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**Thrombosis preventive potential of chicory coffee
consumption: a clinical study**

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ABSTRACT

Protective effects of plant polyphenol intake on cardiovascular morbidity and mortality are widely acknowledged. Caffeine-free chicory coffee is a rich source of plant phenolics, including caffeic acid, which inhibits in vitro platelet aggregation, and also phenylpyruvate tautomerase enzymatic activity of the proinflammatory cytokine, macrophage migration inhibitory factor (MIF). To assess whether chicory coffee consumption might confer cardiovascular benefits we performed a clinical intervention study with 27 healthy volunteers, who consumed 300 mL chicory coffee every day for one week. The dietary intervention produced variable effects on platelet aggregation, depending on the inducer used for the aggregation test. Whole blood and plasma viscosity were both significantly decreased, along with serum MIF levels, after one week of chicory coffee consumption. Moreover, significant improvements were seen in red blood cell deformability. No changes in hematocrit, fibrinogen level or red blood cell counts were detected.

The full spectrum of these effects is unlikely to be attributable to a single compound present in chicory coffee, nevertheless, the phenolics, including caffeic acid, are expected to play a substantial role. In conclusion, our study offers an encouraging starting-point to delineate the antithrombotic and antiinflammatory effects of phenolic compounds found in chicory coffee.

INTRODUCTION

Diverse biological effects of plant polyphenols have been revealed in countless papers published throughout the last decades. Epidemiological studies suggest that consumption of polyphenol-rich food might impede the onset and the progression of certain human diseases (Kris-Etherton *et al.*, 2002). Phenolic compounds are abundant in fruits, vegetables, coffee, wine and olive oil (Huang *et al.*, 1986). The favourable effects of moderate red wine consumption on certain cardiovascular risk factors have been documented (Fuhrman *et al.*, 1995). The possible benefits of coffee, the other widely popular polyphenol-rich beverage, still await clarification. The risk of acute coronary syndromes was found to follow a J-shaped trend dependent on the dose of coffee consumed per day (Panagiotakos *et al.*, 2003). The upsurge with higher doses might be attributable to elevated blood pressure caused by the caffeine content of coffee brewed from *Coffea arabica* beans. Nevertheless, moderate consumption conferred decreased risk compared with non-consumers. The antithrombotic effect of coffee has been reported to be independent from its caffeine, but rather to be attributable to its phenolic acids able to incorporate into platelets (Natella *et al.*, 2008). The influence of coffee consumption on lifetime cardiovascular risk has been analyzed recently in two large cohorts. A dose dependent protective effect has been found that seems to be more pronounced in female consumers (Lopez-Garcia *et al.*, 2008; Lopez-Garcia *et al.*, 2009). These reports also found the protective effect independent from caffeine.

A cup of coffee is an abundant source of chlorogenic acid, i.e. caffeic acid esterified with quinic acid. Caffeic acid becomes available for absorption following hydrolysis. Known to be absorbed almost totally (~95%) from the small intestine caffeic

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4 acid reaches its peak blood level within 2 hours after oral intake (Olthof *et al.*, 2001;
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7 Nardini *et al.*, 2002).

8
9 Macrophage migration inhibitory factor (MIF) has been the first non-
10
11 immunoglobulin immune-mediator described (Bloom and Bennett, 1966; David, 1966).
12
13 The basal serum concentration of this proinflammatory cytokine is 3-5 ng/mL (Metz
14
15 and Bucala, 1997). Its level rises in infectious or non-infectious inflammatory
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17 conditions such as sepsis (Bernhagen *et al.*, 1993) or rheumatoid arthritis (Onodera *et*
18
19 *al.*, 1999). Enzymatic tautomerase (Rosengren *et al.*, 1996; Rosengren *et al.*, 1997) and
20
21 thiol-protein oxidoreductase (Kleemann *et al.*, 1998) activities of MIF have been
22
23 revealed recently. Certain antiinflammatory phytochemicals inhibit the tautomerase
24
25 activity in a concentration dependent manner (Molnar and Garai, 2005). Among these
26
27 molecules caffeic acid exhibits one of the best inhibitory potential concerning either
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29 phenylpyruvate- or dopachrome- tautomerase activities (Molnar and Garai, 2005; Senter
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31 *et al.*, 2002). Although the exact role of the enzymatic activity of this cytokine remains
32
33 to be delineated MIF tautomerase has already attained a reputation as a promising
34
35 pharmacologic target in inflammatory conditions (Lubetsky *et al.*, 2002). Caffeic acid
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37 and its derivatives have been reported to inhibit platelet aggregation *in vitro* and *in vivo*
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39 (Hung *et al.*, 2005; Hsiao *et al.*, 2007) – an effect confirmed in our laboratory as well.
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47 Chicory (*Cichorium intybus*) is one of the richest dietary sources of caffeic acid
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49 and its derivatives e.g. chlorogenic acid. An alcoholic (40%) extract (1:5) of the whole
50
51 plant has been reported to contain 12.98 ± 0.06 g% of caffeic acid derivatives (Kocsis *et*
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53 *al.*, 2003). Of note, coffee brewed from ground roasted chicory roots does not contain
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55 caffeine and has a long history being used as coffee substitute or admixture (Galasko *et*
56
57 *al.*, 1989). In our present study we have addressed the clinical effects of chicory coffee
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4 consumption on platelet aggregation, on hemorheological factors and on serum MIF
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6 levels.
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10 11 **MATERIALS AND METHODS** 12 13

14 15 16 **Study population** 17

18 Our clinical study has been approved by the local Regional Ethical Committee
19 according to the actual amended version of the World Medical Association Declaration
20 of Helsinki. Written informed consent has been obtained from all participants.
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22
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24

25 In our self-controlled study 27 healthy volunteers (13 women, 14 men) were
26 recruited (mostly university students). The average age was 23 ± 0.4 years. Five of them
27 were smokers and 17 of them were habitual coffee drinkers. The volunteers were asked
28 to refrain from consuming (Arabic) coffee or tea one week before and during the whole
29 period of the study, because these also contain caffeic acid. If it became unavoidable
30 caffeine pills were provided. No vitamins or drugs were allowed to be taken one week
31 before and during the study. The volunteers were also asked to refrain from alcohol
32 consumption.
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47 **Intervention and sample collection** 48

49 The volunteers consumed 300 mL coffee in the morning each day prepared from
50 20g (3 tablespoons) ground chicory coffee throughout the one week study period. We
51 aimed to observe the effects of both, a single dose and a week long daily consumption
52 of chicory coffee on the following parameters: platelet aggregation, hemorheological
53 factors, fibrinogen and MIF concentration. Blood samples were collected from 27
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4 volunteers on the first day just before and 2 hours after the first coffee consumption in
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6 fasting conditions. 24 volunteers also donated blood samples on the eighth day
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8 following one week daily chicory coffee consumption. The remaining 3 participants
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10 were dropouts. They were unable to consume daily the requested amount of coffee
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12 because of taste aversion.
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18 19 **Statistical analysis**

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21 Results are expressed as mean \pm SEM. All parameters were normally distributed.
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23 Statistical comparisons between 2 time points were performed by one-way Student's t-
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25 test. The ANOVA Repeated Measures analysis was applied if the parameter was
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27 measured at 3 different time points during the study. *p*-values < 0.05 were accepted as
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29 statistically significant.
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35 36 **Platelet aggregation**

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38 Blood samples were collected from cubital veins in tubes containing 3.8% sodium
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40 citrate, and then incubated at 37°C for 20 minutes. Platelet-rich plasma was prepared
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42 from venous blood by centrifugation at 900g for 10 minutes. After carefully removing
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44 platelet-rich plasma, the remaining specimen was further centrifuged at 3600g for 10
45
46 minutes to obtain platelet-poor plasma. Platelet aggregation was measured in Carat
47
48 TX-4 platelet aggregometer (Carat Ltd., Hungary). Samples were incubated at 37°C and
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50 continuously stirred at 1000 rpm during the measurement. 50 μ L of adenosine
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52 diphosphate (ADP) (5 and 10 μ M), collagen (2 μ g/mL) or epinephrine (10 μ M) was
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54 added to the platelet-rich plasma to induce platelet aggregation. All measurements were
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56 carried out within 2 hours after vein puncture.
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Whole blood and plasma viscosity

Blood samples were collected in tubes containing lithium heparin as anticoagulant. Plasma and whole blood viscosity values were determined with Hevimet 40 capillary viscometer (Hemorex Ltd; Hungary) at 37°C within 2 hours after blood sampling. In this viscosimeter the flow of whole blood (or plasma) is detected optoelectronically along the capillary tube. Apparent whole blood viscosity values were obtained at 90 1/s shear rate (Toth *et al.*, 1994). Plasma fibrinogen concentration was determined by von Clauss's method.

Red blood cell deformability

Red blood cell (RBC) deformability was determined at various shear stresses by laser diffraction analysis, using an ektacytometer, LORCA (Laser-assisted Optical Rotational Cell Analyzer, RR Mechatronics, Hoorn, Netherland). The system has been described elsewhere in details (Hardeman *et al.*, 1994). Twenty five µl of EDTA-anticoagulated blood was suspended in a PBS solution of PVP (Poly-Vinyl-Pyrrolidone 360 kD, Sigma-Aldrich, Budapest, Hungary). The viscosity of the PVP solution was 30 ± 2 mPa x s, all measurements were performed at 37°C (Johnson, 1989). Elongation indexes were calculated from the diffraction patterns for shear stresses between 0.3-30 Pa: a higher elongation index indicates greater red blood cell deformation.

Serum MIF levels

Blood samples were collected in tubes without anticoagulant. Serum MIF levels were assessed by ELISA with a Duo set ELISA Development System from R&D

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4 Systems, Minneapolis, MN, US according to the manufacturer's instructions. The
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6 amount of MIF was expressed as pmol/mL.
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10 11 **RESULTS**

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16 Caffeic acid inhibits platelet aggregation in vitro (Hung *et al.*, 2005). This finding
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18 has been confirmed by us as well: 1.2 mM caffeic acid decreased collagen induced
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20 platelet aggregation in vitro by 50% (data not shown). 10 μ M ADP induced platelet
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22 aggregation has increased after chicory coffee consumption at 2 hours and on the 8th day
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24 as well (Fig. 1A). 5 μ M ADP induced platelet aggregation did not change significantly
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26 (Fig. 1B). Collagen-induced platelet aggregation decreased from baseline at 2 hours
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28 (Fig. 1C), but epinephrine-induced aggregation increased (Fig. 1D).
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34 No changes in basic hematologic parameters like hematocrit or red blood cell
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36 counts were detected (Table 1). Whole blood viscosity significantly decreased after one
37
38 week daily chicory coffee consumption (Fig. 2A). At all fluid shear stresses lower than
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40 30 Pa significant improvements were seen there in RBC deformability, an
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42 acknowledged determinant of whole blood viscosity (Table 2). A significant decrease in
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44 plasma viscosity was evident after one week of chicory coffee consumption (Fig. 2B),
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46 while no changes were detected in fibrinogen levels, one of the main determinants of
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48 plasma viscosity (Table 1).
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52 Daily consumption of chicory coffee for one week significantly decreased serum
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54 MIF levels (Fig. 3).
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DISCUSSION

The participants observed no side effects during the study. Changes in hemorheological parameters detected in the course of this dietary intervention are unlikely to be attributable to only one single compound, but rather to an array of agents absorbed from chicory coffee. The inhibitory effect of chicory consumption on platelet aggregation seems to vary with the inductor used. This might be attributable to a particular mechanism by which agents in chicory coffee interfere with platelet aggregation. Nevertheless, *in vivo* antiplatelet activity was found much weaker than expected from preceding *in vitro* studies with caffeic acid, one of the most abundant plant phenolic of this beverage. Consumption of chicory coffee for one week, however, significantly improved hemorheological parameters, like plasma- and whole blood viscosity and red blood cell deformability. These changes indicate that daily chicory coffee consumption might be preventive against certain microcirculatory pathologies. The decrease in whole blood viscosity might largely be attributable to the increased red blood cell deformability, however, the modest but significant decrease in plasma viscosity in the face of unchanged fibrinogen levels appears perplexing. Further studies are warranted to clarify the exact mechanisms and to identify the compounds behind these effects of chicory coffee.

Baskurt *et al.* (1998) found significantly decreased RBC deformability in septic states at fluid shear stresses less than 5 Pa in humans and rats. Elevated MIF levels are acknowledged indicators of septic state (Bernhagen *et al.*, 1993). Potato peel extract rich in caffeic acid has been reported to protect erythrocytes against oxidative damage, a known cause of impaired cell deformability (Singh and Rajini, 2008). Daily

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4 consumption of chicory coffee rich in antiinflammatory phenols (including caffeic acid)
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6 for one week duration substantially decreased serum MIF levels of healthy volunteers in
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8 our study in parallel with the improved red blood cell deformability. To decide whether
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10 these phenomena are related or are merely coincidental further studies are needed.
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12 Notably MIF upregulates adhesion molecules on endothelial cells (Amin *et al.*, 2006),
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14 hence suppression of MIF levels could be regarded preventive against monocyte
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16 adhesion and emigration, a key element in initiation of the atherosclerotic process.
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21 In conclusion, our study constitutes an encouraging starting point to investigate
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23 further the antithrombotic, antiinflammatory and beneficial hemorheologic effects of
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25 phenolic compounds from chicory coffee.
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30 **Acknowledgement**

31
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35
36 is gratefully acknowledged. The authors have no financial or commercial conflict of
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38 interest.
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Table 1. Effect of chicory coffee consumption on basic hematologic parameters.Mean values \pm SEM.

	Control (n=27)	1 week (n=24)
Red blood cell count (T/L)	4.79 \pm 0.09	4.74 \pm 0.09
Hemoglobin (g/L)	143.81 \pm 2.57	142.33 \pm 2.84
Hematocrit (%)	41.82 \pm 0.59	41.38 \pm 0.65
Fibrinogen (g/L)	2.63 \pm 0.09	2.65 \pm 0.11
Platelet count(G/L)	241.93 \pm 8.04	241.67 \pm 9.25

Table 2. Effect of chicory coffee consumption on red blood cell deformability. The reported elongation indexes represent RBC deformability at different shear stresses.

Mean values \pm SEM.

Shear stress (Pa)	Control (n=27)	2 hours (n=27)	1 week (n=24)
0.3	0.0545 \pm 0.0018	0.0532 \pm 0.0017	0.0594 \pm 0.0018** ##
0.53	0.1094 \pm 0.0027	0.1089 \pm 0.0024	0.1141 \pm 0.0030* #
0.95	0.2067 \pm 0.0027	0.2065 \pm 0.0029	0.2120 \pm 0.0033** #
1.69	0.3176 \pm 0.0027	0.3177 \pm 0.0027	0.3235 \pm 0.0028* #
3	0.4194 \pm 0.0019	0.4186 \pm 0.0023	0.4240 \pm 0.0024* #
5.33	0.4974 \pm 0.0014	0.4966 \pm 0.0020	0.5015 \pm 0.0019** ##
9.49	0.5497 \pm 0.0013	0.5493 \pm 0.0018	0.5533 \pm 0.0016** ##
16.87	0.5961 \pm 0.0013	0.5957 \pm 0.0016	0.5985 \pm 0.0015** ##
30	0.6276 \pm 0.0008	0.6277 \pm 0.0011	0.6290 \pm 0.0011

*difference from baseline $p < 0.05$, **difference from baseline $p < 0.01$

#difference from 2 hours $p < 0.05$, ##difference from 2 hours $p < 0.01$

Schumacher 1 ↑

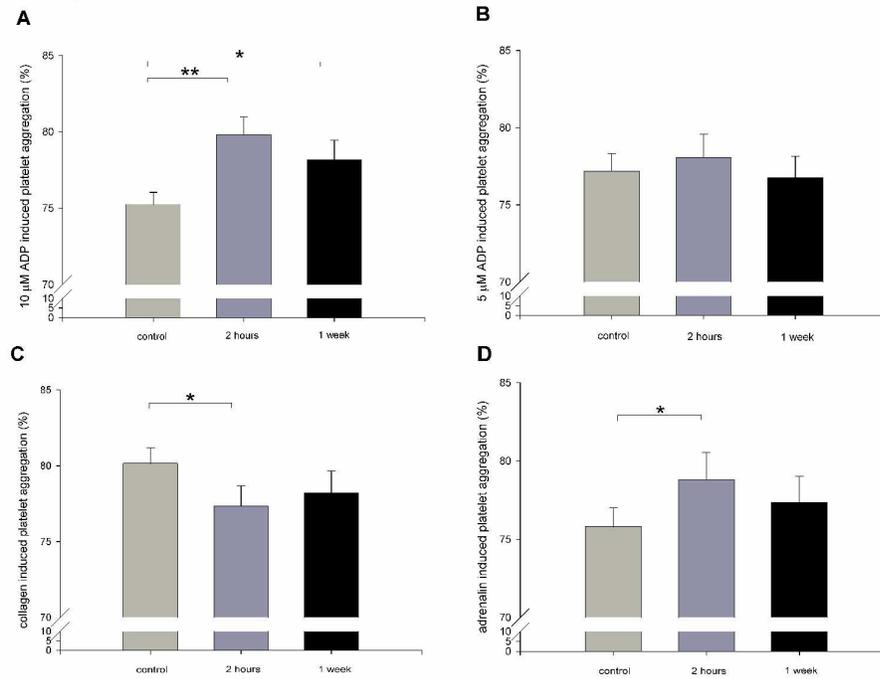


Figure 1.

Effect of chicory coffee consumption on platelet aggregation.

A: Platelet aggregation induced by 10 μ M ADP. B: Platelet aggregation induced by 5 μ M ADP. C: Platelet aggregation induced by 2 μ g/ml collagen. D: Platelet aggregation induced by 10 μ M epinephrine.

Mean values \pm SEM. * p < 0.05, ** p < 0.01
1134x815mm (150 x 150 DPI)

Schumacher 2 ↑

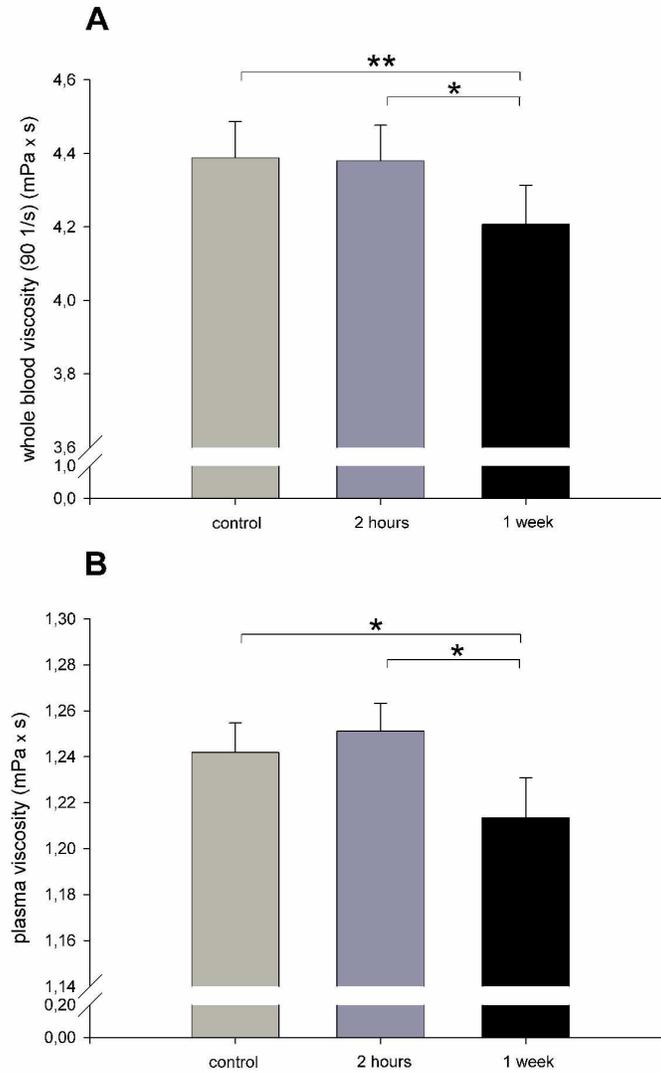


Figure 2.

Effect of chicory coffee consumption on blood and plasma viscosity.

A: Changes in whole blood viscosity. * $p < 0.05$, ** $p < 0.01$; B: Changes in plasma viscosity. * $p < 0.05$ Mean values \pm SEM.

845x1368mm (150 x 150 DPI)

Schumacher 3 ↑

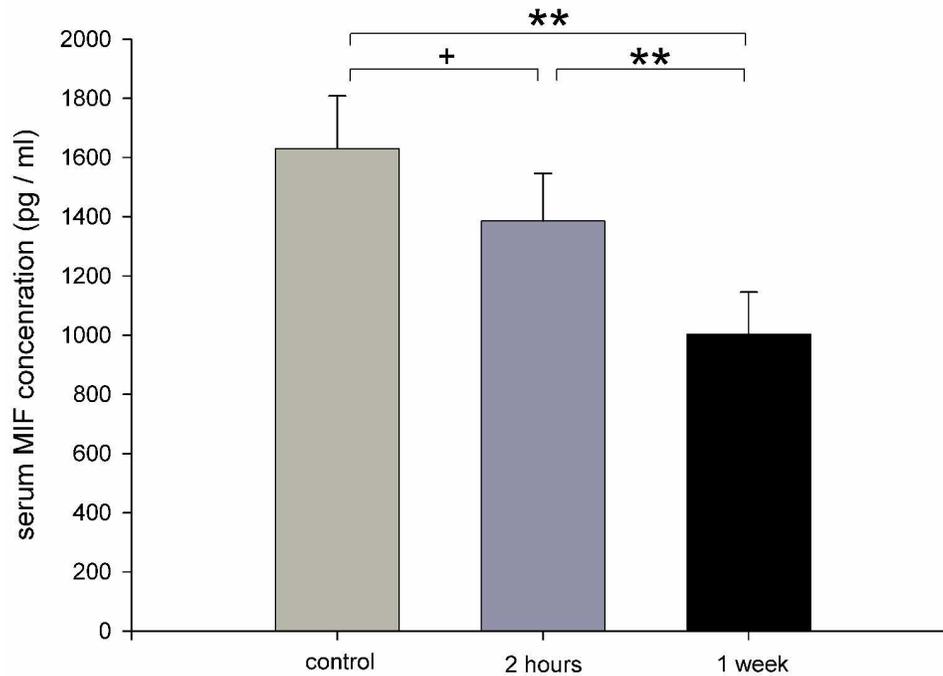


Figure 3.
Effect of chicory coffee consumption on serum MIF levels.
**p < 0.01, +p < 0.06
Mean values \pm SEM
805x690mm (150 x 150 DPI)