

EVALUATION OF A STANDARDIZED EXTRACT OF *GINKGO BILOBA* IN VITILIGO REMEDY

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Received: 3 September 2013, Revised and Accepted: 10 September 2013

ABSTRACT

Introduction Vitiligo is a common acquired, idiopathic skin disorder, this study was conducted to evaluate the effectiveness of *Ginkgo biloba* in patients with vitiligo. **Material and Methods** Fifty persons were included, twelve patients received *Ginkgo biloba* capsule (75mg) twice daily compared with twelve patients received placebo capsule for 8 weeks at Al-Kadhimiya Teaching Hospital. Twenty six healthy volunteers considered as control. Patient's history was taken and measurement of neutrophils percentage, serum Glutathione (Elleman methods), Malonaldehyde (Stocks and Dormandy methods), Interlukine -6 (Elisa) and Vitiligo area scoring index (VASI) at baseline, 4 and 8 weeks interval, as a parameters to indicate the impact of *Ginkgo biloba* on the disease.

Results and discussion showed significant decrease in serum interleukin-6 (S. IL-6) after 4 weeks between *Ginkgo biloba* group and placebo group. No significant difference was found in Neutrophils percent between *Ginkgo biloba* group and placebo group after 4 and 8 weeks. There was a significant increase in serum glutathione (S. GSH) after 4 and 8 weeks in *Ginkgo biloba*. Serum malondialdehyde (S. MDA) showed no significant difference between *Ginkgo biloba* group and placebo group after 4 and 8 weeks and no changes in VASI were found after 8 weeks between *Ginkgo biloba* and placebo group.

Conclusion *Ginkgo biloba* may has good activity in vitiligo treatment.

Keywords: *Ginkgo biloba*, vitiligo, antioxidant.

INTRODUCTION

Vitiligo is a common acquired, idiopathic skin disorder that characterized by one or more patches of depigmented skin because of loss of cutaneous melanocytes [1]. Vitiligo occurs in 1% of the world's population [2] and affects all races [3]. Different studies suggest that there are some genetic mechanisms that involved in the etiology of vitiligo [4], with a positive family history in at least 30% of cases [5]. The disease is unpredictable, and is often associated with periods of remission and exacerbation [6]. An evidence for increased oxidative stress in the entire epidermis of vitiligo patients is reported. [7]. Reactive oxygen species are formed always by many biological processes, and may be considered as a measure of biological inefficiency since they are formed by electron leakage from the membranes and inadequately coupled reactions [8]. Dammak and his research assistants found that antioxidant system are markedly decreased with increased oxidative stress in the blood of active vitiligo patients [9]. Khan and his assistants discovered oxidative system balance changing in vitiligo patients, that there are significant increased levels of malondialdehyde (MDA) and significant decreased levels of Superoxide dismutase (SOD), Glutathione peroxidase (GPx), vitamins C and E and total antioxidant activity in vitiligo patients when compared with controls [10]. *Ginkgo biloba* is a small tree native to China, but widely planted as an ornamental, and cultivated for health use in Korea, France, and the United States. [11]. *Ginkgo biloba* leaf extract was investigated for active constituents by Craig and Stitzel, they found that it contains 24% flavone glycosides and 6% terpene lactones [12].

Parsad points out that *Ginkgo* is known to have anti-inflammatory an immune-modulator, and antioxidant properties [13], thus potentially may affecting the oxidative stress mechanisms of vitiligo. The constituents of *Ginkgo biloba* have been shown to decrease oxidative stress in macrophages and endothelial cells [14]. This study is designed to evaluate the effectiveness of systemic *Ginkgo biloba* in patients with vitiligo and to measure the inflammatory parameters (Interleukin -6) and oxidative stress parameters (glutathione and malondialdehyde) in patients with vitiligo in comparison with healthy volunteers.

Patients and Methods

A prospective, randomized, single blind and placebo controlled designed was conducted in this study. The study was done in the Department of Dermatology and Venereology, Al-Kadhimiya Teaching Hospital between November 2011 and March 2012. The total number of persons sharing in this study was 52. Fifty persons were continued their treatment course successfully while two persons were unable to do so. All included subjects have consented to be enrolled in this study and approval of college council at Al-Nahrain Medical College was taken under order number 1093 in 1/12/2011. All patients were subjected to detailed examination including the general, physical and mainly the skin examination. The diagnosis was made clinically by dermatologist. For all the patients at the initial visit, baseline characteristics had been made and involve age, sex, medical history, family history and drug allergy.

Patients with a new onset (less than 2 years), localized small patches vitiligo of both sexes the affected body surface area of 10-20% with age range 18-58 years were included in this study. Along the course of treatment, each patient should satisfy three visits at 0, 4 and 8weeks. In each visit, the assessment of response of vitiligo lesion toward treatment was performed by using VASI (Vitiligo Area Scoring Index) calculation which is a quantitative parametric score. The total body VASI is calculated using a formula that includes contributions from all body regions [15].

VASI = All Body Sites

[Hand Units] × Residual Depigmentation [15]

A blood sample (5 ml) was obtained to determine Neutrophils percent, Serum Glutathione (S. GSH) (It is based on the reaction of aliphatic compounds with dithio 2-nitrobenzoic acid at ph 8.0. to give p-nitro thiophenol anion which is highly, colored at 412nm) [16], serum Malonaldehyde (S. MDA) (its measurement is based on the reaction of thiobarbituric acid with MDA forming a pink- colored adduct that its light absorbance measured at 535 nm) [17], and Interlukine -6 (IL-6) (Using ELISA, the microtiter plate provided in

the kit has been pre-coated with an antibody specific to IL-6). The patients were allocated into two groups and all the patients were given Vaseline and asked to apply it topically two times daily.

Group I: Included 12 patients (7 females and 5 males) were given *Ginkgo biloba* 1 capsule (75mg) two times daily orally with food for 8 weeks.

Group II: Included 12 patients (8 females and 4 males) were given sucrose as placebo capsule two times daily orally with food for 8 weeks.

Group III: Include 26 healthy subjects (17 females and 9 males) without treatment.

The experiment design used for these studies was Rationalized Complete Block Design (RCBD). The results were reported as means \pm standard deviation (SD).

Unpaired t-test and Wilcoxon Mann -Whitney test for the significance of difference done and was considered statistically significant if the *P* value was less or equal to 0.05 and highly significant if the *P* value was less or equal to 0.001 [18]; [19].

RESULTS

Table (1) shows the descriptive parameters of healthy subjects and patients in this study.

Table1: Descriptive parameters of healthy and patients with vitiligo

Parameters	Sex	patients(n=24)		control group(n=26)	
		Male/Female	Frequency	Frequency	%
			37.5/62.5	34.62/65.38	
		9/15		9/17	
Smoking	Yes/No	5/19	20.83/79.17	4/22	15.38/84.62
Other skin disease	Yes/No	12/12	50/50	0/26	0/100
Family history	Yes/No	12/12	50/50	0/26	0/100
Drug allergy	Yes/No	0/24	0/100	0/26	0/100

Table (2) shows a Significant difference in Neutrophils percent in patients with vitiligo in comparison with healthy group, while high significant were founded in IL-6, S. GSH and S. MDA in patients with vitiligo in comparison with healthy group.

Table2: Comparison between healthy volunteers and patients with vitiligo in relation to Neutrophils, IL-6, S. GSH, and S. MDA.

Parameters	Control (n=26) mean \pm SD	Patients (n=24) mean \pm SD	<i>P</i> value
Neutrophils % baseline	56.74 \pm 6.15	63.58 \pm 9.73	0.0042*
S. IL-6 (pg/ml) baseline	0.93 \pm 0.13	3.41 \pm 1.65	< 0.0001**
S. GSH (mmol/l) baseline	1.88 \pm 0.13	1.45 \pm 0.31	< 0.0001**
S. MDA (mmol/l) baseline	1.9 \pm 0.27	2.64 \pm 0.64	< 0.0001**

* Denote significant difference at *P* value \leq 0.05, ** Denote high significant difference at *P* value \leq 0.001

Table (3) and (4) indicate that both treated groups are comparable with no significant difference in the parameters at baseline (before treatments).

Table 3: The Effect of *Ginkgo biloba* on Neutrophils, S. IL-6, S. GSH and S. MDA concentration in patient with vitiligo in comparison with placebo-treated group

Parameters	Placebo (n=12) mean \pm SD	<i>Ginkgo biloba</i> (n=12) mean \pm SD	<i>P</i> value
Neutrophils % baseline	59.94 \pm 11.31	61.83 \pm 4.23	0.5945
Neutrophils % 4wk	60.46 \pm 11.19	59.97 \pm 6.63	0.897
Neutrophils % 8wk	61.23 \pm 10.95	59.78 \pm 6.66	0.6973
S. IL-6 (pg/ml) baseline	3.52 \pm 1.71	2.31 \pm 1.39	0.0693
S. IL-6 (pg/ml) 4wk	3.47 \pm 1.64	0.91 \pm 1.12	0.0002**
S. GSH (mmol/l) baseline	1.43 \pm 0.22	1.62 \pm 0.38	0.1598
S. GSH (mmol/l) 4wk	1.44 \pm 0.23	1.72 \pm 0.39	0.0391*
S. GSH (mmol/l) 8wk	1.44 \pm 0.23	1.81 \pm 0.4	0.0123*
S. MDA (mmol/l) baseline	2.58 \pm 0.21	2.73 \pm 0.16	0.0725
S. MDA (mmol/l) 4wk	2.58 \pm 0.2	2.42 \pm 0.4	0.2144
S. MDA (mmol/l) 8wk	2.65 \pm 0.24	2.38 \pm 0.38	0.0508

* Denote significant difference at *P* value \leq 0.05, ** Denote high significant difference at *P* value \leq 0.001

Table 4: Effects of *Ginkgo biloba* on clinical feature (VASI score) in patients with vitiligo in comparison with placebo-treated patients

Parameters	Placebo (n=12) mean \pm SD	<i>Ginkgo biloba</i> (n=12) mean \pm SD	<i>P</i> value
VASI baseline	3.75 \pm 2.81	6.42 \pm 4.08	0.069
VASI 4wk	3.75 \pm 2.81	6.42 \pm 4.08	0.069
VASI 8wk	3.88 \pm 2.77	6.17 \pm 4.27	0.126

Table (3) showed that there were no significant difference in Neutrophils percent between *Ginkgo biloba* group and placebo group at baseline, 4 weeks and 8 weeks; Table (3) showed high significant decrease in S. IL-6 was found after 4 weeks between *Ginkgo biloba* group and placebo group and there was a significant increase in S. GSH after 4 and 8 weeks in *Ginkgo biloba* group as shown in Table (3), while Table (3) showed the measurement of S. MDA indicate no significant difference between *Ginkgo biloba* group and placebo group after 4 and 8 weeks was recorded. Table (4) showed the clinical parameters, no significant difference in VASI between *Ginkgo biloba* group and placebo group after 4 and 8 weeks was found.

DISCUSSION

Vitiligo is an acquired pigmentary skin disease characterized by normal hyperpigmented border that contain inside a depigmented white patches [20]. The precise pathogenesis of vitiligo remains unknown although genetic, immune and oxidative stress mechanisms had been suggested recently [21]. In the current study significant increase in Neutrophils is recorded in patients with vitiligo and this is disagreeing with Aksoy *et al.* [22]. In agreement with the *in vitro* study, *in vivo* studies showed that Tissue necrosis factor- alpha, IL (interlukine)-1a, IL-18, IL-6, IL-8, growth related protein a (GRO a), macrophage inflammatory protein (MCP) -1 and IL-1 receptor antagonist (IL-1 Ra), affect the inflammation process and all of these cytokines are released from Neutrophils. [23]. This study showed that the serum level of Interleukin-6 is significantly elevated in patients with vitiligo when compared with healthy volunteer. This is in agreement with studies data of elevated serum level of interleukin-6 in patients with vitiligo [24]; [25], in addition to that when perilesional, non-lesion and healthy skin is investigated for epidermal IL-6, a significant change was founded in vitiligo skin, suggesting that the cytokine production in epidermal surroundings may be occurred. [26]. Moreover, IL-6 plays a role in inhibiting

melanocyte growth [27]. In a study conducted by Yu *et al.*, [25], Yu discovers that IL-6 production of mononuclear cells (MNC) to be significantly increased in vitiligo patients. This study showed the significant decrease level of GSH in patient with vitiligo in comparisons to healthy volunteers and this is agree with the reasonable to assertion of Shin *et al.* [28] that GSH is systemically depleted to counter free radicals. Park *et al.* [29] reported that, melanocyte apoptosis is under arrest by glutathione through inhibition of dopamine. Glutathione is a constituent of nonenzymatic antioxidant system and also it is a substrate for GSHPx. Passi *et al.* [30] showed that the epidermal GSH levels were significantly lower in active vitiligo patients when it is compared with controls. However the present study did not agree with Ozturk *et al.* [31] who showed that plasma glutathione levels did not change significantly in both types of it. Imbalance of Oxidative process can be induced by increased reactive oxygen species (ROS) production. Reactive oxygen species play an important role in cell signaling, but insufficient antioxidant level or increased ROS generation causes damage due to increase oxidant/antioxidant ratio. Reactive oxygen species can then induce a cytotoxic damage in melanocytes via either by initiation of melanocyte apoptosis or by changing melanocytic antigens [32]; [33]. Also the high significant increase in serum level of MDA in patient with vitiligo in compared to healthy volunteers is agree with those of Ozturk *et al.* [31], who discovered a significant increased in MDA, Hydroxyproline and GSHPx levels in plasma of vitiligo patients lead to that H₂O₂ generate free radicals and imbalances in oxidant/antioxidant ratio, which leads to oxidative stress, resulting in increased MDA levels, which is lipid peroxidation end product. Koca *et al.* [34] has also reported increased serum MDA levels in patients with vitiligo. Similar results have been reported by Ines *et al.* [35]. No Significant difference in Neutrophils in the current study is recorded in patients with vitiligo and this is agreeing with Aksoy *et al.* [22]. The current study showed high significant decrease in S. IL-6 in *Ginkgo biloba* treated patients after 4 weeks. This is in agreement with Ching-Hsiang *et al.* [15] who conclude that *Ginkgo biloba* extract used for neurological problems by down-regulation and suppression of IL-6. It was found in this study a significant increase in S. GSH after 4 and 8 weeks in *Ginkgo biloba* treated group, this is agree with Rimbach *et al.* [36] who conclude that when increase the dose of *Ginkgo biloba* extract, an increase in cellular GSH occurred in a human keratinocyte cell culture model, while in this study S. MDA showed no significant difference between *Ginkgo biloba* -treated group and placebo-treated group after 4 and 8 weeks and this is disagree with Zhou *et al.* [37] who conclude that *Ginkgo biloba* extract decrease the MDA level in trinitrobenzene sulfonic acid-induced colitis in rats in relation to increase the dose of *Ginkgo biloba* extract. Also Thanoon *et al.* [38] reported a significant decrease in serum levels of MDA in ischemic stroke patients that received *Ginkgo biloba* (1500 mg/day) for 30 days. The different dose and different pathogenesis use may explain the different results. This study found no significant difference in VASI between *Ginkgo biloba* -treated group and placebo-treated group after 4 and 8 weeks from starting treatment. This result disagree with Szczurko *et al.* [39] who conclude that the total VASI score showed a significant improvement during 12 week. This difference is probably due to the fact that in Szczurko *et al.* [39] study continues for 12 week and it is also possible that genetic, dietary and social differences between the populations of the two studies had an impact on the results. The study concludes that *Ginkgo biloba* may have promising activity in vitiligo remedy.

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