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Effects of Oral Administered *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* Extracts on Oxidative Stress, Lipid Profile and Liver Protein Expressions Hamsters*

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This study has been conducted with the purpose of determining the changes caused by *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* extracts, commonly used for their antioxidant properties, in the plasma, erythrocyte, liver, kidney and heart total oxidant statuses (TOS), total antioxidant statuses (TAS), plasma lipid profiles and liver serum proteins of healthy hamsters. Experimental groups were determined as Control (normal drinking water), *Panax ginseng* (100 mg/kg/day) and *Panax ginseng* + *Ganoderma lucidum* (140 mg/kg/day *Panax ginseng* + 140 mg/kg/day *Ganoderma lucidum*). Applications of drinking water were carried out on animals serving as experimental and control groups for 60 days. As a result of the biochemical analyses, no significant change in the lipid profile was observed (P>0.05). While there were no changes in the TAS rates of plasma, erythrocyte, liver and kidney tissues as well as TOS rates of liver, kidney and heart tissues; it was determined that both *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* extracts reduce TOS rates of plasma (P<0.001). Also *Panax ginseng* + *Ganoderma lucidum* extracts was observed to reduce TOS rates of erythrocyte (P<0.01) samples. Furthermore, it was confirmed with the liver protein electrophoresis that application of these matters led to the thickening in some protein bands in comparison to the control group. Conclusively, it was determined that *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* applications are effective in the prevention of oxidative stress, particularly by reducing the TOS rates in plasma and erythrocyte, and also stimulate to increasing liver protein expressions.

Key Words: *Panax ginseng, Ganoderma lucidum, Phodopus campbelli, oxidative stress, lipid profile, SDS-PAGE.*

Oral Olarak Verilen *Panax ginseng* ve *Panax ginseng* + *Ganoderma lucidum* Ekstraktlarının Hamsterlardaki Oksidatif Stres, Lipit Profili ve Karaciğer Protein Ekspresyonları Üzerine Etkileri

Bu çalışma antioksidan özelliklerinden dolayı yaygın olarak kullanılan *Panax ginseng* ve *Panax ginseng* + *Ganoderma lucidum* eksraktlarının sağlıklı hamsterlerin plazma, eritrosit, karaciğer, böbrek ve kalp total oksidan (TOS), total antioksidan seviyeleri (TAS) ile plazma lipit profili ve karaciğer serum proteinlerindeki değişiklikleri belirlemek amacıyla yapıldı. Deneysel gruplar Kontrol (K, normal içme suyu), *Panax ginseng* (G, 100 mg/kg/gün) ve *Panax ginseng* + *Ganoderma lucidum* (G+GL, 140 mg/kg/gün *Panax ginseng* + 140 mg/kg/gün *Ganoderma lucidum*) olarak belirlendi. Deney ve kontrol grubundaki hayvanlara 60 gün süreyle içme suyu şeklinde uygulama yapıldı. Biyokimyasal analizler neticesinde lipit profili üzerinde önemli bir değişiklik gözlenmedi (P>0.05). Yine plazma, eritrosit, karaciğer ve böbrek TAS ile karaciğer, böbrek ve kalp dokularının TOS düzeylerinde bir değişiklik olmazken; gerek *Panax ginseng* gerekse *Panax ginseng* + *Ganoderma lucidum* eksraktlarının plazma TOS düzeylerini önemli düzeyde azalttığı belirlendi (P<0.001). Ayrıca eritrosit TOS düzeylerinin *Panax ginseng* + *Ganoderma lucidum* grubunda azaldığı gözlemlendi (P<0.01). Ayrıca karaciğer protein elektroforezinde ise kontrol grubuna kıyasla bu maddelerin uygulanmasına bağlı olarak bazı protein bantlarında kalınlaşmalar meydana geldiği saptandı. Sonuç olarak; *Panax ginseng* ve *Panax ginseng* + *Ganoderma lucidum* uygulamasının özellikle plazma ve eritrositte TOS düzeylerini azaltmak suretiyle oksidatif stresi önlemede etkili olduğu ve karaciğer protein ekspresyonlarını artırdığı tespit edildi.

Anahtar Kelimeler: *Panax ginseng, Ganoderma lucidum, Phodopus campbelli, oksidatif stres, lipit profili, SDS-PAGE.*

Introduction

Although plants are used for health care and for a more active life since the very early stages of human being, the fact that the resources allocated for the area of health care have been increasing day by day in the recent years conduced the attention of scientists to be drawn towards herbal products (1). It was realized that the potential protective effects of herbal diets originate from the substances with antioxidant properties that these carry, and these antioxidants protect the cells against the destructive effects of natural oxidation reactions (2). As well as exposure to toxins and

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pathogens, various factors encountered in daily life such as exhaust gas, chemical preservatives in convenience food, smoking, heavy metals in water and high oxygen concentration increase free radical production and accordingly, oxidative stress (3). Increased free oxygen radicals cause many diseases including cardiovascular diseases, neurological disorders, asthma, diabetes mellitus, rheumatologic diseases, cancer and aging (4). Even though humans and other organisms have protective and regenerative defense systems against the oxidative damage caused by free oxygen radicals, these systems are often inadequate in the prevention of oxidative damage (5). In case of situations when these natural defense systems remain incapable, the use of antioxidants produced from specific new sources gain even more importance in fighting oxidative stress (6). Conducted studies indicate that plants such as *Panax ginseng* and *Ganoderma lucidum* are also natural antioxidants. Derived from the Greek word meaning "all-healing", Ginseng is stated to have effects such as supporting antioxidant defense system and stimulating cellular defence systems (7). In the previous studies, it was observed that *Panax ginseng* extract reduces oxidative stress, changes antioxidant enzyme activities to eliminate free radicals and therefore, reduces the end products originating from lipid peroxidation in tissue (8, 9). Known as Lingzhi (mushroom of immortality) in China, *Ganoderma lucidum* has been used in traditional medicine in China, Korea, Japan and other Pacific countries for 2000 years (10). *Ganoderma lucidum* extracts were stated to have various biological activities such as anti-cancer, anti-stress, immunostimulant and antioxidation both in in-vivo and in-vitro studies (11). While *Ganoderma lucidum* includes numerous compounds, the most significant ones of these are *Ganoderma lucidum* polysaccharides (12). Although *Ganoderma lucidum* polysaccharides were demonstrated to have protective effect against the oxidative damage resulting from both physical and chemical agents in tissue culture and animal studies, most of these studies in limited number were obtained by means of non-biological systems or in-vitro cell studies (13, 14).

In this study, the effects of *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* extracts orally administered to hamsters for 60 days on oxidative stress, lipid profile and liver protein expressions were studied.

Materials and Methods

Experimental Design: This study has been conducted under the approval (2012-60) of Kafkas University Animal Experiments Local Ethics Committee. 18 female *Phodopus campbelli* hamsters (25 - 40 g) of 5-6 months old were used in the research. Study groups were determined to include 6 animals as the control group on which no applications were conducted, group to which a daily 100 mg/kg dosage of *Panax ginseng* was administered, and the group to which 140 mg/kg *Panax ginseng* and *Ganoderma lucidum* in similar dose were administered in a combination. The materials were given for 60 days through the drinking water of the animals. *Panax ginseng* (C. A. Meyer) and *Ganoderma lucidum* used in the study were obtained from Shenyang Meheco Foreign Trade Co. Ltd. At the end of trial period, intracardiac blood samples were collected in heparinised tubes from the animals under ether anaesthesia. Subsequent to the cerebral dislocation performed, liver, kidney and heart tissues were collected.

Biochemical Analyses: Routine plasma and erythrocyte samples were prepared with the collected blood. A part of the tissue samples were homogenized by means of homogeniser (Wigen Hauser, Germany) following dilution with phosphate buffer, and the homogenates were centrifuged for 20 minutes at +4°C, with 3000 rpm. HDL, LDL, VLDL, total cholesterol and triglyceride levels were measured in Architect c16000 (Abbott Diagnostics-USA) model autoanalyzer using Abbott Diagnostic branded kits. Levels of Total Oxidant (TOS) and Total Antioxidant (TAS) in plasma, erythrocyte, liver, kidney and heart samples were determined spectrophotometrically using Rel-Assay (Gaziantep-Turkey) Diagnostic test kits.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): A part of the liver samples collected from the animals of electrophoresis of liver tissue were homogenized using a homogeniser for ten minutes in a 7.40 pH Tris-HCl buffer with tenfold higher volume than the liver samples. Later on, the homogenates obtained were centrifuged for 25 minutes at +4 ºC with 4000 rpm to ensure separation of supernatants and phenyl methyl sulfonyl fluoride (PMSF) was added to reach 0.1 mM final concentrations to be kept at -20 ºC until the end of study period with the aim of inhibiting the protolithic activity. Later on, the protein concentrations of the samples were determined through biuret method (15) and SDS-Polyacrylamide Gel Electrophoresis was conducted according to Laemmli (16) and O'Farrell (17). The proteins were separated in 16x10 cm slab gel with 1 mm thickness. Slab gel consists of concentrating gel where the proteins are stocked and the separator gel part where the proteins are separated later on. Resolving gel consisting of 10% acrylamide was prepared 12 hours prior to electrophoresis and kept in the refrigerator overnight. Concentrating gel consisting of 4% acrylamide was prepared 2 hours prior to electrophoresis and polymerised.

By mixing this into each sample and sample buffer consisting of standard 10% glycerol, 2% mercaptoethanol (2-ME), 2% sodium dodecyl sulphate (SDS) and 0.01% bromophenol blue (BPB); the protein concentrations were set for 2 µg/µL and 0.2 µg/µL respectively, and later on the denaturation of the proteins was ensured by keeping the standard proteins in boiling water. 20 µL serum sample and standard protein were applied to each gel slots for concentrator gel, and 200 V power was supplied to the gel until from phenol blue migrated to the lowest part of the gel. Subsequent to the electrophoresis, the gels were stained in water bath at 56 ^{o}C for 20-30 minutes within staining solution consisting of

0.125% commassie blue R-250, 40% methanol and 7% acidic acid. The gels were decoloured in 1 hour in a water bath at 56 ^{o}C with a solution consisting of 5% methanol and 7,5% acetic acid by changing the solution once in every 20 minutes. Finally, the gels were photographed and molecular weight calculations were made according to Weber et al. (15) Protein markers used for SDS-PAGE are bovine albumin (66 kD), egg albumin (45 kD), trypsinogen (24 kD).

Statistical Analysis: For the biostatistical evaluation of the data obtained from the research, SPSS 18 software package was used. Independent Sample T Test was applied for the statistical analyses of the differentiation among the group data. P<0.05 values were accepted to be statistically significant.

Results

While no statistical differentiation was found in respect to total antioxidant levels in the plasma and erythrocyte samples, and liver and kidney tissues of neither the *Panax ginseng* applied group nor *Panax ginseng* and *Ganoderma lucidum* combination applied group in comparison to the control group (p>0.05), it was determined that *Panax ginseng* + *Ganoderma lucidum* combination increased the total antioxidant levels in the heart tissues significantly (P<0.05) (Table 1). Total oxidant level was also observed to decrease in a highly significant manner in the plasma of both groups (P<0.001). It was determined that total oxidant level decreased in erythrocyte samples of both of the groups, however this decrease in significant only in the *Panax ginseng* + *Ganoderma lucidum* combination applied group (P<0.01). Besides, the changes in liver, kidney and heart tissues were not statistically significant (P>0.05) (Table 1). A statistically significant change was not determined among groups in terms of triglyceride, total cholesterol HDL, LDL and VLDL cholesterol parameters (P>0.05) (Table 1).

As for the liver electrophoresis, thickening in 98 kD, 58 kD, 51 kD, 40 kD and 32 kD protein bands in comparison to the control group as a result of *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* applications as well as the fact that thickening was more distinct in *Panax ginseng* applied group were determined (Figure 1).

Control group was compared to *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* groups individually.

Table 1. Total Oxidant (TOS) and Total Antioxidant (TAS) Levels determined in the plasma, erythrocyte, liver, kidney and heart samples and Triglyceride, Total cholesterol, HDL, LDL and VLDL cholesterol levels of the hamsters (average ± standard deviation) (n=6).

Parameters	Experimental groups		
	C (n=6)	G (n=6)	G+GL (n=6)
TAS (mmol Trolox Equiv./L)			
Plasma	2.28±0.12	2.24±0.21	2.14±0.13
Erythrocyte	2.19±0.14	2.11±0.28	2.36±0.05
Liver	1.79±0.19	1.94±0.19	1.62±0.10
Kidney	1.75±0.15	1.80±0.25	1.59±0.18
Heart	1.22±0.08	1.26±0.10	1.38±0.13*
TOS (μmol H_2O_2 Equiv./L)			
Plasma	1.93±0.25	0.86±0.22***	0.63±0.09***
Erythrocyte	124.77±18.53	108.42±4.64	72.26±6.22**
Liver	23.60±3.33	28.76±2.63	21.90±3.18
Kidney	12.81±2.62	15.21±3.78	14.12±1.77
Heart	19.67±1.79	16.26±3.63	24.14±6.22
Triglyceride (mg/dL)	283.75±88.63	328.00±41.58	309.00±78.09
Total cholesterol (mg/dL)	71.20±12.79	69.00±11.5	70.60±15.13
HDL (mg/dL)	49.20±5.81	42.80±2.49	48.60±5.90
LDL (mg/dL)	13.00±3.46	10.33±1.53	10.00±3.46
VLDL (mg/dL)	56.75±17.86	65.67±8.02	61.75±15.33

*: P<0.05 **: P<0.01 ***: P<0.001

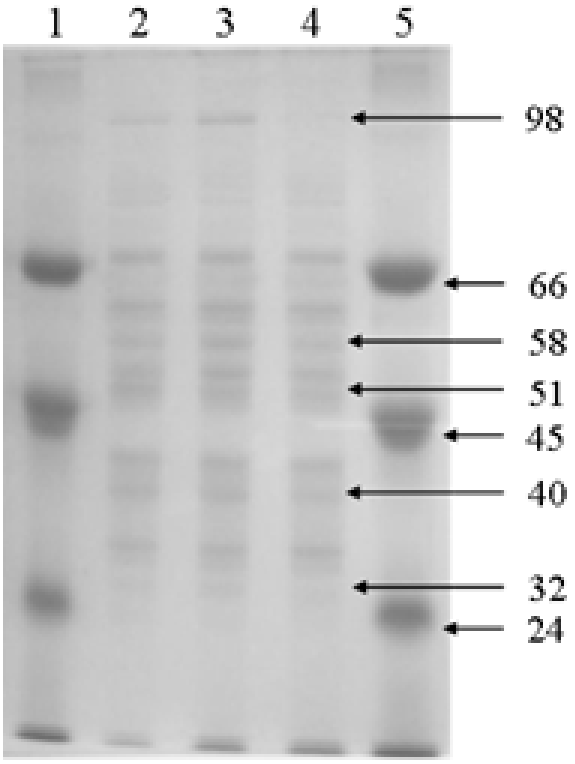

Figure 1. Electropherogram obtained from subjecting liver homogenates collected from animals to SDS-PAGE subsequent to *Panax ginseng, Panax ginseng* + *Ganoderma lucidum* applications. 1-5. lane: Standart proteins, 2. lane: *Panax ginseng* + *Ganoderma lucidum* group, 3. lane: *Panax ginseng* group, 4. lane: Control group.

Discussion

Panax ginseng and *Ganoderma lucidum* are the leading well-known and commonly used herbal products used worldwide, particularly in China and other Asian countries with the purpose of supporting a healthy life and prevent specific diseases (18, 19).

Kim et al. (20) indicated that 1 and 2 g of *Panax ginseng* applied to healthy humans for 1 month reduced serum total ROS (Reactive Oxygen Species) amount as well as Malondialdhyde (MDA) levels, however did not change total antioxidant capacity. Kim and Park (21) stated that giving 2 g of *Panax ginseng* to healthy individuals 3 times a day for 2 months changed plasma MDA levels significantly while increased Superoxide dismutase (SOD) and Catalase (CAT) levels, and additionally reduced Total cholesterol, LDL cholesterol and Triglyceride levels while increased HDL cholesterol levels. Ismail et al. (22) suggested that *Panax ginseng* extract had no effect on hypolipidemic or antioxidant levels of hypercholesterolemic rabbits. *Panax ginseng* was observed to reduce plasma TOS levels significantly while did not lead to a significant change in the TAS levels also in our study. Our findings show resemblance to those reported by Kim et al. (20) and Kim and Park (21) in respect to TOS levels, and to Kim et al. (20) in respect to TAS levels. It is also seen in our study that plasma lipid levels show no changes as a result of *Panax ginseng* application, and is in aggrement with those by Ismail et al. (22) in this regard.

Junk et al. (23) stated that *Panax ginseng* extract applied to rats for 4 weeks with a dosage of 200 mg/kg did not have any effects on the oxidant and antioxidant levels of liver and kidney tissues. Also, Karadeniz et al. (24) was stated that application of *Panax ginseng* do not cause a significant change oxidant-antioxidant balance in testicular tissue. Ramesh et al. (25) stated that application of *Panax ginseng* extract on *Spraque-Dawley* rats with a dosage of 200 mg/kg for 4 months reduced the oxidant levels in liver, kidney and heart tissues, and increased the antioxidant levels significantly. While the increase in the TAS levels of heart tissue as a result of *Panax ginseng* extract application in the current study show resemblance to the study conducted by Ramesh et al. (25) no changes were determined in the TOS and TAS levels of other tissues. This difference is assumed to depend on the species of animals and the duration of application.

Same researchers (26, 27) suggested that *Ganoderma lucidum* extracts increased antioxidant levels while reducing lipid peroxidation and ROS levels. Again, certain researchers (4) suggested that the lipid peroxidation reduced in addition to the significant increase in the antioxidant enzyme levels in blood and liver tissue in rats to which triterpenes isolated from *Ganoderma lucidum* were given. On the contrary to these researches, Wachtel-Galor et al. (28) stated that application of *Ganoderma lucidum* extract on healthy humans for 29 days did not have any effect on plasma oxidant - antioxidant levels and Triglyceride, HDL, LDL and Total cholesterol levels. In this study, it was determined from the observations that *Ganoderma lucidum* combination applied with *Panax ginseng* reduced plasma and erythrocyte TOS levels of healthy hamsters significantly in addition to increasing the TAS levels in heart tissue. Furthermore, it was observed that combined application of *Panax ginseng* + *Ganoderma lucidum* did not have an effect on the lipid profile.

Panax ginseng emphasised by certain studies that certain ginsenosides included by *Panax ginseng* as well as *Ganoderma lucidum* extracts protect erythrocyte membrane against oxidation (11, 29). This study also supports the opinion that combined application of *Panax ginseng* and *Ganoderma lucidum* proves to be more effective than sole application of *Panax ginseng.* Data relevant to the protein electrophoresis of *Panax ginseng* and *Ganoderma lucidum* was not found as a result of the literature researches conducted. A distinct increase in the protein expressions of application group was determined as a result of application of these substances in liver protein electrophoresis.

Consequently; it was concluded that *Panax ginseng* and *Panax ginseng* + *Ganoderma lucidum* do not have a different effects on the lipid profile of hamsters. Furthermore, although *Panax ginseng* application is effective in reducing total oxidant levels, application of this in combination with *Ganoderma lucidum* is assumed to be more effective for reducing oxidant levels and increasing antioxidant levels. In the study, increasing of liver protein expressions has been sourced from albumin production of liver. Increase of liver protein expressions has supported the other parameters of study.

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