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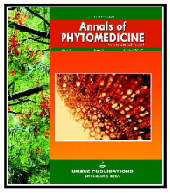

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Effect of orally administered aqueous extract of *Salvia triloba* L. in human volunteers

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Abstract

Salvia triloba L. belongs to the Lamiaceae family, is one in all the vital medicinal plant species. This work aims to study the antioxidant related effects of *S. triloba* in the human body through *in vivo* study and the effects on liver, kidney, and heart function tests. For five days, nine healthy participants consumed 250 ml of *S. triloba* aqueous extract orally. Blood samples were taken before and one hour after the first dosage of water extract (samples I and II, respectively), and again one day after the last dose (*i.e.*, day 6, sample III). Before the first dosage, the first blood sample was taken (*i.e.*, sample I) and was used as a control for the subsequent II and III samples. Subsequent determinations were performed: serum total antioxidant status (TAS), red blood cell reduced glutathione (RBC GSH), red blood cell superoxide dismutation (RBC SOD) activity, red blood cell malondialdehyde (RBC MDA), and serum-selected biochemical tests. After 5 days of oral administration of *S. triloba* extract in healthy volunteers, serum TAS, erythrocyte GSH and erythrocyte SOD activity were significantly increased, and had no influence on serum biochemical examinations of kidney, liver, heart, pancreas, *etc*., contrasted with zero-time dose. *S. triloba* extract has effective antioxidation related effects *in vivo*. Because these findings were obtained in healthy people without oxidative stress, it means that *S. triloba* will enhance the defense system against probable oxidative damage and might be useful in avoiding pathological diseases related to oxidative damage.

1. Introduction

Salvia triloba L. (Lamiaceae), is commonly referred to as Greek sage and the arabic name of *S. triloba* is Meramiyyh (Abu-Rmailah and Afifi, 2000; Ali-Shtayeh *et al.*, 2000). It is commonly used to treat bloating, stomach problems, abdominal pain, oral infections, gingiva and tooth pain, headaches, coughs, flu, common cold, skin diseases, and neurological diseases (Abu-Rmailah and Afifi, 2000; Ali-Shtayeh *et al.*, 2000; Perry *et al.*, 2003; Gali-Muhtasib and Affara, 2000; Salah and Jager, 2005). *S. triloba* extract has antioxidant, anti-inflammatory, antibacterial and anticancer behaviors (Kamatou *et al.*, 2010; Kamatou *et al.*, 2008; Kamatou *et al.*, 2007). *In vitro* pharmacological activity and chemical studies of some sage species are previously evaluated (Kamatou *et al.*, 2005).

In human body, free radicals and reactive oxygen species are continually generated. This oxygen species are the source of cell damage as well as the onset and progression of chronic illnesses. Antioxidants defense mechanisms such as reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and catalase, as well ascarotenoids and polyphenols, protected human body from oxidative damage (Valko *et al.*, 2007).

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The systemic effects are also important to understand if a plant extract affects laboratory analysis, because many patients may go to clinical laboratories for analysis after drinking plant extracts that have become widely available. This work aims to study the antioxidant effects of Meramiyyh in the human body through *in vivo* study and its effects on liver, kidney and heart function tests.

2. Materials and Methods

Nine healthy volunteers were recruited after signing the informed consent form according to the requirements of the ethics committee. Each volunteer took 250 ml of Meramiyyh extract orally every day for five days. Blood samples were collected before and one hour after the first dosage of water extract (samples I and II, respectively), and then one day after the last dose on the fifth day (*i.e.*, day 6, sample III). The initial blood sample (sample I) taken before to the first dosage was utilized as a control for the subsequent samples II and III. This study was carried out in line with the 1964 Declaration of Helsinki's ethical standards.

2.1 Formulation of Meramiyyh water extract

The dried leaves were acquired in Amman, Jordan, at a small herbal shop. 250 grams of leaves was boiled for 10-15 min, then left covered soaking at room temperature for 3-4 h, then 250 ml of soaked aqueous extract was given orally to each individual daily for 5 days.

2.2 Sample of blood

Three blood samples (I, II and III) were collected from each healthy individual in the gel clot activator tubes. These were centrifuged at 3000 rpm for 10 min at 25°C to separate and collect serum. After

that, 2 ml of distilled water added to the cells in the tube under the gel, and tubes were centrifuged at 3000 rpm for 5 min, and the supernatant was collected. Before analysis, all samples were kept frozen at – 20°C until analysis.

2.3 Serum TAS determination

The total antioxidant status of serum was measured by Randox's TAS kit. The results are expressed in millimoles per litre (mmol/l).

2.4 MDA levelsin red blood cells

MDA in red blood cells was determined as a measure of lipid peroxidation according to Stocks and Dormandy's method (1971) using thiobarbituric acid (TBA) as modified by Srour *et al.* (2000). All MDA values are given in nanomoles per gram of hemoglobin (nmol/gHb).

2.5 GSH levels in red blood cells

The Ellman technique, with minor changes, was used to measure red blood cell GSH, as reported elsewhere (Bilto and Alabdallat, 2015a). All GSH values are measured in mg/gHb.

2.6 RBC SOD activity determination

Randox kit (Arthur *et al.*, 1985) was used to test RBC SOD. The result is given in units of U/gHb.

2.7 Biochemical parameters of serum determination

The following biochemical parameters were determined using the Hitachi 902 analyzer: serum sodium (Na), potassium (K), urea nitrogen (BUN), creatinine (CREA), uric acid (UA), albumin (ALB), total protein (TP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK), amylase (AMYL).

2.8 Numerical evaluation

All data are presented as mean ± standard deviation and statistical analysis is carried out with the help of the social science statistical package (SPSS, 17th edition). The results were compared by paired t-test. The results with a value of $p \leq 0.05$ were considered significant.

3. Results

The results are shown in Table 1. Serum total antioxidant status (TAS) (from 1.12 to 1.22), red blood cell reduced glutathione (GSH) (from 0.54 to 0.87) and erythrocyte superoxide dismutation (SOD) (from 868 to 997.5) are all higher on day 6 after oral intake of Meramiyyh extract in healthy volunteers for five days as compared to zero time of oral intake. Meramiyyh had no effect on serum readings that stayed within the reference range (Na, K, BUN, CREA, UA, ALB, TP, ALP, ALT, AST, AMYL, are all tested at zero-time, 1 hour after the first dose and 1 day after the final dose on day 5).

Table 1: Results of *in vivo* research of oral administration of Meramiyyh aqueous extract in healthy volunteers for 5-days. Each statistic reflects the mean ± standard deviation (n = 9), with a *p value ≤ 0.05, compared to 0 time dose. NM stands for "not measured."

Measurement parameters	Sample I	Sample II	Sample III
Serum TAS (mmol/l)	1.12 ± 0.11	1.16 ± 0.15	1.22 ± 0.16*
Erythrocyte GSH (mg/gHb)	0.54 ± 0.09	NM	0.87 ± 0.10*
Erythrocyte SOD (U/gHb)	868.0 ± 167.1	NM	997.5 ± 192.4*
Erythrocyte MDA (nmol/gHb)	17.9 ± 3.4	NM	15.8 ± 3.8
Serum K (ref value=3.7-5.2 mmol/l)	4.38 ± 0.40	4.4 ± 0.2	4.34 ± 0.38
Serum Na (ref value=135-145mmol/l)	146.0 ± 2.2	144 ± 1.2	145.4 ± 1.7
Serum BUN (ref value=6-20 mg/dl)	13.5 ± 3.5	13.0 ± 2.8	9.7 ± 2.0
Serum CREA (ref value= 0.6-1.3 mg/dl)	0.74 ± 0.13	0.72 ± 0.13	0.65 ± 0.16
Serum UA (ref value= 3.5-7.2 mg/dl)	4.92 ± 1.0	5.0 ± 1.0	5.4 ± 1.3
Serum ALB (ref value= 34-54 g/l)	45.2 ± 2.2	44.6 ± 2.5	46.6 ± 3.8
Serum TP (ref value = 60-85 g/l)	77.6 ± 3.9	76.1 ± 2.1	79.7 ± 4.4
Serum ALP (ref value=55-142 U/l)	112.9 ± 71.5	110.3 ± 67.2	112.0 ± 78.3
Serum AST (ref value= 8-40 U/l)	15.8 ± 5.7	16.2 ± 5.0	16.4 ± 5.4
Serum ALT (ref value= 7-55 U/l)	17.4 ± 13.3	17.5 ± 14.1	18.9 ± 15.7
Serum CPK (ref value=38-176 U/l)	90.5 ± 65.7	93.0 ± 64.4	80.5 ± 35.8
SerumLDH (refvalue=200-450 U/l)	307.3 ± 48.0	317 ± 48.2	304.4 ± 39.7
Serum AMY (refvalue=40-140 U/l)	46.8 ± 15.2	45.9 ± 14.7	43.3 ± 16.6

4. Discussion

According to current *in vivo* human research, oral intake of Meramiyyh extract for five days can considerably improve TAS, RBC reduced glutathione (GSH) and RBC superoxide dismutas. Because the current study's findings were achieved in healthy persons who had not been exposed to oxidative stress, it is likely that Meramiyyh might enhance the baseline of the defense system against oxidative stress, lowering the risk of oxidative stress-related illnesses.

The lack of effect of Meramiyyh on serum LDH in healthy individuals might imply that the plant has antihemolytic action and/or no deleterious hemolytic activity *in vivo*. Serum biochemical assays show that oral Meramiyyh extract had no significant impact on the renal function tests (CREA, BUN), liver function enzymes and tests (AST, ALT,ALP, ALB, TP), myocardial enzymes and pancreatic amylase which are all within the reference ranges. This finding agrees with the findings of Moeko *et al.*, 2015; Alabdallat, 2016; Alabdallat, 2019.

Lifestyle factors like nutrition, physical activity, drinking and smoking have been proposed to have a major impact on human oxidative stress and disrupt oxidative balance. As a result of the burden/ antioxidant interaction, it has been shown that a diet high in vegetables and natural antioxidants is good to antioxidants and is most popular among healthy people who live long lives (Dato *et al.*, 2013; Aseervatham *et al.*, 2013).

5. Conclusion

The active antioxidant effects of Meramiyyh on healthy people have no negative effects on the main body system, implying that this plant can be used to prevent the onset or progression of oxidative stress-related pathological conditions by improving the body's defense mechanism's bottom line.

Authors' contributions

NA was in charge of the study's design as well as data collecting, processing, and analysis. The final version of the manuscript was written by NA, who also authorized it.

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Conflict of interest

The author declares no conflicts of interest relevant to this article.

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