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Genotoxicological Evaluation for the Hepanox Drug and its Major Components

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Abstract: Hepanox is widely used as an anti-oxidant drug for treating some liver problems additional to its two main components: selenium and silymarin were evaluated for possible genotoxicity on laboratory albino mice. The evaluation was performed on somatic and germ cells as well as sperm morphology. The mice were orally administrated with the therapeutic dose and its double of Hepanox up to three successive months. The animals were also orally received selenium and silymarin. Their doses were chosen according to their percentages in the Hepanox drug. The results showed that selenium induced a significant reduction in the mitotic and meiotic activity. It also induced chromosomal abnormalities in both somatic and germ cells, but the induced chromosomal abnormalities in the spermatocytes were shown only after the third month of treatment. Silymarin didn't affect the mitotic and meiotic activity, it is also didn't induce chromosomal abnormalities in somatic and germ cells. The hepanox drug didn't affect the mitotic and meiotic activity, it induced some chromosomal abnormalities such as gaps and deletions after the third month of treatment, similarly for spermatocytes the induced abnormalities were X-Y univalents and fragments. Regarding sperm morphology assay, it was found that only selenium which affects the sperm count. All treatments showed significant levels of sperm abnormalities after selenium treatment. Hepanox reduced the rate of sperm shape abnormalities

Key words: Hepanox, selenium, silymarin, mutagenicity, mice, sperm

INTRODUCTION

Egypt has a population of more than 62 million and contains the highest prevalence of hepatitis C in the world. The national prevalence rate of hepatitis C virus (HCV) antibody positively has been estimated to be between 10-13% (Mohamed, 2004). Since 30-40% of individuals clear the infection shortly after exposure based on national studies and village studies in Egypt, the estimated adjusted national prevalence rate of chronic hepatitis C infection is 7.8% or 5.3 million people in 2004 (Mezban and El Wakil, 2003). Hepatitis C infection can lead to serious liver diseases and the population infected decades ago has since spread the virus throughout the country. It is reported that the injections stopped long ago but the pool of carriers keeps spreading, which has led to raise the rates of cirrhosis and liver cancer (Bonkovsky and Mehta, 2001).

The main cause for the widespread of HCV in Egypt is the extensive use of contaminated syringes and transferring the blood or its products form the infected persons to the health persons during the treatment of the Schistosomiasis. Schistosomiasis, also known as bilharzia, is a serious infection of the urinary and intestinal tracts caused by blood flukes tiny flatworms, whose larvae are carried by snails living in stagnant water in the tropics.

The Egyptian government is doing her best to control the HCV by introducing new drugs for improving the liver function. From these drugs, hepanox which is composed mainly from two main components: Selenium and silymarin.

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Selenium is an essential trace mineral needed in the human body. This nutrient is an important part of antioxidant enzymes that protect the cells against the effects of free radicals that are produced during normal oxygen metabolism (National Research Council, 1989). It is also essential for normal functioning of the immune system and thyroid gland (Corvilain *et al*., 1993; Levander, 1997). Conflicting reports about the genotoxicity of selenium. Some reports showed that selenium is not a mutagenic, even after treatment with high doses (Norppa *et al*., 1980a, b). On the other h and some reports proved that selenium is a mutagenic chemical substance (Mousa *et al*., 1988; Khalil, 1989; Moore *et al*., 1996; Biswas *et al*., 1997). In general it is reported that these conflictions came from the selenium salt tested, some salts proved to be more toxic than the others (Biswas *et al*., 1997, 2000; Nuttall, 2006). Selenium was reported as a protective agent against genotoxicity induced by many pollutants (Canoruc *et al*., 2001; El-Bayoumy *et al*., 2001; Bronzetti *et al*., 2003; Zhuo *et al*., 2004).

Silymarin is an extract from a herb called *Silybum marianum* (milk thistle) and found in some parts in USA and Europe (Gruenwald *et al*., 1998). Silymarin has been shown to have positive effects in treating liver diseases of various kinds, including cirrhosis, chronic hepatitis, fatty infiltration of the liver and inflammation of the bile duct (Salmi and Sarna, 1982; Boari *et al*., 1985; Canini *et al*., 1985; Kropacova *et al*., 1998). Miguez *et al*. (1994) reported that, silymarin prevented cell damage in hepatocytes caused by t-butyl hydroperoxide.

The aim of the present study is to evaluate the genotoxicity of the hepanox drugs and two of its main components: selenium and silymarin on both somatic and germ cells as well as sperm morphology assay in albino mice.

MATERIALS AND METHODS

Animals

In this study, one hundred adult male Swiss albino mice (20-25 g) were maintained on feed and water *ad libitium*. Mice were obtained from the animal house of the National Research Center. Five mice were grouped randomly in a cage and kept for treatment; a control group was kept parallel at the same conditions.

Drug and Chemicals

Hepanox drug is a capsule and obtained commercially from Technopharma, Egypt. Sodium selenite is a pure powder and obtained from Prolabo, France. Silymarin was obtained as tablets with sugar coated from Chemical Industries Development (CID), Egypt.

Dosage and Treatment

The Hepanox drug and two of its main components selenium and silymarin were orally administrated to male mice using similar protocol for treatments. The animals were classified to two main groups: the first main group was animals treated with the therapeutic dose of the Hepanox (21.3 mg/kg b.w.), selenium (0.42 mg/kg b.w.) and silymarin (5.6 mg/kg b.w.). A parallel control group was kept at the same time and did not receive any thing. The duration of the treatments was for one, two and three months, so animal were killed at each month end. The second main group was treatment animals treated with the double of the therapeutic dose of Hepanox (42.6 mg/kg b.w.), selenium (0.84 mg/kg b.w.) and silymarin (11.2 mg/kg b.w.). Also a parallel control group was kept at the same time and did not receive any thing. The duration of the treatments also was for one, two and three months, so animal were killed at each month end. All the animals were killed by cervical dislocation. The testis and the bone morrow were taken for preparation of the mitotic and meiotic slides.

The therapeutic dose of the Hepanox was chosen after the prescription of the Egyptian physicians, while the doses of selenium and silymarin were chosen according to their percentages in the Hepanox drug.

Slide Preparation and Analysis

Mitotic and meiotic chromosomes were obtained from the same animal. Animals were injected i.p. 2.5 h before killing with 0.2 mL colchicines (0.05%)/100 g body weight. Bone marrow chromosomes were prepared according to the method of Yosida and Amano (1965), while meiotic chromosome preparations from testicles were prepared following the technique described by Brewen and Preston (1987). For each animal, 50 metaphase spreads were examined for both marrow and spermatocytes. Different types of abnormalities were recorded. The mitotic activity were also calculated. For studying the sperm count and morphology abnormalities, five groups of animals (each five) were treated and used simultaneously. The first group was animals received the high dose of the Hepanox drug, the second group were animals received the high dose of the silymarin, the third group were animals received the high dose of sodium selenite, the fourth group were animals injected with 20 mg/kg cyclophosphamide for 5 days and acts as a positive control, the last group were animals did not receive any treatment and acts as a control group. Animals were scarified after 25 days following the first injection. Sperm were collected for the examination of head and tail abnormalities following the method of Wyrobek and Bruce (1978), more than 500 sperm per animal were examined. The obtained data were analyzed using one way analysis of variance. Significant differences among means were separated using Duncan Multiple Range F-test (Duncan, 1955).

RESULTS

Effect of Selenium

The results showed a significant reduction in mitotic activity for all the treatment tested (Table 1). Regarding structural chromosomal abnormalities observed were: chromatid gaps, breaks, deletions, fragments, centric fusions, end to end associations, centromeric attenuations and endomitosis. Some of these abnormalities were insignificant while others were significant mainly after high dose and starting from the second month (Table 1). Concerning numerical abnormalities no significance was noticed for polyploidy and hypoploidy, while endomitosis and hyperploidy were found to be significant after the third month of treatment (Table 2). It is also noticed a significant reduction in meiotic activity after the different treatments. Some chromosomal abnormalities were significant (breaks, fragments X-Y univalents). Similar to the bone marrow cells the significant abnormalities were clear after the third month of treatment (Table 2). No significance was observed for any type of numerical abnormalities.

Effect of Silymarin

Silymarin didn't significantly reduce the mitotic activity. All types of observed structural and numerical chromosomal abnormalities after silymarin treatments were also insignificant (Table 3). Similarly silymarin didn't significantly reduce the meiotic activity. Concerning chromosomal abnormalities in spermatocytes (Table 4), some figures for some structural abnormalities were significant (breaks, X-Y univalents) especially after the third month of treatment and high dose. All the numerical abnormalities didn't show any significant increase.

Effect of Hepanox

Hepanox didn't affect the mitotic activity of bone marrow cells (Table 5). Some figures of structural abnormalities were significant (gaps, breaks and deletions) while the most were insignificant, similarly some figures of numerical abnormalities were significant (endomitosis and polyploidy). The results didn't show any significant change in mitotic activity after hepanox treatments. Breaks didn't show any significant changes while X-Y univalents and fragments showed some figures of significance. Also hypoploidy and hyperploidy showed some figures of significance especially after the third month of treatment (Table 6).

Table 1: Chromosome aberrations induced by sodium selenite on mice bone marrow cells

		Structural						
Treatments	Gap	Break	Deletion	Fragment	Centric fusion	End to end association	Total	
	Control	0.8±0.4d	0.4±0.2cd	0.0b	0.0c	0.0f	0.0a	1.2±0.4g
Low dose								
	1st month	1.2±0.2cd	0.6±0.4cd	0.2±0.2b	0.4±0.2abc	1.0±0.4def	0.4±0.2a	3.8±0.8def
	2nd month	1.6±0.5bcd	1.6±0.2ab	0.2±0.2b	0.4±0.2abc	2.2±0.7bc	0.4±0.2a	6.4±1.1bc
	3rd month	1.8±0.2bcd	1.8±0.4a	0.4±0.2ab	0.8±0.4ab	1.6±0.5bcd	0.4±0.2a	6.8±0.6b
High dose								
	1st month	0.8±0.6d	0.0d	0.2±0.2b	0.8±0.4ab	1.2±0.6cde	0.4±0.2a	3.4±0.6ef
	2nd month	1.6±0.5bcd	1.6±0.2ab	0.4 ±0.2ab	0.4±0.2abc	2.4±0.4b	0.4±0.2a	6.8±1.0b
	3rd month	2.0±0.3bcd	1.0±0.3abc	0.4±0.2ab	0.4±0.2abc	5.6±0.9a	0.4±0.2a	9.8±0.8a

		Numerical							
Treatments		Centromeric attenuation	Endomitosis	Hypo ploidy	Hyper-ploidy	Polyp loidy	Total	Total aberrations	Meiotic activity
	Control	2.8±0.5c	0.2±0.2ef	2.4±0.4ab	0.2±0.2c	0.0b	5.6±0.7cd	6.8±1.0h	83.6±3.9a
Low dose	1st month	2.6±0.2c	0.2±0.4ef	2.8±0.8ab	0.8±0.4abc	0.0b	6.4±1.0cd	10.2 ±0.8defgh	54.0±1.4b
	2nd month	2.4±0.4c	1.4±0.9abcdef	2.0±0.7ab	0.6±0.4abc	0.2±0.2b	6.6±1.4cd	13.0±1.3cd	50.0±1.4b
	3rd month	5.0±0.7ab	1.2±0.8bcdef	3.4±0.5a	1.0±0.3ab	0.4±0.2b	11.0±0.5ab	17.8±0.6b	50.2±4.4b
High dose									
	1st month	2.8±0.9c	1.4±1.1abcdef	2.0±0.5ab	0.6±0.2abc	0.2±0.2b	7.0±0.8cd	10.4±0.9defg	37.2±3.1c
	2nd month	2.8±0.4c	1.8±1.3abcd	2.2±0.4ab	0.8±0.4abc	0.2±0.2b	7.8±1.2cd	14.6±1.7c	33.6±3.9c
	3rd month	5.8±0.9a	2.6±1.5ab	3.4±0.5a	1.2±0.4a	0.6±0.4ab	13.6±0.7a	23.0±0.9a	29.6±4.4c

Means with different letter(s) (a-h) in the same column are significantly different ($p<0.05$), The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 2: Chromosome aberrations induced by sodium selenite on mice spermatocytes

		Structural				Numerical						
Treatment		Break	XY univalent	Fragment	Total	Hypoploidy	Hyperploidy	Polyploidy	Total	Total aberrations	Meiotic activity	
	Control	1.4±0.4de	1.0±0.4f	0.0d	2.4±0.7g	0.8 ±2.0bc	0.2±0.2b	0.4 ±1.8abcd	± 0.9 4.0bcd	6.4±0.7hi	29.6±1.5a	
Low dose	1st month	1.0±0.3e	1.4±0.2ef	0.4±0.2bcd	2.8±0.2fg	1.0±0.4c	0.0b	0.6±0.4d	1.6±0.5d	4.4±0.7i	25.4±2.2ab	
	2nd month	2.4±0.2cde	2.4±0.4bcdef	0.8±0.4ab	5.6±0.4cde	1.0±0.3c	0.2±0.2b	0.8±0.4cd	2.0±0.8cd	7.6±0.7efghi	23.6±1.0ab	
	3rd month	3.8±0.6abc	3.8±0.4ab	1.0±0.4a	8.6±0.4ab	2.0±0.4bc	0.2±0.2b	1.6±0.2abcd	3.8±0.7bcd	12.6±0.9hi	20.6±0.7b	
High dose												
	1st month	1.6±0.4de	1.4±0.4ef	0.4±0.2bcd	3.4±0.7efg	1.6±0.5bc	0.2±0.2b	1.2±0.4bcd	3.0±0.9bcd	6.4±1.4cde	21.2±1.2b	
	2nd month	2.6±0.2cde	2.0±0.3cdef	0.2±0.2cd	4.8±0.2cdefg	4.0±2.0bc	0.0b	1.2±0.7bcd	5.2±1.9bc	10.0±2.0fgh	20.4±1.6b	
	3rd month	5.0±0.4a	3.8±0.4ab	0.6±0.2abc	9.4±0.9a	1.6±0.5bc	0.6±0.4ab	2.2±0.4abc	4.4±1.2bcd	13.8±1.4abc	20.4±1.2b	

Means with different letter(s) (a-I) in the same column are significantly different ($p<0.05$), The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 3: Chromosomal aberrations induced by silymarin on mice bone marrow cells

		Structural						Numerical							
Teartments		Gap	Break	Deletion	Fragment	Centtric fusion	Total	Centricmeric attenuaation	Endomitosis	hypo-ploidy	Hyper-ploidy	Poly-ploidy	Total	Total aberraation	Meiotic activity
	Control	0.8±0.4d	0.4±0.2cd	0.0b	0.0c	0.0f	1.2±0.4g	2.8±0.5c	0.2±0.2ef	2.4±0.4ab	0.2±0.2c	0.0b	5.6±0.7cd	6.8±1.0h	83.6±3.9a
Low dose	1st month	1.8 ±0.4bcd	0.8±0.4bcd	0.4±0.2ab	0.0c	0.0f	3.0±0.5efg	4.0±0.6abc	1.2±0.6bcdef	2.4±0.7ab	0.0c	0.0b	7.6±1.0cd	10.6±1.4defg	76.6±4.3a
	2nd month	1.2±0.4cd	0.4±0.2cd	0.4±0.2ab	0.2±0.2bc	0.8±0.4def	3.0±0.5efg	2.4±1.3c	0.8±0.4cd	2.6±0.7ab	0.2±0.2c	0.0b	6.0±0.8cd	8.2±0.5fgh	75.8±2.1a
	3rd month	2.2±0.2bcd	0.4±0.2cd	0.0b	0.0c	0.6±0.2def	3.2±0.4ef	2.8±0.5c	0.0f	2.4±0.7ab	0.0c	0.6±0.4ab	5.8±1.1cd	9.0±1.3efgh	74.4±2.7a
Highe dose															
	1st month	2.2±0.2bcd	0.6±0.2cd	0.2±0.2b	0.0c	0.0f	3.0±0.3efg	2.8±0.4c	1.6±0.4abcde	3.0±0.5ab	0.0c	0.4±0.2b	7.8±0.8cd	10.8±1.0defg	79.0±4.3a
	2nd month	1.4±0.4bcd	0.0d	0.4±0.4ab	0.4±0.2abc	0.2±0.2ef	2.4±0.2fg	2.4±0.2c	1.0±0.3cdef	2.8±0.6ab	0.2±0.2c	0.0b	6.4±0.9cd	8.8±1.1fgh	74.6±5.9a
	3rd month	1.2±0.4cd	0.0d	0.6±0.2ab	0.2±0.2bc	0.8±0.4def	2.8±0.4efg	3.0±0.3c	1.6±0.2abcde	2.2±0.2ab	0.8±0.4abc	0.0b	7.6±0.4cd	10.4±0.7defg	73.2±2.9a

Means with different letter(s) (a-h) in the same column are significantly different (p<0.05), The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 4: Chromosomal aberrations induced by silymarin on mice spermatocytes

		Structural				Numerical				
		Break	XY univaalent	Total	Hypoploidy	Hyperploidy	Polyploidy	Total	Total aberrations	Meiotic activity
	Control	1.4 ±0.4de	1.0±0.4f	2.4±0.7g	2.0±0.8bc	0.2±0.2b	1.8±0.4abcd	4.0±0.9bcd	6.4±0.7hi	29.6± 1.5a
Low dose	1st month	2.6 ±0.6cde	1.8±0.4cdef	4.4±0.5defg	4.4±1.6ab	0.2±0.2b	1.6±0.7abcd	6.2±1.7b	10.6±1.4cdefg	27.0±2.2ab
	2nd month	3.0 ±0.8cd	3.8±0.8ab	6.8±1.5abcd	2.2±0.7bc	0.0b	1.6±0.2abcd	3.8±0.7bcd	10.6±2.0cdefg	26.2±1.2ab
	3rd month	4.8 ±0.6ab	3.8±0.4ab	8.6±0.7ab	1.6±0.5bc	0.0b	1.2±0.4bcd	2.8±0.7bcd	11.4±0.9bcde	25.4±2.3ab
High dose										
	1st month	2.2 ±1.0cde	3.0±0.5abcde	5.2±0.7cdef	3.4±0.5bc	0.0b	1.2±0.5bcd	4.6±0.8bcd	9.8±1.0defgh	26.6±2.6ab
	2nd month	3.2 ±0.5bcb	3.8±0.2ab	7.0±0.5abcd	1.8±0.6bc	0.0b	2.2±0.5abc	4.0±0.7bcd	11.0±1.2bcdef	25.4±3.2ab
	3rd month	4.8 ±0.9ab	4.0±0.4ab	8.8±0.9a	4.0±0.6bc	0.6±0.4ab	1.2±0.6b	5.8±0.4b	14.6±0.7ab	23.8±2.5ab

Means with different letter(s) (a-l) in the same column are significantly different (p<0.05), The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 5: Chromosomal aberrations induced by heparox on mice bone marrow cells

		Structural							Numerical							
		Gap	Break	Deletion	Fragment	Centtric fusion	End to end association	Total	Centromeric attenuaation	Endomitosis	Hypoploidy	Hyperploidy	Polyploidy	Total	Total aberraation	Mitotic activity
	Control	0.8±0.4d	0.4±0.2cd	0.0b	0.0c	0.0f	0.0a	1.2±0.4g	2.8±0.5c	0.2±0.2ef	2.4±0.4ab	0.2±0.2c	0.0b	5.6±0.7cd	6.8±1.0h	83.6±3.9a
Low dose	1st month	2.8±1.0ab	0.6±0.4cd	0.2±0.2b	0.6±0.4abc	0.4±0.2ef	0.0a	4.6±1.3cde	3.8±0.6bc	0.4±0.2def	1.8±0.4ab	0.2±0.2c	0.0b	6.2±1.2cd	10.8±0.9defg	76.8±4.2a
	2nd month	2.8±0.4ab	1.2±0.2abc	0.0b	0.0c	0.0f	0.0a	4.0±0.3def	2.8±0.7c	1.2± .2bcdef	2.6±0.5ab	0.0c	0.0b	6.6±1.0cd	10.6±1.0defg	76.4±4.9a
High dose	3rd month	2.6±0.2bc	0.0d	0.4±0.4ab	0.0c	0.6±0.2def	0.4±0.2a	4.0±0.5def	3.8±0.6bc	2.2±0.5abc	1.4±0.2b	0.0c	0.6±0.4ab	8.0±1.0cd	12.0±1.2cdef	74.2±3.9a
	1st month	2.2±0.7bcd	1.2±0.2abc	0.0b	1.0±0.3a	0.2±0.2ef	0.0a	4.6±0.8cde	3.4±0.7bc	2.8±1.1a	1.8±0.9ab	0.2±0.2c	0.2±0.2b	8.4±1.2bc	13.0±0.7cd	75.6 ±6.7a
	2nd month	4.0±0.3a	0.0d	0.0b	0.0c	0.2±0.2ef	0.2±0.2a	4.4±0.4def	2.2±0.4c	1.8±0.9abcd	2.8±0.4ab	0.4±0.2bc	1.0±0.3a	8.2±1.1bcd	12.6±0.9cde	74.0±4.0a
	3rd month	2.8±0.5ab	1.0±0.3abc	1.0±0.5a	0.2±0.2bc	0.6±0.2def	0.0a	5.6±0.4bcd	2.0±0.9c	1.0±0.4cdef	1.8±0.4ab	0.2±0.2c	0.0b	5.0±1.7d	10.6±2.0defg	73.6 ±5.3a

The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 6: Chromosome abnormalities induced by hepanox on mice spermatocytes

		Structural				Numerical				Total	Meiotic
		Break	XY univaalent	Fragment	Total	Hypoploidy	Hyperploidy	Polyploidy	Total	aberrations	activity
	Control	1.4±0.4de	1.0±0.4f	0.0d	2.4±0.7g	2.0±0.8bc	0.2±0.2b	1.8 ±0.4abcd	4.0±0.9bcd	6.4±0.7hi	29.6±1.5a
Low dose	1st month	2.0±0.4cde	1.8±0.7cdef	0.0d	3.8±0.6efg	1.2 ±0.7c	0.0b	1.8 ±0.4abcd	3.0±0.8bcd	6.8 ±0.5ghi	27.2 ±2.8ab
	2nd month	2.0±0.7cde	3.4±1.0abc	0.0d	5.4±1.7cdef	2.8±1.1bc	0.0b	0.6 ±0.2d	3.4±1.0bcd	8.8±2.1defgh	26.0±3.2ab
	3rd month	2.8±0.7cde	3.2±0.6abcd	0.0d	6.0±0.8bcde	3.4±0.7bc	0.0b	2.4±0.7ab	5.8±1.0b	11.8±1.4bcd	23.6 ±1.2ab
Highe dose											
	1st month	2.2±0.2cde	1.6±0.6def	0.0d	3.8±0.7efg	2.2±0.8bc	0.2 ±0.2b	1.0±0.4bcd	3.4±1.1bcd	7.2±1.1fghi	25.6 ±2.3ab
	2nd month	2.8±0.7cde	4.4±0.8a	0.2±0.2cd	7.4±1.2abc	6.8±1.6a	0.0b	2.8 ±0.6a	9.6±1.5a	17.0±1.6a	24.8±2.7ab
	3rd month	3.2±0.4bcd	4.2±0.4a	0.8±0.4ab	8.2±0.6ab	3.4±0.5bc	1.0±0.4a	1.2±0.4bcd	5.6±0.7b	13.8±1.1abc	24.0 ±2.3ab

Means with different letter(s) (a-I) in the same column are significantly different ($p<0.05$), The value in each cell represents mean±standard error, These data were obtained from 250 metaphase/group

Table 7: Effect of different drugs and components on mice sperm morphology

	Head abnormalities						Tail abnormalities	
	Amorphous head	Head without hock	Small head	Big head	Banana head	Total head abnormalities	Coiled tail	Total abnormalities
Control	5.6±0.2C	3.6±0.7b	1.2±0.5b	0.6±0.2a	0.0b	11.0±0.5b	2.4±0.7a	13.4±0.9b
Cyclophos-phamide	10.2±1.0bc	5.8±1.2ab	6.0±2.2a	1.0±0.3a	0.0b	23.0±3.7a	3.8±1.6a	26.8±3.2a
Sodium selenite	16.8±2.9a	6.8±1.4ab	1.6±0.7b	0.6±0.4a	0.0b	25.8±2.3a	0.0b	25.8±2.3a
Silymarin	b15.8±3.4ab	7.8±1.2a	0.8±0.2b	0.2±0.2a	0.6±0.4a	25.2±4.4a	1.4±0.9ab	26.6±4.3a
Hepanox	8.8±0.6c	4.8±0.2ab	0.6±0.2b	0.4±0.2a	0.0b	14.6 ±0.9b	3.8±0.9a	18.4±1.5ab

Means with different letter(s) (a, b, c) in the same column are significantly different ($p<0.05$), The value in each cell represents mean±standard error, These data were obtained from 2500 sperm/group

Effect on Sperm Count and Morphology

It is noticed (Table 7) that the sperm count was decreased after selenium, silymarin and hepanox treatments. Selenium induced the highest rate of total head abnormalities (5.2%), while cyclophosamide produced the highest rate of total head and tail abnormalities (5.4%). Selenium and silymarin induced significant increase in amorphous head sperm. Some figures of head abnormalities were significant while the majority of head abnormalities were not significant (Table 7). The coiled tail abnormality was the only recorded tail abnormality and no significance difference figure was noticed after any treatment.

DISCUSSION

Microscopic examination of bone marrow cells and spermatocytes reveled a significant reduction below the control in mitotic and meiotic activity after selenium treatments. This result agrees with the findings of Frenkel and Falvey (1988) and Jacob *et al*. (2004) who reported that selenium inhibits DNA and RNA synthesis and subsequently affect cell division. For this reason a lot of research activities were focused towards the use of selenium as an inhibitor for tumor cells in general (Melvin *et al*., 1984) and hepatoma specifically (Zheng and Zheng, 2002). The observed ability of selenium in inducing chromosomal abnormalities in the present study agrees with the previous reports by (Kazantzis and Lilly, 1979; Biswas *et al*., 1997, 1999) they found that selenium induced chromosomal abnormalities in human cultured lymphocytes and rats bone marrow. They also observed an increase in the abnormalities with the dose and time increase and that observation was similar to our results. In contrast some other reports didn't show chromosomal abnormalities after selenium treatment (Norppa *et al*., 1980a; Mukherjee *et al*., 1988; Jacob *et al*., 2004). This confliction could be attributed to the type of selenium salt used in the studies. Some salts are very toxic while others are moderate or less toxic. For example selenites are higher in toxicity than selenates (Mihajlovic, 1992; Biswas *et al*., 1999; Nuttall, 2006). The difference of toxicity for the selenium salts may be due to the lower reactivity of the carbon bonded selenol group of selenium than the sulfur bonded selenol of selenopersulfide derived from inorganic selenium compounds (Whiting *et al*., 1980; Khalil, 1989). Concerning the induction of chromosomal abnormalities in spermatocytes, it is noticed that the abnormalities were in the form of breaks and X-Y univalents and were significantly increased after the third month of treatment only. This phenomenon could be explained after the studies of (Watanabe and Endo, 1991; Nam *et al*., 1998), who reported that selenium is a very important in spermatogenesis process, so it is used by the testis and the accumulation of its excess may cause the abnormality. Another explanation, in our opinion is the natural barriers for the testis can minimize the amount of selenium reach to the testis. Although selenium induced some sperm shape abnormalities all these abnormalities were insignificant except amorphous head sperm. Similar results were reported earlier by (Watanabe and Endo, 1991).

Shamberger (1985), reported that selenium has both antimutagenic and mutagenic properties depending on the concentration and its chemical form. Selenium was found to be a protector to the somatic cells in general and the liver cells in specific, against many harmful substances such as heavy metals, mycotoxins and ionizing radiations (Chakraborty *et al*., 1987; Mukherjee *et al*., 1988; Shi *et al*., 1995; Diamond *et al*., 1996; Antunes *et al*., 2000). Additional to that, many reports clarify the potential use of selenium as anticarcinogenic substance against a lot of tumor types (Harr *et al*., 1973; Marchall *et al*., 1979; Medina and Shehered, 1981; Nigro *et al*., 1982).

In this study silymarin treatments didn't show any significant decrease in mitotic or meiotic activity. This could be explained after the observation of (Gakova *et al*., 1992) who reported that silymarin slightly affect the DNA and RNA synthesis. It is also didn't induce any significant increases in chromosomal abnormalities in mitotic or meiotic cells. From another side we found that silymarin induced some increases in amorphous head and sperm without hook. Present results agrees with the

findings of (Pepping, 1999) and (Zhao *et al*., 1999), who found that silymarin is safe after oral administration to human. Silymarin was found to alleviate the changes of nucleic acid changes after radiation exposure in liver, bone marrow and spleen cells (Hakova and Misurova, 1996). Adding silymarin to selenium will be of great importance since silymarin acts as a powerful free radical scavenger (Miguez *et al*., 1994). On the other h and it is able to increase the activity of both superoxide dimutase and glutathione peroxidase (Soto *et al*., 2003), which may explain the protective effect of the hepanox drug to the liver against free radicals and also the stabilizing effect on the blood cell membrane (Altorjay *et al*., 1992). Silymarin was found to be a protector against photocarcinogenesis in mice; it also showed remarkable anti-tumor effect (Katiyar *et al*., 1997). It acts as protective as well as a therapeutic substance against liver damage induced by gamma radiations (Kropacova *et al*., 1998), additional to that silymarin prevented alloxan from the induction of diabetes mellitus in rats (Soto *et al*., 2003). Silymarin is extremely safe, no lethal dose exists for human (Pepping, 1999) or animals (Zhao *et al*., 1999). In general we can conclude that silymarin is safe to use, it is now old alone commercially in having different names such as: Metabotanica and silymarin plus.

Hepanox didn't induce a significant reduction in mitotic or meiotic activity. Gaps and deletions were the only appeared abnormalities in somatic cells, it was recorded after the second and third months of treatment with high the dose only. Gaps as reported by (Evans, 1962) appear to be representing single effect on the chromosome and therefore it indicated damage in the mutational sense. Univalents for X-Y were the only significant germ cell abnormality which observed especially after the second and third months of treatment. X-Y univalent usually arises from non-pairing of X and Y chromosomes, since they must pair in the pseudoautosomal region (PAR), to form chiasmata during male meiosis. The PAR is located terminally at both X and Y chromosomes (Palmer *et al*., 1997; Burgoyne *et al*., 1998). It is well documented that resulted sperm will be either without chromosome or disomy sperm (XY), which may lead to producing intersex offspring or early embryonic death. No sperm shape abnormalities in head or tail were observed after hepanox treatment. In the hepanox drug, additional to the selenium and silymarin, there is some vitamins and minerals such as: vitamins C; E and zinc. Addition of the vitamins such as vitamin C, the hepanox drug will help in protecting the body from harmful chemicals (Gajewska *et al*., 1990). Vitamins also could play a role in minimizing possible genotoxic effects (Kuroda, 1990). Additional to that vitamin E can also help in decreasing the effect of some environmental mutagens (Sivikova *et al*., 2001). Vitamins improve function of selenium in reducing malignancy cells growth (Zheng *et al*., 2002). Additional to the importance of zinc for different types of cell divisions, it is essential for the production of the sex hormones (Underwood, 1981; Hambridge and Krebs, 1986). Zinc is important for the attachment of head to tail in spermatozoa and required for the production of an antibacterial compound released from the prostate gland into the semen (Saaranen *et al*., 1987). Zinc is also important for the testicular growth and spermatogenesis, daily supplementation with zinc increases sperm production and reduces the proportion of abnormal spermatozoa (Somers and Underwood, 1969). Zinc also has antioxidative properties and may also act to reduce the reactive oxygen species and hence increase fertility (Bray *et al*., 1997).

According to the present results and discussion we can confirm that using the hepanox drug for treating liver problems is much better and safer than using its components alone.

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