World Journal of Dairy & Food Sciences 8 (1): 94-99, 2013
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2013.8.1.1122

Comparative Study Between Marjoram and Alpha Lipoic Acid on Potassium Bromide Induced Oxidative Stress in Rats

Waffa Sh. Ali

Nutrition and Food Science Department,
Faculty of Home Economics, Helwan University, Cairo, Egypt

Abstract: The antioxidant activity of marjoram powder, extract and oil against potassium bromated induced oxidative stress in Sprague Dawley strain rats was investigated. The animals were received $KBrO_3$ (20 mg/kg b.w) intragastric twice a week all over period of the experiment. Then, Rats reclassified into five groups which were control positive (untreated) and four treated rat groups that were alpha lipoic acid (10mg/ kg b.w daily), marjoram powder (5% powder in standard diet), marjoram extract (250 mg/kg b.w daily) and marjoram oil (0.5 ml/kg b.w daily by stomach tube). The experiment period was 60 days. The obtained results showed that marjoram either as powder, extract or oil against $KBrO_3$ administration to rats showed increase in nutritional values, antioxidant enzymes, blood hemoglobin (HG) and decrease in serum liver and kidney biomarkers, serum malondialdehyde (MDA)and blood nitric oxide compared to control (+ve) group. There are non significant differences in nutritional results; serum urea, uric acid and MDA; and blood HG and packed cell volume (PCV) and also aspartate amino transferase (AST), catalase and glutathione transferase (GST) in marjoram powder, marjoram extract and marjoram oil groups compared with alpha lipoic acid group enzymes. It can be concluded that potassium bromate administration in rats significantly altered the serum level of liver, kidney biomarkers and activities of antioxidant enzymes. Marjoram could reverse the side effects of potassium bromated by scavenger of free radicals which could be due to the presence of some plant bioactive antioxidant constituents.

Key words: Alpha lipoic acid · Antioxidant enzymes · Marjoram · Potassium bromated · Rats

INTRODUCTION

Oxidative stress can be defined as an imbalance between prooxidant/free radical production and opposing antioxidant defenses. Acute and chronic oxidative stress is implicated in degenerative diseases [1]. It has been reported that potassium bromate ($KBrO_3$) has been used widely for water disinfection, hair-coloring solutions, cosmetics and also in food. Intake of KBrO3 or exposure to it causes production of oxygen free species in living cells. $KBrO_3$ has been reported to be a potent nephrotoxic agent that can mediate renal oxidative stress, toxicity and tumor response in rats. It also enhances renal lipid peroxidation and hydrogen peroxide formation with reduction in renal antioxidant enzymes. Also, potassium bromate contributes to the cellular redox status and impairment of membrane protein activities in rats [2]. An antioxidant is a compound that blocks the action of free radicals, activated oxygen molecules that can damage cells. Alpha-lipoic acid is an antioxidant that is made by the body and is found in every cell, where it helps turn glucose into energy and may help regenerate other antioxidants and make them active again. Alpha-lipoic acid is both fat and water soluble [3]. Several studies have shown that lipoic acid exerts multiple pharmacological actions in different models of diseases characterized by increase in oxidative stress markers. It displays antioxidant effects by scavenging reactive oxygen species and stimulates the synthesis of other antioxidants, such as glutathione [4, 5]. Medicinal plants play important role in the treatment of many degenerative disorders. Several herbs possess bioactive constituents such as phenolic and polyphenolic compounds which regulate various immune systems and possess antioxidant and anti-inflammatory properties [6, 7]. Marjoram (*Origanum majorana* L., Family: Lamiaceae)

Corresponding Author: Waffa Sh. Ali, Nutrition and Food Science Department,
Faculty of Home Economics, Helwan University, Cairo, Egypt.

is used worldwide in food for better flavor, both in dry form and as fresh vegetable It is also a common spicy medicinal herb, used as a home remedy for the treatment of different ailments. It is a well-liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skin care, flatulence and stomach disorders. Marjoram alcoholic, aqueous extracts and essential oil could protect liver and kidney damage [8, 9]. The essential oil obtained from marjoram has aromatic smell and contain high percentage of polyphenols and monoterpenes which are established antioxidants [10, 11].

This study was designed to study the effect of marjoram powder, extract and oil on potassium bromide induced oxidative stress in rats compared to alpha-lipoic acid.

MATERIALS AND METHODS

Materials: The plant material used in this experiment was marjoram plants which purchased from the herbs shop in Cairo, Egypt and scientifically identified at Horticultural Research Institute, Agricultural Research Center, Egypt. Marjoram oil was purchased from Agricultural Research Center. The standard diet was performed according to Reeves *et al.* [12]. BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics. Potassium bromate ($KBrO_3$) and alpha lipoic acid were purchased from El-Gomhoria Company. Forty Sprague Dawley strain rats provided from the National Research Center, Giza, Egypt and weighted 122±5g.

Methods:

Preparation of Marjoram Powder and Extract: Marjoram plants were grinded to give a fine powder. Marjoram extract was prepared from marjoram powdered which was refluxed eight times with an 80% ethanol solution for two times, 1 hr each time. The solvent was removed by evaporation under reduced pressure using Rotary Evaporator and stored at 4° C until further analyzed [13].

Biological Study: After adaptation period (five days), rats received $KBrO_3$ (20 mg/kg b.w) intragastric twice a week all over period of the experiment. Then, rats reclassified into five groups which were control positive (untreated) and four treated rat groups that were alpha lipoic acid (10 mg/kg b.w daily), marjoram powder (5% powder in standard diet), marjoram extract (250 mg/kg b.w daily) and marjoram oil (0.5 ml/kg b.w daily by stomach tube). The food intake was monitored daily and the growth of animals was monitored weekly by recording body weight. Food efficiency ratio (FER) was determined by Chapman *et al.* [14]. The animals were sacrificed after 60 days under light ether anesthesia. Blood was collected for separation of serum for biochemical assays.

Biochemistry Analysis: Serum alanine and aspartate aminotransferase (ALT, AST) activity and alkaline phosphatase (ALP) were estimated according to Bergmeyer and Horder [15] and Kind and King [16], respectively. Serum creatinine, urea and uric acid were determined according to Bonsens and Taussky [17], Kanter [18] and Fossati *et al.* [19], respectively. Briefly, serum superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase and glutathione S-transferase (GST) enzymes were estimated according to the methods of Misra and Frisovich [20], Weiss *et al.* [21], Sinha [22] and Habig *et al.* [23], respectively. The method of Draper and Hadley [24] was employed in determining malondialdehyde (MDA). Blood hemoglobin (HG), packed cell volume (PCV) and nitric oxide (NO) were estimated according to Drabkin [25], Mc Inory [26] and Green *et al.* [27], respectively.

Statistical Analysis: Statistical analysis involved used of the Statistical Analysis System software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P<0.05$ [28].

RESULTS AND DISCUSSION

The influence of marjoram either as powder, extract or oil against $KBrO_3$ administration to rats showed significant increase in body weight gain, food intake and FER at $P<0.01$ &0.001 compared to control (+ve) group. In comparing with alpha lipoic acid group, there are non significant differences in nutritional results among treated groups as shown in Table 1. It is known that, potassium bromate has the ability to induce tissue damage by a free radical mediated reactions and is extremely toxic to tissues especially those of the central nervous system and kidneys and also haemolysis [29]. The obtained results are in agreement with those obtained by Cakatay *et al.* [30] and Packer *et al.* [31], who reported that alpha lipoic acid (ALA) has been found to affect cellular metabolic processes, alter redox status of cells and interact with thiols and other antioxidants. ALA has beneficial effects on energy production and also an essential cofactor of

Table 1: Mean values ± SD of body weight gain (g), food intake (g) and FER of the experimental rat groups

	Groups				
Variables	Control (+ve)	Alpha lipoic	Marjoram powder	Marjoram extract	Marjoram oil
Initial weight	122.41±2.41^{a}	123.71±3.51^{a}	122.40±3.21^{a}	124.11±4.14^{a}	121.19±4.20^{a}
Final weight	186.02±19.19^{b}	243.85±24.11^{a**}	232.01±20.11^{a**}	234.32±21.31^{a**}	236.81±22.21^{a**}
weight gain	63.61±6.31^{b}	120.14±15.14^{a***}	109.61±11.21^{a***}	110.21±10.31^{a***}	115.61±12.11^{a***}
food intake	14.45±1.27^{b}	18.41±1.44^{a*}	17.75±1.25^{a*}	17.95±1.41^{a*}	17.85±1.18^{a*}
FER	0.073±0.001^{c}	0.108±0.003^{a***}	0.102±0.004^{b**}	0.102±0.005^{b**}	0.107±0.007^{a***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd} Mean values in each raw having similar letters were not significantly different.

Table 2: The Mean values ± SD of serum ALT, AST and ALP (µ/ml) of the experimental rat groups.

	Groups				
Variables	Control (+ve)	Alpha lipoic	Marjoram powder	Marjoram extract	Marjoram oil
ALT	77.26±6.91a	33.88±3.11c***	48.21±4.71b**	43.61±5.01b**	45.71±5.11b**
AST	85.11±8.14a	45.11±4.33bc***	55.96±5.11b***	50.19±5.61b***	51.10±5.17b**
ALP	91.61±8.71a	55.11±5.16c***	67.12±7.03b**	57.14±5.19c***	59.31±6.14bc**

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd} Mean values in each raw having similar letters were not significantly different.

Table 3: The Mean values ± SD of serum creatinine, urea and uric acid of the experimental rat groups.

	Groups				
Variables	Control (+ve)	Alpha lipoic	Marjoram powder	Marjoram extract	Marjoram oil
Creatinine (mg/dl)	0.99±0.11a	0.70±0.13b**	0.61±0.01b**	0.58±0.04bc***	0.55±0.06c***
Urea (µ /mg)	71.11±7.71a	60.11±6.12b***	56.11±5.17bc***	58.11±6.14b***	57.14±5.81b***
Uric acid (mg/dl)	4.33±0.41a	3.55±0.47b*	3.11±0.71b*	3.40±0.81b*	3.96±0.45b*

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd} Mean values in each raw having similar letters were not significantly different.

mitochondrial respiratory enzymes. ALA which been used as a nutritional supplementation, has several potential benefits including therapeutic potential and capable of scavenging free radicals. The effect of marjoram was reported by Abdel-Massih *et al*. [32] and Babili *et al*. [33], who reported that majorana herb was cultivated and used as flavoring in foods. The leaves and stems yield an essential oil. The amount of phenolic acids, flavones, flavanones and flavonols were found to play a vital role in the antioxidant capacity of marjoram extracts. Such activities might be held responsible for the protective role observed by marjoram treatment.

Table 2 showed the changes in the ALT, AST and ALP. The treated groups showed significant decrease in serum ALT, AST and ALP at P<0.001 and P<0.01 but Alpha lipoic acid group showed the lowest value of them compared with control (+ve). There was significant increase in ALT and non significant increase in AST in marjoram powder, marjoram extract and marjoram oil groups compared with alpha lipoic acid group. These results are in agreement with those obtained by EL-Ashmawy *et al*. [34] and Rodriguez-Meizoso *et al*. [35], who found that the reduction in the serum levels of AST, ALT as a result of administration of marjoram (volatile oil, alcoholic and aqueous) might probably be due to presence of isoflavones, polyphenols and other antioxidants. It is mentioning that marjoram volatile oil was also found to control oxidative damages induced by ethanol toxicity to brain, liver and fertility. Ursolic acid, carnosic acid and carnosol isolated from marjoram possessed free radical scavenging properties so reduce oxidative injury [36, 37].

Table 3 showed the assessment of kidney function biomarkers in serum. The treated groups showed significant decrease in concentration of creatinine, urea and uric acid at P<0.01, 0.001 &0.05 compared to control (+ve) group. There was a non significant decrease in urea and uric acid in marjoram powder, marjoram extract and marjoram oil groups compared with alpha lipoic acid group. On the other hand, Marjoram oil group showed significant decrease in creatinine compared with alpha lipoic acid group. High levels of creatinine, urea and uric acid reflect the kidney dysfunction and renal injuries induced by $KbrO_3$ treatment. Alpha lipoic acid was

Table 4: The Mean values ± SD of blood HG, PCV and NO of the experimental groups

	Groups				
Variables	Control (+ve)	Alpha lipoic	Marjoram powder	Marjoram extract	Marjoram oil
HG	10.81±1.35b	13.19±1.41a**	12.99±1.95a**	13.26±1.85a**	12.71±1.77a**
PCV	27.11±2.41b	34.21±4.17a**	32.31±3.96a**	33.20±3.51a**	31.19±3.27ab
NO	9.33±1.55a	4.55±0.77c***	6.31±1.01b***	5.11±0.80bc***	5.71±0.87bc**

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

[abcd] Mean values in each raw having similar letters were not significantly different.

Table 5: The Mean values ± SD of serum SOD, GPX, catalase, GST and MDA of the experimental groups.

	Groups				
Variables	Control (+ve)	Alpha lipoic	Marjoram powder	Marjoram extract	Marjoram oil
SOD (mmol/l)	0.38±0.03d	1.01±0.51a***	0.81±0.12bc**	0.96±0.1b**	0.98±0.18b**
GPX (mmol/l)	0.27±0.01d	0.89±0.11a***	0.68±0.05bc**	0.78±0.17b**	0.77±0.15b**
Catalase (µ/l)	0.49±0.05b	0.98±0.18a***	0.87±0.11a***	0.91±0.13a***	0.90±0.11a***
GST (mmol/l)	0.80±0.11c	1.11±0.22a***	0.99±0.05ab**	1.01±0.51a***	1.03±0.45a***
MDA (mmol/l)	11.30±1.57a	6.31±1.01bc***	8.21±1.21b***	7.33±1.11b***	7.14±1.04b***

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

[abcd] Mean values in each raw having similar letters were not significantly different.

effective in normalizing the antioxidant levels, as well as levels of creatinine and blood urea nitrogen in acetaminophen-induced renal damage in rats [9, 38]. Marjoram/Origanum species exerted antimalarial, antioxidant and protective activities. Such properties of marjoram might be attributed to the presence of terpenes, polyphenols, phenolic glycosides, phenolic derivatives, flavonoids, tannins, sitosterol and essential oil [33].

Table 4 showed the changes in the blood HG, PCV and NO. The treated groups showed significant increase in blood HG at P<0.01 and significant decrease in blood NO P<0.001 compared with control (+ve). Alpha lipoic acid, marjoram powder and marjoram extract groups showed significant increase in blood PCV compared with control (+ve). There is a non significant difference in the effect of marjoram powder, marjoram extract and marjoram oil groups in blood HG and PCV but marjoram powder showed significant increase in blood NO compared with alpha lipoic acid group. Potassium bromate-induced methemoglobinemia resulted from the reduction of glutathione peroxidase activity due to increases in superoxide, nitric oxide and peroxynitrite [39]. In addition, it has been demonstrated that NO may enhance cellular injury by decreasing intracellular GSH levels. Nitric oxide promotes oxidative stress-induced cell injury by formation of peroxynitrite anion, a potent prooxidant and cytotoxic intermediate that causes protein nitration and tissue injury [40, 41]. Alpha lipoic acid is capable to scavenge reactive oxygen species generated during the lipid peroxidation and protects the cell structure against damage. ALA is functionally efficient in helping cells to recover from oxidative damage. Alpha lipoic acid supplementation exerts the vascular morphology is probably through its antioxidant properties and the effects on carbohydrate and lipid metabolism [42]. Alpha lipoic acid inhibits lipopolysaccharide induced NO production in isolated rat Kupffer's cells and in murine macrophages. Another possible explanation is the direct scavenging effect of NO by the sulphydryl group [43].

The antioxidant effect of marjoram is illustrated in Table 5. Alpha lipoic acid group showed significant increase in SOD, GPX, catalase and GST at P<0.001 but showed significant decrease in MDA at P<0.001 compared to control (+ve) group. Marjoram powder, extract and oil groups showed also significant increase in SOD, GPX (P<0.01), catalase and GST at P< 0.001 but showed significant decrease in MDA at P<0.001 compared to control (+ve) group. On the other side, marjoram powder, extract and oil groups showed significant decreased in SOD and GPX and non significant decrease in catalase and GST and also non significant increase in MDA compared with alpha lipoic acid group. Potassium bromate induced the formation of free radicals which react with some cellular components such as membrane lipids and produce lipid peroxidation products. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation [9]. The results regarding the effect of alpha lipoic acid against the toxic affect of $KBrO_3$ in rat on the activities of antioxidant enzymes. Alpha Lipoic acid is a disulfide compound that functions as a coenzyme in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase mitochondrial reactions, leading to the production of cellular energy (ATP).α-

Lipoic acid and its reduced form, dihydrolipoic acid, reduce oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains, by chelating transition metals in biological systems, by preventing membrane lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as vitamins C and E and by increasing intracellular glutathione [44,45]. The findings of the present experiment are fully in agreement with earlier reports demonstrating that marjoram were shown to possess different biological activities including antioxidant potential and free radical scavenging properties. Generally, the high antioxidant capacity of marjoram and other *Origano* species were found depending on their total phenol contents [10].

In conclusion, marjoram has antioxidant activity nearly as alpha lipoic acid even in powder or extract or oil in potassium bromide toxicity in rats.

REFERANCES

1. Sun, Y., 1990. Free radicals, antioxidant enzymes and carcinogenesis. Free Rad. Biol. Med., 8: 583-599.
2. Chipman, J.K., J.E. Davies, J.L. Parsons, J. Nair, G. O'Neill and J.K. Fawell, 1998. DNA oxidation by potassium bromate; a direct mechanism or linked to lipid peroxidation? Toxicology, 126: 93-102.
3. Thirunavukkarasu, V. and C.V. Anuradha, 2004. Influence of alpha-lipoic acid on lipid peroxidation and antioxidant defence system in blood of insulin-resistant rats. Diabetes Obes. Metab., 6(3): 200-7.
4. Bharat, S., B.C. Cochran, M. Hsu, J. Liu, B.N. Ames and J.K. Andersen, 2002 Pre-treatment with R-lipoic acid alleviates the effects of GSH depletion in PC12 cells: Implications for Parkinson's disease therapy. Neurotoxicology, 23: 479-486.
5. Ahmed, H.H., 2010. Modulatory effects of vitamin E, acetyl-lcarnitine and α-lipoic acid on new potential biomarkers for Alzheimer's disease in rat model. Exp. Toxicol. Pathol., in press. doi:10.1016/j.etp.2010.11.012
6. Youdim, K., J. McDonald, W. Kalt and J. Joseph, 2002. Potential role of dietary flavonoids in reducing micro-vascular endothelium volubility to oxidative and inflammatory. Results J. Nut. Biochem., 13(5): 282-288.
7. Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat. Res., 523-524: 9-20.
8. Faleiro, L., G. Miguel, S. Gomes, L. Costa, F. Venancio, A. Teixeira, A. Cristina-Figueiredo, J.G. Barroso and L.G. Pedro, 2005. Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. J. Agric. Food Chem., 53(21): 8162-8168.
9. Abd El-Ghany, M.A. and N.Y. El-Metwally, 2010. Effect of marjoram leaves on injured liver in experimental rats. Report and Opinion, 2(12): 181-191.
10. Novak, J., C. Bitsch, J. Langbehn, F. Pank, M. Skoula, Y. Gotsiou, C.M. Franz, 2000. Ratios of cis-and trans-sabinene hydrate in *Origanum majorana* L. and *Origanum microphyllum* (Bentham) Vogel. Biochem. System Ecol., 28(7): 697-704.
11. Naif, O., 2011. Effect of marjoram extract treatment on the cytological and biochemical changes induced by cyclophosphamide in mice. Journal of Medicinal Plants Research, 5(23): 5479-5485.
12. Reeves, P.G., F.H. Nielsen and G.G. Fahmy, 1993. AIN-93. Purified diets for laboratory rodents: Final report of the American Institute of Nutrition Adhoc Writing Committee on the Reformulation of the AIN-76 A Rodent Diet. J. Nutrition, 123: 1939-151.
13. WHO, 1983. Protocol CG-04: Preparation of Alcoholic Extract for Bioassay and Phytochemical Studies (APJF/IP, 1001A). Geneva, World Health Organization.
14. Chapman, D.G., R. Castilla and J.A. Campbell, 1959. Evaluation of protein in food. Determination of protein and food efficiency ratio. Can. J. Biochem. Physio., 1(37): 679-686.
15. Bergmeyer, H.U. and M. Horder, 1980. Methods for the measurement of catalyuc concentration of enzymes. J. Clin. Chem. Clin. Biochem., 18: 521-534.
16. Kind, P.R. and E.J. King, 1954. Estimation of alkaline phosphatase activity by determination of hydrolyzed phenol with aminoantipyrene. J. Clin. Path., 7(4): 322-326.
17. Bonsens, K.E. and D.H. Taussky, 1984. Determination of serum creatinine. J. Chem. Inv., 27: 648-660.
18. Kanter, M.W., 1975. Clinical Chemistry. The Bobber Merrill Company Inc., USA, pp: 80.
19. Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3, 5 dichloro-2-hydroxybenzene sulfonic acid /4-amlnophenazon chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.
20. Misra, H. and I. Fridovich, 1972. The role of superoxide anion in the antioxidant epinephrine and a single assay of superoxide dismutase. J. Biol. Chem., 247: 3170-5.

21. Weiss, C., H.S. Marker and G.M. Lehrer, 1980. Sensitive fluorometric assays for glutathione peroxidase and reductase. Anal Biochem, 106: 512-516.
22. Sinha, A.K., 1972. Colorimetric assay of catalase. Analytical Biochemistry, 47: 389-394.
23. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-9.
24. Draper, H.H. and M. Hadley, 1990.Malondialdehyde determination as index of lipid peroxideation. Methods Enzymol., 186: 421-431.
25. Drabkin, D.I., 1949. The standardization of heamoglobin measurements. Am. J. Med. Sci., 21(7): 710-711.
26. Mc Inory, R., 1954. A micro heamatocrit for determining the packed cell and hemoglobin concentration on capillary blood. J. Clin. Path., (7): 32-33.
27. Green, L., D. Wagner, J. Glokowski, P. Skipper, J. Wishnok and S. Tannenbaum, 1981. Analysis of nitrite, nitrate and [15N] nitrite in biological fluids. Anal. Biocheml., 126: 131-138.
28. Abo-Allam, R.M., 2003. Data Statistical Analysis using SPSS Program. 1st Ed., Publication for Universities, Cairo.
29. Parsons, J.L. and J.K. Chipman, 2000. The role of glutathione in DNA damage by potassium bromate *in vitro*. Mutagenesis, 15(4): 311-316.
30. Cakatay, U., A. Telci, S. Salman, L. Satman and A. Sivas, 2000. Oxidative protein damage in type I diabetic patients with and without complications. Endocr Res., 26(3): 365-379.
31. Packer, L., K. Kraemer and G. Rimbach, 2001. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition, 17(10): 888-895.
32. Abdel-Massih, R.M., R. Fares, S. Bazzi, N. El-Chami and E. Baydoun, 2010. The apoptotic and anti-proferative activity of *Origanum majorana* extracts on human leukemic cell line. Leuk. Res., 34(8): 1052-1056.
33. Babili, F.E., J. Bouajila, J.P. Souchard, C. Bertrand, F. Bellvert, I. Fouraste, C. Moulis and A. Valentin, 2011. Oregano: chemical analysis and evaluation of its antimalarial, antioxidant and cytotoxic activities. J. Food Sci., 76(3): C512-C518.
34. EL-Ashmawy, I.M., A.F. EL-Nahas and O.M. Salama, 2005. Protective effect of volatile oil, alcoholic and aqueous extract of Origanum majorana on lead acetate toxicity in mice. Basic Clin. Pharmacol. Toxicol., 97(4): 238-243.
35. Rodriguez-Meizoso, I., F.R. Marin, M. Herrero, F.J. Senorans, G. Reglero, A. Cifuentes and E. Ibanez, 2006. Subcritical water extraction of nutraceuticals with antioxidant activity from oregano: Chemical and functional characterization. J. Pharm. Biomed. Anal., 41(5): 1560-1565.
36. Chung, Y.K., H.J. Heo, E.K. Kim, H.K. Kim, T.L. Huh, Y. Lim, S.K. Kim and D.H. Shin, 2001. Inhibitory effect of ursolic acid furified from *Origanum majorana* L. on the acetylcholinesterase. Mol. Cells, 11(2): 137-143.
37. El-Ashmawy, I.M., S. Amal and O.M. Salama, 2007. Acute and long term safety evaluation of *Origanum majorana* essential oil. Alex. J. Pharm. Sci., 21: 29- 35.
38. Abdel-Zaher, A.O., R.H. Abdel-Hady, M.M. Mahmoud and M.M. Farrag, 2008. The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. Toxicology, 243: 261-270.
39. Watanabe, S., S. Togashi and T. Fukui, 2002. Contribution of nitric oxide to potassium bromate-induced elevation of methaemoglobin concentration in mouse blood. Biol. Pharm. Bull., 25(10): 1315-9.
40. Zhang, C., L.M. Walker and P.R. Mayeux, 2000. Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochem. Pharmacol., 59: 203-209.
41. Massion, P.B., O. Feron, C. Dessy and J.L. Balligand, 2003. Nitric oxide and cardiac function: Ten years after and continuing. Circ. Res., 93: 388-398.
42. Balkis, B.S., F. Othman, S.R. Louis, M. Abu Bakar, M. Radzi, K. Osman, S. Das and J. Mohamed, 2009. Effect of alpha lipoic acid on oxidative stress and vascular wall of diabetic rats. Rom. J. Morphol. Embryol., 50(1): 23-30.
43. Biewenga, G.P., G.R. Haenen and A. Bast, 1997. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol., 29: 315-331.
44. Packer, L., 1998. α-Lipoic acid: A metabolic antioxidant which regulates NF-kappa B signal transduction and protects against oxidative injury. Drug Metab. Rev., 30: 245-275.
45. Çiftçi, H., A. Özkaya, A. Dayangaç, A. Ölçücü, S. Çelik, Z. Şahin and S. Ateş, 2009. Effect of lipoic acid on the some elements in brain tissue of DMBA-induced Guinea Pigs. Kafkas Univ. Vet. Fak. Derg., 15(4): 569-573.