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Effects of commonly used medicinal herbs in Jordan on serum total antioxidant status and clinical laboratory testing

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ABSTRACT

The use of medicinal herbs is widespread and growing. We tested the *in-vivo* effects of commonly used medicinal herbs in Jordan, on normal human volunteers after oral administration of aqueous extracts for several days, to see whether the *in-vitro* chemical antioxidant activity of a given herb replicates *in-vivo* and to see whether a given medicinal herb affects the results of selected clinical chemistry tests performed on the serum. Healthy volunteers were given orally aqueous herbal extracts daily for five days. Venous blood samples were taken before and one hour after the first dose of aqueous extract and then one day after the last dose of day five. Total antioxidant status and 13 of routine clinical chemistry tests were assayed on the serum. The tested herbs caused a significant increase in serum total antioxidant status. The studied herbs can be arranged in decreasing order of their *in-vivo* antioxidant strength after one hour of first consumption as follows: *Nigella sativum* > *Rosmarinus officinalis* > *Zingiber officinale* > *Verbena triphylla* > *Origanum syriacum* > *Salvia triloba.* The studied herbs except *for Rosmarinus officinalis* also caused a significant increase and/or decrease in 10 out of 13 laboratory tests performed on the serum. These results indicate that oral administration of aqueous extracts of medicinal herbs although improve the serum total antioxidant status, they could also significantly alter some laboratory results used for diagnosis or evaluation of diseases and this may negatively affect patient's care. The present study highlights the importance of obtaining information regarding patients' uptake of herbal products that might interfere with some laboratory testing.

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INTRODUCTION

Medicinal herbs play an important role in folk medicine in Jordan (Issa and Basheti, 2017, 2016; Abu-Irmaileh and Afifi, 2003; Abdelhalim *et al.*, 2017). Medicinal herbs are sold in the Jordanian market through ordinary food shops and supermarkets in addition to herberian shops. They are widely used by people in Jordan, and one can hardly imagine a person in Jordan not taking a cup of herbal extract once or more daily, in fact, herbal products are being served in Jordan as much as they drink tea or coffee. Whereas many people replaced the habit

of tea drinking with these herbal extracts.

The most common herbs used by Jordanians in a daily basis are extracts of *Zingiber officinale, Rosmarinus officinalis, Verbena triphylla*, *Origanum syriacum* and *Salvia triloba*, and the seeds of *Nigella sativum*. The consumption of these herbal extracts by the Jordanians is not related to any of their recommended uses, but rather due to it is becoming a common public habit among populations. Therefore, it is very likely that a patient going to a clinical laboratory for analyses of being drank a cup or more of the above herbal extracts either the same day or a day before. Its well known that medicinal herbs posse's antioxidant properties that contribute to their therapeutic benefits (Chirag *et al.*, 2013; Serafini and Peluso, 2017).

But, despite the epidemiological evidence that consumption of various herbs is associated with positive health benefits, there is limited information on their mechanisms of action, yet many of them were found to have an adverse effects, one of which their interference in laboratory testing (Narayanan and Young, 2016; Barrett *et al.*, 1999; Staines, 2011; Ernst, 2002). However, there are very few reports dealt with the effects of medicinal herbs on laboratory testing, which indicated that they can cause abnormal or alter test results and even confusion in patient's care (Narayanan and Young, 2016; Staines, 2011; Corns, 2003; Dasgupta, 2007; Dasgupta and Bernard, 2006; Dasgupta, 2003).

These reports proposed three mechanisms for the effects of medicinal herbs on laboratory testing which included a direct interference by herbal component with certain laboratory assays, Drug-herb interactions leading to unexpected concentrations of therapeutic drugs and herbal toxicity altering the normal physiology, thus leading to abnormal test results. To our knowledge, the reports in the literature that are showing the effects of herbal medicines on laboratory testing were mostly clinical case reports found incidentally that lead to the above-proposed mechanisms.

As none of these reports included any of the commonly used herbs in Jordan nor they were studied on healthy individuals, therefore this study was carried out in order to find whether consumption of the commonly used medicinal herbs in Jordan by healthy individuals such as *Zingiber officinale, Rosmarinus officinalis, Verbena triphylla*, *Origanum syriacum, Salvia triloba* and *Nigella sativum* affect clinical chemistry tests that are used routinely for the assessment of liver, renal, cardiac and pancreatic functions, such as Sodium (Na), Potassium (K), Blood Urea Nitrogen (BUN), Creatinine (CREA), Uric Acid (UA), Albumin (ALB), Total Protein (TP), Lactate Dehydrogenase (LDH), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Creatinine Phosphokinase (CPK) and Amylase (AMYL). In addition, it was important to measure serum total antioxidant status (TAS) in the same individuals as an indicator for the absorption of given medicinal herbs and their appearances in the blood.

The present study was designed by giving orally aqueous extracts to healthy individuals for five days. All selected herbal extracts were prepared as being administrated by the public to reflect the actual effect on tested laboratory parameters. Three blood samples were collected from each individual were sample I was taken before drinking or administration of aqueous extract as a reference, sample II after one hour of the first dose and sample III was collected at day 6 (i.e. 24 hours after the last dose to allow wash out the herb from the body). The results of this study identified the effects of commonly used herbal products on some clinical laboratory parameters. We think that patient's sample collection criteria should be updated to include history information of patients undergoing laboratory screening whether they have taken any herbal products to be considered by a physician for accurate laboratory results interpretation.

MATERIALS AND METHODS

Herbal material

Dried leaves from the following medicinal herbs (*Rosmarinus officinalis, Verbena triphylla*, *Origanum syriacum and Salvia triloba*), rhizomes of *Zingiber officinale* and seeds of *Nigella sativum* were purchased from the local herbal stores in Amman, Jordan. Table 1 summarizes the medicinal herbs included in this study according to their Families, Scientific, English and Arabic names with the respected recommended use by sellers.

Preparation of aqueous extracts of tested herbs

The process of aqueous extract preparation was performed similar to the way of usual preparation performed in public to reflect the exact effect on the measured parameters rather than using a chemical concentrated extract that could observe an exaggerated effect that could not be seen in regular population administration dose. 250 g dried leaves of each herb was boiled in 12.50 L water for 10-15 min and then left covered soaking for 3-4 hrs at room temperature. An amount of 1.25 L of soaked aqueous extracts were filled in clean bottles for each individual to be consumed in the morning with 250 ml dose

Table 1: List of medicinal herbs used in this study according to their Families, Scientific, English and Arabic names with the recommended uses by sellers

Family	Scientific Name	English Name	Arabic Name	Part used	Recommended uses
Lamiaceae	Rosmarinus officinalis L.	Rosemary	Ikleel al-Jabal	Leaves	Obesity, constipation, kidney stones, hypertension, common cold, abdominal pains, ulcer, flatulence, toothache, edema, gynecological disorders, nervosity.
	Salvia triloba L.	Greek sage	Meramiyyh	Leaves	Headache, flatulence, toothache, abdominal pain & common cold.
	Origanum syriacum L.	Thyme	Zaatar	Leaves	Blood coagulation, common cold, cough, influenza, abdominal pain, constipation.
	Verbena triphylla L.	Lemon verbena	Melissa	Leaves	Abdominal pain, gynecological disorders, arthritis.
Zingiberaceae	Zingiber officinale	Ginger	Zanjabil	Rhizomes	Anemia, common cold, abdominal pains, indigestion, gynecological disorders, impotence, general weakness.
Ranunculaceae	Nigella sativa L.	Black seed	Habbat al-barakah	Seeds	Arthritis, general weakness, gynecological disorders, lactation deficiency, gastrointestinal problems, obesity, hypercholesterolemia, common cold, inflammations.

Table 2: Age (years) and sex of participants groups distribution

Group	Age (mean±S.D.)	Female/Male
Zingiber officinale	41.8±7.6	7/2
Rosmarinus officinalis	35.4±13.5	4/5
Verbena triphylla	34±18.6	4/5
Origanum syriacum	35.8±14.7	4/5
Salvia triloba	42.8±14.6	6/3
Nigella sativum	36.7±14.1	8/1

Table 3: Serum total Antioxidant Status (TAS) before and after oral administration of tested medicinal herbs. Each value represents the mean value ± S.D., (n =9), *P value ≤ 0.05, compared to 0 time administration

	TAS (mmol/l)		
	0 time	1hr (day 1) level (% change)	Day 6 level (% change)
Zingiber officinale	1.08±0.16	1.21±0.10 (+12%)	1.24±0.12* (+15%)
Rosmarinus officinalis	1.14±0.3	1.31±0.27*(+15%)	1.30±0.20* (+14%)
Verbena triphylla	1.23±0.2	1.36±0.2* (+11%)	1.38±0.22* (+12%)
Origanum syriacum	1.14±0.10	1.21±0.11 (+6%)	1.28±0.09* (+12%)
Salvia triloba	1.12±0.11	1.16±0.15 (+4%)	1.22±0.16* (+9%)
Nigella sativum	0.89±0.2	1.1±0.25* (+24%)	0.7±0.13

per each treatment period.

Blood samples

60 Healthy volunteers were recruited in this study after they signed an informed consent according to the ethics committee requirements. They were grouped into six groups, (each group n=9-10), their age in years and sex are shown in Table 2. 5 groups drank 200-250 ml of aqueous extract from the following medicinal herbs (*Zingiber officinale, Rosmarinus officinalis, Verbena triphylla*, *Origanum syriacum, Salvia triloba*) respectively daily in the morning for 5 days, group 6 individuals received one spoon from grinded seeds of *Nigella sativum* daily for 5 days. 3 blood samples were collected in gel clot activator tubes from each healthy volunteer (sample I before drinking or administration of the aqueous extract, sample II after one hour of the first dose (i.e. drinking aqueous extract) on day one and sample III next day following the last dose of day five. Blood tubes were then centrifuged for 10 min at 3000xg at room temperature after each collection period, and serum samples were obtained. All samples were stored frozen at -20°C until analysis.

Ethical Issue: Inclusion and Exclusion Criteria

This study was approved by the committee of the University of Jordan, and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. All healthy volunteers were recruited in the study after they signed informed consent for publication of this study. Individuals were excluded if they had (i) a disease condition, such as liver, renal, or heart dysfunction; (ii) a history of cancer; (iii) allergies to any drug or food ingredient. Furthermore, women were excluded if they were pregnant or lactating. Smokers were also excluded. This was considered in order to exclude any results of interference that might be caused by the above-mentioned conditions.

Serum total antioxidant status (TAS) assay

TAS was measured by using the commercially available kit obtained from Randox. In this kit, the 2.2'-Azino-di[3-ethylbenzthiazoline sulphonate (ABTS) is incubated with peroxidase (metmyoglibin) and H_2O_2 to produce the radical $ABTS^{\cdot+}$. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

Serum clinical chemistry assays

The kits for the determination of serum clinical chemistry assays were purchased commercially from Roche. The 902 Hitachi analyzer was used to perform the following assays: serum sodium (Na), potassium (K), blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), albumin (ALB), total

protein (TP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine phosphokinase (CPK) and amylase (AMYL).

Statistical analysis

All data are reported as the mean ± S.D., statistical analysis was performed using SPSS statistics 17. The results were compared by paired *t*-test. The results with a value of $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Total serum antioxidant status (TAS) results are shown in Table 3. It was found that oral administration of aqueous extracts of tested herbs by healthy individuals for 5 days increased significantly serum TAS at either 1 hour after the first dose on day one and/or at the sixth day (i.e. one day following the last dose of day five) compared to 0 time administration. Accordingly, *Nigella sativum* was the strongest at 1 hour after administration causing 24% significant increase followed by *Rosmarinus officinalis* (15%) and *Verbena triphylla* (11%), whereas at the sixth day (i.e. one day following the last dose of day five) *Zingiber officinale* was the strongest causing 15% significant increase followed by *Rosmarinus officinalis* (14%), *Verbena triphylla* (12%), *Origanum syriacum* (12%) and *Salvia triloba* (9%). Altogether, our results indicated that consumption of the tested medicinal herbs can significantly increase serum TAS levels.

This result coincides with the findings of other researchers who have showed that the serum TAS increased in humans after consuming selected herbal products such as green tea, nut, spices, fruits, vegetables and their extracts that are known to have antioxidant property (Serafini and Peluso, 2017; Li *et al.*, 2014; Torabian *et al.*, 2009; Pecorari *et al.*, 2010). There was also another study that showed a linear correlation between green tea *in-vitro* antioxidant activity and the extent of the antioxidant effect *in-vivo* that appeared in plasma after one hour from consumption (Pecorari *et al.*, 2010). The studied herbs in the present study can be arranged in decreasing order of their *in-vivo* antioxidant strength after one hour of first consumption as follows: *Nigella sativum* > *Rosmarinus officinalis* > *Zingiber officinale* > *Verbena triphylla* > *Origanum syriacum* > *Salvia triloba.* A previous report from our laboratory (Bilto *et al.*, 2015) showed that *Nigella sativum* extract was the strongest in iron-chelating ability and the weakest in free radical scavenging abilities that were tested chemically *in-vitro*, compared with the other herbs tested in the present study. And also showed that *Rosmarinus officinalis* and *Zingiber officinale* were the strongest in free radical scavenging abilities and the weakest in iron-chelating ability compared with the other herbs tested in the present study.

As *Nigella sativum, Rosmarinus officinalis* and *Zingiber officinale* were the strongest in the present *in-vivo* study, the results of the present study,, therefore,, confirm that both metal-chelating ability (that intercepts the generation of free radicals via fenton reaction) and free radical scavenging abilities are both important for the *in-vivo* antioxidant capacity. The serum concentration of the absorbed antioxidant compounds could be dependent on their absorption rate and renal clearance which supposed to be dependent on the hydration state of the body and the extent of their binding to plasma proteins and lipids which is varied according to the chemical structure from one compound to another (Li *et al.*, 2014; Pérez-Fons *et al.*, 2010; García-Alonso *et al.*, 2006). This could explain the variation between the tested herbs in regard to the time taken after the dose to increase serum TAS or to disappear after 24 hours of the last dose, possibly being washed out of the body quickly. The effects of medicinal herbs under investigation in this study on serum clinical chemistries are shown in Table 4. The results showed that *Zingiber officinale* caused a significant decrease in K and BUN reaching 7% and 24.6% respectively and a significant increase in CPK activity reaching 31.1% at day 6 (i.e. one day following the last dose of day five).*Rosmarinus officinalis* had no significant effect on any of the given clinical laboratory tests. *Verbena triphylla* caused a significant decrease in BUN and CREA reaching 28.1% and 12.2% respectively and a significant increase in UA, reaching 9.8%.

Origanum syriacum caused a significant decrease in BUN and LDH activity, reaching 27.3% and 8% respectively and a significant increase in ALP activity, reaching 4.4%. *Salvia triloba* caused a significant increase in K and UA reaching 7.5% and 8.1% respectively and a significant decrease in BUN and LDH reaching 21.1% and 10.4% respectively. *Nigella sativum* caused a significant increase in K, ALP, ALB, TP and AMYL, reaching 17.3%, 6.2%, 8.1%, 8.5% and 11.7 respectively. However, serum Na, AST and ALT were not affected by any of the tested herbs (data not shown). Overall, the most significant effect was shown on the level of BUN after administration of all herbal products except for *Rosmarinus officinalis and* Nigella *sativum* causing a considerable decrease in its level ranging from 21.1% to 28.1%. The exact mechanisms of these laboratory results alterations are not known.

Table 4: The effect of medicinal herbs administrations on serum chemistries at different times (1hr and day 6) compared to 0 time administration. Each value represents the mean value ± SD, (n = 8-10), *P value ≤ 0.05, compared to 0 time administration. For the significant changes the (% change) was shown

Measured Parameter		Zingiber officinale	Rosmarinus officinalis	Verbena triphylla	Origanum syriacum	Saliva triloba	Nigella sativum
K	0 time	4.3± 0.47	4.4± 0.39	4.38± 0.40	4.2± 0.43	4.0± 0.47	3.98± 0.27
	1hr	4.3± 0.53	4.6± 0.35	4.4± 0.21	4.4± 0.27	4.0± 0.39	4.15± 0.31
	Day 6	4.0± 0.26* (- 7.0 %)	4.4± 0.29	4.34± 0.38	4.4± 0.25	4.3± 0.3* (+ 7.5 %)	4.67± 0.48* (+ 17.3 %)
BUN	0 time	17.9± 4.5	12.6± 2.2	13.5± 3.5	15.4± 3.8	14.7± 4.7	11.7± 2.8
	1hr	16.6± 4.5* (-7.3 %)	12.6± 1.5	13.0± 2.8	14.3± 3.8* (- 7.1 %)	14.1± 4.7* (- 4.1 %)	11.8± 2.8
	Day 6	13.5± 3.6* (- 24.6%)	10.2± 1.9	9.7± 2.0* (- 28.1 %)	11.2± 2.1* (- 27.3 %)	11.6± 3.7* (- 21.1 %)	11.0± 2.0
CREA	0 time	0.67± 0.14	0.78± 0.20	0.74± 0.13	0.78± 0.18	0.71± 0.20	0.63± 0.11
	1hr	0.67± 0.13	0.78± 0.21	0.72± 0.13	0.73± 0.17	0.70± 0.19	0.63± 0.10
	Day 6	0.62± 0.12	0.76± 0.26	0.65± 0.16* (- 12.2 %)	0. 73± 0.0.14	0.69± 0.19	0.65± 0.10
UA	0 time	4.6± 1.1	5.4± 1.8	4.92± 1.0	5.0± 1.1	3.95± 1.7	4.55± 1.3
	1hr	4.7± 1.1	5.4± 1.7	5.0± 1.0* (+ 1.6 %)	5.0± 1.0	3.90± 1.7	4.55± 1.3
	Day 6	4.6± 0.84	5.6± 1.8	5.4± 1.3* (+ 9.8 %)	5.0± 1.0	4.27± 1.8* (+ 8.1 %)	4.6± 1.2
ALP	0 time	76.1± 22.8	130.2± 86.9	112.9± 71.5	67.6± 24.0	98.5± 64.8	84.4± 59.8
	1hr	75.8± 23.0	126.8± 84.0	110.3± 67.2	65.9± 21.2	97.2± 64.8	83.7± 55.0
	Day 6	77.9± 22.8	126.3± 82.8	112.0± 78.3	70.6± 23.2* (+ 4.4 %)	96.1± 61.0	89.6± 64.7* (+ 6.2 %)
CPK	0 time	63.1± 23.9	124.0± 71.8	90.5± 65.7	100.7± 31.2	100.0± 58.4	103.5± 23.2

Continued on next page

Table 4 continued

Measured Parameter		Zingiber officinale	Rosmarinus officinalis	Verbena triphylla	Origanum syriacum	Saliva triloba	Nigella sativum
	1hr	67.6± 22.6* (+ 7.0 %)	121.1± 64.8	93.0± 64.4	106.8± 34.8	102.9± 59.0	106.5± 25.5
	Day 6	82.7± 32.5* (+ 31.1 %)	121.0± 50.0	80.5± 35.8	121.7± 57.2	118.7± 81.0	115.7± 65.4
LDH	0 time	375.0± 48.7	403.5± 105.9	307.3± 48.0	350.5± 54.5	355.2± 38..5	366.9± 61.15
	1hr	371.9± 42.6	374.6± 79.7	317± 48.2	350.2± 66.5	346.1± 37.4	343.9± 57.5
	Day 6	370.5± 37.4	358.9± 66.9	304.4± 39.7	322.5± 39.9* (- 8.0 %)	318.3± 40.5* (- 10.4 %)	352.1± 52.2
ALB	0 time	44.8± 2.8	47.2± 4.6	45.2± 2.2	46.6± 3.8	47.0± 3.5	44.5± 2.7
	1hr	45.4± 2.2	45.3± 3.5	44.6± 2.5	45.9± 3.9	46.6± 3.0	43.5± 2.5
	Day 6	44.5± 2.3	46.6± 3.4	46.6± 3.8	47.7± 4.0	46.2± 3.6	48.1± 3.6* (+ 8.1 %)
TP	0 time	77.7± 4.9	79.4± 6.7	77.6± 3.9	77.3± 6.1	80.0± 4.6	77.2± 4.5
	1 hr	78.1± 3.6	77.4± 4.8	76.1± 2.1	75.8± 5.4	79.3± 4.7	76.3± 4.0
	Day 6	77.9± 4.9	81.5± 5.5	79.7± 4.4	80.2± 6.4	79.8± 3.0	83.8± 2.9* (+ 8.5 %)
AMYL	0 time	57.3± 14.3	51.2± 16.3	46.8± 15.2	58.1± 12.8	67.4± 20.2	60.0± 21.8
	1hr	55.7± 13.7	49.3± 14.1	45.9± 14.7	55.9± 13.4	65.3± 16.3	59.9± 21.5
	Day 6	55.8± 15.8	50.2± 15.0	43.3± 16.6	63.1± 16.0	66.8± 19.3	67.0± 22.6* (+ 11.7 %)

However, some proposed mechanisms involve one or more of the following mechanisms (Dasgupta and Bernard, 2006; Dasgupta, 2003); (i) Direct interference of a component of the medicinal herb with the assay (ii) Physiologic effects either through toxicity or enzyme induction or inhibition due to an herbal component (iii) Herb-drug interactions when the herb was being consumed by a patient under therapy with some drugs which could result in exaggerated or suppressed drug effects and/or false drug concentrations in laboratory measurements. Since the present study was performed on healthy individuals, one could speculate that the altered laboratory test results by a given herb could be considered as a beneficial effect and/or a side/adverse effect. This phenomenon was obvious in the present study with some tests, showing a beneficial effect of reducing some blood parameters such as BUN and CREA, especially when dealing with uremic patients, and a side/adverse effect of increasing or decreasing K that could have an effect on heart performance. The effects of tested herbs on the following tests; increased UA, ALP, CPK, ALP, ALB, TP and AMYL and decreased LDH activity cannot be explained as neither positive or negative effects, but overall speaking they should be considered as confusing effects that could lead to confusion in patient's diagnosis and/or treatment. It doesn't go without saying that phenolic compounds in plants are responsible for the various colors inflected in these plants and thus presence of these phenolics in serum samples could somehow affect the absorption spectra for a given test when spectrometers are used for laboratory measurements. Its noteworthy, therefore, for future studies to test the effects of these herbal extracts on laboratory methods by direct addition of these extracts to test tubes along with the serum samples.

To our knowledge, the design of the present *in-vivo* study regarding the effects of given medicinal herbs on clinical laboratory testing on healthy individuals has not been reported before. It should be pointed out that the present study is meant to be a preliminary study involving a small number of participants for a short period. Thus further studies may be needed to clarify the effects of long-term use of these herbs and the related mechanisms on laboratory testing.

CONCLUSION

Consuming medicinal herbs have both beneficial effects for intended use as antioxidants and side effects concerning clinical laboratory testing that could lead to mis-interpretation and confusion in patient's care. Use of medicinal herbs as an alternative medicines may significantly alter laboratory results, and thus it is important to ask patients about herbal medicine use and to check for any possible interactions with their medications and laboratory testing. Overall, medicinal herbs can improve the base line of the defense mechanisms against possible oxidative stress and possibly inhibit pathological conditions related to oxidative stress. In the light of the present results, the reported conclusions in the literature that showed some herbal products improved disease states based on measuring serum chemistries could be questionable in the absence of data regarding their effects on serums of healthy individuals.

Conflict of interest

The final manuscript has been seen and approved by all authors. The authors declared that they have no competing interests.

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