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IN VIVO EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT EXTRACTS OF DATE FRUITS IN ADJUVANT ARTHRITIS

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Key words: antioxidant activity, anti-inflammatory activity, toxicity, rats, date (*Phoenix dactylifera*)

This study was carried out to evaluate the antioxidant and anti-inflammatory activity of methanolic and water extracts of the edible portion of date fruits (*Phoenix dactylifera* L., Family Palmae) and methanolic extract of date seeds in adjuvant arthritis in rats, a model of chronic inflammation. Acute oral toxicity of the different extracts was carried out to determine their safety as functional foods. The results revealed that oral administration of the methanolic and aqueous extracts of edible portion of date fruits suppressed the swelling in the foot significantly by 67.8 and 61.3% respectively, while the methanolic extract of date seeds showed significant reduction by 35.5%. Antioxidant state (plasma vitamin C, E and A and β-carotene) increased significantly on administration of different extracts, while plasma level of MDA reduced significantly. Methanolic extract of edible portion of the fruit was the best in reducing foot swelling, ESR and plasma fibrinogen and in normalizing the plasma level of antioxidants.

Adjuvant arthritic rats showed significant reduction in body weight gain and food efficiency ratio. Administration of any of the extracts produced significant increase in body weight gain and food efficiency ratio.

Acute lethal toxicity test showed that the safest extract was the methanolic of edible portion of the fruit followed by the water extract that showed complete safety up to 6 g/kg mice weight. Methanolic extract of the seed showed LD50 equal to 6.75 g/kg mice.

INTRODUCTION

Free radicals are implicated in chronic inflammatory diseases including rheumatoid arthritis. Free radicals play an important role in the severity of rheumatoid arthritis and patients usually suffer high oxidative stress. Antioxidants either synthetic or natural are potent scavengers of free radicals and have beneficial effects on human health and disease prevention [Bagchi *et al.*, 2000]. They may have a possible role in improving inflammatory condition in rheumatoid arthritis patients [Wittenborg *et al.*, 1998]. Fruits of the date palm (*Phoenix dactylifera* L. Palmae) are commonly consumed in many parts of the world especially the Arabian countries [Vayalil, 2002]. Date fruit are used as nutrient while the pollen grains used in the treatment of infertility [Rendle, 1959]. In a previous study, we have shown that edible portion of date fruits and its methanolic extract had antioxidant activity *in vitro* due to the presence of phenolic compounds [Mohamed & Al-Okbi, 2004]. Phenolic compounds are prototypic chain breaking antioxidant; their protective effect against lipoperoxidative damage depends on the hydrogen-donating capacity of hydroxyl group in each molecule [Kahl, 1991]. Vayalil [2002] proved that the aqueous extract of date fruit had *in vitro* antioxidant activity due to the presence of compounds with potent free-radical-scavenging activity. So it was of importance to study the antioxidant activity of edible portion of date fruits extracts *in vivo*, so as to confirm their activity in intact biological system. Since the edible portion of fruits has been proved to contain phenolic compounds, so the methanolic and water extracts may show anti-inflammatory activity according to Cook and Samman [1996]. Although the methanolic extract of seed showed no antioxidant activity *in vitro*, however, we have decided to study its antioxidant activity *in vivo* to conclude to what extent we can rely on the *in vitro* test.

The aim of the present work is to study the antioxidant and anti-inflammatory activity of methanolic and water extracts of edible portion of date fruits, and methanolic extract of date seeds on rheumatoid arthritis using adjuvant arthritis model in rats. The aim included toxicity study of the different extracts to determine their safety as functional foods.

MATERIALS AND METHODS

Preparation of extracts. Date fruits (*Phoenix dactylifera* L., Palmae) Zaghlool were purchased from local market in Egypt. The defatted powder of edible portion of date fruits (Zaghlool) were extracted successively with methyl alcohol then distilled water in a continuous extraction apparatus. The defatted powder of date seeds was extracted with methyl alcohol at the same condition of date fruits extraction. The extracts were evaporated under vacuum and dried to a constant weight using a freeze-drier. The dry extracts of fruits and seeds were dissolved in distilled water instantaneously before given to rats.

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DESIGN OF EXPERIMENTAL WORK

Adjuvant arthritis experiment. White male albino rats of 164 g average body weight were used in the adjuvant arthritis experiment. The animals were kept individually in wire bottomed cages at room temperature. Water and food were given *ad-libitum*. Rats were divided into five groups, each comprised eight rats. Two groups served as control (one healthy control and the other was arthritic control), where rats received no extracts. The other three groups were the test groups where rats of different groups were given daily oral dose of 500 mg of either methanolic or water extracts of edible portion of date fruits or methanolic extract of date seeds /kg rat body weight. Rats of both control groups were given only equal amounts of distilled water daily. One day after starting dosage, adjuvant arthritis was induced in all rats (except one of the control groups which is the normal healthy group) by injection of Freund's complete adjuvant (Sigma, USA) into the subplanter region of the right hind--foot paw [Singh *et al.*, 1992]. All the oral supplements continued for 14 days. Rats were maintained on the balanced diet (Table 1) allover the experiment. Paw thickness was measured before induction of arthritis and three times per week using vernier calipers to assess the degree of inflammation and were presented in Figure 1. At the end of the experiment, the increase in the thickness of injected foot (inflammation) of rats of tests groups was compared with that of the arthritic control. During the experiment, body weights and food intake were recorded twice daily. At the end of the experiment; total food intake, body weight gain and food efficiency ratio were calculated. After elapse of experimental period, rats were fasted 16-18 h and blood samples were drawn and divided into three parts one mixed with trisodium citrate (0.14 mol/L) for the determination of fibrinogen [Toro & Ackerman, 1975], the second part mixed with trisodium citrate (109 mmol/L) for the determination of erythrocyte sedimentation rate (ESR) [Westergren, 1921], and the third mixed with heparin for separation of plasma for the determination of vitamin C [Jagota & Dani, 1982], vitamin E [Desia & Machlin, 1985], vitamin A [Neeld & Pearson, 1963], β-carotene [Neeld & Pearson, 1963], and malondialdehyde (MDA) as indicator for lipid peroxidation [Satoh, 1978].

TABLE 1. Composition of the diet (%).

Ingredients	%
Casein	10
Corn oil	10
Sucrose	25.2
Starch	50.3
Salt mixture [Briggs & Williams, 1963]	3.5
Vitamin mixture [Morcos, 1967]	1

Acute toxicity test. Acute lethal toxicity test of different extracts was carried out according to Goodman *et al.* [1980]. Adult normal male and female, albino mice of 21–25 g body weight were used. The 24 h mortality counts among equal sized groups of mice (8 animals/group) receiving progressively increasing oral dose levels of the different extracts were recorded. LD50 of extracts that have appreciable toxicity have been calculated according to Paget and Barnes [1974].

Statistical analysis of student's *t-test* (2 tailed) was applied to the results. Pearson correlation test was used to study the correlation of the thickness of inflammation and different biochemical parameters of control arthritic rats with each other. Duncan's test was applied between control arthritic and different experimental groups.

RESULTS AND DISCUSSION

The thickness of the injected foot paw during the period of experiment is presented in Figure 1. The injection of Freund's complete adjuvant into the right hind-foot paw of rats produced an inflammation which reached its maximum during the first 3 days, thereafter, the swelling slowly subsided until the ninth day and remained almost steady to the thirteenth day. It was noticed that oral administration of different extracts produced marked reduction of the hind paw inflammation allover the experiment.

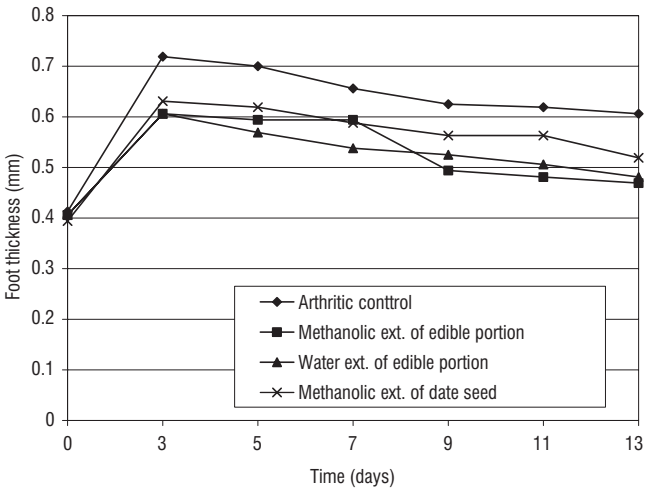

FIGURE 1. The foot thickness of rats of different experimental groups in relation to time.

The increase in foot thickness (Thickness of inflammation) of the control arthritic rats at the end of the experiment compared with that of rats given the different extracts are present in Table 2. To deduce the thickness of inflammation in rats of each group, the thickness of foot at the start of the experiment was subtracted from that at the end of the experiment. It was noticed that oral administration of

TABLE 2. The increase in the thickness of the injected foot (after 13 days of adjuvant arthritis induction) of arthritic rats given a daily oral dose of the different date fruit extracts (500 mg/kg rat body weight).

Groups	Mean ± SEM	% Inhibition of inflammation
Arthritic control	0.1938 ± 0.0113^{a}	-
Methanolic extract of edible portion of fruits	0.0625 ± 0.0082^{b} *****	67.8
Water extract of edible portion of fruits	0.075 ± 0.0095^{b} *****	61.3
Methanloic extract of seeds	0.125 ± 0.0164^{b} ****	35.5

Values significantly differ from the control according to T-test: ****: $p<0.005$, *****: $p<0.001$; ^{a,b} Mean values within a column with unlike superscript letters were significantly different ($p<0.05$) according to Duncan's test.

the methanolic and water extracts of an edible portion of date fruits suppressed the swelling in the foot significantly by 67.8 and 61.3% respectively ($p<0.001$), while the methanolic extract of date seeds showed significant reduction in swelling of the foot by 35.5% ($p<0.005$).

Biochemical parameters of arthritic rats

The results are shown in Table 3. It was noticed that ESR and plasma fibrinogen were significantly higher in arthritic control rats compared with normal control rats. This result agreed with that of Glenn [1966] and Glenn and Kooyers [1966]. ESR has been reported to be an excellent index of assessing the therapeutic effect of anti-inflammatory agents [Arvidsson *et al.*, 1998].

Antioxidant state showed that plasma vitamin E, vitamin C, vitamin A and β-carotene were significantly lower in arthritic control rats than normal rats ($p<0.001$). Reduction in antioxidant state indicating reduced antioxidant capacity and elevation of oxidative stress during adjuvant arthritis which is similar to rheumatoid arthritis in human [Ialenti *et al.*, 1993]. The low concentration of plasma vitamin C has been ascribed to the destruction of this vitamin by oxygen free radicals formed by activated phagocytes [Rowley & Halliwell, 1983]. Reduction of plasma vitamin C may also be attributed to its consumption in the process of collagen synthesis since collagen is destructed in rheumatoid arthritis [Mc Alindon *et al.*, 1996]. Simoes *et al.* [2003] have reported that plasma vitamin C can be used as biomarker in the inflammatory phase of rat adjuvant arthritis and that it correlated with other already established disease activity parameters. Vitamin C can also be used as a tool to evaluate the anti-inflammatory effects of new anti-inflammatory agents. In the present work, statistical test of correlation showed that plasma vitamin C had significant negative correlation with the thickness of inflammation ($r=-0.7559$, $p<0.05$), which agreed with previous study [Simoes *et al.*, 2003]. Vitamin E is an antioxidant for superoxide, singlet oxygen and peroxyl free radicals [Mayes, 1996]. It is effective in breaking lipid peroxidation chain by making the stable vitamin E radical which then can be recycled back to its reduced form by reduction with ascorbate [Bankson *et al.*, 1993]. In addition, vitamin C is a good scavenger of oxidants including superoxide, hydroxyl and peroxyl free radical [Lunee, 1992]. It was reported previously that blood retinol level and β-carotene reduced significantly during chronic inflammatory rheumatic diseases [Dougados *et al.*, 1988; Comstock *et al.*, 1997]. In the present study plasma retinol had significant positive correlation with vitamin C ($r=+0.940$, $p<0.001$), other parameters of arthritic rats showed non-significant correlation. Low antioxidant level is a risk factor for rheumatoid arthritis and it worsen the severity of the condition [Heliovaara *et al.*, 1994; Comstock *et al.*, 1997]. Our results revealed that plasma level of MDA is significantly higher in arthritic control rats than normal control. Gambhir *et al.* [1997] and Kiziltunc *et al.* [1998] reported that lipid peroxidation measured as MDA was significantly higher in rheumatoid arthritis compared to a healthy individual. High MDA levels were an indication of reduced antioxidant capacity and increased oxidative stress in rheumatoid arthritis [Ozturk *et al.*, 1999].

Erythrocyte sedimentation rate and plasma fibrinogen of arthritic rats decreased significantly when any of the extracts were administered but still, they were higher than normal control. Mean while methanolic extract of date seed showed only significant increase in fibrinogen. Methanolic extract of edible portion of date fruits was the best in this concern.

Antioxidant state (plasma vitamin C, E and A and β-carotene) increased on administration of different extracts, while plasma level of MDA reduced significantly. This result showed the reduction of oxidative stress and increase in antioxidant state. Again methanolic extract of an edible portion of the fruit is the best in this respect and even it normalized the level of the majority of these parameters, which became comparable to those of control rats.

It can be noticed that although methanolic extract of seeds showed previously to have non-antioxidant activity *in vitro* test [Mohamed & Al-Okbi, 2004], however it showed antioxidant activity in the present study. This let us recommend that *in vitro* and *in vivo* test must be applied to confirm the activity and do not depend completely on *in vitro* test.

In vitro test coincides with *in vivo* only in the relative potency of different extract to each other, where methanolic extract of edible portion of date fruits was the best in both *in vitro* and *in vivo*. This means that *in vitro* test could be used only as a preliminary test and must be confirmed by an *in vivo* study.

TABLE 3. Biochemical parameters of different experimental groups.

Groups		ESR (mm/h)	Fibrinogen (g/100 mL)	Vit. C (mg/dL)	Vit. E (mg/dL)	β-carotene (μg/100 mL)	Retinol (μg/100 mL)	MDA (nmol/mL)
Normal control	Mean	1.25	0.38	1.30	1.24	2.04	33.2	2.7
	±SEM	0.164	0.009	0.086	0.043	0.011	1.321	0.075
Arthritic control	Mean	4.38^{*a}	0.67^{*a}	0.65^{*a}	0.64^{*a}	0.70^{*a}	13.1^{*a}	3.6^{*a}
	±SEM	0.2631	0.011	0.06	0.049	0.008	0.553	0.149
Methanolic extract of edible portion of fruits	Mean	$1.63^{\bullet\bullet\bullet\bullet\bullet b}$	$0.42^{\bullet\bullet\bullet\bullet\bullet b}$	$1.53^{\bullet\bullet\bullet\bullet\bullet b}$	$1.07^{\bullet\bullet\bullet\bullet b}$	1.20^{a}	$35.7^{\bullet\bullet\bullet\bullet\bullet b}$	$2.84^{\bullet\bullet\bullet\bullet\bullet b}$
	±SEM	0.26	0.03	0.06	0.103	0.022	1.738	0.06
Water ext. of edible portion of date fruits	Mean	$3.13^{\bullet\bullet\bullet b}$	$0.49^{\bullet\bullet\bullet\bullet\bullet b}$	$0.86^{\bullet\bullet b}$	$0.98^{\bullet\bullet\bullet\bullet b}$	0.81^{a}	$27.9^{\bullet\bullet\bullet\bullet\bullet b}$	$3.0^{\bullet\bullet\bullet\bullet b}$
	±SEM	0.295	0.039	0.044	0.087	0.013	1.391	0.056
Methanolic ext. of date seeds	Mean	3.90^{a}	$0.47^{\bullet\bullet\bullet\bullet\bullet b}$	0.76^{a}	0.75^{a}	$1.12^{\bullet\bullet\bullet\bullet b}$	$19.5^{\bullet\bullet\bullet\bullet b}$	$3.2^{\bullet b}$
	±SEM	0.227	0.019	0.354	0.045	0.008	1.659	0.094

Values significantly differ from normal control (T-test): *: $p< 0.001$. Values significantly differ from arthritic control (T-test): •: $p<0.05$, ••: $p<0.025$, •••: $p<0.010$, ••••: $p<0.005$, •••••: $p<0.001$; $^{a, b}$ – Mean values within a column with unlike superscript letters were significantly different ($p<0.05$) according to Duncan's test.

TABLE 4. Nutritional parameters of different experimental groups.

Groups		Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food intake (g/day)	Food efficiency ratio
Normal control	Mean	163.8	193.9	28.9	190.5	13.6	0.152
	±SEM	1.567	2.099	1.092	5.819	0.416	0.004
Arthritic control	Mean	164.6^{a}	172.5^{*****a}	8.375^{*****a}	180.3^{a}	12.9^{a}	0.046^{*****a}
	±SEM	3.876	4.192	0.625	4.329	0.310	0.003
Methanolic ext. of date fruits	Mean	164.8^{a}	188.9$^{•••••b}$	24.1$^{•••••b}$	195$^{•b}$	13.9$^{•b}$	0.123$^{•••••b}$
	±SEM	2.617	2.125	1.159	4.158	0.297	0.004
Water ext. of date fruits	Mean	163.5^{a}	181.1^{a}	17.6$^{•••••b}$	208.9$^{•••b}$	14.9$^{•••b}$	0.084$^{•••••b}$
	±SEM	5.504	6.789	1.401	7.509	0.536	0.005
Methanolic ext. of date seeds	Mean	164.4^{a}	182.8$^{•b}$	18.4$^{•••••b}$	192.5^{a}	13.75^{a}	0.095$^{•••••b}$
	±SEM	2.204	1.555	1.742	6.542	0.467	0.007

Values significantly differ from normal control (T-test): *****: $p<0.001$. Values significantly differ from arthritic control (T-test): $^{•}$: $p<0.05$, $^{•••}$: $p<0.010$, $^{••••}$: $p<0.005$, $^{•••••}$: $p<0.001$; ^{a,b} – Mean values within a column with unlike superscript letters were significantly different ($p<0.05$) according to Duncan's test.

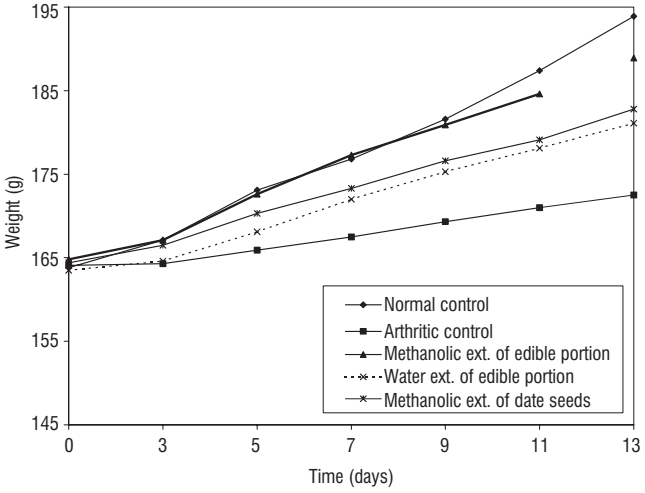

FIGURE 2. Growth curves of different experimental groups.

The results of nutritional parameters are shown in Table 4, whereas growth curves are presented in Figure 2.

Body weight gain and food efficiency ratio decreased significantly in control rats with adjuvant arthritis compared to normal control. It has been reported that rheumatoid arthritis is usually associated with loss of lean tissues, which contain most of the body's protein [Bistrian & Blackburn, 1983]. This decrease in body weight gain may be due to tissue destruction in adjuvant arthritic rats. Rates of protease-mediated degradation of muscle protein were accelerated without changes in protein synthesis in experimental arthritis [Fagan *et al.*, 1987]. Intracellular proteolysis of muscle proteins by lysosomal proteases is mediated by PGE_2 the later increased during inflammation [Fagan *et al.*, 1987]. It was noticed that food intake was also reduced which may share in the reduction of body weight gain. It has been also reported that rheumatoid arthritis is associated with anorexia [Bistrian & Blackburn, 1983].

Administration of methanolic extracts of an edible portion of date fruits and seeds produced a significant increase in the final body weight of arthritic rats, while water extract of date fruits showed a non-significant increase in the final body weight of arthritic rats. Administration of any of the extracts produced a significant increase in body weight gain. The improvement of body weight gain is an indicator for improvement of the adjuvant arthritis [Glenn & Kooyers, 1966]. Food efficiency ratio increased significantly on administration of any of the extracts, which is a good finding towards the disease.

Figure 2 showed that mean body weight of control arthritic rats was the lowest all over the experiment compared to the other experimental groups followed by those given water extract of an edible portion then methanolic extract of seeds. The mean body weight of groups of rats given methanolic extract of edible portion was almost similar to control normal till the ninth day then showed lower weights till the end of the experiment.

TABLE 5. Acute oral lethal toxicity of dried methanolic extract and water extract of edible portion of *Phoenix dactylifera* fruits.

Group No.	Doses of extracts g/kg mice body weight	Observed mortality data			
		Methanolic extract of edible portion of fruit		Water extract of edible portion of fruit	
		Dead tested	% Observed mortality	Dead tested	% Observed mortality
1	1	0/8	0	0/8	0
2	2	0/8	0	0/8	0
3	4	0/8	0	0/8	0
4	6	0/8	0	0/8	0
5	8	0/8	0	1/8	12.5
6	10	0/8	0	1/8	12.5
7	12	0/8	0	1/8	12.5

TABLE 6. Acute oral lethal toxicity of dried methanolic extract of *Phoenix dactylifera* seeds.

Group No.	Dose (g/kg)	No. of animals/group	No. of dead animals	Z	D	Z.D
1	1	8	0	0	1	0
2	2	8	0	0	2	0
3	4	8	0	2	2	4
4	6	8	4	5	2	10
5	8	8	6	6.5	2	13
6	10	8	7	7.5	2	15
7	12	8	8	4	0	0

Z = Half the sum of dead mice from two successive doses. D = The difference between the two successive doses. ZD = Product of Z and D.

Acute lethal toxicity test (Tables 5 and 6) revealed that the most safe extract was the methanolic of edible portion of the fruit followed by the water extract that showed complete safety up to 6 g/kg mice weight that is corresponding to 46.5 g/70 kg man body weight for human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme [Paget & Barnes, 1974], which is also considered as safe when it is used within the safe doses. Methanolic extract of the seed showed that LD50 calculated from Table 6 was equal to 6.75 g/kg mice body weight which corresponds to 52.4 g for man weighing 70 kg.

CONCLUSIONS

It can be concluded that all the tested extracts of date fruits have anti-inflammatory and anti-oxidant activity, methanolic extract of edible portion of fruit was the best.

Concerning nutritional parameters again methanolic extract of edible portion was the superior in improving food efficiency ratio and body weight gain.

Acute oral toxicity test showed also methanolic extract of edible portion to be the most safe one. So methanolic extract of edible portion can be recommended as functional food ingredient.

REFRENCES

1. Arvidsson N.G., Gudjornsson B., Hallgren R., Larsson A., Concordant message of different inflammatory markers in patients with rheumatoid arthritis. Upsala J. Med. Sci., 1998, 103, 35–42.
2. Bagchi D., Bagchi M., Stohs S.J., Das D.K., Ray S.D., Kuszynski C.A., Joshi S.S., Pruess H.G., Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology, 2000, 148, 187–197.
3. Bankson D.D., Kestin M., Fai N., Role of free radicals in cancer and atherosclerosis. Clin. Lab. Med., 1993, 13, 463–470.
4. Bistrian BR., Blackburn G.L., Assessment of protein--calorie malnutrition in the hospitalized patient. 1983, *in*: Nutritional Support of Medical Practice. (eds. H.A. Schneider, C.E. Anderson, D.B. Coursin). Harper and Row, New York, pp. 128–153.
5. Briggs G.M., Williams M.A., A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. Fed. Proc., 1963, 22, 261–266.
6. Comstock G.W., Burke A.E., Hoffman S.C., Helzlsouer K.J., Bendich A., Masi A.T., Norkus E.P., Malament R.L., Gershwin M.E., Serum concentrations of alpha tocopherol, beta carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythrematosis. Ann. Rheum. Dis., 1997, 56, 323–325.
7. Cook N.C., Samman S., Flavonoids – Chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem., 1996, 7, 66–76.
8. Desia I.D., Machlin L.J.,Vitamin E. 1985, *in*: Methods of Vitamin Assay. (eds. J. Augustin, B.P. Klein, D. Becker, P.B. Venugopal). 4th edition. A widely Interscience Publication John Wiley & Sons, New York, pp. 255–275.
9. Dougados M., Leporho M.A., Esmilaire L., Elaiba F., Amor B., Plasma levels of vitamins A and E in hyperostosis, ankylosing spondylarthritis and rheumatoid polyarthritis. Rev. Rhum. Mal. Osteoartic., 1988, 55, 251–254.
10. Fagan J.M., Waxman L., Goldberg A.L., Skeletal muscle and liver contain a soluble ATP + ubiqutin-dependent protedytic system. Biochem. J., 1987, 243, 335–340.
11. Gambhir J. K., Lali P., Jain A. K., Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin. Biochem., 1997, 30, 351–355.
12. Glenn E.M., Adjuvant-induced arthritis: Effects of certain drugs on incidence, clinical severity, and biochemical changes. Am. J. Vet. Res., 1966, 27, 339–352.
13. Glenn E.M., Kooyers W.M., Plasma inflammation units: An objective method for investigating effects of drugs on experimental inflammation. Life Sci., 1966, 5, 619–628.
14. Goodman A.G., Goodman L.S., Gilman A., Principles of toxicology. 1980, *in*: The Pharmacological Basis of Therapeutics (eds. Goodman and Gilman). 6th ed. Macmillan, New York, pp. 1602–1615.
15. Heliovaara M., Knet P., Aho K., Aaran R.K., Alfthan G., Aromaa A., Serum antioxidants and risk of rheumatoid arthritis. Ann. Rheum. Dis., 1994, 53, 51–53.
16. Ialenti A., Moncada S., DiRosa M., Modulation of adjuvant arthritis by endogenous nitric oxide. Br. J. Pharmacol., 1993, 110, 701–706.
17. Jagota S.K., Dani H.M., A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. Analytical Biochem., 1982, 127, 178–182.
18. Kahl R., Protective and adverse biological actions of phenolic antioxidants. 1991, *in*: Oxidative Stress, Oxidants and antioxidants. (eds. H. Sies). Academic Press, London, San Diego, pp. 245–260.

19. Kiziltunc A., Cogalgil S., Cerrahoglu L., Carnitine and antioxidants levels in patients with rheumatoid arthritis. Scand J. Rheumatol., 1998, 27, 441–445.
20. Lunee J., Oxygen radicals: Their measurement and role in major disease. J. Intern. Fed. Clin. Chem., 1992, 4, 58–65.
21. Mayes P.A., Structure and function of the lipid-soluble vitamins. 1996, *in*: Harper's Biochemistry. (eds. R.K. Murray, D.K. Granner, P.A. Mayes, V.W. Rodwell). 23rd edition, Appleton and Lange, California, pp. 614–624.
22. Mc Alindon T.E., Jacques P., Zhang Y., Do antioxidant micronutrients protect against the development and progression of Knee OA. Arth. Rheum., 1996, 39, 648–656.
23. Mohamed D.A., Al-Okbi S.Y., *In vitro* evaluation of antioxidant activity of different extracts of *Phoenix dactylifera* L. fruits as functional foods. Deutsche Lebensmittel-Rundschau. 2004 (in press).
24. Morcos S.R., The effect of protein value of the diet on the neurological manifestations produced in rats by β-immodipropionitrile. Br. J. Nutr., 1967, 21, 269–274.
25. Neeld J.B., Pearson W.N., Macro and micromethods for the determination of serum vitamin A using trifluroacetic acid. J. Nutr., 1963, 79, 454–462.
26. Ozturk H.S., Cimen M.Y., Cimen O.B., Kacmaz M., Durak I., Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatol. Int., 1999, 19, 35–37.
27. Paget P., Barnes T., Evaluation of Drug Activities Pharmacometrics, 1974, Vol. (1), (eds. D. R. Laurrence, A.L. Bacharach). Academic Press, London and New York, pp. 135–140.
28. Rendle B., The Classification of Flowering Plants, 1959, Cambridge University Press, pp. 68–75.
29. Rowley D.A., Halliwell B., Formation of hydroxyl radicals from hydrogen peroxide and iron salt by superoxide-and ascorbate-dependent mechanisms: relevance to the pathology of rheumatoid disease. Clin. Sci., 1983, 64, 649–653.
30. Satoh K., Serum lipid- peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 1978, 20, 37–43.
31. Simoes S.I., Eleuterio C.V., Cruz M.E., Corvo M.L., Martins M.B., Biochemical changes in arthritic rats: dehydroascorbic and ascorbic acid levels. Eur. J. Pharm. Sci. 2003, 18, 185–189.
32. Singh G.B., Singh S., Bani S., Gupta B.D., Banerje S.K., Anti-inflammatory activity of oleanolic acid in rats and mice. J. Pharm. Pharmacol., 1992, 44, 456–458.
33. Toro G., Ackerman P.G., Practical Clinical Chemistry, 1975, 1st edition, Little, Brown and Company, Boston, USA, pp. 188–192.
34. Vayalil P.K., Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). J. Agric. Food Chem., 2002, 50, 610–617.
35. Westergren A., Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Medica Scandinavica, 1921, 54, 247–249.
36. Wittenborg A., Petersen G., Lorkowski G., Brabant T., Effectiveness of vitamin E in comparison with diclofenac sodium in treatment of patients with chronic polyarthritis. Z. Rheumatol., 1998, 57, 215–221.