Effects of Plant Essential Oils on Vitamin C, Malondialdehyde and Some Biochemical Parameters of Rats*

ABSTRACT

The aim of this study was to determine the LD₃₀ values of the essential oils of *Origanum minutiflorum* He Schwarz-PH Davis (OM) and *Juniperus excelca* by Bieb.subsp. Excel (JE) in vivo investigation on the effects of malondialdehyde (MDA), vitamin C and some biochemical parameters. In this study, the essential oils of OM and JE plants were used. LD₃₀ values of the essential oils were determined by using rats. OM (n=10), JE (n=10), carvacrol (n=10) which dissolve in olive oil were used as experimental group and as control group saline (SF) (n=8) and solvent Olive oil (n=10) were used and applied intraperitoneal on rats for 12 days in LD₃₀ dosages. In the end of 12 days, Vit-C, malondialdehyde (MDA) and routine biochemical analyses were studied on their heart bloods. The difference in the levels of MDA and Vit-C was found significant among the groups (p<0.005). When the biochemical parameters of the groups were compared, all differences in all test were found significant (p<0.005) except for creatinine (p>0.005). When the values are put to paired comparison, the differences between groups were found statistically significant (p<0.005). OM and JE has led to significant changes in all lipid peroxidation and enzyme levels.

INTRODUCTION

Although different types of plant extracts have already been studied, essential oils of plants have recently attracted the attention of researchers due to their antimicrobial and antioxidant characteristics for possible use for pharmaceutical purposes and as preservatives for fresh and processed foods (Sökmen *et al.*, 2004).

The genus *Origanum* belongs to the Lamiaceae family, and has 41 species representing 52 taxa globally, and 23 species representing 32 taxa in Turkey (Başer, 2002). The most common chemical component of *Origanum* spp. essential oil is carvacrol (Kirimer *et al.*, 1995; Başer, 2002; Baydar, 2005). *Origanum minutiflorum* O Schwarz-PH Davis (OM) is found worldwide and it is an endemic species in mountainous regions of Turkey, where it is commonly used in folk medicine (Baydar, 2005; Davis *et al.*, 1998). *Origanum* has been found to have antimicrobial (Vardar-Ünlü *et al.*, 2007), antigenotoxic (Ipek *et al.*, 2005), antibacterial, antifungal, and anti-angiogenic properties (Paster *et al.*, 1995; Göze *et al.*, 2016). In addition, it is used in the pharmaceutical, cosmetic, and soap industries (Farag *et al.*, 1989; Souleles, 1991).

There are about 70 species of *Juniperus* L. (Cupressaceae) in the world. *Juniperus* species are represented by 10 taxa, with 7 subtypes of *Juniperus excelca* Bieb.subsp.excelca (JE)(gray tall juniper) identified in Turkey. Studies showed that one of its active ingredients is alpha-pinene (Ataş *et al.*, 2012; Göze *et al.*, 2015). *Juniperus* spp essential oils have been used in the cosmetic industry as tar and it is commonly used
Hemoglobin analysis. The cyanmethemoglobin method was applied (Tietz, 1994) for hemoglobin analysis in erythrocyte suspension of the experimental and control groups.

Vitamin C analysis. The method of 2,4-dinitrophenylhydrazine method was applied (Tietz, 1994). The absorbance of all tubes was read at 520 nm via spectrophotometer. Vitamin C concentration is expressed as mg/dL plasma.

Biochemical parameters. Heart blood samples collected from rats both in control and experimental groups were centrifuged at 4000 rpm/min to obtain serum samples. Fasting blood glucose, cholesterol, high-density lipoprotein (HDL), blood urea nitrogen (BUN), creatinine, total protein, albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), gamma glutamyl transferase (GGT), total bilirubin, direct bilirubin, chloride (Cl), sodium (Na), and potassium (K) levels were measured using reagents (Beckman Synchron) of the autoanalyzer Beckman Synchron LX20.

The obtained data were entered in the statistical software SPSS (version 13.0) One-way analysis of variance (ANOVA) was used to determine the differences between multiple groups and to test the results of homogeneity. Kruskal Wallis was used in the test results that did not provide both at the same time. Wilcoxon Rank test and paired samples T test were used for binary comparison (Aygül, 2005).

RESULTS AND DISCUSSION

The chemical composition of the OM oil was determined only the essential oil part defined by GC-MS as 96.15%, in which 34 compounds were found, out of which the highest concentration was carvacrol represented 79.34%. The remaining part could not be defined by GC-MS. The chemical composition of JE was defined only the essential oil part by GC-MS as 91.35% and the rest of part could not be defined GC-MS. The major component with the highest concentration was α-pinene (55.53%).

All biochemical parameters were significantly influenced (p<0.005) the treatments, except for creatinine (p>0.005). When the group values were compared, the difference between the groups was statistically significant (p<0.005). The results are shown in Table 1.

When the results of rats injected with OM, carvacrol and JE (Groups 3, 4, and 5, respectively) were compared...
with those obtained in the group given saline solution (Group 1), all three groups showed lower fasting blood glucose, and higher AST, ALT, AST/ALT, GGT and vitamin C levels. Cholesterol, HDL, total protein, albumin, total bilirubin and direct bilirubin levels were lower in Groups 3 and 5, and higher in Group 4. BUN and creatinine levels were lower in Groups 3 and 5, and higher in Group 4. Group 4 showed higher LDH and MDA levels and Groups 4 and 5 lower levels.

Compared with the results obtained in the group fed olive oil, fasting blood glucose, HDL, LDH, T. Bil., D. Bil., T. protein and albumin levels decreased, while AST, ALT, AST/ALT, and vitamin C levels increased in the three groups. The levels of LDH, T. bilirubin and D. bilirubin increased only in Group 4 and 5. Groups 3 and 5 showed lower cholesterol, BUN, and creatinine levels, whereas higher levels of these indicators were determined in Group 4. Higher MDA levels were obtained in Group 3 and 5, but lower MDA levels in Group 4.

Carvacrol, the active ingredient of the OM injected in Group 3, was also administered in the Group 4. However, the different results obtained in Group 4 compared with Group 3 may be explained by the fact that the active agent was applied directly.

The water-soluble vitamin C can turn the tocopheroxyl radical into α-tocopherol again and trolox radicals into trolox. In other words, vitamin C recovers the antioxidant properties of vitamin E, having an indirect antioxidant activity (Doba et al., 1985; Barclay et al., 1985). In the present study, vitamin C levels significantly increased in the groups injected with OM, carvacrol, and JE oils.

MDA is an indicator of oxidative stress, and its levels were statistically lower in the Groups 3 and 4 compared with Group 1 (p<0.005). This finding supports the strong antioxidant activity of carvacrol and OM, which main compound is carvacrol. High vitamin C levels and low MDA levels were determined the rats injected with carvacrol. Carvacrol, the main compound of OM essential oil, is a phenolic compound used in traditional medicine and as a spice, and has been shown to have strong antioxidant activity (Teissedre, Waterhouse, 2000; Botsoglou et al., 2000; Skerget et al., 2000; Botsoglou et al., 2003b; Skerget et al., 2005). Phenolic compounds release/donate the hydrogen of the hydroxyl groups located in aromatic rings to prevent lipids and biomolecules to be oxidized by free radicals. These compounds are strong antioxidants that eliminate free radicals (Rice-Evans et al., 1995), form complexes with metal ions (metal chelation), as prevent or minimize the formation of singlet oxygen (Farag et al., 1989; Aeschbach et al., 1994).

In a review on antidiabetic medicinal plants, Patel et al. (2012) stated that blood glucose level may decrease as a function of reduction in glucose absorption or in insulin sensitivity. Lemhadri et al. (2004) found out that Origanum vulgare extract significantly decreased blood glucose levels of diabetic rats without changing basal insulin level. The present study showed that blood glucose levels were reduced in the rats injected with OM, carvacrol, and JE, supporting those findings (p<0.005).

The parameters AST/ALT ratio, albumin, and bilirubin are important for evaluating liver basal steatosis and initial fibrosis scoring. (Konerman et al., 2014). Aspartate aminotransferase (AST)/alanine
aminotransferase (ALT) ratios higher than 2 and high gamma glutamyl transpeptidase (GGT) activity levels indicate liver disease (Leung et al., 1986; De Bruyn, Graviss, 2001; Giannini et al., 2003). In the present study, the high AST, ALT, and GGT activity levels and AST/ALT ratios higher than 2 observed in Groups 3 to 5 suggest that the rats injected with OM, carvacrol, and JE experienced liver damage.

Among essential oils, thyme oil is the most widely used in traditional medicine. The active compounds of thyme oil, like thymol and carvacrol, have strong antimicrobial effects (Marino et al., 1999; Dorman & Deans, 2000). Carvacrol breaks down proteins of the bacterial cell wall, deforming it, and disrupt the pH gradient, causing other ions, primarily K+, to flow into extracellular fluid. Cymene, a biological precursor of carvacrol, accumulates in cytoplasmic membrane and excessively expands the cell wall, creating gaps in the phospholipid layers, allowing ions to flow into intercellular fluids. As a result, the bacterial cells, which cannot synthesize ATP, die. According to Ultee et al. (2002), this mode of action does not pose risk of residues in the foods from animals fed carvacrol, because no metabolic waste is generated.

It is difficult to use as essential oils as antibacterial food preservatives because of their unique and sharp smell. As required by the law, antibiotics and synthetic preservers cannot be added to animal feeds, and therefore, OM and JE, which are widely grown in our country, may be used as an alternative that will also contribute to our economy. However, in order to prevent metabolic problems that may arise from excessive consumption of these types of herbs and oils, suitable dosages should be designated and used (Karma, 2005a; Tuncer, 2007).

Considering the results of previous research and of the present study, OM and JE plant oils may be effective and beneficial as animal feed additives or as a food preservative because they reduce blood glucose and cholesterol levels. However, the fact that they may cause liver damage and metabolic disorders cannot be disregarded.

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