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The protective effect of garlic on changes in liver and brain proteins occurring in albino rats exposed to 900 MHz (GSM) microwaves

Sharaf-Eldeen, Kh. M.; Zowail, M. E. M. and Abdel-Kareim, A. M.

Department of Zoology, Faculty of Science, Benha University, Benha, Egypt

ABSTRACT

Garlic (*Allium sativum*) has been used as a medicinal agent for thousands of years. In the present work the radioprotection of this agent will be investigated. Fourty five mature female albino rats, *Rattus norvegicus* were allotted into groups and exposed to mobile phone microwave (MP) of 900 MHz for 14, 30 or 45 days. The effect of garlic treatment in association with the MP exposure was also studied. Protein expression was examined in both liver and brain tissues by using SDS-polyacrylamide gel electrophoresis. The results showed that MP exposure caused induction of newly synthesized polypeptides in liver and brain tissues. On the other hand, the daily administration of 20mg/kg garlic decreased the number of protein fractions in liver while such fractions were increased in brain tissue and this effect was associated especially in liver tissue with expression of new proteins which maybe stress proteins; these proteins have low molecular weight. Also, garlic prevented significantly the depression of mitotic index (MI) that occurred under the effect of MP exposure. The present work suggested the *in vivo* radioprotective role, antimutagenic and anticarcinogenic effect of garlic.

Key Words: *Mobile phone, electrophoresis, protein, liver, brain, garlic*

INTRODUCTION

Leszczynski *et al*.[1] investigated the response of the human endothelial cell line EA.hy926 exposed to 900 MHz radio frequency electromagnetic field (RFEMF) emitted from a GSM mobile phone. They noted that 324 new phosphoproteins appeared in the exposed cells. The same study was done by **Nylund and Leszczynski**[2] that found up to 38 various proteins were statistically altered their expression levels following the irradiation. **Fritze *et al*.**[3] using rat as a model, have shown an increase in expression of stress protein hsp70 in brains of animals exposed for 4 hr to RF-EMF (890-915 MHz). **De Pomerai *et al*.**[4] have shown that *in vivo* irradiation of nematode worms overnight with RF-EMF (750 MHz) caused an increase in expression of heat shock protein. **Kwee *et al*.**[5] have shown an induction of stress protein hsp70, but not hsp27, in cultures of transformed human epithelial amnion cells exposed for 20 min to RF-EMF (960 MHz). Also, evidences for rapid cellular and molecular alterations in the rat brain after an acute exposure to high power GSM 900-MHz microwaves were provided [6].

The protective effects of garlic has been attributed to the presence of organosulphur compounds like diallyl sulphide (DAS), diallyl disulphide (DADS),

ajoene, allixin, allyl mercaptans and allyl methyl sulphides[7]. **Shukla and Taneja**[8] revealed that garlic extract has chemopreventive potential against cyclophosphamide (CP)-induced chromosomal mutations in Swiss albino mice.

MATERIALS AND METHODS

Experimental animals :

In the present study about 45 mature female albino rats, *Rattus norvegicus* weighing 80-100 g, obtained from the National Research Center in Dukki, Cairo, were used for the experiments. The animals were divided into three groups. Group 1 included twenty rats were exposed to mobile phone radiation of 900 MHz for 14, 30, 45 days (5 rats were studied each interval) while the last 5 rats were left 30 days post exposure as a recovery group after 45 days of MP exposure. Group 2 included twenty rats that were exposed to mobile phone radiation of 900 MHz for 14, 30, 45 days and were simultaneously administered orally with 0.1 ml garlic as Tomex (ATOS, Cairo, Egypt) (20 mg/kg) daily during the exposure while 5 rats were also left for other 30 days after 45 days post exposure as a recovery group. Group 3 (control), includes 5 rats that were kept in the normal conditions of the laboratory, with no exposure to mobile phone radiation, to be used as control.

Chromosome Preparations

The mitotic activity of bone marrow cells was investigated in each animal by recording the number of dividing cells per 1000 cells. It was expressed as the mitotic index (MI).

Electrophoretic Studies

Protein fractionation was done using 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to **Laemmli**[9]. Liver and brain samples were excised and frozen at –20ºC until use. Tissue sample of 0.2 g was homogenized in 1ml of 1% SDS. The mixture was heated to 90 – 95ºC for 5 minutes and centrifuged at 14000 rpm for 5 minutes. The supernatant containing the protein was carefully removed to a clean Eppendorf tube. The samples were loaded into wells and run at 15 – 20 mA/gel. Similarly 5µl of standard protein marker (205.000-7.600 kDa) (Bio-Rad Laboratories, USA) was loaded onto the same gels. Samples were run on electrophoresis unit (Hoefer mighty small П, SE 250, USA). The gels were stained with Coomassie Brilliant Blue R 220 dye. Proteins were detected as blue stained bands against a clear background. The gel was preserved in 10% acetic acid and photographed. The gel bands were scanned using the Alpha Ease TM software (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis

The data were statistically analyzed using the student's "*t*" test **(Snedecor and Cochran**[10] and also by using one-way analysis of variance ANOVA and Tukey test, by using the statistical software SPSS. Data were considered significant at $P \leq 0.05$.

RESULTS

In the present study the mitotic index (MI), which is equal to the number of metaphases per 1000 cell, was examined and showed that the MI was decreased significantly in mobile phone groups if compared to the control (Table 1). The administration of garlic in association with MP

returned the MI percentage to the control values.

Electrophoretic studies of proteins:

Liver of rats exposed to mobile phone of 900 MHz for 14, 30, 45 days recorded an increased the number of fractions by 4 to 6 bands (Table 2, Fig.1). The irradiated animals revealed 9 new synthesized proteins with molecular weight of 20, 25, 29, 31, 33, 36, 38, 67 and 70 kDa. Protein polymorphism was screened in rats liver exposed to mobile phone radiation of 900 MHz in combination with garlic. Data showed that the number of fractions was increased by 2 to 3 bands than in the control animals and decreased by 2 to 3 bands than in MP group (Table 3, Fig.1). Proteins of 27, 55, 71, 88 and 104 kDa were appeared by the exposure to MP/T.

The analysis of proteins in Table (4) showed the banding pattern of proteins extracted from brain of rats exposed to MP mobile phone radiation. The total number of bands in the exposed groups was increased by 5 fractions when compared to the control. Eight new polypeptides (24, 29, 34, 42, 46, 57, 77 and 85 kDa) appeared clearly on exposure to MP radiation. The association of garlic with MP radiation revealed an increase of the number of fractions by 5 to 7 bands than in the control group (Table 5). The proteinogram displayed 13 new synthesized polypeptides.

DISCUSSION

The frequencies of microwave radiations have a wide range from extremely low frequency (ELF, 50-60Hz) to radiofrequencies and microwave (300 kHz to 300 GHz)[11]. The electric fields have little effect on the growth of cells as measured by the proportion of cells undergoing mitosis at a given time (the mitotic index)[12]. In the present study, there was a significant decrease in the mean value of mitotic index prior to the exposure to mobile phone radiation in all the exposed groups compared with the control one. These results are in agreement with the data reported by **Dyshlovoi *et al.*[13]** they postulated that the alternating current electric field 50 Hz causes depression in mitotic index at 24 and 48 hr in human fibroblasts *in vitro*. Also, **Khalil and Qassem**[14] found significant decreases in mitotic index in human lymphocytes exposed to alternating current electric field 50/60 Hz. These authors suggested that exposure to an electromagnetic field may lead to cell death and retardation of cell division as indicated by the reduction of the mitotic index and the cell proliferation index, respectively. In contrast, **Livingston *et al*.** [15] declared that the alternating current of electric field 50/60 Hz has no effect on the mitotic index of human lymphocytes exposed *in vitro* for 69 hr.

In non-irradiated control exposed cells, 110 phospho-proteins were detected, whereas in exposed cells some 372 phospho-proteins were detected. The observed broad change in the pattern of global protein phosphor-ylation has suggested that cells respond to RF-EMF and that possibly any of the hundreds of phosphoproteins that have altered their phosphorylation status could at least potentially affect cell physiology. Protein electrophoresis of liver and brain in MP group revealed an increase of the total

number of fractions. Liver showed 9 new synthesized polypeptides that may act as stress proteins. These all disappeared in the recovery period except the protein of 31 kDa. Contrarily, 26 and 34 kDa, proper proteins were downregulated by the MP exposure and returned again by the recovery. Polypeptide 86 kDa was observed as resistant protein in all groups. On the other hand, brain proteinogram fractions showed 4 new synthesized proteins appeared only in the MP exposed group. However, there were other 4 polypeptides appeared by the MP exposure and remained in the recovery group. On the contrary, 25 and 37 kDa were the only proper proteins that downregulated by the MP exposure and returned by the recovery. Twenty seven and 40 kDa were proteins that persisted in all the tested groups. All these new synthesized proteins in both liver and brain may be represented as stress proteins for protein misfolding. This hypothesis may strengthened by **Lim *et al*.** [16] who revealed that cells respond to some abnormal physiological conditions by producing cytoprotective heat-shock (or stress) proteins. **Leszczynski *et al*.** [1] investigated the response of the human endotheliel cell line EA.hy926 exposed for 1 hr to 900 MHz GSM mobile phone. **Harvey and French**[17] concluded that low power microwave (864.3 MHz) exposure may act on human mast cell line HMC-1 by altering gene expression *via* a mechanism involving activation of protein kinase C, and at temperatures well below those known to induce a heat shock response. **Fritze *et al*.** [3], using rat as a model, have shown an increase in expression of stress protein hsp70 in brains of animals exposed for 4 hr to radio frequency electromagnetic field (RF-EMF) 890-915 MHz. **Kwee *et al*.** [5] have shown an induction of stress protein hsp70, but not hsp27, in cultures of transformed human epithelial amnion cells exposed for 20 min to RF-EMF (960 MHz). **Leszczynski *et al*.** [18] identified heat shock protein 27 (hsp27) as one of the phospho-proteins responding to RF-EMF. Induction of the increased expression and phosphorylation of hsp27 by the RF-EMF exposure might lead to inhibition of the apoptotic pathway that involves apoptosome and caspase-3. This event, when occurring in RF-EMF exposed brain cells that underwent either spontaneous or external factor-induced transformation/damage, could support survival of the transformed/damaged cells which, in favorable circumstances, could help clonal expansion of the trasnsformed/damaged cells – a prerequisite for the tumour development. **French *et al*.**[19] proposed that repeated exposure to mobile phone radiation acts as a repetitive stress leading to continuous expression of stress protein in exposed cells and tissues as the heat shock proteins, (Hsps) is a normal defence response to a cellular stress. However, chronic expression of Hsps is known to induce or promote oncogenesis, metastasis and/or resistance to anticancer drugs, which in turn affects their normal regulation, and cancer results. This hypothesis provides the possibility of a direct association between mobile phone use and cancer[20].

The administration of garlic in association with MP returned the MI percentage to the control

values. There is increasing evidence that garlic and compounds isolated from garlic have significant antiproliferative effects on human cancer cells[20]. The effects shown by garlic derivatives include induction of apoptosis, regulation of cell cycle progression and modification of pathways of signal transduction. Additionally, authors reported that garlic derivatives appear to regulate nuclear factors associated with immune function and inflammation.

The administration of garlic in combination with MP radiation increased the number of resistant fractions to 4 proteins in liver when compared to MP group. Simultaneously, the new synthesized polypeptides decreased from 9 in MP group to only 5 proteins. On the contrary, brain showed a decrease of the number of resistant proteins from 3 in MP group to only one protein by garlic administration. This was paralleled by the increase of new synthesized polypeptides from 8 to 13 proteins. Comparing the results with the MP exposed group, data showed that the numbers of protein fractions in all the exposed groups were decreased with the effect of garlic in liver while it increased in brain tissue. The protective effects of garlic has been attributed to the presence of organosulphur compounds like diallyl sulphide (DAS), diallyl disulphide (DADS), ajoene, allixin, allyl mercaptans and allyl methyl sulphides[8]. **Shukla and Taneja**[8] revealed that garlic extract has chemopreventive potential against cyclophosphamide (CP) induced chromosomal mutations in Swiss albino mice. **Das *et al.***[21] studied the anticlastogenic activity of crude extract of garlic (*Allium sativum* L.) in bone marrow cells of mice. They found that garlic extract alone induced a low level of chromosomal damage. The clastogenicity of all the three mutagens were reduced significantly in the animals, which had been given garlic extract as dietary supplement. The extent of reduction was different for the three clastogens and may be attributed to the interaction with the different components of the extract.

There is evidence that at least part of the chemopreventive action of garlic is due to the induction of phase Π detoxification enzymes including glutathione transferases; quinone reductase; epoxide hydrolase and glucuronosyl transferase that inactivate toxic substances and facilitate their excretion[22]. **Manson *et al*.**[23] studied the effect of oral administration of garlic oil to rats on a number of drug metabolizing enzymes in liver tissues. The authors reported that garlic oil induced phase Π enzymes such as Glutathione S-transferases (GSTs). GSTs are important detoxifying enzymes that remove harmful electrophiles by conjugating them with glutathione[24]. The effect of oral administration of three garlic-derived compounds (allyl methyl trisulphide, diallyl trisulphide and diallyl disulphide) stimulated GST activity in the liver and lung of mice[25].

The present findings have strengthened the earlier reports on the possible role of realistic dose of garlic in exerting *in vivo* radioprotective effects.

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Table (1): The mean values of mitotic index in bone marrow cells of rats exposed to mobile phone radiation.

Exposure	Exposure/ day	Mean ± SD	%	*T*- Test Treated X Control	*T*- Test for the same exposure days Radiation X Radiation & Tomex	ANOVA Test
Control	0	470.40 ± 42.6	47.04%			
Mobile phone	1) 14 days 2) 30 days 3) 45 days 4) R	391.00 ± 41.8* 422.20 ± 58.6 334.20 ± 52.6* 257.60 ±20.9*	39.10% 42.22% 33.42% 25.76%	1.76 1.24 3.83* 8.74*		0.005*
Mobile phone & Tomex	1) 14 days 2) 30 days 3) 45 days 4) R	433.00 ± 74.7 460.20 ± 68.7 452.60 ± 110 372.40 ± 70.4*	43.30% 46.02% 45.26% 37.24%	1.47 0.28 0.27 3.99*	0.71 1.68 2.89* 3.10*	0.359

*** Significant (P ≤ 0.05)**

R = Recovery (30 days post exposure)

Table (2): SDS-PAGE banding pattern of proteins measured in liver cells of rats exposed to mobile phone radiation.

M.Wt (kDa) / Band No.	Protein Marker	Lane 1 (Control)	Lane 2 (14 days)	Lane 3 (30 days)	Lane 4 (45 days)	Lane 5 (Recovery)
1	205					
2			132		132	
3					120	
4	119					
5						104
6						99
7	98				98	
8			97			
9		86	86	86	86	86.6
10			70	70	70.7	
11				69		
12			67		67	
13		66				
14						65
15			64			
16						53
17	52.3					
18				50		
19					39	
20			38	38		
21						37
22	36.8		36	36	36.7	
23		34				34
24			33	33	33	
25		32.5		32		
26			31	31	31	31
27	30.1	30				
28			29		29	
29		26				26
30			25	25	25	
31		23				
32	22					22
33			20	20	20	
No. of Fractions	**7**	**7**	**13**	**11**	**13**	**10**

Table (3): SDS-PAGE banding pattern of proteins measured in liver cells of rats exposed to mobile phone & Tomex.

M.Wt (kDa) / Band No.	Protein Marker	Lane 1 (Control)	Lane 2 (14 days)	Lane 3 (30 days)	Lane 4 (45 days)	Lane 5 (Recovery)
1	205					
2			104	104	104	
3	119					
4	98					
5			88.8	88		
6					87	
7		86				
8						85
9			78			
10					72	
11			71	71.5		
12						67
13		66				
14			55	55	55	
15						53
16	52.3		52			
17						48
18						44
19						42
20						39
21			38			
22				37		37
23	36.8					
24					35	
25		34	34.7	34	34	34.8
26						33
27		32.5		32	32	32
28	30.1	30		30		30
29				27	27	
30		26	26.8			26.6
31		23	23	23	23	23
32	22					
No. of Fractions	**7**	**7**	**10**	**10**	**9**	**14**

Table (4): SDS-PAGE banding pattern of proteins measured in brain cells of rats exposed to mobile phone radiation.

M.Wt (kDa) / Band No.	Protein Marker	Lane 1 (Control)	Lane 2 (14 days)	Lane 3 (30 days)	Lane 4 (45 days)	Lane 5 (Recovery)
1	205		205			
2					198	
3				191		
4	119					
5			114			
6					112	
7				111.7		111
8			106.6			
9					105.5	
10	98					
11			96.8			
12				85	85	
13			78			
14				77	77.6	
15		64				
16			62.5			
17				61.4		
18			58.8			
19				57.7	57.7	57.4
20	52.3					
21		49.9				
22			47			
23				46	46	
24						45.9
25			42.5		42	42
26				41.8		
27		40.9		40.3	40	40
28		37.4				37
29	36.8					
30			34.9	34.4	34	34.5
31		31.7				
32	30.1	30				
33				29	29	29.2
34		27.4		27.6	27.4	27.6
35		25.7				25.2
36				24.9	24.8	
37		23.8		23.4	23.5	
38	22	22		22		
39					20.9	

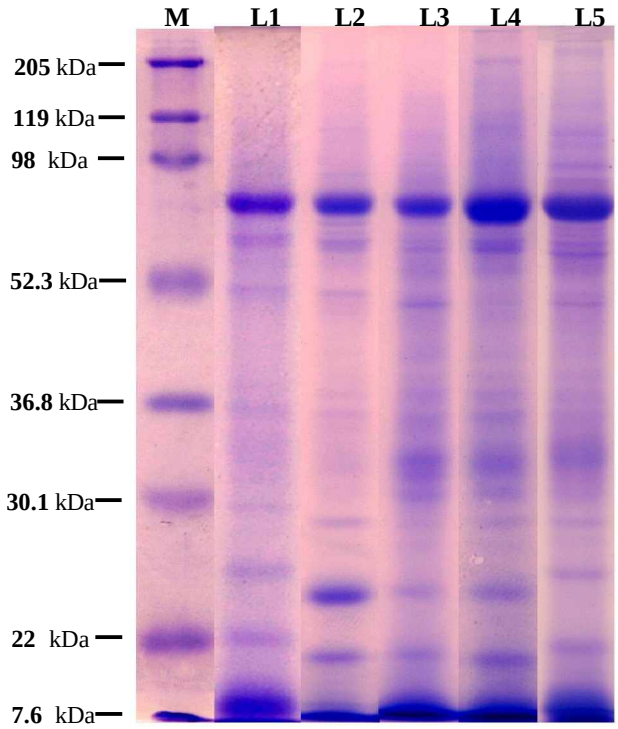
40		18				
No. of Fractions	**7**	**11**	**10**	**15**	**15**	**10**

Table (5): SDS-PAGE banding pattern of proteins measured in the brain cells of rats expsed to mobile phone & Tomex.

M.Wt (kDa) / Band No.	Protein Marker	Lane 1 (Control)	Lane 2 (14 days)	Lane 3 (30 days)	Lane 4 (45 days)	Lane 5 (Recovery)
1	205					
2			200			
3					195.7	
4						191
5				189		
6	119			119		
7			112	111.7	111.7	
8						108.8
9	98					
10					84.5	84.5
11			82.5	82.9		
12					78.5	78.5
13				77		
14			76.6			
15						72
16			71		71.6	
17				70.7		
18		64				
19			62.9			
20				58	58.8	58.8
21			57			
22				54.9	55	
23	52.3			51.9	51.9	
24				50.7	50	
25		49.9				
26			46	46.7	46.7	46.8
27				42	42.6	42.9
28			41.9			
29		40.9				
30		37.4				
31	36.8		36.3			
32			34	34.4	34.5	34.6
33					33	33
34			32.7	32.9		
35		31.7				
36	30.1	30				30
37			29.6	29.8	29.8	
38					28.4	28.7
39		27.4	27.9	27.9		
40						26
41		25.7	25.6	25.6	25.9	

42			24		24	24
43		23.8	23.9			
44	22	22				
45		18				
No. of Fractions	**7**	**11**	**16**	**18**	**17**	**12**

(1)



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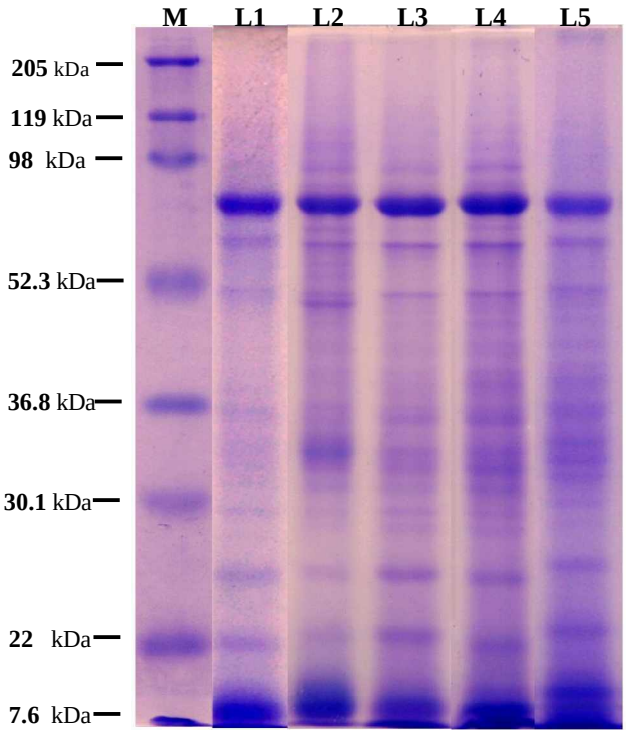

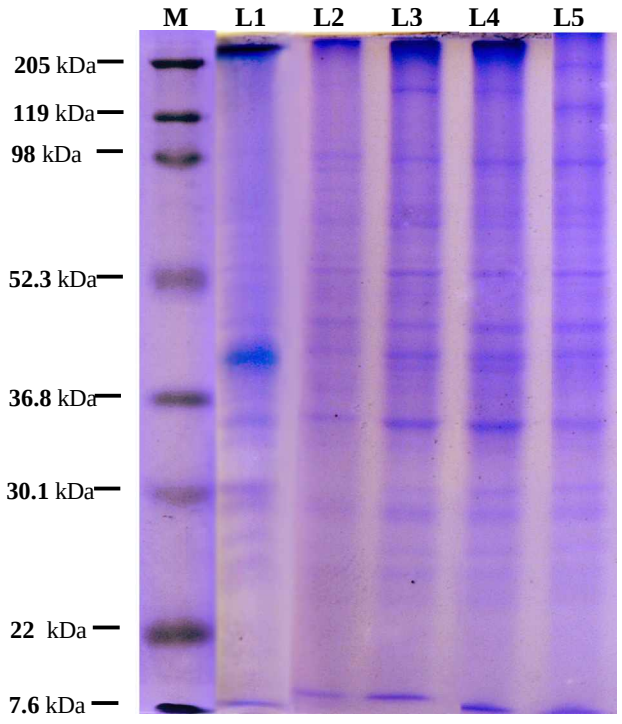

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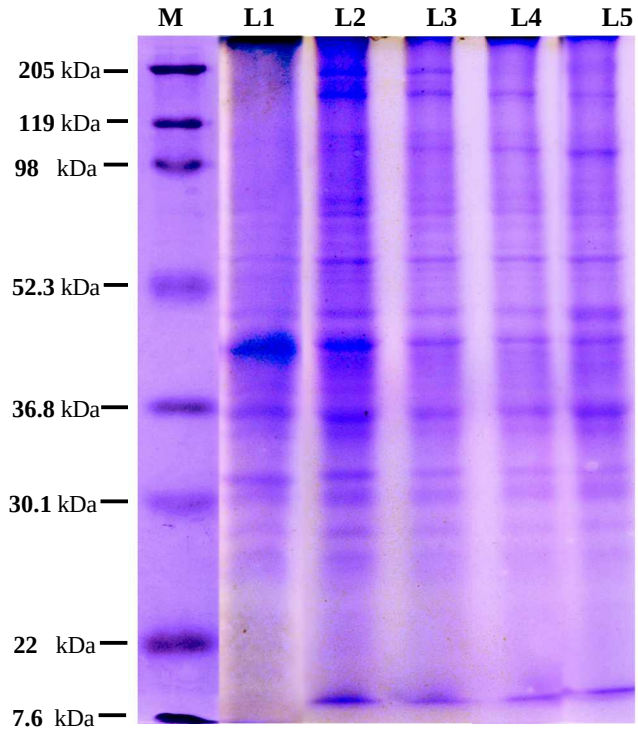

(4)

Fig (1): Polyacrylamide gel electrophoresis of liver and brain proteins extracted from rats exposed to mobile phone radiation (1&3) and were given 0.1 ml Tomex (2&4). L: Lane, M: Protein molecular weight marker, L1: Control, L2: 14 days, L3: 30 days, L4: 45 days, L5: Recovery.

التأثير البروتينى للثوم المضاد لموجات (المحمول) الجوال على كبد و مخ الجرذان البيضاء

خالد محمد شرف الدين – محمد السيد زويل – امل محمد عبد الكريم

قسم علم الحيوان – كلية العلوم – جامعة بنها – بنها – القليوبية - مصر

الملخص العربي

لقد استعمل الثوم كنبات طبى منذ آلاف السنين . فى الدراسة الحالية استخدم هذا النبات للوقاية من موجات الإشعاع. ولذا تمت هذه الدراسة على ٤٥ جرذ أبيض بالغ (إناث) ، تم تعريض البعض منها الى موجات الجوال ، ذات تردد ٩٠٠ ميجاهرتز لمدة ١٤ و ٣٠ و ٤٥يوم على التوالى . كما تم دراسة التأثير الوقائى للثوم عند تناوله مع تعرض الجرذان لموجات الجوال . وقد أُستخدم تحليل التفريد الكهربى لجل البولى أكريلامايد فى اختبار ظهور بروتينات انسجة الكبد و المخ فى الجرذان. وبينت النتائج ظهور بروتينات جديدة مختلفة فى أنسجة الكبد والمخ عند التعرض لموجات الجوال. كما أظهرت نتائج الدراسة أن تناول ٢٠ ملجم/كجم يوميا من الثوم قد خفض عدد حزم البروتينات فى الكبد بينما زادت فى المخ . وصاحب هذه الظاهرة ، ظهور بروتينات جديدة (ربما تكون بروتينات إجهاد) ذات الوزن الجزيئى المنخفض. كذلك منع تناول الثوم بصورة ملحوظة الإنخفاض فى معامل الانقسام الميتوزى الناتج عند التعرض لموجات الجوال . تعضد الدراسة الحالية الدور الوقائى للثوم ضد الإشعاع والطفرات الجينية التى ربما تؤدى الى ظهور الأورام.