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The Review of Certain In Vivo Antioxidant Effects on Essential Oils of Origanum Minutiflorum O Schwarz-Ph Davis, Juniperus Excelsa Bieb.subsp. Excelsa and Histopathologic Changes

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ABSTRACT

Essential oil of plants called *Juniperus excelsa* Bieb. (JE), *Origanum minutiflorum* O. Schwarz and P.H. Davis (OM) were used in this study. In order to determine experimental doses, LD_{50} values of essential oils were determined on mice. Taking into consideration the LD_{30} range, the experimental toxic doses were calculated for each rat (rat/kg). The toxic dosages thus determined were adapted to rats for active substances (rat/kg). Using commercially available pure virgin olive oil (VOO) as the solvent and diluting agent, OM oil (n=10), JE fruitoil (n=10), carvacrol (CRV) (n=10), VOO (n=10) and normal saline SF (n=8) were administered on the basis of 12 days into intraperitoneal (IP). Enzyme activities of Glucose-6-Phosphate dehydrogenase (G6PDH), malate dehydrogenase (MDH), Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Adenosine-deaminase (ADA) and Catalase were studied in isolates of kidney, brain and liver tissues. The data was statistically analyzed through Kruskal Wallis variance analysis. Elevated levels of GST and catalase have been found statistically important, as have both essential oil activities of OM and JE in the kidney tissue ($p<0.005$). All of the enzymes except the levels of ADA and SOD led to a statistically significant change in the brain and liver. There was sinusoidal hyperemia and capsular adhesion in the liver as histopathological were found to be statistically significant ($p<0.005$). It did not observe any important changes in the other organs. Findings were scored and analyzed by using x^2(chi-square) test and Fisher's definite variance analysis.

INTRODUCTION

There are about 70 kinds of Juniperus L. (Cupressaceae) in the world. The type of Juniperus is represented by 10 taxons with seven sub types in Turkey. *Juniperus excelsa* Bieb (gray tall juniper), (Cupressaceae) which is an evergreen and coniferous tree, covers 82% of juniper forests in Turkey. It has disseminated to all countries within Iranian-Turan climate zone. The fruits of this tree are registered as juniper berries in Turkish codex (Demirhan, 2011); and the researches conveyed show that it involves alpha-pinene as active ingredient in its compound (Adams *et al.,* 2013).

The fruits of *Juniperus excelsa* Bieb (J.E.) are being used as diuretic, stimulant, antiseptic and for treatment of injuries by Anatolians since the ancient ages. It is known that, its extracts bear the effects of being anti-inflammatory, anti-microbial, insecticide, anti-termite, hypotensive, diuretic; and also used as antiseptic for urinal system (Adams 1990a; Adams 1990b; Adams 2011; Demirhan 2001; Adams *et al.,* 2013). Also

it is used as flavor or fragrance material in some foods and beverages, by distilling the wood and leafs of some kinds of juniper, the juniper essence which is produced is used in perfumery and medicine industry (Adams *et al.*, 2013; Yesenofski 1996). In various studies activities of antioxidant, antibacterial, antispasmodic and chemical composition of JE were found (Dadalıoğlu & Evrendilek, 2004; Sokovic *et al.*, 2004; Topçu *et al.*, 2005; Asilli *et al.*, 2008; Moein *et al.*, 2010; Emami *et al.*, 2011; Orhan *et al.*, 2011; Taviano *et al.*, 2011; Ataş *et al.*, 2012; Ehsani *et al.*, 2012; Khan *et al.*, 2012; Orhan *et al.*, 2012; Karapandzova *et al.*, 2014; Moein, 2014). The type of Origanum is represented by 23 kinds 32 taxons in Turkey and 41 kinds 52 taxons in the world (Baser *et al.*, 1993; Baser, 2002).

Thyme *Origanum minutiflorum* O. Schwarz and P.H. Davis (O.M.), which is known as 'Dairy thyme' and 'Tote thyme' in the thyme world market, is an endemic kind that has disseminated only at Dairy (Sütçüler) region in Isparta city and contains 80 % of carvacrol and 4.0 & p-cimen (Baser *et al.*, 1993; Baser, 2002; Dadalıoğlu & Evrendilek, 2004; Goze *et al,*2010).

Some kinds of Origanum are also used as spice in food, and in medicine among people against indigestion, inappetency and cough. It bears the features such as being antiseptic, sedative, expectorant and cramp healing. It is used in cosmetics, alcoholic or alcohol free beverages. Due to its anti-bacterial effect on bacteria which causes food spoil and food poisoning, it is a most wanted medicinal plant. It is generally known for its rich essential oils, antioxidant, antibacterial, antiviral and antifungal activities (Baser *et al.*, 1993; Davis *et al.*, 1998; Baytop 1999; Baser, 2002; Dadalıoğlu & Evrendilek, 2004; Baydar, 2005; Baser, 2008; Goze *et al.*, 2010; Oke 2010; Kılıçgün & Korkmaz 2014). Owing to this fact, for the natural active ingredients to be defined in plants, which are used for medication or food, it is important to convey researches that examine the biological activity, toxicological and pharmacological dose-response relation.

This study aims to determine the in vivo antioxidant activity of O.M. and J.E. on kidneys which are used orally and pursuit possible tissue changes histopathologically. Although there are many studies about in vitro antioxidant and antibacterial activities of active ingredients of O.M. and J.E., there are not many studies of in vivo. Therefore, the research findings are the first one in this field.

MATERIAL AND METHOD

JE fruit and OM were harvested from Isparta, Sütçüler flora. The samples of the plant were registered under JE (CUFH Voucher No. ED11003) and OM (CUFH Voucher No. ED 1101) numbers at the Herbarium of Cumhuriyet University Faculty of Science and Literature Department of Biology.

Plant samples which were dried in shade and pulverized, were held in Clevenger apparatus for approximately 3 hours being exposed to hydro distillation. The oil yield of 500gr dried plant was determined as 2.1 ml for JE and 10 ml for OM.

In the study, 40 healthy 180gr and approximately 4 weeks old adult albino Wistar rats (Rattus Norvegicusvar. Albino) were used; and in order to determine the LD_{50} dose, again, 40 healthily 25-30gr, 4 weeks old rats (MusMusculusvar. Albino) were used. The experimental animals were provided from Experimentary Animals Laboratory at Cumhuriyet University. Experimental Animals Ethical Committee's approval was gained (approval date and no: 1/9/2005-116). The animals were preserved under standard laboratory circumstances, with free feed and water at room temperature (21 ± 3 °C).

During literature searches no studies about OM and JE, LD_{50} doses were found. Therefore, LD_{50} dose of oils was determined by using rats. The toxic dose was calculated as rat/kg for each and found as LD_{30} (for JE –LD_{30}:160 ul). The animals which were tried on with Pure OM and pure carvacrol died at all the levels of doses given. Thus, OM and carvacrol (CRV) were mixed into olive oil and a depot/store solution was prepared (1 OM+3 Olive oil); every rat was given 100u (LD_{30}) i.p. from this solution and this dose was determined as LD_{30} for OM. In the same way, by applying 100ul to each rat from the store solution which was prepared by using carvacrol (1 carvacrol +6 olive oil), LD_{30} value was found for the carvacrol.

This experiment was made on rats. The rats were divided into 5 groups. To the 1st group of rats JE (160ul), to the 2nd group only olive oil (40 ul), to the 3rd group OM (100ul), to the 4th group carvacrol (100ul) were injected for 12 days with i.p. and 10 rats were used as control group (SF). At the end of 12 days the rats were killed with dislocation and their organs were taken into cooled boxes.

Preparation of Homogenates: Tissues were taken and washed with 0.15 KCl. These pieces of tissue were cut on a glass; kidney, brain and liver were homogenized by using rotating 1000 rpm/min via glass homogenizer (B. Braun) after added 0.15 M KCl ratio of 1:3(w/u)

per 1 gram. The homogenates were centrifuged (Beckman Model J2) for 15 min at 4800 rpm. Glucose 6 phosphate dehydrogenase (G_6PDH), (Beutler 1971); malate dehydrogenase (MDH), (Warburg & Christian 1931); Glutathione S-transferase (GST), (Habig *et al.*, 1974); Superoxide dismutase (SOD), (Sun *et al.*, 1988); Adenosine deaminase (ADA), (Giuisti, 1974); and catalase enzymes activities were studied in tissue as spectrophotometrically (Spectro UV-VIS Double Beam PC Scanning), (Aebi,1983). The data was statistically analyzed through Kruskal Wallis variance analysis by SPSS (Ver: 13.0) program. Standard error level is taken as 0.05 (Akgül, 2005).

The tissues samples which were sent to a pathology laboratory for routine follow up in automatic tracking device via handon Path Centre (Thermo Electron Corporation, UK). It observed adhesions among the intestine kidney, brain and liver of rats which were given JE in the macroscopic examination. After scored of findings analyzed with X^2 test and precise analysis of variance Fisher (Akgül, 2005).

RESULTS

Results of kidney homogenates

The activities of GST and catalase were found important as a statistics for OM and JE ($p<0.005$). The difference between groups was not significant as G_6PDH, MDH, ADA and SOD enzymes values compared with in pairs ($p>0.05$) Apart from these, there were significant difference in values ($p<0.05$). Between groups of OM and CRV, JE and CRV, JE and olive found significant differences to pairwise comparisons are made in terms of value in GST ($p<0.005$).

When catalase activity compared in pairs of groups OM and CRV, OM and VOO, JE and CRV, JE and olive oil values difference was found statistically significant as shown in Table1 and Table 2.

Results of brain homogenates

The level of G6PDH between groups of VOO /JE and JE/SF ($p<0.005$); the level of GST between groups of JE-CRV ($p<0.005$); the level of MDH between groups of OM-JE and CRV-JE ($p<0.005$); the level of catalase between groups of OM- VOO and JE-VOO-SF, CRV-JE and SF were found important if statistically compared in pairs (Table 2).

Results of liver homogenates

The activity of G6PDH in between groups of OM-SF and JE-VOO and CRV/VOO/SF; the activity of GST between groups of JE–CRV and JE-VOO and JE-SF; the activity of MDH between groups of OM and VOO, the activity of catalase between groups of OM/VOO and SF were found statistically important.

Table1 – Analyzes of enzymes activities in kidney homogenates

	G6PDH	GST	MDH	ADA	SOD	CATALASE
OM	0.000165±0.0001	0.00150±0.0012	0.00023±0.0003	0.00047±0.0003	97.80±0.57	97.58±32.02
JE	0.00043±0.0003	0.00027±0.0002	0.00021±0.0001	0.00037±0.0000	97.96±0.84	117.95±21.80
CRV	0.00064±0.0004	0.00308±0.0019	0.00023±0.0001	0.00035±0.0001	98.13±0.45	117.75±34.76
VOO	0.00040±0.000	0.00230±0.0004	0.00022±0.0001	0.0027±0.0010	97.77±0.26	171.51±17.44
SF	0.00125±0.00012	0.00136±0.0001	0.00024±0.0001	0.00027±0.0001	98±0.60	129.48±32.87
Kw	10.19	22.69	4.35	4.44	3.34	18.20
p Value	0.037	0.000	0.003	0.366	0.502	0.001

GP6DH U/g pro; GST U/mg pro; MDH U/mg pro; ADA U/mg pro; SOD U/g pro; CATALASE U/mg pro

Table2 – Analyzes of enzymes activities in brain homogenates

	G6PDH	GST	MDH	ADA	SOD	CATALASE
OM	0.00124±0.0012	0.00167±0.0015	0.0001±0.0001	0.00038±0.0007	97.45±0.56	2.07±0.75
JE	0.00032±0.0002	0.00096±0.0004	0.00025±0.0001	0.00017±0.0001	97.52±0.58	2.79±0.81
CRV	0.00088±0.0002	0.00470±0.0050	0.00013±0.0001	0.00005±0.0001	97.13±0.15	2.62±0.75
VOO	0.0019±0.0004	0.0027±0.0002	0.00014±0.0001	0.00006±0.0000	97.67±0.67	9.76±0.60
SF	0.00151±0.0003	0.00276±0.0003	0.00016±0.000	0.00026±0.0002	96.10±2.60	10.38±0.44
Kw	19.21	18.270	17.01	11.73	5.81	14.89
p Value	0.001	0.001	0.002	0.009	0.213	0.005

GP6DH U/g pro; GST U/mg pro; MDH U/mg pro; ADA U/mg pro; SOD U/g pro; CATALASE U/mg pro

The enzymes of G6PDH, MDH, ADA and SOD did not show significant differences between the groups compared in pairs ($p>0.005$) (Table3).

In histopathological examination, a finding was not seen in kidney tubule epithelia cell except cloudy swelling ($p>0.05$). After scored of findings analyzed

Table 3 – Analyzes of enzymes activities in liver homogenates

	G6PDH	GST	MDH	ADA	SOD	CATALASE
OM	0.00099±0.00016	0.0017±0.0016	0.00032±0.00025	0.00006±0.0001	90.81±4.44	116.17±10.2
JE	0.0021±0.0015	0.00335±0.0023	0.00024±0.0001	0.0001±0.0001	93.52±0.89	98.13±8.49
CRV	0.0034±0.0004	0.00020±0.0001	0.00016±0.000	0.0001±0.00001	93.11±1.72	103±20.4
VOO	0.0039±0.0025	0.00021±0.0003	0.00001±0.000	0.00001±0.0000	92.61±2.68	90.21±2.465
SF	0.00448±0.001	0.0001±0000	0.00014±0.0011	0.00017±0.0002	91.83±2.07	90±3.368
Kw	18.87	20.12	12.89	19.94	4.32	14.89
p Value	0.000	0.000	0.012	0.364	0.364	0.005

GP6DH U/g pro; GST U/mg pro; MDH U/mg pro; ADA U/mg pro; SOD U/g pro; CATALASE U/mg pro

with X^2 test and precise analysis of variance Fisher. Fuzzy swelling (FS) in surface of tissue sample according to where tissue is turned off as semi quantitative (%), focal necrosis (FN) and the group of cell necrosis as a number (present or absent), Kupffer cell hyperplasia (present or absent), periportal inflammation (present or absent), sinusoidal hyperemia (SHR) (present or absent) were evaluated (Table 4). The sinusoidal hyperemia (SH) and capsular adhesion (CA) in liver were found statistically important ($p<0.05$) (Figure1). There are not significant changes which in other organs were observed.

Table 4 – Evaluation of tissue samples in statistically

GROUPS		OM		JE		RESULTS
		S	%	s	%	
SCN	0	11	68.8	3	100	P=0.530
	1	5	31.3	-	-	
KOL	0	15	93.8	3	100	P=1.00
	1	1	6.3	-	-	
PPI	0	2	12.5	-	-	X2=3.20 P=0.361
	1	7	43.8	3	100	
	2	5	31.3	-	-	
	3	2	12.5	-	-	
CA	0	7	43.8	2	66.7	X2=6.69 P=0.025
	1	9	56.0	7	33.3	
FS	0	5	31.3	3	100	X2=4.89 P=0.179
	1	1	6.3	-	-	
	2	2	12.5	-	-	
	3	8	50.0	-	-	
SH	0	10	62.5	2	66.6	X2=7.71 P=0.021
	1	6	37.5	1	33.3	
	2	-				

SCN (Single Cell Necrosis), PPI (Periportal inflammation), KOL (Kollaps), FS (Fuzzy swelling), SH (Sinusoidal hyperemia), CA (Capsular Adhesion)

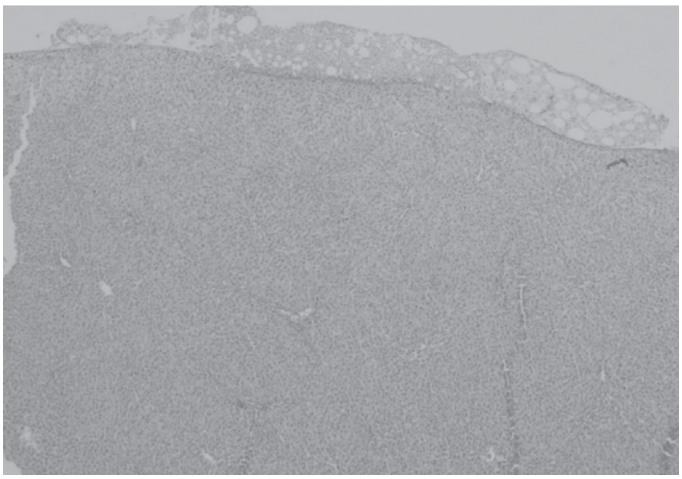

Figure1 – A general view of adhesions in the liver capsule (HEx40).

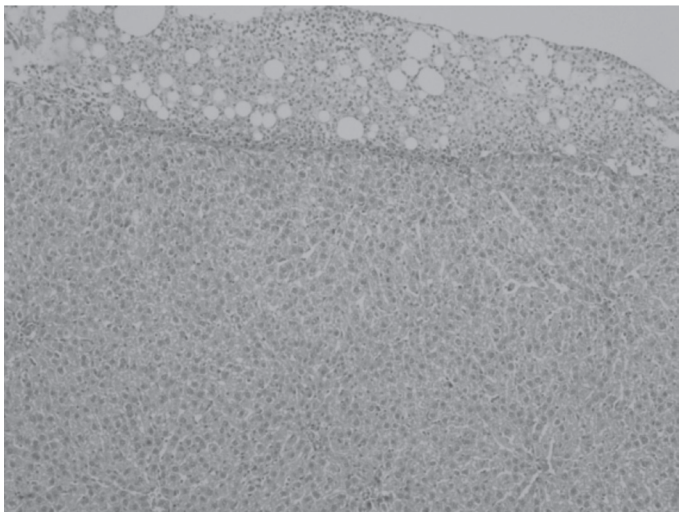

Figure 2 – The breakdown in the liver capsule adhesions: fibroadipose hipogralunom to similar tissue infiltration (HEx100).

DISCUSSION

Serum enzyme activities are widely used in determining of organ functions in mammals. Also these are very important for environmental biology, searching for the effects of environmental pollutants on living organisms, the toxic effects of orally consumed plants and for bioavailability in recent years. These tests have been of great importance in recent years, particularly in the context of bioavailability, to explore the aquatic effects of environmental biology and environmental pollutants and the toxic effects of orally consumed plants.

The changes that may occur in the activities of these important enzymes working in the energy metabolic pathways may affect the formation of energy by the

organism and affect the functioning of other metabolic pathways.

Superoxide dismutase (SOD), catalase (CAT), Glutathione reductase (GST) and malate dehydrogenase (MDH) are enzymatic compounds. Superoxide dismutase (EC 1.15.1.1, EC-SOD), as a superoxide anion is a form that part of the cellular antioxidant defense system by catalyzing the conversion in hydrogen peroxide to molecular oxygen. It plays an important role in controlling cellular superoxide levels in the compartment. It consists in high concentrations in the brain, liver, heart, kidney and erythrocytes (Fridovich, 1989).

In this study, SOD was found stable in all of the tissues. The mature erythrocytes don't include ribosome, mitochondria and nucleus. Enzymes of SOD, catalase, GSH-Px and pentose phosphate pathway protected against O_2 radicals. It can consider that OM and JE don't cause inhibition of the metabolic pathway.

Glucose-6-phosphate dehydrogenase (G-6-PD) (D-glucose-6-phosphate: NADP+ 1-oxidoreductase, E.C. 1.1.1.49) is the first and control enzyme of the pentose phosphate pathway.G_6PHD; it is an enzyme that plays a key role in the pentose, phosphate and glycolysis (Scott 1975). Also it has an anti-oxidant activity. G_6PDH enzyme activity was found to decrease in homogenates of all tissues. According to these results, it can be observed that essential oils may generate inhibiting change.

Adenosine deaminase is purine metabolism enzyme which is responsible for immune mechanism (Wilson *et al.*, 1991). Significant changes weren't observed in ADA activity in all organs except for the brain. In this case, the tested substances aren't effective in purine metabolism and suggest no creating damage.

MDH; is taken office in the maintenance of cell membrane integrity and the regulation of enzyme activity (Musrati *et al.*, 1998). In this research, the levels of MDH was high in the liver and in the brain but was stabile in the kidney.

GST, glutathione plays an important role against sourced interior and exterior toxic chemicals in the cellular defense system (Armstrong, 1990). Reversible damage may have been created during the period of JE and OM application by various mechanisms in all tissues. In this case, it can be considered to change their enzyme activations to maintain the redox state of cells and especially repairing damage membranes by keeping to a minimum damage. It is very important for all of tissues.

Catalase (H2O2: H2O2 oxidoreductase, EC 1.11.1.6) has 4 units with a group hemoprotein and localized in intracellular organelles as peroxisomes. Its task is to destroy hydrogen peroxide to oxygen and water (Kono & Fridovich, 1982). This is quite low compared to the catalase activity in the kidneys of the control group that can cause essential oils erythrocyte catalase activity by inhibiting the accumulation of H_2O_2 and consequently hemolysis which is very important for all of tissues.

As a result, the activity of catalase was very meaningful in all tissues while the level of SOD was stable. The levels of GST and catalase were in kidney, ADA and SOD were important in liver. Statistical differences were found in all enzymes except for SOD. It can be observed that these types of plants consumed as a food of toxic effects should have more comprehensive studies. Additionally, the LD value for each study which uses the plant´s essential oils, need to be determinate. Because the composition of the plant essential oils vary depending on many reasons such as seasonal, droughts and temperature difference.

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