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Antimutagenic Activity of Some Natural supplements on Ivermectin genotoxicity in Lymphocytes of Buffalo

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Abstract

Ivermectin is a veterinary anthelminthic drug, highly effective against a number of arthropod and nematode infestations in vertebrates. The literature reported that ivermectin have mutagenic activities. The extensive use of ivermectin in food producing animals can cause potential hazard to humanity by causing gene mutation or chromosomal aberrations. Recently, there have been considerable efforts to search for naturally occurring substances that can inhibit, reverse, retard or prevent mutagenicity. A wide array of substances derived from edibles and medicinal plants reported to possess anticarcinogenic and antimutagenic activities. Therefore, the aim of this study was to evaluate the modulator role of each of garlic, L-carnitine and wheat germ oil (WGO) against genotoxicity of ivermectin in buffalo lymphocyte cultures by using the cytokinesis block micronucleus assay and chromosomal aberrations test as cytogenetic end-points**.** Results showed that ivermectin induced dose dependent increase in the frequencies of the binucleated lymphocytes with micronuclei as well as the number of micronuclei in lymphocytes of river buffalo, while the number of binucleated lymphocytes significantly decreased. In addition, ivermectin low dose caused non-significant increase in the frequency of total chromosomal aberrations in lymphocytes of river buffalo as compared to control. Whereas, in medium and high doses the frequencies of aberrant cells increased at a significant level *(*$P \leq 0.001$) than control. Meanwhile, the treatment with the three natural supplements (garlic, L-carnitine, wheat germ oil) in continuous with ivermectin significantly reduced the frequencies of binucleated lymphocytes with micronuclei, the number of micronuclei and the frequencies of total chromosomal aberrations induced by ivermectin tested doses, and increase the number of binucleated lymphocytes. In addition, the results indicated that there were non-significant differences between the modulator role of garlic, L-carnitine or wheat germ oil against the mutagenicity of ivermectin in blood lymphocytes. Finally, we can concluded that regular consumption of natural antioxidants, antimutagenic compounds is inversely related to the risk of cancer, since the free radicals scavenger activities of antioxidants are very important due to the deleterious role of free radicals in foods and in biological systems.

Keywords: Ivermectin – Garlic - L-carnitine - Wheat Germ Oil – peripheral blood lymphocytes- Chromosomal aberrations -Micronucleus formation.

Introduction

Veterinary medicines used widely to protect animal health, prevent economic loss, and to help ensure a safe food supply. Veterinary medicine active ingredients detected in various environmental media, including surface and groundwater, sugges-ting the potential for indirect human exposure from such residues (Boxall *et al.,* 2006 and Capleton *et al*., 2006). In addition, the extensive use of veterinary drugs in food producing animals can cause the presence of drugs residues in food; thus, consumers of foods drive from animals exposed to veterinary drug residues (Galer and Monro, 1998). In recent years, there has been increasing concern that veterinary drugs may present a potential hazard to humanity by causing gene mutation or chromosomal aberrations and considered as potential developmental toxicants (Ardito *et al*., 1996; Mailhes *et al*., 1997; Crebelli, 2000; Adler *et al*., 2002 and El-makawy & Radwan, 2003). Ivermectin is a veterinary anthelminthic drug, highly effective against a number of arthropod and nematode infestations in vertebrates (Grant and Briggs, 1998). Chemically, it is a form of abam-ectin, origi-

nally isolated from the actino-mycete Streptomyces avermitilis (Tway *et al*., 1981). The literatures reported that ivermectin have mutagenic activities in bone marrow cells of mice (El-makawy and Radwan, 2003). In addition, Lankas *et al*. (1989) studied the effects of ivermectin on reproduction and neonatal toxicity in rats, they determined that doses of ivermectin as low as 400 µg/kg /day were toxic to neonatal rats. Recently, there have been considerable efforts to search for naturally occurring substances that can inhibit, reverse, retard or prevent mutagenicity. Nowadays, naturally occurring compounds with the potential antimutagenic and anticarcinogenic effects are of great importance for their prospective use in cancer chemoprevention and treatment (Miadokova *et al*., 2005 and Shukla & Kalra, 2007). A wide array of substances derived from edibles and medicinal plants reported to possess anticarcinogenic and antimutagenic activities (Surh *et al*., 2001). Garlic known for its medicinal qualities and its uses as antibiotic, antithrombotic and antineoplastic agent (Guyonnet *et al*., 2000). The anticarcinogenic property of garlic has been attributed to its organosulfur constituents out of whom the major one reported is Diallyl Sulfide, which exhibit strong antioxidant properties (Jung *et al*., 2001; Robert *et al*., 2001; Wu *et al*., 2002 and Lohani *et al*., 2003). In addition, in recent years, dietary supplements such as L-carnitine reported to influence the development and amelioration of numerous disease states. The biochemical active amino acid L-carnitine influences fatty acid dependent energy use and prevents oxygen free radical-induced cellular damage. This antioxidant activity may explain many L-carnitine beneficial effects that do not appear to be directly associated with enhanced fatty acid beta-oxidation (Maher, 2001). Recent literatures reported that L-carnitine might modulate DNA damage and cell proliferation (Boerringter *et al*., 1993; Vanella *et al*., 2000 and Santoro *et al*., 2005). Wheat germ considered as a foodstuff interacting with the immune system due to its content in wheat germ agglutinin, a lectin known to influence several immune functions *in vivo* and *in vitro* (Kilpatrick, 1999). Ethanolic extract of wheat germ indicated the presence of classes I and II antioxidants (Krings *et al*., 2000). Recent research confirms that wheat germ oil can reduce oxidative stress (Alessandri *et al*., 2006). Wheat germ oil also contains policosanol, a substance that can be helpful in lowering raised blood sugar and/or cholesterol levels, as well as octacosonal, a substance reported to improve human fitness (Irmak and Dunford, 2005). Recently, interest has increased in the occurrence, importance and consequences of potential genotoxic activity of a variety of drugs and chemicals. At the same time, to combat parasite infections in a variety of animals, large doses of therapeutic agents are required (De Silva *et al*., 1997) and the same antiparasitics chemicals may also have mutagenic effects on the organisms they are designed for protection. To date, for example, no antihelmintic agents shown to be risk-free (Otubanjo and Mosuro, 2001). Therefore, This work aims to evaluate the modular role of each of garlic, L-carnitine and wheat germ oil against genotoxicity induced by ivermectin in river buffalo lymphocytes by using the Cytokinesis block micronucleus and the chromosomal aberrations assay as cytogenetic end-points.

Materials and methods

Cells used

The study was carried out using buffalo peripheral lymphocytes from fresh blood samples. Blood samples were taken from five healthy animals for each group, which had no treatment during the last 3 months. Approximately 10 ml of blood was collected into syringes containing sodium heparin as anticoagulant.

Chemicals

Ivermectin (CAS no 1119-97-7) was purchased from Pfizer-Egypt-S.A.E. - Cairo-ARE. Under license from Pfizer Inc– Corporate- USA. Garlic (CAS no.240/2000) purchased from ATOS pharma, Cairo-Egypt. L-carnitine (CAS no. 20679/99) and wheat germ oil (CAS no.451/2) purchased from Arab Co. for pharm and medicinal plants (MEPACO) Egypt.

Doses

The concentrations of ivermectin (250, 500 and 1000 µg/ml) were chosen on

the basis of a cytotoxicity test (Dean and Danford, 1994), eight blood lymphocyte cultures were prepared. The first considered as a control. The other seven cultures were treated with different concentrations of the drug: 1, 10, 100, 200 and 500µg/ml, and 1 and 2 mg/ml. The mitotic index frequency scored in each of the eight cultures. 1000 µg/ml is the concentration that reduced the mitotic index to about 50 % and used as the highest dose. The drug doses of 500 and 250 µg/ml were taken as medium and low doses, respectively. Garlic, L-carnitine and wheat germ oil were used at concentration of 20µg/ ml.

Cytokinesis block micronucleus assay

The CBMN assay was carried out using the standard technique proposed by Fenech (1993), with slight modifications according to Surralle´s *et al*. (1994). Briefly lymphocyte cultures were set up by adding 0.5 ml of heparinized whole blood to 4.5 ml of RPMI 1640 chromosome medium supplemented with 20% heat-inactivated fetal calf serum, antibiotics (penicillin and streptomycin) and L-glutamine (all obtained from Gibco, Eragny, France). Lymphocytes were stimulated by 1% phytohaem-agglutinin (PHA, Gibco). Iver-mectin at the three tested concentrations (250, 500 and 1000 µg/ml.) and the different natural products (garlic, L-carnitine and wheat germ oil) at concentration of 20µg/ ml were added to the cultures 24 h after phytohaemm-aglutinin stimulation. Cytochalasin-B (Cyt-B, Sigma), at a final concentration of 6 µg/ml was added at 44 h after the cultures were established, to arrest cytokinesis of dividing cells. This concentration of Cyt-B was selected because it gives a higher percentage of binucleated cells and a lower baseline MN frequency. Binucleated lymphocytes were harvested 72 h after culture setting. The cells were collected by centrifugation and washed with hypotonic solution (0.075 M KCl) at room temperature using vortex. Next, the cells were centrifuged, and a methanol/acetic acid (3:1 v/v) solution was gently added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution. The cells were spread onto cold slides dipped in 70% ethyl alcohol. The slides were air-dried. All the slides were coded prior to scoring.

Chromosomal aberrations assay

For chromosomal aberrations 1 ml of whole heparinized blood was added to 4 ml RPMI [1640 medium, Sigma), containing 20% fetal calf serum (Sigma), 4% phyto-haem-aglutinin (PHA, Sigma) and streptomycin (250µg/ml), culturing took place at 38.5 c and lasted for 72hr. After 24 h from culture initiation, the test chemicals was added. Colchicines (Sigma) at a concentration of 100 µg/ ml was added 1½ h before harvesting. Hypotonic performed with (0.075M KCl) for 20 min. The cells were fixed with methanol-acetic acid (3-1). Slides were stained with 10% Giemsa diluted with phosphate buffer (PH 6.8) for 35 min. in each culture 100 good metaphase examined for scoring of chromosomal aberrations.

Statistical analysis

Data were compared by one-way analysis of variance (ANOVA). Statistical analysis was performed using SPSS for Windows. Multiple comparisons were performed by the least significant difference Duncan ´s test. P≤0:05 were considered as the level of significance.

Results

Table (1) represents the mean values of binucleated lymphocytes, binucleated lymph-ocytes with MN and the number of micron-uclei induced by different drug concentrations with or without natural supplements. Results showed that iverme-ctin induced dose depe-ndent increase in the frequencies of the binucleated lymphocytes with micronuclei as well as the number of micronuclei in lymphocytes of river buffalo, while the number of binucleated lymphocytes significantly decreased. Meanwhile, co- treatment of the different natural supplements with ivermectin significantly reduced the frequencies of binucleated lymphocytes with MN and micronuclei induced by ivermectin and increase the number of

binucleated lymphocytes as shown in chart (1).

Tables 2, 3 and 4 represent the mean frequencies of chromosomal aberrations recorded in blood cultures of river buffalo treated with ivermectin at the three-tested concentrations alone or with the natural supplements as well as in control. The structural chromosomal aberrations studied in the present work included chromatid gaps, chromatid breaks, deletions, fragments and centromeric attenuations. In cultures treated with low dose ivermectin, there was non-significant increase in the frequency of total chromosomal aberrations as compared to control. Whereas, medium and high doses caused a significant increase in the frequencies of aberrant cells at the level ($P \leq 0.001$) when compared with control. Meanwhile, the data of this study showed that the co-treatment with the three natural supplements significantly reduced the frequencies of total chromosomal aberrat-ions induced by all tested doses as shown in the tables (2, 3 and 4) and chart (2).

The current results clearly indicated that treatment with garlic, L-carnitine or wheat germ oil showed non-significant decrease in the frequencies of micronuclei and total chromos-omal aberrations when compared with control.

Table (1): Effect of natural products on frequencies of binucleated cells and micronuclei in ivermactin treated lymphocyte cultures of the river buffalo.

Treatments	No. of examined cells	Binucleated lymphocytes	Binucleated lymphocytes with Micronuclei	Micronuclei
Control	10000	98.80 ± 2.39 b	4.20 ±0.84 h	4.20 ±0.84 h
250µg/ml ivermectin	10000	89.60±1.14 d	8.60±1.14 de	10.80±0.84 d
500µgl/ml ivermectin	10000	80.40±1.14 e	13.80±1.30 b	16.20±1.30b
1000µg/ml ivermectin	10000	61.60±1.82 h	20.40±2.88 a	25.20±3.11 a
Garlic 20µg/ml	10000	108.60±3.85 a	3.40±0.55 h	3.40±0.55 h
250µg/ml ivermectin + garlic	10000	99.00±1.00 b	6.40±1.14 g	8.00±1.00 g
500µg/ml ivermectin + garlic	10000	87.40±7.02 d	8.20±0.84 def	10.00±0.71 def
1000µg/ml ivermectin + garlic	10000	79.60±1.14ef	9.60±0.55 d	12.60±1.14 c
L-carnitine 20µg/ml	10000	106.20±3.56 a	3.80±0.84h	3.80±0.84 h
250µg/ml ivermectin +l-carnitine	10000	94.00± 1.00 c	6.80±0.84 fg	8.60±0.89fg
500µg/ml ivermectin + l- carnitine	10000	81.40±1.67 e	9.00±1.00 de	10.40±1.14 de
1000µg/ml ivermectin + l-carnitine	10000	76.40±1.67 f	11.40±1.67 c	12.80±1.64 c
Wheat germ oil 20µg/ml	10000	99.60±1.14 b	4.00±0.71 h	4.00±0.71h
250µg/ml ivermectin + wheat germ oil	10000	90.00±1.41 d	7.60±1.14 efg	9.00±0.71 efg
500µg/ml ivermectin + wheat germ oil	10000	80.20±1.10 e	9.40±0.55 d	10.80±0.84 de
1000µg/ml ivermectin + wheat germ oil	10000	72.40±2.07g	11.80±0.84 c	13.20±1.30 c

Means with different letters within each column are significant at 5% level.

Table (2): Mean values of different chromosomal aberrations induced by ivermectin with or without garlic in lymphocytes of the river buffalo.

Experimental Groups	*Structural chromosomal aberrations*					*Total excluded gaps*	*Total With gaps*	*Poly-ploidy*
	gaps	breaks	deletions	fragments	centromeric attenuations			
Control	1.80 cd ± 0.45	0.80 d ± 0.45	0.00 c ± 0.00	0.00 b ± 0.00	1.20 b ± 0.84	2.00 de ± 0.71	3.80 d ± 0.84	0.00 c ± 0.00
250µg/ml ivermectin	1.80 cd ± 0.45	2.00 b ± 0.71	0.60 bc ± 0.55	0.00 b ± 0.00	0.60 cd ± 0.55	3.20 d ± 0.84	5.00 c ± 0.71	0.20 b ± 0.45
500µg/ml ivermectin	3.00 b ± 0.71	3.20 a ± 0.45	0.60 bc ± 0.55	0.00 b ± 0.00	1.20 bc ± 0.45	5.00 b ± 0.71	8.00 b ± 0.71	0.80 ab ± 0.84
1000µg/ml ivermectin	5.00 a ± 0.71	3.80 a ± 0.45	1.40 a ± 0.55	0.80 a ± 0.45	2.20 a ± 0.84	8.20 a ± 0.45	13.20 a ± 0.84	1.20 a ± 0.84
Garlic (20µg/ml)	1.40 d ± 0.55	1.00 cd ± 0.00	0.00 c ± 0.00	0.00 b ± 0.00	0.20 b ± 0.45	1.20 e ± 0.45	2.60 e ± 0.55	0.00 c ± 0.00
250µg/ml ivermectin + garlic	1.20 d ± 0.45	0.80 d ± 0.45	0.40 c ± 0.55	0.00 b ± 0.00	1.00 b ± 0.71	2.00 de ± 0.00	3.20 de ± 0.45	0.00 c ± 0.00
500µg/ml ivermectin + garlic	2.20 c ± 0.45	1.40 bcd ± 0.55	0.60 bc ± 0.55	0.00 b ± 0.00	1.00 b ± 1.00	2.60 cd ± 0.89	4.80 c ± 0.45	0.40 bc ± 0.55
1000µg/ml ivermectin + garlic	3.00 b ± 0.00	1.60 bc ± 0.55	1.20 ab ± 0.45	0.20 b ± 0.00	1.20 b ± 0.45	4.20 b ± 0.45	7.20 b ± 0.45	0.60 abc ± 0.55

Means with different letters within each column are significant at 5% level.

Table (3): Mean values of different chromosomal aberrations induced by Ivermectin with or without L-carnitine in lymphocytes of the river buffalo.

Experimental Groups	*Structural chromosomal aberrations*					*Total ex-cluded gaps*	*Total With gaps*	*polyploidy*
	gaps	breaks	deletions	fragments	centromeric attenuations			
Control	1.80 d ± 0.45	0.80 c ± 0.45	0.00 c ± 0.00	0.00 b ± 0.00	1.20 b ± 0.84	2.00 c ± 0.71	3.80 c ± 0.84	0.00 c ± 0.00
250µg/ml ivermectin	1.80 d ± 0.45	2.00 b ± 0.71	0.60 bc ± 0.55	0.00 b ± 0.00	0.60 bc ± 0.55	3.20 c ± 0.84	5.00 c ± 0.71	0.20 b ± 0.45
500µg/ml ivermectin	3.00 b ± 0.71	3.20 a ± 0.45	0.60 bc ± 0.55	0.00 b ± 0.00	1.20 b ± 0.45	5.00 b ± 0.71	8.00 b ± 0.71	0.80 ab ± 0.84
1000µg/ml ivermectin	5.00 a ± 0.71	3.80 a ± 0.45	1.40 a ± 0.55	0.80 a ± 0.45	2.20 a ± 0.84	8.20 a ± 0.45	13.20 a ± 0.84	1.20 a ± 0.84
L-carnitine (20µg/ml)	1.40 d ± 0.55	1.00 c ± 0.00	0.00 c ± 0.00	0.00 b ± 0.00	0.00 c ± 0.00	1.00 cd ± 0.71	2.40 d ± 0.55	0.00 c ± 0.00
250µg/ml ivermectin + L-carnitine	1.20 d ± 0.45	0.80 c ± 0.45	0.40 c ± 0.55	0.00 b ± 0.00	0.60 bc ± 0.55	1.80 de ± 0.89	3.00 d ± 1.00	0.00 c ± 0.00
500µg/ml ivermectin + L-carnitine	2.00 cd ± 0.71	1.40 bc ± 0.55	0.40 c ± 0.55	0.00 b ± 0.00	0.60 bc ± 0.55	2.40 bc ± 0.87	4.40 c ± 0.89	0.40 bc ± 0.55
1000µg/ml ivermectin + L-carnitine	2.80 bc ± 0.84	1.80 b ± 0.45	1.20 ab ± 0.45	0.00 b ± 0.00	1.20 b ± 0.45	4.20 b ± 0.45	7.00 b ± 0.45	0.60 abs ± 0.55

Means with different letters within each column are significant at 5% level.

Table (4): Mean values of different chromosomal aberrations induced by Ivermectin with or without wheat germ oil in lymphocytes of the river buffalo

Treatment groups	*Structural chromosomal aberrations*					*Total ex-cluding gaps*	*Total With gaps*	*polyploidy*
	gaps	breaks	deletions	fragments	centromeric attenuations			
Control	.80 cd ± .45	0.80 d ± 0.45	0.00 c ± 0.00	0.00 b ± 0.00	1.20 bc ± 0.84	2.00 e ± 0.71	3.80 e ± 0.84	0.00 b ± 0.00
250µg/ml ivermectin	1.80 cd ± 0.45	2.00 b ± 0.71	0.60 bc ± 0.55	0.00 b ± 0.00	0.60 cd ± 0.55	3.20 d ± 0.84	5.00 d ± 0.71	0.20 b ± 0.45
500µg/ml ivermectin	3.00 b ± 0.71	3.20 a ± 0.45	0.60 bc ± 0.55	0.00 b ± 0.00	1.20 bc ± 0.45	5.00 b ± 0.71	8.00 b ± 0.71	0.80 ab ± 0.84
1000µg/ml ivermectin	5.00 a ± 0.71	3.80 a ± 0.45	1.40 a ± 0.55	0.80 a ± 0.45	2.20 a ± 0.84	8.20 a ± 0.45	13.20 a ± 0.84	1.20 a ± 0.84
Wheat germ oil (20µg/ml)	1.40 d ± 0.55	1.00 cd ± 0.00	0.00 c ± 0.00	0.00 b ± 0.00	0.20 d ± 0.45	1.20 e ± 0.45	2.60 f ± 0.55	0.00 b ± 0.00
250µg/ml ivermectin + wheat germ oil	1.60 d ± 0.55	0.60 d ± 0.55	0.20 bc ± 0.45	0.00 b ± 0.00	0.80 cd ± 0.45	1.60 e ± 0.89	3.20 e f ± 0.84	0.20 b ± 0.45
500µg/ml ivermectin + wheat germ oil	1.80 cd ± 0.45	1.60 bc ± 0.55	0.80 b ± 0.45	0.20 b ± 0.45	1.00 bcd ± 0.71	3.60 cd ± 1.14	5.40 d ± 1.34	0.60 ab ± 0.55
1000µg/ml ivermectin + wheat germ oil	2.40 bc ± 0.55	1.80 b ± 0.45	0.40 bc ± 0.55	0.40 b ± 0.55	1.80 ab ± 0..55	4.40 bc ± 0.89	6.80 c ± 0.85	0.60 b ± 0.55

Means with different letters within each column are significant at 5% level.

Chart (1):Effect of three natural products on the frequencies of micronuclei induced by ivermectin in the river buffalo lymphocytes.

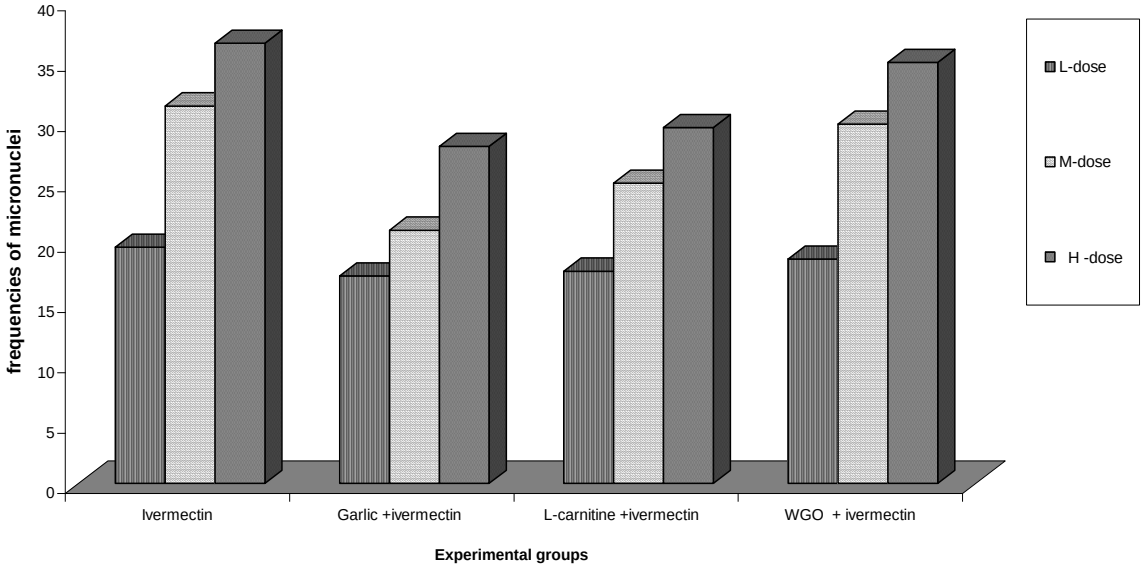
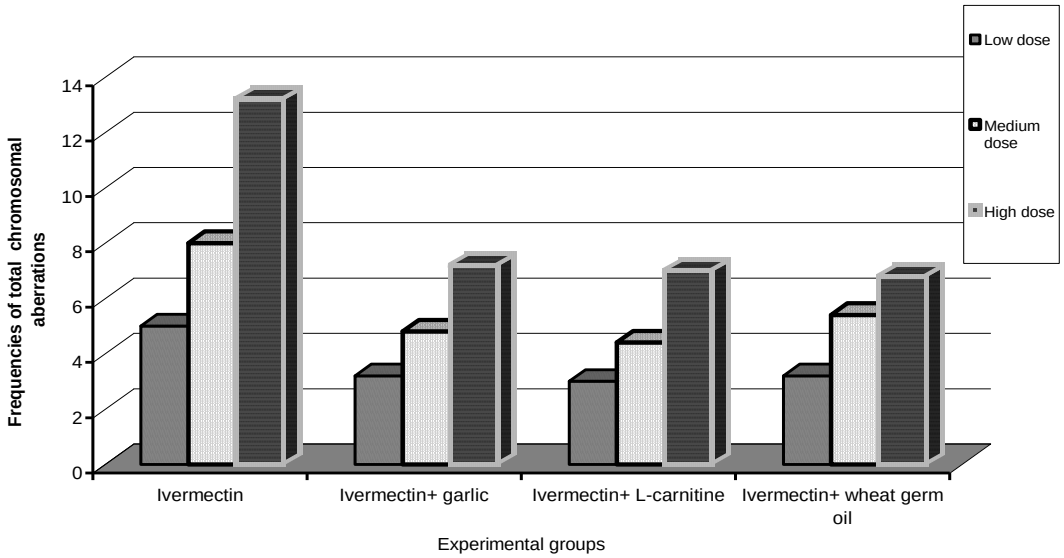


Chart (2): The effects of the three natural products on the frequencies of total chromosomal aberrations induced by ivermectin in lymphocytes of the river buffalo.



Discussion

The present investigation was carried out to explore the possible modulator role of garlic, L-carnitine and wheat germ oil on ivermectin genotoxicity in mammalian cells. Ivermectin is a highly active antiparasitic animal drug utilized in a variety of injectable, oral and topical formulations. Residues of this drug may reach the environment through manufacturing and animal wastes and may potentially have effects on terrestrial and aquatic organisms (Bloom and Matheson, 1993). Meanwhile, previous literature reported that ivermectin has mutagenic activities. El-makawy and Radwan, (2003) indicated that ivermectin induced statistically significant increase in the number of micronucleated polychro-matic erythrocytes. This is in agreement with the results of the present study, which showed that ivermectin induced dose dependent significantly increase in the number of binucleated lymphocytes with micronuclei and also the frequencies of total chromosomal aberrations. In addition, the numbers of binucleated lymphocytes showed dose dependent decrease than control. These results revealed that the drug has a cytotoxic effect on the number of cell divisions. As the micronuclei are small chromatin-containing bodies arising from chromosome fragmentation by breaks or deletion, the results of MN formation confirmed our results of chromosomal aberrations indicating the clastogenic effects of ivermectin. Nowadays, naturally occurring compounds with the potential antimutagenic and anticarcinogenic effects are of great importance for their prospective use in cancer chemoprevention and treatment (Miadokova *et al.*, 2005). A wide array of substances derived from edibles and medicinal plants reported to possess anticarcinogenic and antimutagenic activities (Surh *et al.*, 2001). Natural antioxidants closely related to their bio-functionalities, such as the reduction of chronic diseases like DNA damage, mutagenesis, carcinogenesis and inhibition of pathogenic bacteria growth that is often associated with the termination of free radical propagation in biological systems (Bloom and Matheson, 1993; Covacci *et al.*, 2001 and Zhu *et al.*, 2002). The present investigation revealed the antimutagenic potential of garlic, L-carnitine and wheat germ oil against the mutagenic effect of ivermectin in the buffalo blood lymph-ocytes. These results were in agreement with Gulcin (2006) who reported that the protective effect of garlic towards cyclophosphamide induced cytotoxic and cytogenetic damage implies as a good marker of its antimutagenic and anti-neo-plastic activity. In addition, previous literatures showed that continuous daily dietary uptake of raw garlic extract modulated the mutagenicity of sodium arsenate and aflatoxin B (1) **(**Choudhury *et*

al., 1997 and Shukla & Taneja, 2002). Treatment of male Swiss albino mice with garlic significantly reduced the frequency of micronuclei induced by 7, 12-dimethylbenz-[a]anthracene (DMBA) in bone marrow (Guyonnet *et al*., 2002). In addition, garlic showed a clear antimut-agenic and immunomodulatory activities on mutagenicity and immunosu-ppression induced by different mutagens (Bhuvaneswari *et al*., 2004). Chemical analysis has indicated that protective effects of garlic appear to be related to the presence of organosulfur compounds mainly allyl derivatives. Garlic mechanisms to cancer chemopreventive effects include modul-ation in activity of several metabolizing enzymes that activate and detoxify carcinogens and inhibit DNA adduct formation, antioxidative properties, regulation of cell proliferation, apoptosis and immune responses (Shukla and Kalra, 2007). Meanwhile, Barta *et al*. (2006) demonstrated that L-carnitine treatment decreased DNA damage induced with fumonisin B1 in liver and spleen of rats. In addition, Atroshi *et al.* (1999) concluded that L-carnitine might act as positive modulator of cytotoxic anticancer agents. In addition, Santoro *et al*. (2005) stated that L-carnitine reduced hydrogen peroxide formation which induces the chromosomal aberrations. Wheat germ oil posses an antioxidative potential that may protect the body against He-Ne laser irradiation due to the amelioration of oxidative stress of free radicals (EL-makawy and El-Ashmaoui, 2003). On the same time, (Omran, 2006 and Zhu *et al*., 2006) investigated the antioxidant and free radical-scavenging activities of wheat germ oil by employing several *in vitro* assay systems, including the linoleic acid emulsion model system, 1,1-diphenyl-2-picrylhydrazyl (DPPH)/ superoxide/hydroxyl radical-scavenging, reducing power, and ferrous ion-chelating activity. WGO showed scavenging activity against free radicals such as DPPH, superoxide, and hydroxyl radicals. These previous findings suggested the protective role of the used natural supplements and confirmed the report of Zhu *et al*. (2006) in which they suggested that a diet containing even low levels of different naturally occu-rring compounds is effective in exerting antigenotoxic effects by modulating oxidative stress. In conclusion, the current results revealed that ivermectin induced genotoxic effect in the lympho-cytes of the buffalo, included, increase in the number of micronuclei and chromo-somal abnorm-alities, decrease in the number of binuc-leated lymphocytes. Garlic, L-carnitine and wheat germ oil proved to have antimut-agenic effect against iverm-ectin mutagen-icity. Their modulator role may be attribu-ted to the ability to scavange free radicals and protection of the cell membranes from lipid peroxidation. Generally, the modulator role of these compounds can be explained by their mech-anism in enhancing cellular antioxidant activity by free radical scavenging and augmentation of endogenous antioxidants via prevention of GSH depletion and alteration of GSH dependent enzymes activity and/or their gene expression (Sener *et al*., 2004; Dokmeci, 2005; Dokmeci *et al*., 2005 and Sabayan *et al.*, 2007).

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التأثير المضاد للطفرات لبعض المكملات الطبيعية ضد التسمم الوراثى لعقار الايفرمكتين فى الخلايا اللمفية لدم الجاموس

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الايفرمكتين أحد الأدوية البيطرية طاردات الديدان واسعة الانتشار ، والتى تستخدم فى علاج و وقاية حيوانات المنتجة للغذاء من الإصابة بالطفيليات. وقد وجد أن الاسراف فى استخدام عقار الايفرمكتين من الممكن أن يؤدى الى اضرار صحية خطيرة للانسان. كما ثبت علميا أن عقار الايفرمكتين له تأثير مطفر. وحديثا تبذل جهود كثيرة للبحث عن العديد من المنتجات الطبيعية والتى من الممكن ان يكون لها القدرة على تقليل أو منع التأثير المطفر للعديد من المطفرات. لذلك كان الهدف من هذه الدراسة تقييم التأثير المضاد للطفرات لبعض من المركبات الطبيعية مثل الثوم،ال –كارنتين وزيت جنين القمح ضد التسمم الوراثى لعقار الايفرمكتين فى الخلايا اللمفية لدم الجاموس. وفى هذه الدراسة تم أخذ خمس عينات من دم الجاموس المصرى لكل مجموعة. أجريت التجربة على16 مجموعة، شملت المجموعة الضابطة والمجموعات المعاملة بكل من الثوم ، ال-كرنيتين وزيت جنين القمح(20 ميكروجرام/ مللى). كذلك المجموعات المعالجة بالجرعات المختلفة من عقار الايفرمكتين (1000،500،250 ميكروجرام/ مللى) بدون المركبات الطبيعية أو بإضافتها. وقد تم اجراء اختبار *Cytokinesis block micronucleus* assay لكل المجموعات لتقدير معدلات الانوية الصغيرة باستخدام مادة السيتوكلازين-ب *وكذلك* تم تحضير الكروموسومات لتقدير معدلات التشوهات الكروموسومية فى الخلايا اللمفية لكل المجموعات. أوضحت النتائج ان المعاملة بالايفرمكتين نتج عنها زيادة معنوية فى كلا من معدلات الأنوية الصغيرة والتشوهات الكروموسومية فى الخلايا اللمفية لدم الجاموس، وهذه الزيادة تتناسب طرديا مع زيادة الجرعات. بينما سببت نقصاً فى أعداد الخلايا ثنائية الأنوية .ولقد أثبتت النتائج أيضا ان المعاملة بالمركبات الطبيعية بمفردها نتج عنها نقص غير معنوى فى أعداد الخلايا ذات الأنوية الصغيرة وكذلك التشوهات الكروموسومية مقارنة بالمجموعة الضابطة . كما أثبتت النتائج أن المعاملة بالايفرمكتين مع المركبات سابقة الذكر أدت الى حدوث تحسنا معنويا فى القياسات تحت الدراسة. نستخلص من هذه الدراسة أن كل من الثوم ، ال- كرنيتين وزيت جنين القمح لها تأثير محسن فعال ضد السمية الوراثية الناتجة عن عقار الايفرمكتين .