kg/day by intestinal tube for five consecutive days and is given ifosfamide at the same period at dose 30, 60 and 150 mg/day via injection into peritoneal cavity for five consecutive days.

Table 1: Shows the number of mice and the amount of doses of different groups experience.

Group I (C)		Group II (P)		Group III (T1)		Group IV (P+T1)	
Number of mice	Dose	Number of mice	Dose	Number of mice	Dose	Number of mice	Dose
5	Physiological solution	5	(0.4 ml / kg) Pineapple	5	T1A 1.0 mg /kg	5	T2A P 0.4 ml / kg + 1.0 mg / kg
				5	T1B 2.0 mg /kg	5	T2B P 0.4 ml / kg + 2.0 mg / kg
				5	T1C 10.0 mg /kg	5	T2C P 0.4 ml / kg + 10.0 mg / kg

C: Control , P Treatment with Pineapple, T1 Treatment with Ifosfamide, T1+P Treatment with Pineapple & Ifosfamide.

Method of Heddle [28], Schmid [29] and Weber *et al.* [30] were used in the preparation of the bone marrow. After the death of the animal were extracted the bone marrow and placed in a centrifuge tube with about 1 ml of fetal calf serum, has been mixing the contents of the bone marrow with serum by pipette, the centrifuge for 10 minutes at a rate of 1000 rpm, liquid surface disposed and added about 0.5 ml of serum and then turned well, draws from the preparation on clean slides already prepared and dried then fixed in absolute methyl alcohol then paint with eosin [31], and then examine 1000 cell of polychromatic erythrocytes (PECs) from each sample, inventory of how nuclei presence and micro-nuclei observed when examining the rules recommended of Schmid [32], Hayashi *et al.* [33] and Albanese and Middleton [34].

Statistical analysis:

The data were statistically analyzed with completely randomized design. Significant differences between treatments were tested with student's T test and (ANOVA) analysis. [35]

Results:

Effect of treatment with pineapple:

Microscopic examination showed easily identify polychromatic erythrocytes either does not contain or with a micronucleus (Figure 1). The results obtained after 24 hours from the last treatment to male rats with dose 0.40 ml/kg of pineapple that there was a slight increase in numbers of micronucleus but no statistically significant morale in the emergence of polychromatic erythrocytes cells containing micronucleus compared to the control group, where the result was 3.60 ± 0.40 (0.36%) and 2.20 ± 0.37 (0.22%), respectively (Table 1 and Figure 2).

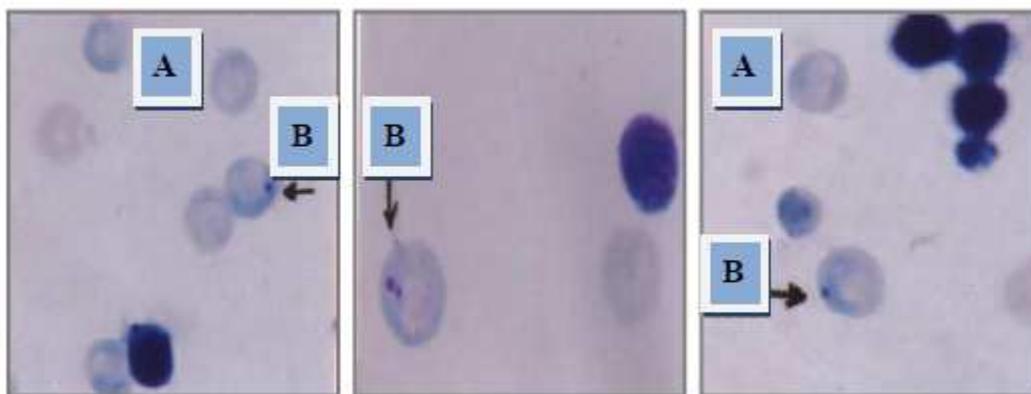


Fig. 1: Micrographs show Bone marrow cells in treated male mice and show where
 A. polychromatic erythrocytes "normal"
 B. polychromatic erythrocytes" with micronucleus" (1000 X)

Table 2: Effect of Pineapple Juice, Treatments of (30, 60,150 mg/kg) of Ifosfamide and The Dual Treatment with Pineapple and Ifosfamide on the mean of polychromatic erythrocytes with micronucleus in Male Mice.

Groups Treatment	No. animal	The calculated number of polychromatic erythrocytes	The number of polychromatic erythrocytes with micronucleus	The rate of development of micronucleus	The rate of antimutagens Effects
Control (C)	1	1000	2	0.22 %	—
	2	1000	3		
	3	1000	3		
	4	1000	2		
	5	1000	1		
	Mean ± Std.Error		37,0 ± 20.2		
Pineapple Juice (0.4 ml / kg) (P)	1	1000	3	0.36 %	—
	2	1000	3		
	3	1000	3		
	4	1000	4		
	5	1000	5		
	Mean ± Std.Error		3,60 ± 0,40		
Ifosfamide (30 mg/kg) (T1A)	1	1000	25	1.5 %	—
	2	1000	15		
	3	1000	13		
	4	1000	10		
	5	1000	12		
	Mean ± Std.Error		15,00 ± 2,62**		
Ifosfamide (60 mg/kg) (T1B)	1	1000	32	2.14 %	—
	2	1000	20		
	3	1000	17		
	4	1000	21		
	5	1000	17		
	Mean ± Std.Error		21,40 ± 2,77**		
Ifosfamide (150 mg/kg) (T1C)	1	1000	30	2.98 %	—
	2	1000	36		
	3	1000	22		
	4	1000	32		
	5	1000	29		
	Mean ± Std.Error		29,80 ± 2,29**		
Pineapple and Ifosfamide (0.4 ml/kg +30 mg/kg) (T2A)	1	1000	8	0.66 %	056.00 %
	2	1000	7		
	3	1000	7		
	4	1000	5		
	5	1000	6		
	Mean ± Std.Error		6,60 ± 0,51**		
Pineapple and Ifosfamide (0.4 ml/kg +60 mg/kg) (T2B)	1	1000	9	0.72 %	66.36 %
	2	1000	4		
	3	1000	8		
	4	1000	5		
	5	1000	10		
	Mean ± Std.Error		7,20 ± 1,16**		
Pineapple and Ifosfamide (0.4 ml/kg +150 mg/kg) (T2C)	1	1000	6	0.76 %	74.50 %
	2	1000	8		
	3	1000	6		
	4	1000	9		
	5	1000	9		
	Mean ± Std.Error		7,60 ± 0,68**		

p* significant<0.05

p** highly significant<0.01

p*** extremely significant<0.001

Effect of treatment with Ifosfamide:

The findings obtained from male rats treated with different doses of the drug Ifosfamide to toxic capacity of this property which appeared evident on bone marrow cells and 24 hours after the last dose, as noted from Table 1 that treatment doses of the drug have shown a response with the micronucleus test, and median emergence of polychromatic erythrocytes cells containing micronucleus high statistically significant rise high morale as a result of treatment with 30 mg/kg of the drug was worth 15.00 ± 2.62 (1.50%), while statistically significant was higher in treatment doses of 60 and 150 mg/kg of the drug were worth 21.40 ± 2.77 and 29.80 ± 2.29 (2.14 and 2.98%), respectively, compared to the control sample mean value 2.20 ± 0.37 (0.22%). This shows previous results a linear relationship, where the average number of micronucleus formed per minute increases with increasing the dose of the drug (Figure 2).

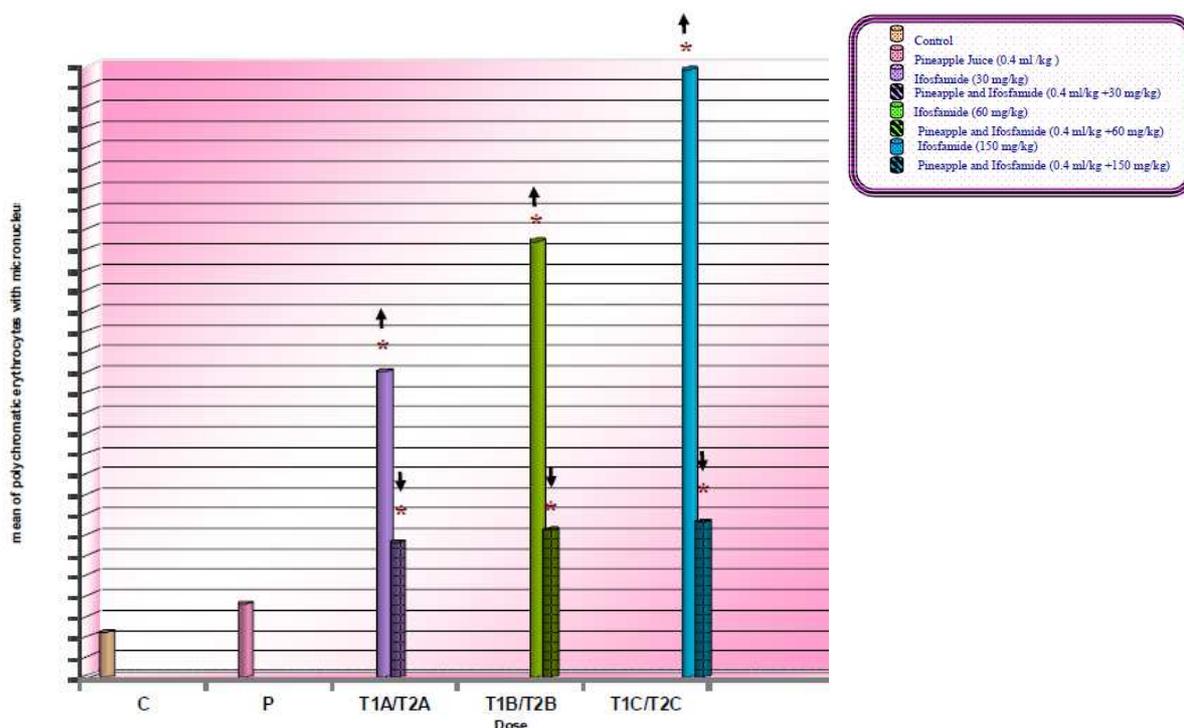


Fig. 2: Effect of Pineapple Juice (30,150 mg/kg) of Ifosfamide and The Dual Treatment with Pineapple and Ifosfamide on the mean of polychromatic erythrocytes with micronucleus in Male Mice.

Effect of treatment with pineapple and Ifosfamide:

Examination of polychromatic erythrocytes containing micronucleus in bone marrow cells of mice treated with pineapple and different doses of Ifosfamide it 24 hours after taking the last dose of pineapple and drug treatment with 0.40 ml/kg of pineapples have had a statistically significant decrease of average appearance of high polychromatic erythrocytes cells containing micronucleus was worth 6.60 ± 0.51 and 7.20 ± 1.16 (0.66% and 0.72%), respectively when compared with 15.00 ± 2.62 and 21.40 ± 2.77 (1.50% and 2.14%), that had resulted from single-dose treatment amounting to 30 and 60 mg/kg of the drug, while the average was remarkably low, statistically significant moral double dose of 0.40 + 150 ml of pineapple and property were worth 7.60 ± 0.68 (0.76%), compared to 29.8 ± 2.29 (2.98%) when treated in single-dose with 150 mg/kg of the drug (Table 1 and Figure 2).

When calculating the effect of occurrence of antimutagenic on development of micronucleus that transactions with pineapple and different doses (30, 60, 150 mg/kg) of the drug Ifosfamide has made marked improvement was 56.00, 66.36 and 74.50%, respectively.

Results obtained from Table 2 showed statistically superior ($P \leq 0.001$) in the emergence of polychromatic erythrocytes containing micronucleus amount ($F=34.33$) between treatment with pineapple and various doses of Ifosfamide compared to the control sample.

Statistically significant ($P \leq 0.001$) in the emergence of polychromatic erythrocytes containing micronucleus of minutes as a result of treatment with various doses of the drug Ifosfamide, while not registered treatment pineapple statistically significant difference in the appearance of polychromatic erythrocytes containing micronucleus (Figure 3), we can possible arrangement of the brightest of transactions in terms of their impact on the average height of the highest development of minute micronucleus are as follows:

$$P < T1A < T1B < T1C$$

As is evident from the results Table 2, that there is a statistically significant difference of high moral integrity ($P \leq 0.01$) in the emergence of polychromatic erythrocytes containing micronucleus amount ($F=14.31$) between treatment with 0.40 ml/kg of pineapple and double-dose treatment 0.40 ml/kg + 30 mg/kg of pineapple and the drug compared to single dose (30 mg/kg) of the drug.

Results indicated that superior moral statistical significance ($P \leq 0.001$) in the emergence of polychromatic erythrocytes containing micronucleus of minutes as a result of treatment with pineapple while teams with high statistical significance of moral consequence dual treatment pineapple property (Figure 3), and are a possible arrangement of the brightest of transactions in terms of their impact on average higher development of micronucleus as follows: $T2A < P$.

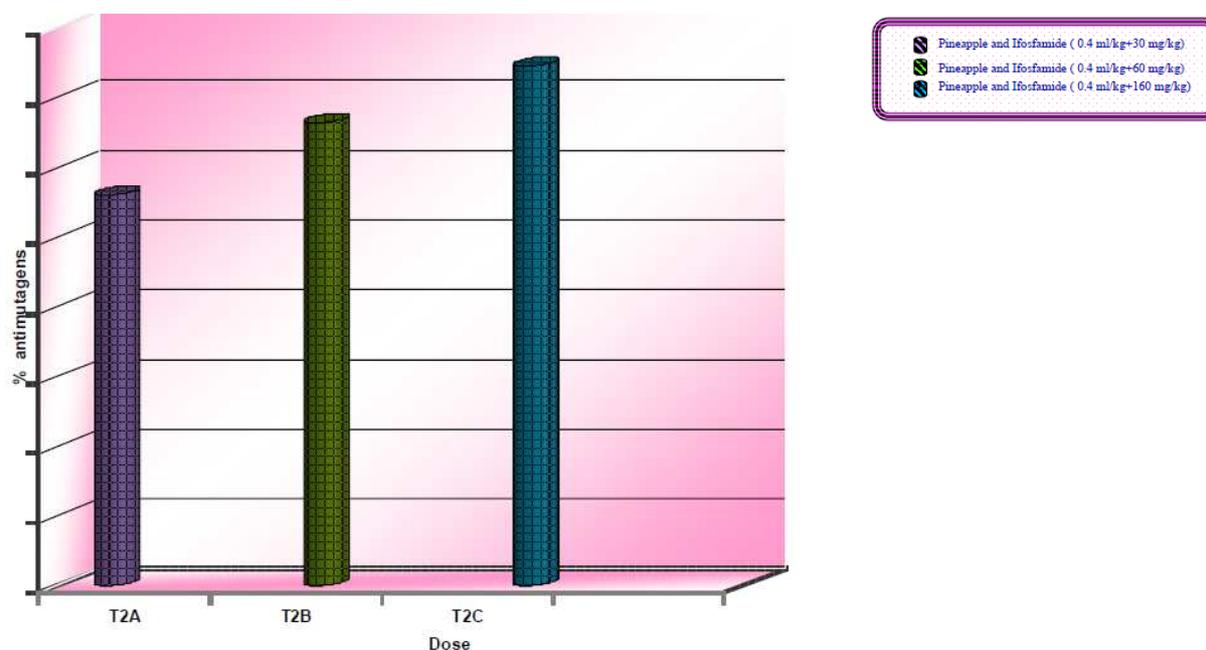


Fig. 2: Effect of Pineapple Juice, (30,150 mg/kg) of Ifosfamide and The Dual Treatment with Pineapple and Ifosfamide on the rate antimutagens on induce micronucleus in Male Mice.

Table 3: ANOVA and LSD between The Effect of Treatment of (30,60,150 mg/kg) of Ifosfamide, Pineapple Juice and The Dual Treatment with Pineapple and Ifosfamide on the mean of polychromatic erythrocytes with micronucleus.

	ANOVA)((LSD)(
	(F)	(Sig)	Groups Treatment	Mean Difference	(Sig)
Control (C)	33,34	**	Pineapple Juice (0.4 ml / kg) (P)	40 -,1	
			Ifosfamide (30 mg/kg) (T1A)	80 -,12	**
			Ifosfamide (60 mg/kg) (T1B)	20 -,19	**
			Ifosfamide (150 mg/kg) (T1C)	60 -,27	**
Ifosfamide (30 mg/kg) (T1A)	31,14	**	Pineapple Juice (0.4 ml / kg) (P)	40,11	**
			Pineapple and Ifosfamide (0.4 ml/kg +30 mg/kg) (T2A)	80,8	**
Ifosfamide (60 mg/kg) (T1B)	01,29	**	Pineapple Juice (0.4 ml / kg) (P)	80,17	**
			Pineapple and Ifosfamide (0.4 ml/kg +60 mg/kg) (T2B)	20,14	**
Ifosfamide (150 mg/kg) (T1C)	99,101	**	Pineapple Juice (0.4 ml / kg) (P)	20,26	**
			Pineapple and Ifosfamide (0.4 ml/kg +150 mg/kg) (T2C)	20,22	**

p* significant<0.05

p** highly significant<0.01

p*** extremely significant<0.001

As the results of analysis of variance in Table 2 the existence of statistically significant difference ($P \leq 0.001$) in the emergence of polychromatic erythrocytes containing micronucleus amount ($F=29.01$) between each dose treatment 0.40 ml/kg of pineapple and double-dose treatment 0.40 ml/kg + 60 mg/kg of pineapple and the drug compared to single-dose 60 mg/kg of the drug.

Statistical significance ($P \leq 0.001$) was obtained from the transaction with pineapple and pineapple and double treatment, compared with single-drug treatment, and through the Figure(3) if possible, arrange transactions in terms of their impact on average higher development of micronucleus as follows: $T2B < P$.

Date in Table 2 showed the existence of statistically significant difference ($P \leq 0.001$) in the emergence of polychromatic erythrocytes containing micronucleus amount ($F=101.99$) between each dose treatment 0.40 ml/kg of pineapple and double-dose treatment 0.40 ml/kg + 150 mg/kg of pineapple, real estate, compared to single-dose 150 mg/kg of the drug.

Statistically significant difference ($P \leq 0.001$) the result of each transaction with pineapple and pineapple and double treatment, compared with single-drug treatment, and through the Figure (3) if possible, arrange the brightest of transactions in terms of their impact on average higher development of micronucleus as follows: $T2C < P$.

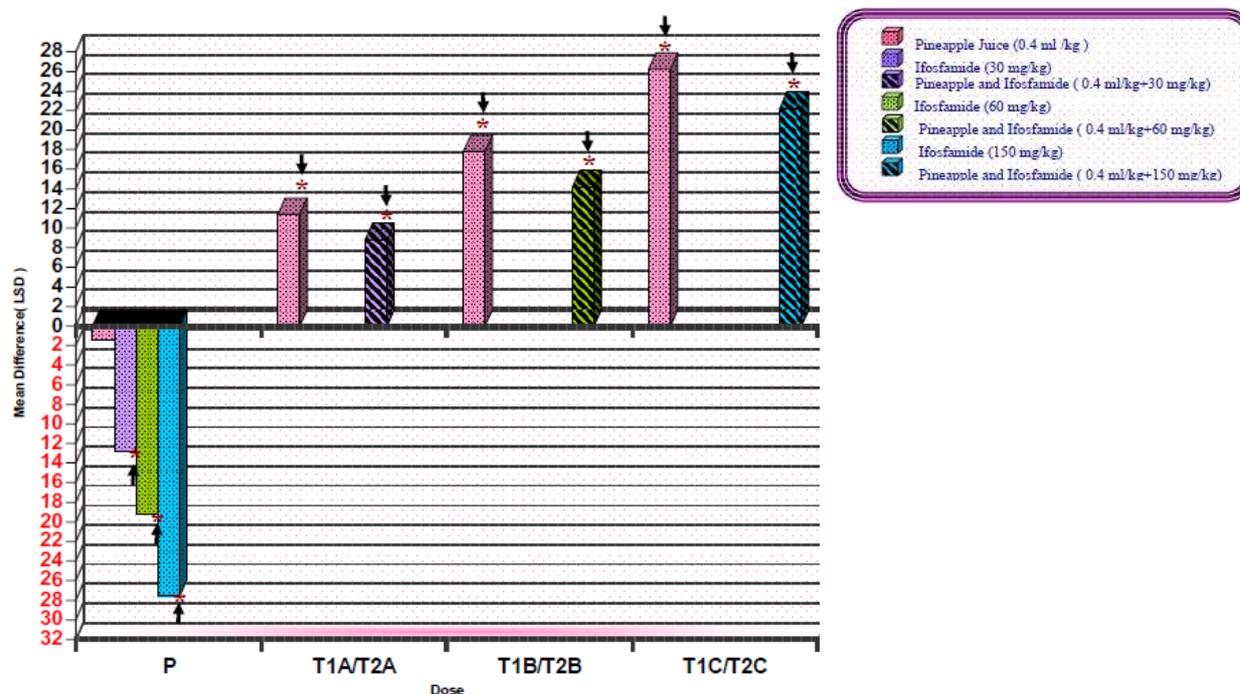


Fig. 3: Comparison between The Effect of Treatment of Pineapple Juice, (30,60,150 mg/kg) of Ifosfamide and The Dual Treatment with Pineapple and Ifosfamide on the mean of polychromatic erythrocytes with micronucleus by LSD.

Discussion:

If the previous indicators show that Ifosfamide drug side effects on genetic material so that this study is aimed at how to reduce those risks by eating a natural material such as fresh pineapple juice.

It has been used cytogenetic test which are of short-term tests and have the possibility to show the property of toxic effects and cellular: Testing the micronucleus in polychromatic erythrocytes in the bone marrow.

Ifosfamide property is one of the most commonly used chemotherapy drugs in the treatment of many human cancers, it is drugs that require metabolic activation by Cytochrome P_{450} hepatic effect to appear *in vivo* or *in vitro*. The metabolic of Ifosfamide beginning of vital ways; I: ring active oxidation to produce 4-OH-IF (active Ifosfamide), II: oxidation of the side chain- oxidation and release Chloroacetaldehyde component outputs inactive metabolites 3-dechloroethylifosfamide (3-DCE-IF), 2-dechloroethylifosfamide (2-DCE-IF), which is believed to derive from acrolein is responsible for urinary toxicity and composition of blood bags, while CAA is responsible for Nephrotoxicity [36; 37]. Also, the property Ifosfamide is alkylation agent which is not linked in the effectiveness certain stage of cell cycle Phase known as Non-Specific Drugs [38; 39].

The main factor was attributed in cellular toxicity of most effective medical strong cross-linkage which between two template DNA produced this tape is idle so it stops the production of DNA, leading ultimately to apoptosis [40; 41]; and not only that, but some alkaline factors with detrimental impact on tissues with low rate of division such as liver, kidney and mature lymphocytes, but may be highly toxic to tissue fast division such as bone marrow cells [42].

Hall and Tilby [43] and Latz and Weber [44] reported that Ifosfamide cellular toxic effects and that this toxin believed to be produced by covalent links between aggregates of alkyl and DNA which lead to links between his crossings DNA interstrand cross-linking.

The obtained results shown the exact kernel ability real estate Ifosfamide to develop micronucleated polychromatic erythrocytes (MN-PCEs) and statistically in bone marrow cells of mice treated with different doses of the drug as compared to the control sample (Table 1 and Figure 2), and found a positive relationship between the presence of a drug dose Ifosfamide and resulting impact dose related effect where the proportion of

PCEs cells containing micronucleus increased the dose coefficient, this Consistent with its many previous researchers; the result treated bone marrow [45; 46] or spleen cells in mice [47] as well as in the cells of the Ifosfamide as a result of the treatment of rats with anti-cancer drugs [48] and also in Chinese hamster ovary cells [49] and in peripheral blood lymphocytes of human [50; 51; 52].

Goldberg *et al.* [53] and Albanese [54] showed that when male rats injected with different doses of the drug cyclophosphamide, which is an anti-cancer drug bilateral post which is similar in its impact effect property cyclophosphamide into peritoneal, therapists, and taken samples of bone marrow after different times of taking a dose, the top response for property cyclophosphamide at 24 hours after taking a dose, where the highest proportion of the cells (MN-PCEs) containing micronucleus. So that we in the present study further sampling of bone marrow cells after 24 hours to give another dose. We found that there was an increase in moral (MN-PCEs) when the amount of sample officer at this time after 24 hours of treatment doses (150, 60, 30 mg/kg) of the drug. These results are consistent with a finding of Alvarez-Gonzalez *et al.* [11] when treated male mice with a dose of 60 mg/kg of the drug Ifosfamide has found that this increase in dose rate configuration micronucleus.

Since the micronucleus consisting of parts of chromosomes or chromosomes were delayed and remained in the cytoplasm [55], the emergence of micronucleus is a strong indication of the impact of chemicals that harm the DNA and which in fact reflects the process broken chromosomes, as well as damage to the threaded spindle [56; 57; 58; 33], and this became the test kernel rpm and an essential part of chromosomes clastogenic and aneugenic effects which are usually on the bone marrow cells in rodents, particularly mice [55; 57; 58], and is therefore used to identify chemicals that have the potential to cause damage to chromosomes or spindle in cells mother that are red blood cells [56; 60; 61], many have made clear in previous research the capacity of many chemical produced micronucleus in polychromatic erythrocytes cells in rats and humans, a real anti-leukemia [61; 62; 63; 64; 65], therefore the micronucleus test is good evidence of cytogenetic toxicity, and features a quick and simple test short-term Test for this evaluation as it is inexpensive.

Hence it is clear that the emergence of micronucleus in the cells (PECs) in mice treated with different doses of Ifosfamide, obtained from the current study is strong evidence on the impact of toxic cellular genetic property and an indicator of its ability mutagens. As noted also that treatment with pineapple and the estate had statistically significant decrease morale of average appearance of micronucleated polychromatic erythrocytes.

Can explain the preventive role played pineapple in reducing pathological effects resulting from treatment with the drug Ifosfamide that pineapple juice possesses an antioxidant activity [66] because it contains bromelain, which is one of the most active compounds in the fruit because it contains critical enzyme known as Cysteine proteinase which is used in medical treatments as a facilitator for digestion and in vaccine formulation, attributes and has an Antitumor & Antimetastatic Activity [20; 21; 67], Vitamin C and c6-Hydroxy-1-Methyl-1-2-3-4-Tetrahydro-Beta-Carboline, which are believed to function as antioxidants after absorption and accumulation in the body as well as free scavengers [66; 68; 69; 70; 71; 72 73; 74].

The present study therefore recommends further studies that show the relationship between the pineapple from the stimulation of these antioxidants in the body and its ability to reduce the toxic effects of antiretroviral drugs for cancer tumor.

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