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ISSN 2320 -5083

Journal of International
Academic Research for Multidisciplinary

A Scholarly, Peer Reviewed, Monthly, Open Access, Online Research Journal

Impact Factor – 1.393

VOLUME 1 ISSUE 9 OCTOBER 2013

A GLOBAL SOCIETY FOR MULTIDISCIPLINARY RESEARCH

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THE PROTECTIVE EFFECT OF DIETARY SUPPLEMENTS AGAINST THE CYTOGENOTOXIC EFFECT OF CYCLOPHASMIDE IN MICE (MUSMUSCULUS)

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ABSTRACT

Endoxanknown as cyclophasmide used to treat various cancerous dieases (breast and ovarian cancers and leukaemia). 180 healthy adult male Swiss albino mice (Musmusculus), were allotted among three groups. The animal were given daily (0.9 mg/20g mice) of Endoxanintraperitonealy followed by injection of Omega-3 plus at dose (8.58 mg/20g mice), Apple at dose (1.43 mg/20 g mice) and Psyllium at dose (18.2mg /20 g mice)for five days. Various Structural and numerical chromosomal aberrations in bone marrow cells; mitotic activity and sperm head abnormality were recorded, quantitated, and statistically analyzed. Also DNA extraction and apoptosis detection in liver was done. The intensity of apoptotic bands located at 200 bp; 400bp; 600 bp;800bp and intact DNA measured by software Gel Pro program as maximum optical density values. Endoxan had adverse effect on chromosomal and sperm head structure, also it induce apoptosis, necrosis and decrease total DNA in mice liver.Omega-3 plus, Apple, and Psylliumhave a protective effect against Endoxan.

KEYWORDS: Endoxan–Omega-3 Plus- Apple- Psyllium-Chromosomal Aberrations-Sperm- DNA-Liver

INTRODUCTION

Endoxanknown as Cyclophosphamide (CP) (N, N-bis(2-chloroethyl) tetrahydro-2H-1,3,2-oxaphosphorin-2-amine, 2-oxide monohydrate; INN, trand names are Endoxan, cytoxan, Neosar, procytox, Revimmune), also known as cytophosphane,(National Cancer Dictionary). Endoxan is a drug used to treat various cancerous diseases (breast and ovarian cansers and leukaemia), disorders of the immune system (such as systemic lupus erythematosus and vasculitis). Endoxan is also used to prevent transplant rejection in some instances (Anderson et al., 1995).

Patients with cancer take numerous alternative products to protect themselves from cancer. The review article of (Muriel, 2004)provides information about 47 herbs and natural

products that have the potential to protect humans against cancer. The majority of these herbs and natural products are fruits, vegetables, animal or fish products, grains, and molecular components of plants or herbs that are found in human diets. Several grains such as barley, rice bran, and wheat bran protect against cancer. Various vegetables, fruits, and plants also show promise as protection against cancer: apple, asparagus, blueberry, cabbage, cranberry, green tea, lavender tea, olive oil, peanut oil, and spinach (Muriel, 2004).

Omega-3 Plus is composed of 1000gm Fish oil (EPA/ DHA 30%) and 100 gm Wheat germ oil. Fish oil has scientific name of N-3 fatty acid, N-3polyunsaturated fatty acids and others. Salmon, Albacore, Tuna and Mackerel are just a few of the fish with high concentrations of omega three in their system. These fatty acids seem to decrease rates of prostate cancer (Terryet al., 2001) and prolong cancer remissions as well as decrease the production of lactic acid in tumor cells(Ogilvie et al., 2000).

Wheat germ oil obtained from the embryo or kernel of the wheat grain, wheat germ oil is a light yellow, fat soluble natural oil. The germ is the most nutritious portion of the wheat and it makes up about 2.5 % of the weight. Wheat germ oil also contains alpha- and gamma-tocotrienols (Leenhardtet al., 2008; Hassanein and Abedel-Razek, 2009).

Wheat germ oil Protects material in the cell nucleus and DNA from damaging by free radicals. There are some studies have shown that vitamin E protects guanosine amino acid, which is a component of DNA, from damaged by hydroxyl and superoxide radicals. On the other hand, it destroys peroxynitrite, which is a substance similar to the nitrogen dioxide compounds present in cigarette smoke. Wheat germ oil not only prevents autoxidation of unsaturated fatty acids but also generates DNA protective properties, (Gelmezaet al., 2009).

Apple has a scientific name of Malussylvestris. Individuals use apples for many conditions from cleaning their teeth to treating diarrhea, constipation, fever, and cancer. The antioxidant flavonoid quercetin in apples seems to have a protective property against lung cancer. Apple's antioxidant power is not simply due to its content of Vitamin C. In fact, the vitamin C content of apples contributes just 0.4% to their total antioxidant activity (Boyer and Liu, 2004). Fresh apples have been reported to suppress mammary carcinogenesis and proliferative activity and induce apoptosis in mammary tumours in rats (Liu et al., 2009).

Psyllium (PlantagoovataForsskaol) which is synonymous with Plantagodecumbens and Plantagoisphagula. Psylliumis a herbaceous low-growing annual plant native to India and Iran, and is also referred to as Ispaghul (Blumenthal et al., 2000). The seed husks of this plant, Plantagoovata, are commonly referred to as ispaghula husks (Leng-Peschlow, 1991) or

psyllium (Washington et al., 1998).Psyllium husk is a gel-forming, water-soluble fiber (McCall et al., 1992; Sierra etal., 2002).Psyllium is an effective blood cholesterol-lowering agent in human studies (Sierra et al., 2002).

Adding high fiber foods (such as psyllium enriched cereals) to the diet may help lower heart disease risk. In fact, studies show that a diet high in water soluble fiber is associated with lower triglyceride levels, and a lower risk of cardiovascular disease (Cicero et al., 2007).Psyllium, may have a protective role for helping lower blood pressure, by adding fiber (12 g of soluble fiber per day) to your diet (Cicero et al., 2007).

The aim of the present work is to investigate the side effect of Endoxan (cyclophasmide) on chromosomes and sperms head morphology by cytogenetic methods. Also, detection of its side effect on DNA in liver by molecular biology method and the protective role of Omega - 3Plus, Apple and Psyllium against Endoxan effects.

MATERIAL AND METHODS

In this study 180 healthy adult male Swiss albino mice (Musmusculus), approximately three months old and weighting (± 20 g) were used in the present study. These mice were obtained from the National Research Center in Dukki, Cairo (N.R.C).The animals were kept in individual special rodents cages in the laboratory under constant condition of temperature (25 ± 3ºC) with a reveres natural dark – light cycle 12 / 12 hrs. Animals were maintained on a standard rodent diet, obtained from Egyptian company of Oils and Soap Kafr-Elzayat Egypt, manufactured especially for laboratory purposes. The diet composed of 20% casein, 15% corn oil, 55% corn starch 5% salt mixture and 5% vitaminized starch. Water was available ad libitum. Maintenance of animals and experimental procedures was approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals of BenhaUniversity, Egypt.

Endoxan available as 200mg dry powder for solution for intravenous injection, produced by Industrias Farmaceuticas Baxter Oncology GmbH, Germany.

To prepare solution for injection 10 ml of physiological saline solution is added to dry powder. Endoxan was injected intraperiotenally at dose (0.9 mg/20g mice) calculated according to therapeutic dose of human Paget and Barnes (1964) daily for 5 days. Apple available as Apple-lite tablets, each tablet contains; 500 mg of apple fibers (apple cuticle) and 50 mg of pure apple gel pectin (from apple pulp), produced by Arab Co. for pharmaceuticals& Medicinal plants (MEPACO-MEDIFOOD) Enshas El Raml-Shakeya, Egypt. The tablet was ground and dissolved in 100ml distilled water. It is used as a dose level

of (1.43 mg/20 g mice) modified according to therapeutic dose of human Paget and Barnes (1964) and each animal was injected intraperiotenally 0.26 ml daily for 5 days.

Omega-3-plus available as soft gelatin capsules, each capsule contains; 1000 mg Fish Oil and 100mg wheat germ oil.Omega-3-plus produced by South Egypt Drug industries Co.(SEDICO),6 October City, Egypt.

It is used as a dose level of (8.58 mg/20 g mice) modified according to therapeutic dose of human Paget and Barnes (1964) and each animal was injected intraperiotenally 0.1 ml daily for 5 days. Psyllium available as Regumucil Powder, each 100 gm contains 49gm psyllium husk powder, produced by Kahira Pharm.& Chem. Ind. Co. for multipharma, Cairo, Egypt. Each package contains 7 gmregumucil powder, dissolved in 250 ml distilled water. It is used as dose level (18.2mg /20 g mice) modified according to therapeutic dose of human Paget and Barnes (1964) and each animal was injected intraperitonally 0.65 daily for 5 days.

The animals divided into three groups, group A, B and C; each group contains 60 male mice. Group A used to study chromosomal abnormalities. Group B used to study sperm head morphology by classical methods of cytogenetic, while group C contain 60 male mice used to molecular studies. Each group divided into 12 sub group, 5 mice were allotted to each sub group. Sub group (1): Control Group: - 5 mice were injected intraperitoneally with 0.9% sterile saline solution. Sub group (2): Endoxan Group: - 5 mice were injected intraperitoneally with endoxan at dose (0.9mg/20g mice) daily for 5 days. Sub group (3): Omega-3-plus Group: - 5 mice were injected intraperitoneally with Omega-3-plus at dose (8.58 mg/20g mice) daily for 5 days. Sub group (4): Apple Group: - 5 mice were injected intraperitoneally with Apple-lite at dose (1.43 mg/20g mice) daily for 5 days. Sub group (5): Psyllium Group: - 5 mice were injected intraperitoneally with Regumucil at dose (18.2mg/20g mice) daily for 5 days. Sub group (6): (Endo+ Omega) Group:- 5 mice were injected intraperitoneally with Endoxan and omega-3-plus at dose ((0.9mg/20g mice) + (8.58 mg/20g mice), respectively) daily for 5 days. Sub group (7): (Endo+Apple) Group: - 5 mice were injected intraperitoneally with Endoxan and Apple at dose ((0.9mg/20g mice) + (1.43 mg/20g mice), respectively) daily for 5 days. Sub group (8):(Endo+Psyllium) Group:-5 mice were injected intraperitoneally with Endoxan and Psyllium at dose ((0.9mg/20g mice) + (18.2mg/20g mice), respectively) daily for 5 days. Sub group (9): (Endo after omega) Group:- 5 mice were injected intraperitoneally with omega-3-plus followed by Endoxan at dose ((8.58 mg/20g mice) then (0.9mg/20g mice), respectively) daily for 5 days. Sub group (10): (Endo after Apple) Group:-5 mice were injected intraperitoneally with Apple followed by

Methotrexate at dose ((1.43 mg/20g mice) then(0.9mg/20g mice), respectively) daily for 5 days.Sub group (11): (Endo after Psyllium) Group: - 5 mice were injected intraperitoneally with psyllium followed by Endoxan at dose ((18.2mg/20g mice) then (0.9mg/20g mice) respectively) daily for 5 days. Sub group (12): (Endo before Omega) Group: - 5 mice were injected intraperitoneally with Endoxan followed by Omega -3-Plus at dose((0.9mg/20g mice) then (8.58 mg/20g mice), respectively) daily for 5 days. Sub group (13):(Endo before Apple) Group:-5 mice were injected intraperitoneally with Endoxan followed by Apple at dose ((0.9mg/20g mice) then (1.43 mg/20g mice), respectively) daily for 5 days. Sub group (14):(Endo before Psyllium) Group:-5 mice wereinjected intraperitoneally with Endoxan followed by Psyllium at dose ((0.9mg/20g mice) then (18.2mg/20g mice), respectively) daily for 5 days.

Metaphase spreads were prepared according to Yosida and Amano (1965).Fifty well metaphase spreads were examined / each animal. The type and frequency of chromosomal aberrations were recorded and photographed. Mitotic activity of the cells was calculated as the number of dividing cells including prophase and metaphase per 1000 cells. Cells with stickiness were considered as dividing cells.

Smears for sperm morphology were prepared and stained with eosin according to (Mukherjee et al., 1988). One thousand sperms were counted for each animal, and the abnormal shape involving the head was recorded. Statistical analysis was carried out using the student (t) test (Snedecor, 1946).

DNA extraction and apoptosis detection in tissues (liver) was done according to" salting out extraction method of (Aljanabi and Martinez, 1997) and modification introduced by (Hassab El-Nabi, 2004). DNA damage was detected in lysate tissue (Hassab EL-Nabiet al., 2002). Gel Was prepared using 1.8% electrophoretic grade agarose. All the gels of DNA were photographed with digital camera while the DNA were visualized at 312 nm UV light under a transilluminator. Apoptotic bands appeared and located at 200 bp; 400bp; 600 bp and 800 bp. The intensity of apoptotic bands and intact DNA could be measured by software Gel program as maximum optical density values.

RESULTS

Various chromosomal aberrations are observed in the bone marrow cells of male mice treated with Methotrexate and protected with Omega-3 plus, Apple and psyllium. Both Structural and numerical types of aberrations and chromosomal stickiness are observed.

The Structural aberrations included chromatid deletion (Fig.1a), fragmentation (Fig.1b), centromeric attenuation (Fig.1c), Centric fusion (Fig.1d), chromosomal ring (Fig.1e), end to end association (Fig.1f), break (Fig.1g) and gap (Fig.1h).The numerical aberrations included monosomy, trisomy and polyploidy(Fig.1i). Stickiness may give arise to sticky adhesions between two or more chromosomes, and formation of sticky bridges at metaphase (Fig. 1j). Table (1), fig. (2) Showed the average of chromosomal abnormalities of bone marrow cells of mice treated with Endoxan and protected with Omega-3plus, Apple and psyllium. It represented a very high significant increase in treated group with endoxan(99.40± 14.85) than control group (7.40±6.28), on the other hand it indicated that omega-3 plus made a high protective role against Endoxan especially when injected with Endoxan(28.20 ± 7.37) and when injected after administration of Endoxan(24.20 ± 10.81). As well as, the result indicated that pre-treatment with Apple and Psyllium is more protective than post-treatment .Marked decrease in the mean values of structural chromosome aberrations in bone marrow cells (23.80 ± 9.80)and (37.00± 10.85) when pre treated with apple and psyllium respectively.

Table (1): Average of structural abnormalities observed in bone marrow cells of mice treated with Endoxanand protected with Ap, Omega, and Ps.

	Groups	Mean ± SD																					
		Deletion			Fragmentation			Centromeric attenuation			Centric Fusion			Chromosomal Ring			End to end association			Breaks			Gaps			Total		
1	Control	2.2	±	1.48	2	±	1.22	0.8	±	0.84	0	±	0.55	1	±	0.55	0.4	±	0.55	1	±	0.55	0	±	0.55	7.40	±	6.28
2	Endo	16	±	3.56	41	±	1.30	5.2	±	1.30	4	±	2.19	4	±	0.84	10.8	±	1.64	6	±	1.14	12	±	2.86	99.40	±	14.85
3	Omega	1	±	1.00	3	±	0.89	0.8	±	0.84	0	±	0.55	0	±	0.55	0.6	±	0.55	1	±	0.55	0	±	0.55	7.60	±	5.47
4	Apple	1.6	±	0.55	3	±	1.92	1.8	±	0.45	1	±	0.00	1	±	0.84	1.8	±	0.84	1	±	0.84	1	±	0.55	11.60	±	5.98
5	Psyllium	2.4	±	0.55	4	±	2.59	2.6	±	0.55	1	±	0.00	1	±	0.84	2.8	±	0.84	1	±	0.71	1	±	1.10	16.00	±	7.16
6	Endo+Omega	6.4	±	1.14	7	±	1.48	2.8	±	0.84	4	±	0.84	3	±	1.14	1.8	±	0.84	2	±	0.55	2	±	0.55	28.20	±	7.37
7	Endo+Apple	7.2	±	1.30	7	±	1.48	5.2	±	0.84	3	±	0.84	3	±	0.84	2.8	±	0.84	3	±	1.10	3	±	0.71	34.20	±	7.94
8	Endo+Psyllium	11	±	2.07	9	±	1.00	3.8	±	0.84	4	±	1.14	3	±	1.82	3.4	±	0.55	3	±	1.73	2	±	1.14	38.40	±	10.29
9	Endo after Omega	15	±	2.07	28	±	2.39	4.4	±	0.89	6	±	1.30	5	±	1.30	6.2	±	2.39	5	±	1.30	5	±	1.48	75.20	±	13.14
10	Endo after Apple	6.6	±	1.14	7	±	1.58	2.4	±	0.89	2	±	1.92	2	±	1.14	0.8	±	0.84	2	±	1.14	2	±	1.14	23.80	±	9.80
11	Endo after Psyllium	9	±	1.58	9	±	1.92	4.8	±	1.48	5	±	1.14	3	±	0.84	1.8	±	1.48	3	±	1.30	1	±	1.10	37.00	±	10.85
12	Endo before Omega	5.8	±	1.48	6	±	1.58	2.2	±	1.48	3	±	1.14	2	±	1.22	2	±	1.58	2	±	1.10	1	±	1.22	24.20	±	10.81
13	Endo before Apple	15	±	4.12	35	±	3.81	4	±	1.58	4	±	1.30	4	±	1.92	10.2	±	2.39	4	±	1.58	4	±	3.03	80.00	±	19.74
14	Endo before psyllium	17	±	2.88	35	±	3.27	3.8	±	0.84	4	±	1.58	4	±	2.30	3.4	±	1.52	4	±	1.48	3	±	1.92	74.00	±	15.80

* Significant (p<=0.05), ** Highly Significant (p<=0.01), *** Very Highly Significant (p<=0.001) Endo = Endoxan, Omega = Omega-3-Plus, Ps = Psyllium, Ap = Apple

Tables (2) fig. (3) Showed that the mean values of numerical chromosomal abnormalities and mitotic index. Data represented a considerable increase in average of numerical aberration of chromosomes when animals treated with Endoxan by (12.00 ± 3.78)in comparison with control. Omega -3-plus exhibited a noticeable reduction of the mean values of numerical aberration appeared in animal groups administrated with Omega-3- plus and its effect is the greatest especially in post-treatment with the anticancer drug administration. On the other side, the Apple and Psyllium revealed a sharp decline in numerical aberration of

chromosomes when treated with Endoxan. Moreover the Apple exhibited a great role to repair the numerical aberration in mice bone marrow cells especially when administrated before Endoxan. There is a very highly significant reduction in the mean values of mitotic index in group injected with Endoxan by239.8, in compare with 570 of control group. These result indicated that the treatment with Endoxanreduced the mean values of mitotic index in compare with control group. While the three natural products (Apple, Omega-3-plus and Psyllium) repair the decrease in mitotic index caused by Endoxan.

Table (3), fig. (5) represented the incidence of abnormality in the shape of sperms per 1000 for each mice treated with Endoxan and protected with Omega-3-plus, Apple and psyllium, which include without hook shape (fig. 4b), banana shape (fig.4c) and amorphous shape (fig.4d).Endoxan group showed a very high significance increase in the average of total abnormality (110.2± 12.90) compared with control group (11 ± 3.62).The highest range of abnormality in the shape of sperms of mice treated with Endoxan was amorphous while Banana shape was the lowest frequency. On the other hand the three natural products showing a protective effect when mixed with pre-treated and post-treated with Endoxan. Omega-3-plus is the best protective natural product in a pretreatment with anticancer drug used.

Table (2): Average of numerical chromosomal abnormalities and mitotic index observed in bone marrow cells of mice treated with Endoxan and protected with Ap, Omega, and Ps.

	Groups	Mean ± SD												Mitotic Index		
		Monosomy			Trisomy			Polyolidy			Total					
1	Control	0	±	0.00	0.2	±	0.45	0	±	0.00	0.20	±	0.45	570	±	23.72
2	Endo	2.8	±	1.30	3.4	±	0.55	5.8	±	1.92	12.00	±	3.78	239.8	±	10.03
3	Omega	0	±	0.00	0	±	0.00	0	±	0.00	0.00	±	0.00	567.4	±	24.06
4	Apple	0	±	0.00	0	±	0.00	0	±	0.00	0.00	±	0.00	588.8	±	25.84
5	Psyllium	0	±	0.00	0	±	0.00	0	±	0.00	0.00	±	0.00	599	±	31.88
6	Endo+Omega	1.4	±	0.55	0.8	±	0.84	3.8	±	0.84	6.00	±	2.22	587.8	±	5.12
7	Endo+Apple	3	±	1.00	2	±	1.87	5	±	1.22	10.00	±	4.10	582.8	±	4.44
8	Endo+Psyllium	2	±	1.58	4.2	±	0.84	4.8	±	2.77	11.00	±	5.19	560.4	±	3.21
9	Endo after Omega	3.8	±	0.84	2.6	±	1.82	8.2	±	1.30	14.60	±	3.96	280.6	±	3.05
10	Endo after Apple	0.4	±	0.55	0.8	±	0.84	2.8	±	1.92	4.00	±	3.31	451.4	±	9.86
11	Endo after Psyllium	1.6	±	1.14	2	±	1.58	3.8	±	1.30	7.40	±	4.03	416.8	±	7.50
12	Endo before Omega	1	±	0.71	1.2	±	1.30	3.4	±	1.14	5.60	±	3.15	588.2	±	6.30
13	Endo before Apple	3.2	±	1.92	3	±	2.24	4.8	±	1.48	11.00	±	5.64	250	±	3.61
14	Endo before psyllium	3.4	±	1.14	3.4	±	1.14	5.2	±	1.48	12.00	±	3.76	250.2	±	5.07

*Significant (p<=0.05), **Highly Significant (p<=0.01),***Very Highly Significant (p<=0.001) Endo = Endoxan, Omega = Omega-3-Plus,Ps = Psyllium,Ap = Apple

Table (3): Average of sperm head abnormalities observed in bone marrow cells of mice treated with Endoxanand protected with Ap, Omega, and Ps.

	Groups	Sperm head abnormalities											
		Without hook			Banana			Amorphous			Total		
1	Control	4.4	±	0.55	3	±	1.00	3.6	±	2.07	11	±	3.62
2	Endo	12	±	2.55	19.8	±	3.96	78.4	±	6.39	110.2	±	12.90
3	Omega	3	±	1.00	2.8	±	1.48	2.8	±	1.30	8.6	±	3.79
4	Apple	3	±	1.87	2.8	±	1.10	4.2	±	0.45	10	±	3.41
5	Psyllium	4.2	±	1.64	4.2	±	1.10	3.6	±	0.89	12	±	3.63
6	Endo+Omega	2	±	1.00	6.4	±	2.07	42	±	1.58	50.4	±	4.65
7	Endo+Apple	1.4	±	1.14	3.2	±	1.30	46.4	±	2.30	51	±	4.75
8	Endo+Psyllium	3	±	1.00	5.4	±	1.14	49.8	±	1.92	58.2	±	4.06
9	Endo after Omega	1.4	±	1.14	2.4	±	0.55	34.2	±	1.64	38	±	3.33
10	Endo after Apple	1.4	±	1.14	1.2	±	0.84	42.2	±	1.48	44.8	±	3.46
11	Endo after Psyllium	2.2	±	1.48	9	±	13.53	46.6	±	3.05	57.8	±	18.06
12	Endo before Omega	2	±	1.00	7	±	1.58	46.2	±	3.19	55.2	±	5.77
13	Endo before Apple	9.2	±	1.92	18	±	2.92	57.8	±	3.35	85	±	8.19
14	Endo before psyllium	14.6	±	2.07	22.8	±	3.35	69.8	±	2.95	107.2	±	8.37

* Significant (p<=0.05), ** Highly Significant (p<=0.01), *** Very Highly Significant (p<=0.001)Endo = Endoxan, Omega = Omega-3-Plus, Ps = Psyllium,Ap = Apple

Damage and optical density of DNA in mice treated with Endoxan and protected with Omega-3-plus, Apple and Psyllium observed in liver (Fig. 6&7 and table 4), it represents that:the Damage in DNA of mice treated with Endoxan increased when was compared with control so the optical density of apoptotic bands of DNA at 200; 400; 600 and 800 bp showed a very significant increase than control. On the other hand intact DNA decreased sharply than control. The obtained results showed that the natural products (Omeg-3 plus, Apple, and Psyllium) reduce DNA damage induced by Endoxan.

Table (4) Optical density of intact and apoptotic fragments of DNA at 200, 400, 600 and 800 bp in liver of mice treated with Endoxan and protected with Omega, Ap, Ps.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
	Control	Endo	Omega	Ap	Ps
Intact DNA	152.22	17.161	218.15	189.2	175.3
DNA at 800 bp	20.2	54.494	25.294	50.294	64.77
DNA at 600 bp	8.37	94.08	13.247	37.541	40.77
DNA at 400 bp	6.45	123.91	5.152	20.153	32.851
DNA at 200 bp	4.23	137.16	0.58824	6.988	12.184
Sum of apoptotic fragments	39.3	409.644	44.2	115	150.6

Table (5): Optical density of intact and apoptotic fragments of DNA at 200,400,600,800bp in liver of mice treated and protected together with Endoxan and with Omega, Ap and Ps.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
	Control	Endo	Endo+Omega	Endo+Ap	Endo+PS
Intact DNA	152.22	17.161	154.7	138.69	138.99
DNA at 800 bp	20.2	54.494	65.529	52.356	56.782
DNA at 600 bp	8.37	94.08	49.747	41.46	47.793
DNA at 400 bp	6.45	123.91	34.138	29.977	38.644
DNA at 200 bp	4.23	137.16	15.759	19.45	18.69
Sum of apoptotic fragments	39.3	409.644	165.173	143.243	161.909

Table (6): Optical density of intact and apoptotic fragments of DNA at 200,400,600,800bp in liver of mice treated with Endo after Omega, Ap and Ps.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
	Control	Endo	Endo after omega	Endo after Ap	Endo after Ps
Intact DNA	152.22	17.161	43.271	113.9	99.2
DNA at 800 bp	20.2	54.494	51.4	125.56	75.682
DNA at 600 bp	8.37	94.08	64.224	73.218	90.24
DNA at 400 bp	6.45	123.91	100.58	36.253	115.86
DNA at 200 bp	4.23	137.16	112.8	16.69	90.54
Sum of apoptotic fragments	39.3	409.644	329.004	251.721	372.322

Table (7): Optical density of intact and apoptotic fragments of DNA at 200,400,600,800bp in liver of mice treated with Endo before Omega, Ap and Ps.

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
	Control	Endo	Endo before omega	Endo before Ap	Endo before Ps
Intact DNA	152.22	17.161	113.38	33.678	32.835
DNA at 800 bp	20.2	54.494	124.48	59.287	44.2
DNA at 600 bp	8.37	94.08	93.54	79.057	58.212
DNA at 400 bp	6.45	123.91	46.264	90.95	97.67
DNA at 200 bp	4.23	137.16	20.736	110.6	165.14
Sum of apoptotic fragments	39.3	409.644	285.02	339.894	365.222

Table (5, 6, 7) showing the optical density of intact and apoptic fragments of DNA at 200, 400, 600, 800 bp in liver of mice treated with Endoxan and the natural products together; Endoxan after natural products and Endoxan before natural products. It is clear that the intact DNA increase sharply in the liver of mice treated with Endoxan and the natural products (Omega-3 plus; Apple and Psyllium)together than the mice treated with just Endoxan as demonstrated in figure (8 and 9). Besides, there is a protection action of natural products in pre-treatment and post-treatment as shown in figure (10, 11, 12, 13). There is a great protection action Apple and psyllium when mice injected with Endoxan after Apple or Psyllium. On the other hand Omega- 3 plus has the best protection action in the post-treatment.

DISCUSSION

The present study aimed to investigate the side effects of Endoxan as anticancer drug on the chromosomes; sperm head abnormalities and the DNA damage in male mice (MusMusculus), and the protection action by Omega-3Plus, Apple and Psyllium. In the present study Endoxan was target because, the main use of Endoxan as chemotherapy to treat various cancerous diseases (breast and ovarian cancers, and leukaemia), disorders of the

immune system (such as systemic lupus erythematosus and vasculitis). It is a chemotherapy drug that works by slowing or stopping cell growth. Cyclophosphamide also decreases the immune system's response to various diseases and conditions. Although Endoxan has proved to be a very effective chemotherapeutic agent, its use has been linked to the onset of some secondary cancers (IARC, 1981).

At the same time, using Endoxanin cancer chemotherapy has various side effects. Oral administration of cyclophosphamide resulted in skin tumours in transgenic mice (Yamamoto et al., 1996; Eastinet al., 2001), and in urinary bladder carcinoma, leukaemia, and nervous system tumours in rats. The use of plants for the prevention of diseases is an ancient practice. Recently, there is a direction to use plants, herbs, composition of fruits, vegetables and grains in medicinal manufactures for their medicinal and protection abilities against many illnesses especially in case of cancer patients. Lifestyle and especially diet can also serve as important sources of antioxidants. In vitro studies demonstrate that certain micronutrients may reduce DNA oxidation (Djuricet al., 2001) as well as mutagenicity as (Poharet al., 2003).

The aim of the present study using three different natural products as a try to minimize the side effects of chemotherapy. In the present study we used Omega -3 Plus, Apple and Psyllium as trials testing the effectiveness of antioxidants as cancer prevention agents. The fatty acids of fish oil seem to decrease rates of prostate cancer (Terry et al.;2001).The omega-3 fatty acids in fish oil seem to be able to expand blood vessels, and this brings blood pressure down (Djousséet al., 2012; Huand Manson, 2012). The second natural product that we used in the present study is Apple which has a scientific name of Malussylvestris. Apples are a rich source of nutrient as well as non-nutrient components and contain high levels of polyphenols and other phytochemicals. (Shia et al., 2009). There is some evidence for effects of flavonoid intake on reduced risk of lung cancer in some populations (Cui et al., 2008). Fresh apples have been reported to suppress mammary carcinogenesis and proliferative activity and induce apoptosis (programmed cell death) in mammary tumours in rats (Liu et al., 2009).

Psyllium as kind of prebiotics was associated with a reduced incidence of colon cancer in various populations (Segal et al., 1995). Prebiotics have been shown to deactivate genotoxic carcinogens. DNA damage had been prevented and chemopreventive systems may be stimulated invivo in colon tissues (Albertset al., 2000).

In the present study, the increases in chromosomal aberrations were observed in the male Musmusculus (significant and highly significant difference) after treatment with Endoxan.These aberrations are structural (include deletion, fragmentation, centric fusion, chromatid break, centromeric attenuation, gape, end to end association and stickiness) and numerical (included monosomy, trisomy and polyploidy). Mitotic index also affected after treatment with Endoxan.

It is obvious from the present study that the statistical analysis of chromosomal fragmentation, deletion, break, gap and stickiness of chromosomes of bone marrow cells of mice treated with Endoxan showed a very highly significant increase in the mean value than that of control. These result agreed with, Anderson et al.,(1995) who investigated that Endoxan produce gene mutations, chromosome aberrations, micronuclei and sister chromatid exchanges in a variety of cultured cells in the presence of metabolic activation as well as sister chromatid exchanges without metabolic activation. Also in (IARC, 1987) indicated that Endoxan induces chromosomal aberrations, sister chromatid exchange, and gene conversions. Significantly increased of gap was observed in this study in treated animals with Endoxan than control and this was due to the local loss of both DNA and chromosomal basic protein, this loss occur on a chromatid in the locus and doesn't represent real discontinuities in the chromosome (Stoian and Raicu, 1975). It was occurred as a result of primary lesions which disrepair to give aberrations (Evan, 1977).

Endoxan cause a remarkable increase in the mean values of numerical aberration specially (Monosomy, Trisomy and polyploidy) in the bone marrow cells of mice. Our result agreed with (Barekatiet al., 2008) who indicated that previous maternal chemotherapy by cyclophosphamide causes numerical chromosome abnormalities in preimplantation mouse embryos.

Aneuploidy in general arise as a non disjunction of homologous chromosomes at meiosis, or by non disjunction of sister chromatids at mitosis. The failure of disjoin or separate accurately can occur at any nuclear division in which the event occurs at the time occurrence. Non disjunction at meiosis gives rise to gametes with one more or less chromosome than usual. If such gametes are viable and fuse to produce a zygote, the zygote will be trisomic or monosomic for non disjunction chromosomes (Avers, 1980).

The value of chromosome stickiness increased in bone marrow cells of mice treated with Endoxan than control. Stickiness is due to the process of depolymerization of DNA, thus making the chromosome surface becoming sticky. Stickiness has been regarded later as

physiological and unspecific disturbance attributed to the action of proteins on chromosome or form improper folding of the chromosome fibers into chromatids and thus chromosome become attached to each other by means of sub chromatid bridges (Brogger, 1974).

The mean values of mitotic index decreased in mice after treated with Endoxan. Mitotic index is a measure for the proliferation status of a cell population. The apparent lower values for mitotic index reported in the present study may be due to decrease in cell number arrested in metaphase (Wissmuller, 1971). The results of the present study showed that Endoxan induced genotoxic effects including DNA damage (apoptosis and necrosis) in the treated mice. Our result agreed with (Souliotiset al., 2003)and (Hartmann et al., 1995) who confirm that Cyclophasmide cause increased in DNA damage (comet formation) was also observed in the lymphocytes of patients administered cyclophosphamide.

As well as, the previous IARC Monograph (IARC, 1987) states that cyclophosphamide induced chromosomal aberrations, sister chromatid exchange, and DNA damage in human cells invitro. The cytotoxic effects of Endoxan are generally considered to be the result of DNA crosslink formation through covalent bonding of highly reactive alkyl groups of the alkylating nitrogen mustards (Zhang et al., 2005). The alkylation of the 7-nitrogen atom of guanine in DNA molecules takes place by phosphoramide mustard resulting from cyclophasmide activation (Petteet al., 1995). At alkaline or neutral pH, the nitrogen mustard is converted to chemically reactive carbonium ion through imonium ion. Carboinium ions react with the N7 of guanine residues in DNA to form a covalent linkage. The second arm in the phosphoramide mustard can react with a second guanine moiety in an opposite DNA stand or in the same stand to form cross links (Fleer and Brendal, 1983; Springer et al., 1998). Following crosslink formation, the cells will undergo apoptosis initiated by DNA damage and inhibition of DNA replication, modulation of cell cycle, and other anti-proliferative (Bhatia et al., 1995 and Mastaet al., 1995).

Laboratory animals exposed to Cyclophasmide by various routes of administration develop benign and malignant tumors of the bladder, breast, lungs, liver, and injection site (IARC, 1981). In addition, rats treated with Cyclophasmide developed leukemia and lymphoma (IARC, 1981 and 1987).

Apoptosis seems to be induced by mild genotoxic stimuli; the strength of stimuli increases the cell death mode shifting it towards necrosis. This seems to be due to the fact that most the intense genotoxic stimuli damage the proteins or genes that make these proteins and other cellular macromolecules which may be required for apoptosis (Singh, 2000).

Apoptosis is mediated by members of the caspase family of proteases, and eventually causes the degradation of chromosomal DNA.

In the present study Omega-3 Plus acts as highly protective agent so aberrations decreased in all protected groups. As well as the result showed the best and very highly significant protective effect of Omega-3- Plus in post-treatment than pre-treatment with Endoxan.

These result agreed with (Gelmezaet al., 2009) who indicated the protective effect of wheat germ oil whereas wheat germ oil not only prevents autoxidation of unsaturated fatty acids but also generates DNA protective properties.

Moreover (Hong et al.,2005) who observed the protective effect of fish oil against oxidatively induced colon cancer. Whereas their study confirmed that fish oil protects against colon cancer by decreasing oxidative DNA damage at the initiation stage of colon tumorigenesis, oxidative DNA damage, proliferation, and apoptosis. On the other hand, (Manna et al., 2010) reported that Fish oil regulates cell proliferation, protect DNA damages and decrease HER-2/neu and c-Myc protein expression in rat mammary carcinogenesis. The data in the present study indicated that Apple with Endoxan showing a protective effect, when compared with animal groups treated with just Endoxanlonely. As well as the result indicated that the protective effect of Apple after Endoxan is more useful than before. These result agreed with (Liuet al., 2005) who indicated that, Apples are one of the very few individual foods specifically identified in population studies as having the capacity to reduce cancer risk and more specifically lung cancer (Liu et al., 2005). Fresh apples have been reported to suppress mammary carcinogenesis and proliferative activity and induce apoptosis (programmed cell death) in mammary tumours in rats (Liu et al., 2009).Besides, (Adhamiet al., 2012), they studied Fisetin is commonly found in many fruits and vegetables such as apples, and they evaluated that the effects of fisetin against melanoma and cancers of the prostate, pancreas and the lungs.

There is a significant decrease in the chromosome aberration and DNA damage due to protective effect of Psyllium was observed clearly on our data. Moreover, the protective effect of psyllium was the best in the post-treatment.

These result agreed with (Segal et al., 1995) who explained that Psyllium as kind of prebiotics was associated with a reduced incidence of colon cancer in various populations (Segal et al., 1995).Slavin, (2003)indicated that whole-grain intake is protective against cancer, cardiovascular disease, diabetes and obesity.

Three types of sperm head abnormalities were recorded. Amorphous, banana like and without hook shape. Amorphous and without hook like were the highest incidence of aberration in groups treated with Endoxan, while banana shape the lowest frequency in both treated groups. On the other hand the three natural products we used in the experiment (Omega -3 plus, Apple and Psyllium) showing a protective effect in the mean values of the sperm head abnormalities of animals Musmusculus. Our result agreed with Selvakumaret al., (2006) whoindicated that cyclophasmide-treated rats showed a significant decrease in sperm count and motility with an increase in dead and abnormal sperms. These changes were associated with significant increase in DNAdamage and in the sperm as evidenced by increased single strand breaks in fluorimetric analysis of DNAunwinding (FADU). As well as in rats treated with Cyclophasmide, abnormal changes in the activities/levels of enzymic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymic (reduced glutathione, ascorbate and α-tocopherol) antioxidants, were also observed. Rezvanfaret al., (2008) found that treatment of rats with cyclophasmide cause, a decrease in sperm quality and associated with increased in DNA damage and decreased in chromatin quality. As well as thehistopathological analysis of testes and epididymides and staining of mast cells indicated that Cyclophasmide-induced toxic effects on androgenesis and spermatogenesis are mediated by free radicals.

In an attempt to explain the different mechanisms involved in the induction of the abnormal morphology of the sperm heads, Kaczmarski (1972) stated that in complete condensation of chromatin and the presence of large vacuoles and canals containing remnants of cytoplasm in various regions of the head is the cause of failure of sperm to pass through the final steps of maturations occurring normally during spermatogenesis. Moreover, Topham (1980) mentioned the agents which accumulate in the testis can cause alterations in testicular DNA and disrupt the process of differentiation of spermatozoa directly.

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Figures

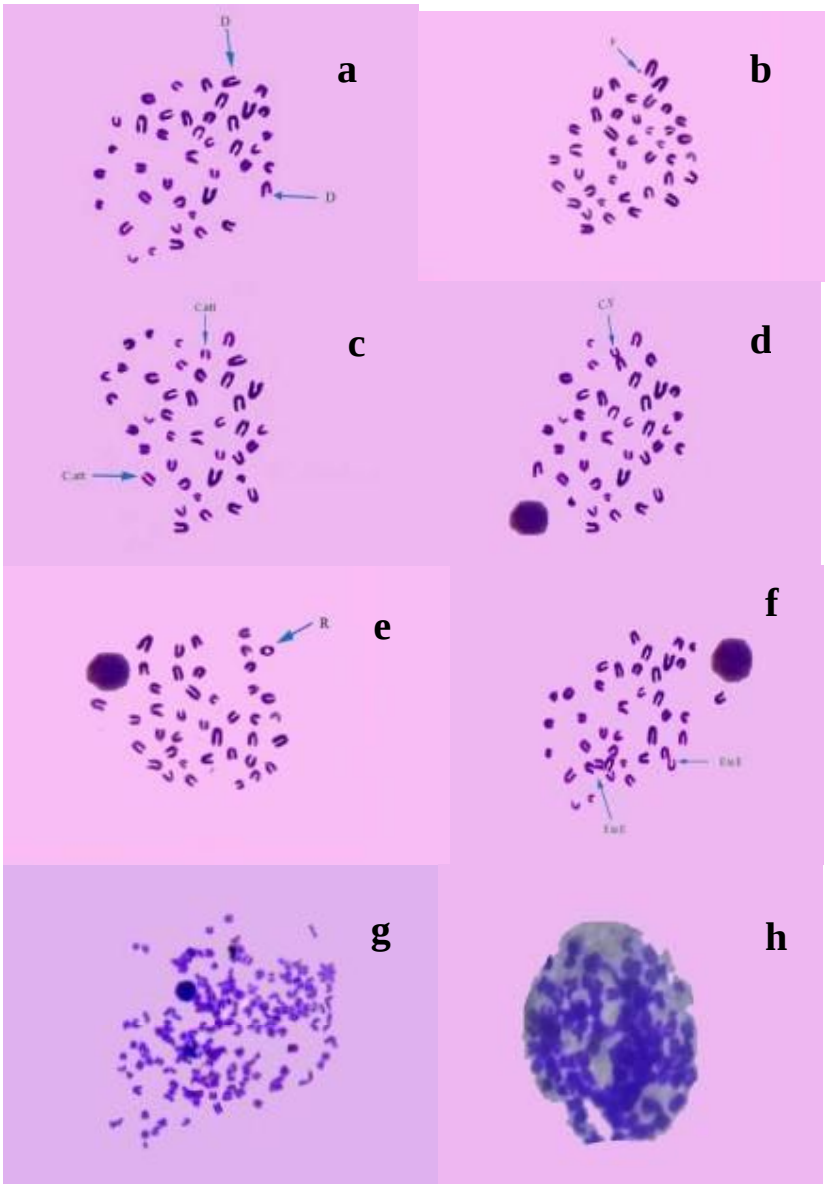


Figure (1): Types of chromosomal aberrations in rat bone marrow cells treated with Endoxan.(a): deletion (D); b:fragmentation (F); c: centromeric attenuation (C att); d: centric fusion (CF); e:chromosomal ring; f: end to end association (E to E); g: break (B); h: gap (G); i: Polyploidy and j: Sticky.

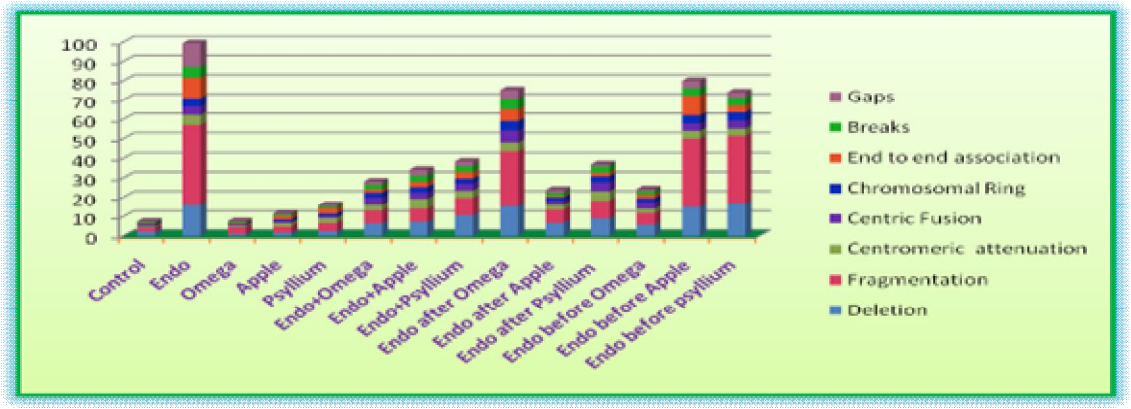
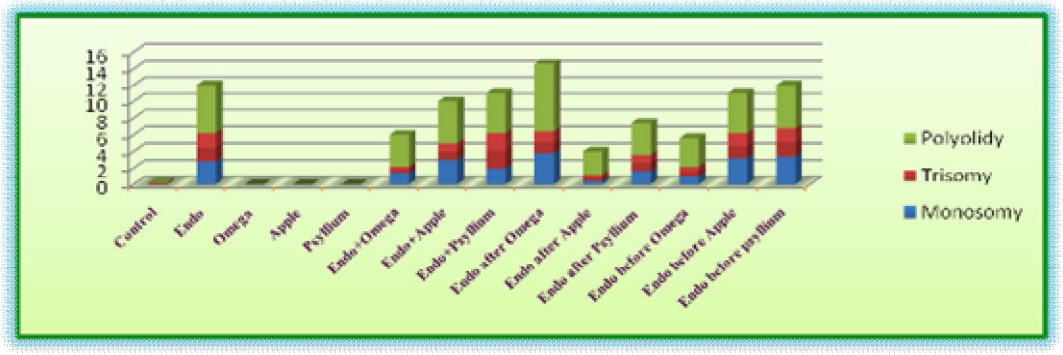


Figure (2): Average of structural abnormalities observed in bone marrow cells of mice treated with Endoxan and protected with Ap, Omega, and Ps.



Figure(3): Average of numerical chromosomal abnormalities observed in bone marrow cells of mice treated with Endoxan and protected with Omega, Ap and ps

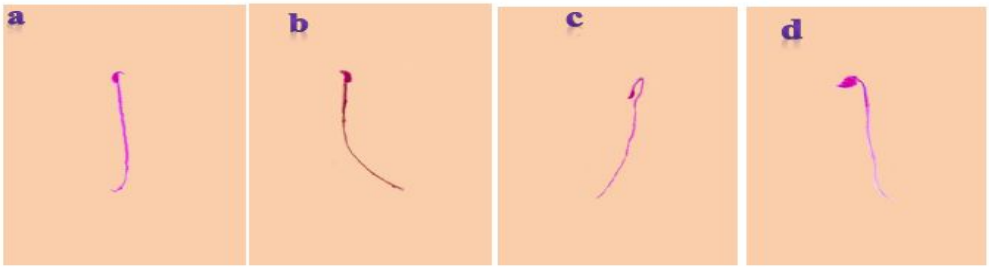


Figure (4): Average of sperm head abnormalities observed in bone marrow cells of mice treated with Endoxan and protected with Ap, Omega, and Ps. a: normal sperm; b: without hook; c: banana shape and d: amorphous.

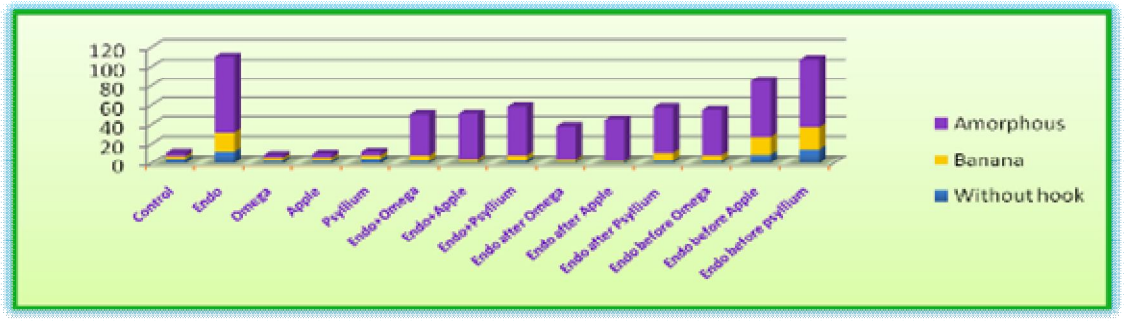


Figure (5): Average of sperm head abnormalities observed in bone marrow cells of mice treated with Endoxan and protected with Ap, Omega, and Ps

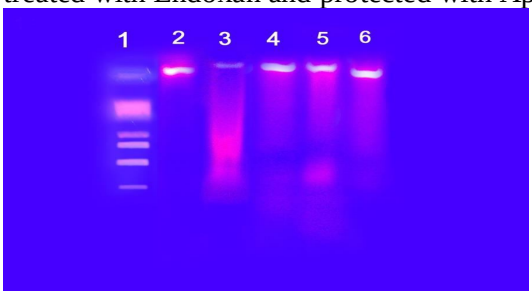


Figure (6): DNA damage in liver of mices treated with Endoxan and protected with Omega, Ap and Ps.lane1: DNA ladder; lane 2: Control; Lane 3: Endo; lane 4: Omega; lane 5: Ap; lane 6:Ps.

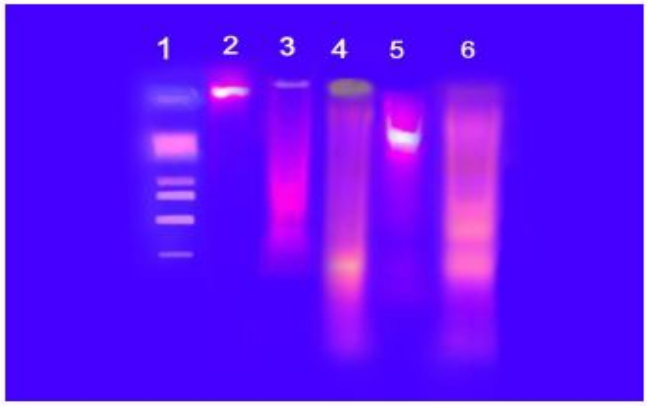
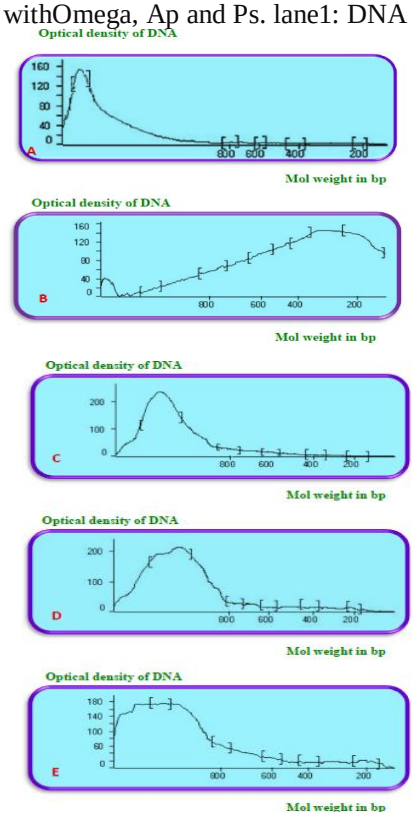
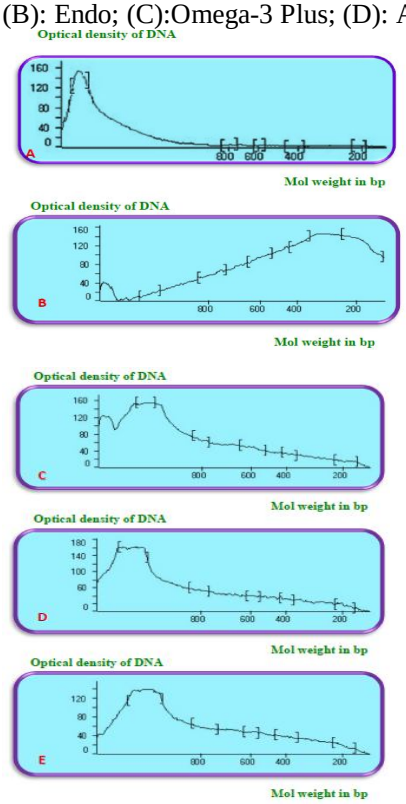


Figure (8): DNA damage in liver of mices treated and protected together with Endoxan and withOmega, Ap and Ps. lane1: DNA ladder; lane 2: Control; Lane 3: Endo; lane



Figure(7): Histograms of optical density of intact and apoptic fragments of DNA at 200, 400, 600 and 800 bp in liver of mices treated with Endoxan and protected with Omega, Ap and Ps.(A): Control; (B): Endo; (C):Omega-3 Plus; (D): Apple; (E): Psyllium.



Figure(9): Histograms of optical density of intact and apoptic fragments of DNA at 200, 400, 600 and 800 bp in liver of mices treated and protected together with Endoxan and with Omega, Ap and Ps. (A): Control; (B): Endo; (C):Endo+Omega; (D):Endo+ Apple; (E):Endo+ Psyllium.

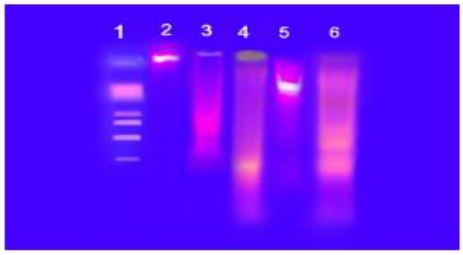
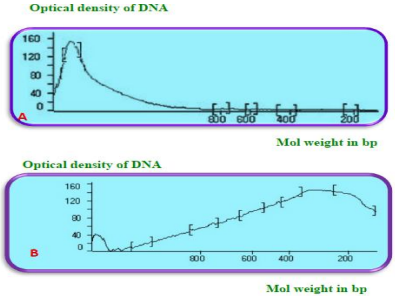
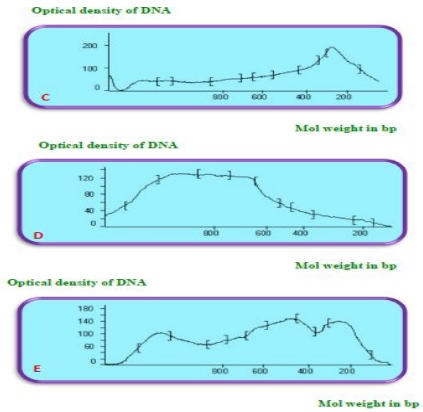


Figure (10): DNA damage in liver of mices treated with Endoxan after Omega, Ap and Ps. lane1: DNA ladder; lane 2: Control; Lane 3: Endo; lane 4: Endo after Omega; lane 5: Endo after Ap; lane 6:Endo after Ps.





Figure(11): Histograms of optical density of intact and apoptic fragments of DNA at 200, 400, 600 and 800 bp in liver of mices treated with Endoxan after Omega, Ap and Ps. (A): Control; (B): Endo; (C):Endo after Omega; (D):Endoafter Apple; (E):Endo after Psyllium.

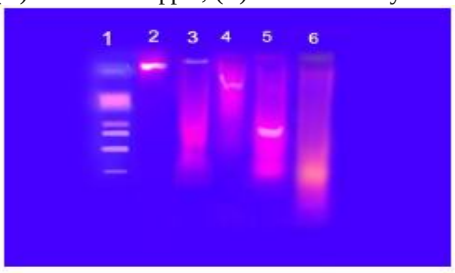
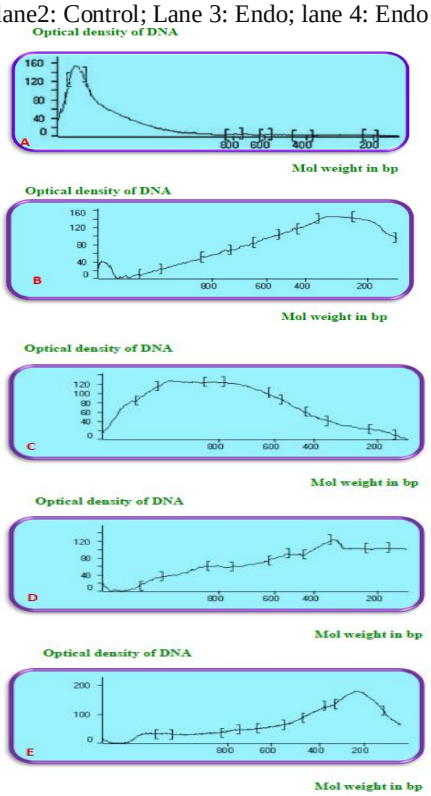


Figure (12): DNA damage in liver of mices treated with Endoxan before Omega, Ap and Ps. lane1: DNA ladder; lane2: Control; Lane 3: Endo; lane 4: Endo before Omega; lane 5: Endo before Ap; lane 6:Endo before Ps.



Figure(13): Histograms of optical density of intact and apoptic fragments of DNA at 200, 400, 600 and 800 bp in liver of mices treatedwith Endoxan before Omega, Ap and Ps. (A): Control; (B): Endo;(C):Endo before Omega; (D):Endobefore Apple; (E):Endo before Psyllium.