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ORIGINAL ARTICLE

Improved haemorrheological properties by *Ginkgo biloba* extract (Egb 761) in type 2 diabetes mellitus complicated with retinopathy

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Ginkgo biloba extract;
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Summary *Background & Aims*: Abnormal haemorrheological property changes in erythrocyte deformability, plasma and blood viscosity, and blood viscoelasticity may play very important roles in the development of microangiopathies in diabetes mellitus (DM). In this study, we demonstrate the improvement in abnormal haemorrheological parameters in DM with ingestion of *Ginkgo biloba* extract 761 (Egb 761).

Methods: Haemorrheological parameters before and 3 months after Egb 761 oral ingestion were determined in 25 type 2 DM patients with retinopathy. These parameters included lipid peroxidation stress of erythrocytes, erythrocyte deformability, plasma and blood viscosity, blood viscoelasticity, and retinal capillary blood flow velocity.

Results: After taking Egb 761 orally for 3 months, the blood viscosity was significantly reduced at different shear rates, by 0.44 ± 0.10 ($\gamma = 400$), 0.52 ± 0.09 ($\gamma = 150$) and 2.88 ± 0.57 ($\gamma = 5$). Viscoelasticity was significantly reduced in diabetic patients by 3.08 ± 0.78 (0.1 Hz). The level of erythrocyte malondialdehyde (MDA) was reduced by 30%; however, the deformability of erythrocyte was increased by 20%. And lastly, retinal capillary blood flow rate was increased from 3.23 ± 0.12 to 3.67 ± 0.24 cm min^{-1}.

Conclusion: In this preliminary clinical study, 3 months of oral administration of Egb 761 significantly reduced MDA levels of erythrocytes membranes, decreased fibrinogen levels, promoted erythrocytes deformability, and improved blood viscosity and viscoelasticity, which may facilitate blood perfusion. Furthermore, it effectively improved retinal capillary blood flow rate in type 2 diabetic patients with retinopathy.

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Introduction

Therapy of diabetic patients has centred on controlling blood sugar. However, diseases in peripheral circulation, retinopathy and foot ulcer, could still develop in the long run. In general, abnormalities of haemorrheological parameters in diabetic patients have usually been attributed to factors such as increments in plasma and blood viscosity,[1,2] erythrocyte aggregation,[2] erythrocyte rigidity (T_K),[3] and lipid peroxidation,[4,5] and reduction in the deformability of erythrocytes and leucocytes.[2,6,7]

Ginkgo biloba extract 761 (Egb 761) has been widely used to improve peripheral blood circulation.[8] It has been reported that Egb 761 could increase peripheral circulation,[9] decrease erythrocyte aggregation,[10] and enhance deformability of erythrocyte.[9] Furthermore, for Alzheimer's disease, Egb 761 has also been demonstrated to increase blood flow rate and cerebral blood perfusion[11] and even has been ingested in diabetes mellitus DM based on the same rationale. However, some critical issues need to be considered if the Egb 761 is given; first of all, how does Egb 761 improve erythrocyte deformability; secondly, what are the effects of Egb 761 on blood viscosity and viscoelasticity; and thirdly, how the blood viscosity and the viscoelasticity of diabetic patients correlate to retinal capillary blood flow velocity. Unfortunately, there is no report yet regarding the change in haemorrheologic parameters in diabetic patients. Thus, this study was conducted to determine the effectiveness of the Egb 761 for 3 months in type 2 diabetic patients. The assessments of whole blood viscosity of steady flow and viscoelasticity of oscillatory flow, erythrocyte deformability and oxygen transport efficiency (T_E) of erythrocytes in vitro were examined. The retinal capillary blood flow velocity also was examined by the Scanning Laser Ophthalmoscope (SLO) to evaluate the improvements of peripheral circulation in diabetic patients after *Egb 761 administration*.

Subjects and methods

Patients

This open-labelled self-controlled pilot study was conducted from March to August of 2002 in an ophthalmology clinic. Subjects were outpatients of the Department of Ophthalmology, Taipei Municipal Ho-Ping Hospital in Taipei, Taiwan. Subjects diagnosed with type 2 DM by the 1998 American Diabetes Association (ADA) guideline, aged from 54 to 64 years old and followed up for diabetic retinopathy, were enrolled in this study. Oral hypoglycaemic agents (5 mg Euglucon and 500 mg Glucophage 1# t.i.d) were given for each type 2 DM patient and all type 2 DM patients were under well diabetic controlled. The exclusion criteria include history of hypertension, other systemic diseases, hypersensitivity to the test drug, and not being able to comply with the study protocol.

All subjects gave informed consent for this study. Haemorrheological characteristics and biochemical measurements were determined at the baseline. Each patient received a daily dose of 240 mg Egb 761 (three times with two film-coated tablets, 40 mg Egb 761/per tablet). A film-coated table of the active product contains 40 mg of dry extract of *Ginkgo biloba* leaves, adjusted to 9.6 mg *Ginkgo* flavone glycosides and 2.4 mg terpene lactones (ginkgolides, bilobalide). The baseline measurements were repeated after 3 months of active treatment.

Methods

Haematological measurements

Fresh blood samples were collected from patients by venipuncture into EDTA-contained test tubes. Blood cell counts and other haematological data such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haematocrit (Hct), etc. were determined by an automatic cell counter (SYSMEX NE-800, TOA Medical Electronic Co., Kobe, Japan). Plasma was separated under centrifugation at 1500*g* for 10 min. Plasma fibrinogen and glycosylated haemoglobin (HbA_{1c}) were determined by the thrombin clot technique[12] and by agar gel electrophoresis,[13] respectively.

Haemorrheological measurements

Plasma and blood viscosity were measured using a Rheostress 1 double cone viscometer (HAAKE Mess-Technik, Karlsruhe, Germany), with a cone angle of 1° at 310 K. The serial blood viscosity under various shear rates was determined via a computer controlled testing program. We provide data measured at shear rates of 400, 150, and 5 s^{-1} that reflect high, medium, and low shear rates, respectively.

In terms of the viscoelastic properties of blood, the viscoelasticity of blood was tested in an oscillatory mode. The oscillatory shear force was

set at a constant 0.01 Pa and the frequencies ranged from 0.3 to 0.1 Hz.

For erythrocyte deformability, we used constant flow rate filtration methods to prepare the erythrocyte suspensions.[14,15] After being separated from plasma by centrifuging the sample at 1500*g* for 10 min, erythrocytes were washed three times in PBS. And then, erythrocyte suspensions at 5% Hct, in which leucocyte concentrations were usually less than 100 cells mm^{-3}, were filtered through a 5 μm pore size Nuclepore® membrane with a disc diameter of 13 mm and an effective area of 0.8 cm^2 at a constant flow rate of 1.6 ml min^{-1}.

The pressure–time data were measured with a pressure transducer (Model DP45, Validyne Engineering Corp, Northridge, USA) connected to a Validyne digital transducer indicator (Model CD-23). The continuous output data of the indicator were digitized and recorded on a computer. Recorded data were played back off-line, and P_o values for ringer solutions and P_i values for erythrocyte suspensions were determined as reported.[14] β values were calculated using the data of P_i/P_o and were indexed as the resistance of erythrocytes when flowing through the pores. The value of $1/\beta$ was selected as an index of erythrocyte deformability. Erythrocyte rigidity (T_K) was calculated at a shear rate of 400 s^{-1} by the equation of Dintenfass.[16] Furthermore, the oxygen transport efficiency (T_E) of the blood was calculated as the ratio of the Hct to blood viscosity at a fixed shear rate.[17]

Erythrocyte malondialdehyde (MDA) analysis

To measure the oxidative stress of erythrocyte membranes, the level of MDA, a product of lipid peroxidation which reacts with thiobarbituric acid (TBA), was examined by determining the quantities of the MDA–TBA complex at 532 nm with a spectrophotometer (Hitachi U2000, Hitachi Corp. Japan);[18] the detailed preparation procedures for measuring the MDA–TBA complex are described elsewhere.[19] Quantities of MDA presented in the results are based on 10^{10} erythrocytes.[19] Both biochemical analyses were determined in a blinded manner.

Retinal capillary blood flow velocity measurements

Retinal capillary blood flow velocity was examined by using a scanning laser technique.[20,21] Video fluorescent angiography with an SLO (Rodenstock SLO 101, Rodenstock Instrument Corp., Germany) was performed on all patients. The video image was generated and recorded in digital form by a frame grabber and was saved in an image storage unit. The blood flow velocity in capillaries was calculated off-line using frame-to-frame analysis. Detailed descriptions of the method are available elsewhere.[21] The retinal capillary blood flow velocity for each patient was a mean value of velocities of four different perifoveal vessels in different quadrants.

Statistical analysis

Data are presented as the mean ± SD. All data were normally distributed; pre- and post-treatment data were compared by paired *t*-tests ($\alpha = 0.05$). Linear regressions with higher than a 95% confidence level were also calculated. All calculations were analysed by SigmaStat® Statistical Software (Jandel Scientific, San Rafael, CA, USA).

Results

Twenty-five (14 males, 11 females) type 2 DM patients who had neither a history of nor evidence of systemic hypertension, but only background retinopathy. Retinopathy was diagnosed by opthalmoscopic and fluorescent angiographic examinations. Their mean weight was 72 ± 5 kg (male), 58 ± 4 kg (female) and mean age was 60 (range, 54–64) years with a mean duration of diabetes of 8 (range 6–11) years and patients had a mean HbA_{1c} of 5.1 ± 0.3%.

There were no significant changes in haemoglobin contents, Hct, and HbA_{1c} in these type 2 DM patients after taking 3 months of oral Egb 761 (Table 1). Moreover, except for a significant improvement in fibrinogen levels, neither total proteins nor lipoproteins significantly differed after treatment (Table 1).

However, erythrocyte rigidity T_K (or internal viscosity of red cells), and plasma viscosity were decreased and blood viscosity was reduced at all tested shear rates after treatment (Table 2). Furthermore, both the dynamic viscosity (η') and elasticity (η'') of the blood viscoelasticity had significantly improved after treatment (Table 2). In terms of lipid peroxidation stress of erythrocyte membranes, the results indicate that there was a significant reduction in MDA of erythrocyte membranes after treatment (Table 2). As for erythrocytes deformability, we used the flow resistance (β) model, and results indicate that the£value significantly decreased (i.e. erythrocyte deformability index increased by $1/\beta$) after treatment. This phenomenon might associate with the decrease in erythrocyte rigidity and MDA in erythrocyte membranes.

To assess peripheral circulation in type 2 DM patients, SLO video fluorescent angiography was used to observe retinal capillary blood flow velocity. The results indicate that retinal capillary blood flow velocity in diabetic patients increased from 3.2 to 3.7 mm s^{-1} after treatment (Table 2). Lastly, the oxygen transport efficiency (T_E) of blood, we found that the T_E value in type 2 DM patients was increased at high, medium, and low shear rates (Table 2).

Discussion

Impairment of haemorrheological properties has been documented in diabetes.[1–7] Some studies reported abnormalities in erythrocyte membrane properties of diabetic patients.[2,3,6] The concurrence of blood abnormality was partially attributable to lipid peroxidation stress or reduced vitamin E content of cell membranes. The MDA is one of lipid peroxidation products and may play a role as an oxidative trigger that contributed to the haemorrheological alternation. Concerning the relationship between MDA values and erythrocyte deformability in diabetic patients, it has been reported that higher HbA_{1c} may increase the quantities of phospholipid–MDA adduction, and that HbA_{1c} levels were inversely correlated with erythrocyte deformability in diabetic patients.[22] However, in our study, there were no significant changes in HbA_{1c} after the supplementation; the range of

Table 1 Haematogical parameter of type 2 DM patients before and after *Egb 761* supplementation.

Parameter	Before treatment	After treatment	Differences	Paired *t*-test *P* value
MCV (f1)	90.90±0.95	90.85±0.99	−0.05±0.38	NS
MCHC (g dl^{-1})	33.17±1.18	33.10±1.25	−0.06±0.32	NS
Hct (%)	42.25±1.27	42.11±1.17	−0.14±1.21	NS
Globulin (mg l^{-1})	3.43±0.09	3.43±0.08	0.00±0.05	NS
Albumin (g l^{-1})	4.39±0.09	4.41±0.08	0.01±0.05	NS
Fibrinogen (mg l^{-1})	326.3±42.3	283.6±25.5	−42.7±23.6	<0.001
HBA_{1c} (%)	5.04±0.29	5.06±0.22	0.01±0.13	NS

Results are mean±SD ($n = 25$). Differences between before and after treatment determined by *t*-test. Significantly different from control, $P < 0.001$.
NS: non-significant.

Table 2 Haemorrheological characteristics and oxygen transport efficiency of blood, MDA of erythrocyte membranes, and retinal capillary blood flow velocities for the pre- and post-treated on type 2 DM.

Parameter	Before treatment	After treatment	Difference	Paired *t*-test *P* value
η plasma (cp)	1.31±0.02	1.23±0.03	−0.08±0.03	<0.05
η blood (cp)[a] ($\gamma = 400\,S^{-1}$)	4.32±0.04	3.88±0.11	−0.44±0.10	<0.05
η blood (cp)[a] ($\gamma = 150\,S^{-1}$)	4.74±0.05	4.22±0.07	−0.52±0.09	<0.05
η blood (cp)[a] ($\gamma = 5\,S^{-1}$)	11.23±0.37	8.35±0.57	−2.88±0.57	<0.01
η' blood (cp)[b]	11.54±0.46	8.46±0.89	−3.08±0.79	<0.01
η'' blood (cp)[b]	3.65±0.07	3.13±0.33	−0.52±0.35	<0.05
β	1.41±0.06	0.96±0.10	−0.45±0.12	<0.01
MDA ($\times 10^{10}$ nmol $cell^{-1}$)	4.89±0.07	3.97±0.14	−0.92±0.16	<0.05
Blood flow (mm s^{-1})	3.23±0.12	3.67±0.24	0.44±0.24	<0.05
T_K ($\gamma = 400\,S^{-1}$)	0.90±0.03	0.89±0.03	−0.01±0.04	NS
T_K ($\gamma = 150\,S^{-1}$)	0.96±0.03	0.94±0.03	−0.02±0.04	<0.05
T_K ($\gamma = 5\,S^{-1}$)	0.96±0.03	0.94±0.03	−0.02±0.04	<0.05
T_E ($\gamma = 400\,S^{-1}$)	9.81±0.31	10.60±0.43	0.79±0.49	<0.05
T_E ($\gamma = 150\,S^{-1}$)	8.81±0.33	9.74±0.36	0.93±0.39	<0.05
T_E ($\gamma = 5\,S^{-1}$)	3.76±0.25	4.78±0.67	1.02±0.59	<0.01

Results are mean±SD ($n = 25$). Differences between before and after treatment determined by *t*-test. Significantly different from control, $P < 0.05$. NS: non-significant.
η': the whole blood dynamic viscosity; η'': the whole blood elasticity viscosity; γ: shear rate.
[a]The steadily flow model of blood.
[b]The oscillatory flow model of blood (0.1 Hz).

HbA_{1c} is an index of blood glucose levels. Therefore the stable glucose levels could have played a minor role in improving erythrocyte deformability.

From the nutrition point of view, vitamin E, an antioxidant located in the cellular membrane structure, plays an important role by acting as a physiological antioxidant and membrane stabilizer. Malnutrition of vitamin E reduces erythrocyte deformability and survival, as well as increases susceptibility to oxidan.t damage and adhesiveness.[23] In patients with chronic diabetes, long-term hyperglycaemia may result in an increase in both lipid peroxidation in erythrocyte membranes[19] and oxidation of membrane spectrin,[22] and the oxidative stress may cause abnormalities in erythrocyte membranes and alter the haemorrheological properties of erythrocytes.[24] We have found blood vitamin E concentration declined in DM patients received placebo in an ongoing 13-week vitamin E interventional study; furthermore, the activity of erythrocyte glutathione peroxidase in DM patients showed inversed correlation to RBC vitamin E content (unpublished data). Sushil et al.[25] demonstrated that supplementation with vitamin E ($100\,IU\,day^{-1}$) significantly increased glutathione and lowered lipid peroxidation in erythrocytes of type 1 diabetic patients. In our study, we observed that oral ingestion of Egb 761 significantly reduced the level of MDA at the cell membrane of erythrocytes. These phenomena implied that the lipid oxidation in type 2 DM patients could be lowered by the Egb 761 ingestion attributed to its antioxidative property.

In this study, we correlated the erythrocyte deformability index ($1/\beta$) with the value of MDA before and after treatment. The data demonstrated that there was an inverse correlation between $1/\beta$ and the MDA value (Fig. 1). These results showed a solid correlation between erythrocyte deformability and lipid peroxidation stress of erythrocyte membranes in type 2 DM patients. Meanwhile, the results also indicated the erythrocyte T_K value significantly decreased after treatment. Thus, we logically summarize that the improvement in erythrocyte deformability might attribute to molecular structure of Egb 761 that presents antioxidative capability, such as the radical scavenger effectiveness[26–28] and the transited metal ion quenching property[29] and improves the internal viscosity of red cells in type 2 DM patients.

Concerning the effect of Egb 761 on blood viscosity, Witte's study reported that Egb 761 decreased blood viscosity in type 2 DM patients with arterial circulatory disorders.[30] In order to discuss the effects of Egb 761 on blood viscosity and viscoelasticity, we designed two in vitro blood flow models such as steady flow and oscillatory flow study. In steady flow assessment, blood viscosity of diabetic patients decreased under high, medium, and low shear rates after ingestion. It implied that the decrease of blood viscosity should highly relate to erythrocyte rheological property, especially in lipid profile characteristics, e.g. the membrane integrity. Under a high shear rate, the decreases of blood viscosity might attribute to the decreases of erythrocyte rigidity and the increases of erythrocyte deformability, vice versa under a low shear rate. Therefore, the lowering blood viscosity in treated subjects highly correlated to decreases in erythrocyte aggregation and decreasing fibrinogen levels in plasma may play a critical factor (Table 1).

Limited literature in oscillatory blood flow of diabetic patients is available.[31–33] Thus, in order to obtain a better illustration of interaction between blood stream and stimulator in vivo, we designed an oscillatory assessment to illustrate the simulation of blood stream. In this test, we measured the blood viscoelasticity (dynamic viscosity and dynamic elasticity) in diabetic patients under oscillatory shear force at the constant 0.01 Pa and with different frequencies which ranged from 0.3 to 0.1 Hz. In general, the viscoelasticity of blood is primarily determined by the aggregation and disaggregation of erythrocytes at low shear rates. The parameters η' (dynamic viscosity) and η'' (dynamic elasticity) of blood reflect the ability of erythrocytes to aggregate and adjust their shape in low shear flow rate and the elastic properties of erythrocytes as they aggregated, respectively. It is possible to obtain qualitative information on blood when it flows in large vessels in pulsation,[33] and of rouleaux formation in microcirculation. Our studies not only decreased the value of η' and η'' but also reduced the status of erythrocyte aggregation after Egb 761 treatment in type 2 DM patients (Table 2). Based on these data, we logically hypothesized that the Egb 761 improves the fibrinogen levels in plasma, decreases the interaction between erythrocytes and fibrinogen, and avoids the erythrocytes aggregation in blood stream.

According to the results of the SLO study, an increasing trend of retinal capillary blood flow velocity was observed after Egb 761 treatment. One of the possible reasons for these results could be the reduction of the blood viscosity and viscoelasticity or the induction of the extension of the blood vessels. Clinically, Hct is a useful factor to judge the oxygen capacity in a certain blood unit. In our study, the result indicates no significant difference in Hct levels after treatment (Table 1).

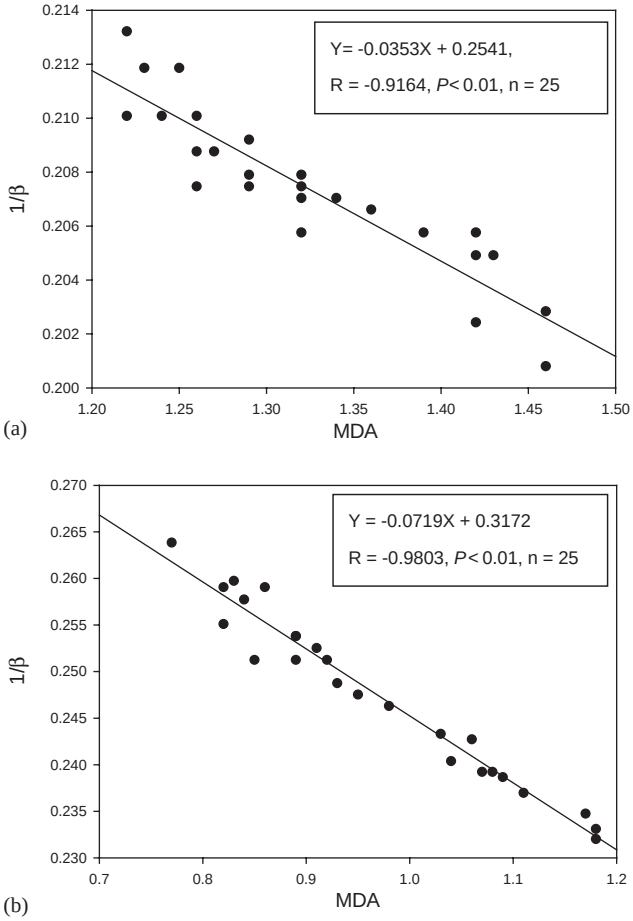

Figure 1 Linear correlation between the erythrocyte deformability index ($1/\beta$) and MDA of erythrocyte membranes for type 2 DM patients before (a) and after (b) treatment.

However, since erythrocyte deformability was increased and the viscosity was decreased after treatment, these factors may further enhance the oxygen transport efficiency of blood to tissues. Therefore, enhancement of the retinal capillary blood flow velocity and oxygen transport efficiency of blood can decelerate the deterioration of retinopathy or microangiopathy in DM.

In our study, we concluded that 3 months of oral ingestion of Egb 761 for type 2 DM patients with retinopathy can reduce the MDA (i.e. reduce lipid peroxidation stress) of erythrocyte membranes, fibrinogen levels, and plasma viscosity, and increase erythrocyte deformability, which improves the blood viscosity and viscoelasticity and further increases blood flow velocity and the T_E value of the blood. These factors could all contribute to improving or lowering peripheral circulation disorders among diabetic patients. Therefore, we believe that providing Egb 761 to type 2 DM patients could result in a meaningful impact on their blood circulation and reduction of retinopathy occurrence.

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References

1. Ziegler O, Guerci B, Muller S, et al. Increased erythrocyte aggregation in insulin-dependent diabetes mellitus and its relationship to plasma factors: a multivariate analysis. *Metabolism* 1994;**43**:1182–6.
2. Tsukada K, Sekizuka E, Oshio C, Minamitani H. Direct measurement of erythrocyte deformability in diabetes mellitus with a transparent microchannel capillary model and high-speed video camera system. *Microvascular Res* 2001;**61**:231–9.
3. Dintenfass L. Blood viscosity factors in severe non-diabetic and diabetic retinopathy. *Biorheology* 1977;**14**:151–7.
4. Kesavulu MM, Rao BK, Giri R, Vijaya J, Subramanyam G, Apparao C. Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease. *Diabetes Res Clin Pract* 2001;**53**:33–9.

5. Sekeroglu MR, Sahin H, Dulger H, Algun E. The effect of dietary treatment on erythrocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase, and serum lipid peroxidation in patients with type 2 diabetes mellitus. *Clin Biochem* 2000;**33**:669–74.
6. Petit KI, Hunt WB, George SJ, Barnes AJ. Is impaired red cell filtration in diabetes due to a small abnormal sub-population of cells? *Clin Hemorheol* 1996;**16**:479–85.
7. Ernst E, Matrai A. Altered red and white blood cell rheology in type II diabetes. *Diabetes* 1986;**35**:1412–5.
8. Clostre F. Protective effects of a *Ginkgo biloba* extract (EGb761) on ischemia-reperfusion injury. *Therapie* 2001;**56**: 595–600.
9. Jung F, Mrowietz C, Kiesewetter H, Wenzel E. Effect of *Ginkgo biloba* on fluidity of blood and peripheral microcirculation in volunteers. *Arzneim-Forsch/Drug Res* 1990; **40**:589–93.
10. Plotnikov MB, Aliev OI, Vasil'ev AS, Maslov Miu, Zibareva LN, Dmitruk SE, Kalinkina GI. The hemoreological effects of *Lychnis chalcedonica* L. extracts. *Eksp Klin Farmakol* 2000;**63**:54–6.
11. Itil TM, Eralp E, Ahmed I, Itil KZ. The pharmacological effects of *Ginkgo biloba*, a plant extract, on the brain of dementia patients in comparison with tacrine. *Psychopharmacol Bull* 1998;**34**:391–7.
12. Rampling MW, Gaffney PJ. The sulfate precipitation method for fidrinogen measurement. *Clin Chim Acta* 1976;**67**:43–52.
13. Menard L, Dempsey ME, Blankstein LA, Aleyassine H, Wacks M, Soeldner JS. Quantitative determination of glycosylated hemoglobin A1 by agar gel electrophoresis. *Clin Chem* 1980; **26**:1598–602.
14. Schmaizer EA, Skalak R, Usami S, Vago M, Chen S. Influence of red cell concentration on filtration of blood cell suspensions. *Biorheology* 1983;**20**:29–40.
15. Chung TW, O Rear EA. Assessing erythrocyte filterability with 3 μm pore size polycarbonate membrane at constant cell flux. *Clin Hemorheol* 1990;**10**:505–14.
16. Dintenfass L. Problems associated with definition of plasma viscosity and effect volume of red cells in blood viscoity equation. *Biorheology* 1975;**12**:1480–6.
17. Chien S. Present state of blood rheology. In: Messmer K, Schmid-Schmid SH, editors. *Hemodilution. Theoretical basis and clinical application*. Basel: Karger; 1972. p. 1–45.
18. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br J Haematol* 1971;**20**:95–111.
19. Jain SK, Mcvie R, Duett J, Herst JJ. Erythrocyte membrane lipid peroxidation and glycoslyated hemoglobin in diabetes. *Diabetes* 1989;**38**:1539–43.
20. Plesch A, Klingbeil U, Bille J. Digital laser scanning fundus camera. *Appl Opt* 1987;**26**:1480–6.
21. Wolf S, Arend O, Toonen H, Bertram B, Jung F, Reim M. Retinal capillary blood flow measurement with a scanning laser ophthalmoscope. *Ophthalmology* 1991;**98**: 996–1000.
22. Schwartz RS, Madsen JW, Rybicki AC, Nagel RL. Oxidation of spectrin and deformability defects in diabetic erythrocytes. *Diabetes* 1991;**40**:701–8.
23. Tamai H, Miki M, Mino M. Hemolysis and membrane lipid changes induced by xanthine oxidase in vitamin E deficient erythrocytes. *Indian J Biochem Biophys* 1986;**21**: 361–4.
24. Chung TW, Yu-Hau JJ, Liu DZ. Reducing lipid peroxidation stress of erythrocyte membrane by α-tocopherol nicotinate plays an important role in improving blood rheological properties in type 2 diabetic patients with retinopathy. *Diabetes Med* 1998;**15**:380–5.
25. Sushil KJ, Robert M, Jiney S. Vitamin E supplementation restores glutathione and malondialdehyde to normal concentrations in erythrocytes of type 1 diabetic children. *Diabetes Care* 2000;**23**:1389–94.
26. Xin W, Wei T, Chen C, Ni Y, Zhao B, Hou J. Mechanisms of apoptosis in rat cerebellar granule cells induced by hydroxyl radicals and the effects of EGb761 and its constituents. *Toxicology* 2000;**148**:103–10.
27. Chen JX, Chen WZ, Huang HL, Chen LX, Xie ZZ, Zhu BY. Protective effects of *Ginkgo biloba* extract against lysophosphatidylcholine-induced vascular endothelial cell damage. *Chung-Kuo Yao Li Hsueh Pao- Acta Pharmacol Sini* 1998;**19**: 359–63.
28. Christen Y. Oxidative stress and alzheimer disease. *Am J Clin Nutr* 2000;**71**:621–9.
29. Dumont E, D'Arbigny P, Nouvelot A. Protection of polyunsaturated fatty acids against iron-dependent lipid peroxidation by a *Ginkgo biloba* extract (EGb761). *Methods Find Exp Clin Pharmacol* 1995;**17**:83–8.
30. Witte S, Anadere I, Walitza E. Improvement of hemorheology with *Ginkgo biloba* extract. Decreasing a cardiovascular risk factor. *Fortschri Med* 1992;**110**:247–50.
31. Schneditz D, Ribitsch V, Kenner T. Rheological discrimination between native, rigid and aggregated red blood cells in oscillatory flow. *Biorheology* 1985;**22**:209–19.
32. Thurston GB. Rheological parameters for the viscosity, viscoelasticity and thixotropy of blood. *Biorheology* 1979; **16**:149–62.
33. Stoltz JF, Lucius M. Viscoelasticity and thixotropy of human blood. *Biorheology* 1981;**18**:453–73.