Hindawi Publishing Corporation
ISRN Oxidative Medicine
Volume 2014, Article ID 364836, 4 pages
http://dx.doi.org/10.1155/2014/364836

Clinical Study

Tualang Honey Supplementation Reduces Blood Oxidative Stress Levels/Activities in Postmenopausal Women

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Received 22 January 2014; Accepted 3 March 2014; Published 9 March 2014

Academic Editors: N. Ercal and J. Moskovitz

This study aimed to investigate the ability of Tualang honey (TH), a phytoestrogen, to reduce blood oxidative stress levels/activities in postmenopausal women and compared the effects with the standard estrogen progestin therapy (EPT). A total of 78 healthy postmenopausal women were randomly assigned to one of two groups; EPT group received Femoston conti 1/5 (1 mg 17β-estradiol and 5 mg dydrogesterone), and TH group, received 20 g of TH supplement daily for 16 weeks. The reduced glutathione to oxidized glutathione ratio (GSH : GSSG), plasma glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and 4-hydroxynonenal (4-HNE) levels/activities were measured at pre- and postinterventions using commercially available kits. The plasma GPx and CAT activities were notably increased, and plasma 4-HNE level was significantly decreased in postmenopausal women who received EPT and TH supplementation. TH supplementation for 16 weeks was able to reduce blood oxidative stress levels/activities of postmenopausal women comparable to those who received EPT. Thus, TH has a potential to be used as antioxidant therapy to combat oxidative stress-induced neurodegenerative diseases. This trial is registered with NCT01300676.

1. Introduction

Studies have shown that oxidative stress is increased in postmenopausal women, and it was reversed by antioxidant action of estrogen [1–3]. The antioxidant action of estrogens *in vivo* is due not to their chemical phenolic structure but rather to their interaction with estrogen receptors in cells which eventually lead to the activation of mitogen activated protein kinases (MAPK) and nuclear factor kappa B (NFκB) [4, 5]. Despite the proven benefit of hormone replacement therapy (HRT), beliefs about side effects and concerns about safety hinder the acceptance of HRT among patients even in recent researches [6]. Hence, there is a need to find a comparable alternative treatment to alleviate postmenopausal symptoms.

Honey contains various kinds of phytochemicals with high phenolic and flavonoid content which contribute to its high antioxidant activity [7, 8]. Flavonoids and other polyphenols, common constituents of honey, have the potential to impound metal ions in complexes, preventing the formation of free radicals [9]. Malaysian Tualang honey (TH) is reported to have the highest antiradical activity compared to Gelam honey, Indian forest honey, and Pineapple honey [10] and a total of six phenolic acids and five flavonoids [10–12]. Recently, our research group has reported the beneficial effects of estrogen progestin therapy (EPT) and TH on verbal memory of postmenopausal women [13]. These led us to hypothesize that both EPT and TH improved verbal memory of postmenopausal women by reducing blood oxidative stress levels/activities. Therefore, the aim of this study was to compare the blood oxidative stress levels/activities of postmenopausal women receiving EPT and TH supplementation.

2. Materials and Methods

2.1. Study Subjects. This study involved 78 postmenopausal women aged between 45 and 60 years, recruited from the

Family Medicine and the Obstetrics and Gynecology Clinic, Hospital Universiti Sains Malaysia (USM). Participants were excluded if they took any other herbal or hormone replacement therapy 3 months prior to the study, smoked, had a history of drug or alcohol abuse, or had a history of serious medical, surgical, mental, or gynecological diseases. Selected participants underwent physical examinations including pelvic ultrasonography to exclude any gynecological problems.

2.2. Study Procedure and Design. The study protocol was approved by the Research and Ethics Committee of USM. Participants were briefed on the nature of the study, and informed consent was obtained at the initial visit. Randomization was computer generated, and participants were identified by their hospital registration numbers and were randomly assigned to one of two groups: TH and EPT groups. The TH group received oral TH (Agro Mas) at a daily dose of 20 g. The EPT group received oral Femoston 1/5 (1 mg 17β estradiol and 5 mg dydrogesterone) daily. Participants took the treatment for 16 weeks, and their health status, compliance, and possible adverse effects were monitored every 8 weeks.

2.3. Determination of Blood Oxidative Stress Levels/Activities. The blood GSH to GSSG ratio, plasma catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were determined using a commercially available kit (Calbiochem, Darmstadt, Germany). Plasma 4-hydroxynonenal (4-HNE) and total protein were determined using the OxiSelect HNE-His adduct Elisa kit (Cell Biolabs, Inc., San Diego, USA) and Protein Assay kit (Bio-Rad, Hercules, CA, USA), respectively. Procedures for the verification of antioxidant enzymes lipid oxidative damage and total protein were in accordance with the manufacturer's protocol.

2.4. Statistical Analysis. The results were analyzed using PASW Statistics version 20 software. Values of outcome data are expressed as mean and standard error of mean (SEM). Demographic data were characterized using descriptive statistics and group comparisons were conducted using repeated measures ANOVA. The differences in blood oxidative stress values between pre- and postinterventions were analyzed using paired t-test when within-group difference in repeated measures ANOVA was significant. $P < 0.05$ was considered statistically significant.

3. Results

A total of 78 postmenopausal women successfully completed this study and the participants' characteristics are summarized in Table 1. There was no significant difference in the mean age of participant, mean age of menarche, mean age of menopause, and duration of menopause between EPT and TH groups. EPT was used as active comparator since it is the standard-of-care therapy. In the present study, there were no group differences in the preintervention values of blood oxidative markers. These data reflect that the baseline values were similar in both groups at the start of the study and were used as control values.

Table 1: Characteristics of the participants.

Characteristics	Group	
	TH	EPT
Age (years)	55.44 (0.52)	55.31 (0.49)
Menarche age (years)	13.28 (0.25)	13.87 (0.27)
Menopausal age (years)	50.08 (0.46)	49.21 (0.79)
Duration of menopause (years)	5.33 (0.53)	6.28 (0.73)

Values are expressed as mean (SEM). TH: Tualang honey; EPT: estrogen progestin therapy. Statistical comparisons were made between groups using independent t-test. [a]$P < 0.05$ was considered statistically significant.

The level/activity of oxidative stress markers between the two groups was analyzed using repeated measures ANOVA. There were significant differences between the two groups in mean CAT activity ($F_{1,76} = 36.73$ and $P \leq 0.001$), GPx activity ($F_{1,76} = 10.309$ and $P = 0.02$), and 4-HNE level ($F_{1,76} = 7.587$ and $P = 0.007$). However, there were no significant differences between the two groups in mean SOD activity ($F_{1,76} = 1.045$ and $P = 0.310$) and GSH/GSSG ratio ($F_{1,76} = 0.062$ and $P = 0.804$). The differences in mean CAT activity, GPx activity, and 4-HNE level between pre- and postinterventions were further analyzed using paired t-test. Differences in the levels/activities of oxidative stress marker between pre- and postinterventions were summarized in Table 2.

4. Discussion

There was a significant increase in CAT activity after 16 weeks of EPT, and these results were in contrast with previous studies conducted on postmenopausal women. Previous studies reported that CAT activity displayed minute differences between treated and nontreated postmenopausal women [14] and between premenopausal and postmenopausal women [15]. The discrepancy could be due to different age groups and duration of HRT used in the studies.

The increased CAT activity in TH group could be explained by its antioxidant contents such as glucose oxidase, diastase, invertase, catalase, and peroxidase and other bioactive constituents [16]. Our finding was also supported by previous animal studies that showed restoration of CAT activity in the erythrocytes of young and middle-aged rats [17], pancreas of diabetic rats [18], and liver of male BALB/c mice administered trichlorfon [19] following honey supplementation.

GPx activity was found to be significantly increased after 16 weeks of EPT and was consistent with previous studies [1, 20]. There was a significant increase in GPx activity after 3 and 6 months of Premarin and Provera therapy in postmenopausal women [1]. In addition, the erythrocytes GPx were positively correlated with serum estrogen levels in both premenopausal women and HRT-treated postmenopausal women [20]. Estrogen has been suggested to exert its antioxidant effects via the modulation of intracellular GPx activity [20].

Table 2: Blood oxidative stress level/activity of the participants at pre- and postinterventions.

Markers	TH ($n = 39$)		EPT ($n = 39$)	
	Pre	Post	Pre	Post
Blood GSH : GSSG ratio	25.2 (7.6)	39.7 (11.5)	32.7 (8.0)	22.4 (6.9)
Plasma SOD (U/mL)	0.068 (0.005)	0.060 (0.005)	0.068 (0.006)	0.069 (0.005)
Plasma CAT (nmol/min/mL)	4.1 (0.5)	5.6 (0.5)[a]	4.1 (0.4)	6.4 (0.5)[a]
Plasma GPx (nmol/min/mL)	75.3 (5.5)	90.1 (6.5)[a]	83.5 (5.7)	105.8 (8.3)[a]
Plasma 4-HNE (μg/mL)	3.6 (0.23)	3.1 (0.23)[a]	3.4 (0.28)	2.6 (0.24)[a]

Values are expressed as mean (SEM). TH: Tualang honey; EPT: estrogen progestin therapy; GSH : GSSG: ratio of reduced to oxidized glutathione; GPx: glutathione peroxidase; 4-HNE: 4-hydroxynonenal; CAT: catalase; SOD: superoxide dismutase. Statistical comparisons were made between pre- and postinterventions using paired t-test, and [a]$P < 0.05$ was considered statistically significant.

The increase in GPx activity following TH supplementation could be explained by its vitamin contents which reduce the imbalance between uncontrolled ROS generation and scavenging enzyme activities [21]. This finding was also supported by studies in the erythrocytes of young and middle-aged rats [17] as well as in male BALB/c mice administered trichlorfon [19] which showed honey supplementation restored GPx activity.

GSH : GSSG ratio represents the glutathione redox status, an index of oxidative status in all type of cells. There were no significant changes in blood GSH to GSSG ratios after EPT and TH supplementation. This could be explained by inadequate antioxidant capability of EPT and TH to restore intracellular GSH [18] or improper sample handling resulting in GSH which is available in RBC to be oxidized to GSSG.

The plasma SOD level was almost unchanged after 16 weeks of EPT or TH as supported by previous finding [22]. Our results are inconsistent, however, with those of previous studies who showed either increased SOD levels in postmenopausal women with HRT [14] or decreased SOD levels in pancreas of diabetic rats [18]. These inconsistencies could be explained by difference in response between animal and human following honey supplementation.

The present study showed a reduction in plasma level of 4-HNE, a marker for lipid peroxidation, after 16 weeks of EPT and TH supplementation. This could be explained by the capability of both EPT and TH supplementation to increase the blood antioxidant capacity and offset oxidative stress as evidenced by the increased CAT and GPx activities.

The level of 4-HNE was earlier reported to be higher in postmenopausal women compared to fertile or premenopausal women [23]. The reduction in oxidative damage was evident by a decrease in 4-HNE level in the EPT group and this could be explained by direct effect of estrogen as antioxidant or its indirect effect via induction of GPx and CAT activities.

Our finding in TH group could be supported by several animal studies which showed reduction in lipid peroxidation after honey supplementation. Honey supplementation significantly reduced malondialdehyde (MDA) levels in the chemically induced colitis [24] and bile duct ligated [25] rats. In other studies, honey supplementation significantly reduced MDA levels and restored SOD and CAT activities in pancreas of diabetic rats [18] and prevented MDA formation in kidney of spontaneously hypertensive rats [26].

A study on buckwheat honey discovered that phenolic antioxidants from processed honey are bioavailable and could increase plasma antioxidant activity and augment defenses against oxidative stress [27]. These findings suggest that phenolic content of TH may also be bioavailable and responsible for its observed antioxidant effects as supported by a recent finding by Khalil and colleague [11] who revealed that irradiated TH had the highest total phenolic content among other types of Malaysian honey, the nearest to the total phenolic content in Manuka honey. In addition to its phenolic compounds, other constituents in honey may act synergistically and contribute to its antioxidant effects [28]. These include vitamins (C and E), enzymes (catalase, peroxidase, and glucose oxidase), flavonoids, carotenoids, and products of the Maillard reaction [28, 29]. Therefore, the results of the present study were the first to demonstrate that honey supplementation reduces blood oxidative stress comparable to that of EPT.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgment

This work was supported by Research University Grant 1001/PPSP/811083.

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