

## EFFECTS OF CHRYSIN ON HAEMATOLOGICAL PARAMETERS IN RATS

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### Abstract

Chrysin (5,7-dihydroxyflavone) is a natural flavonoid found in plant extracts, which has significant biological and pharmacological properties. In this study, we tested the influence of chrysin on haematological parameters in the blood of rats treated with various doses of chrysin (25, 50, 100 mg/kg bw) for one or 7 days. Total red blood cell (RBC), haemoglobin (HGB), and haematocrit (HTC) values decreased markedly in rats exposed for one day to 25 mg/kg bw ( $p < 0.01$ ), 50 mg/kg bw ( $p < 0.05$ ), and 100 mg/kg bw ( $p < 0.01$ ) of chrysin. There was an increase in RBC, HGB, and HTC values in rats after administration of 100 mg/kg bw of chrysin once a day over a 7-day period as well as in RBC and HTC values in rats exposed to 25 and 50 mg/kg bw of chrysin over the same period of time. The average corpuscular haemoglobin concentration (MCHC) value decreased significantly when rats were exposed to chrysin once, at a dose of 50 mg/kg bw, or for 7 days, a doses of 25 or 50 mg/kg bw. After 7 days of chrysin administration the body weight of rats increased visibly. Chrysin may significantly influence haematological parameters and the differences between a single and multiple administrations have strong clinical implications. According to the results of our study, chrysin given more than once may beneficially influence haematological parameters and increase body weight in rats. It opens new perspectives for the use of such a flavonoid in anaemia.

### Rezumat

Crisinan (5,7-dihidroxiflavonă) este un flavonoid natural care se găsește în extracte de plante, cu proprietăți biologice și farmacologice semnificative. În acest studiu, am testat influența crisinei asupra unor parametri hematologici la șobolani. Au fost determinați parametrii hematologici în sângele șobolanilor tratați cu diferite doze de crisină (25, 50, 100 mg/kgc) timp de 24 ore, respectiv 7 zile. Valorile totale ale eritrocitelor (RBC), hemoglobinei (HGB) și hematocritului (HTC) au scăzut semnificativ la șobolanii expuși pentru 24 ore la 25 mg/kgc ( $p < 0,01$ ), 50 mg/kgc ( $p < 0,05$ ), și 100 mg/kgc ( $p < 0,01$ ) de crisină. S-a observat creșterea valorilor RBC, HGB și HTC la șobolani după administrarea a 100 mg/kgc de crisină o dată pe zi pe o perioadă de 7 zile, precum și a valorilor RBC și HTC la șobolanii expuși la 25 și 50 mg/kgc de crisină în aceeași perioadă de timp. Valoarea

concentrației de hemoglobină corpusculară medie (MCHC) a scăzut semnificativ atunci când șobolanii au fost expuși la crisină o singură dată, la o doză de 50 mg/kgc, sau timp de 7 zile, la dozele de 25 sau 50 mg/kgc. După 7 zile de administrare a crisinei, greutatea corporală a șobolanilor a crescut vizibil. Crisina poate influența în mod semnificativ parametrii hematologici, iar diferențele dintre administrarea dozei unice și dozelor multiple are implicații clinice puternice. Conform rezultatelor studiului nostru, crisina, administrată în mai multe doze poate influența benefic parametrii hematologici și creșterea greutății corporale la șobolani. Aceste observații deschid noi perspective pentru utilizarea unui astfel de flavonoid în anemie.

**Keywords:** chrysin, rats, haematological parameters.

## Introduction

Chrysin, a natural flavonoid found in plant extracts (*Passifloraceae: Passiflora caerulea, Salicaceae: Populus tremula*) [1] is one of the most abundant components of propolis and honey [2-5]. The chrysin concentration varies among different types of propolis; for example, propolis collected during the summer of 2010 from Podlasie region (Poland) contains 2.3% of chrysin (manuscript under review in *Oncology Reports*). Like other flavonoids, chrysin has significant biological and pharmacological properties: antioxidant and anti-inflammatory effects [6, 7] as well as antihypertensive activity [8] and anticancer properties. This particular flavonoid induces apoptotic pathways [9-12] and inhibits cell proliferation [13] in many types of tumour cell lines. Chrysin also has the potential for clinical and therapeutic applications against the physiological and biochemical effects of aging [14]. Although chrysin possesses potential therapeutic applications, there is still not enough data concerning its influence on haematological parameters. Some studies show that propolis, as a main source of chrysin, does not significantly affect the biochemical and haematological parameters in rats [15-17]. Jasprica *et al.* [18] showed a significant decrease in red blood cell number and a concentration of haemoglobin in men after a 30-day period of propolis supplementation. Talas and Gulhan [19] presented that propolis given once at various concentrations significantly decreased red blood cell count (RBC), haemoglobin (HGB), and haematocrit (HCT) values in contrast to an increase of the average corpuscular haemoglobin concentration (MCHC) of rainbow trout (*Oncorhynchus mykiss*). These contradicting data about the influence of propolis on haematological parameters probably depend on the chemical composition of propolis and the used doses. Potential therapeutic applications of chrysin require knowledge about its effects on the basic haematological parameters in order to avoid disadvantageous influences. In this study, we test the influence of chrysin in different doses on

haematological parameters in rats. The body weight of rats was used as an additional indicator useful in measurement effects of chrysin. We decided to administer chrysin once or for 7 days in three doses to show the effect of a single and multiple doses of this flavonoid.

## Materials and Methods

### *Chemicals*

Chrysin (Sigma, St. Luis, USA) dissolved in 1% methylcellulose was administered orally by gavage cannula (not implanted), at doses of 25, 50, 100 mg/kg bw in a volume of 1 mL/kg bw [20-24] 30 minutes before the lethal anaesthesia when chrysin was administered once [25, 26] or multiple times during seven days of the experiment [20]. One-percent methylcellulose (pH 7.4) was administered as a vehicle of suspension by gastric canula in the volume of 1 mL/kg bw per rat.

### *Animals*

On the first day of the study, 64 male Wistar Han (Hannover) rats weighing 130-180 g (mean weight was  $145.75 \pm 10.36$  g) were used. They were obtained from The Centre of Experimental Medicine, Medical University in Bialystok, Poland. Rats were housed in an individually ventilated cage (IVC) system exposed to 12 h light/dark cycle beginning at 7.00 h, temperature ( $22 \pm 2^\circ\text{C}$ ), and 55% relative humidity. Animals were fed with a standard rat diet and they had free access to drinking water. Measurements of food and water intake were performed each day. The experiment was carried out in accordance with the EU Directive 86/609/EEC and the International Guidelines on the Ethical Use of Animals. Moreover, the Ethics Committee of Medical University in Bialystok, Poland, approved this work [No.1/2010].

### *Experimental design*

In order to determine the effect of chrysin on haematological parameters, the 64 rats were divided into two individual groups, "single" and "multiple", each consisting of 32 animals. Next, each of these two groups was randomly divided into four equal groups. The control group received 1% methylcellulose as a vehicle. Methylcellulose is a chemical compound derived from cellulose which helps stabilize emulsions and foams, but does not contribute as any significant nourishment or calories as they pass through the human digestive system. The treatment groups were administered chrysin in three different doses (25, 50, 100 mg/kg bw). Rats from the "single" group received the vehicle or chrysin once. Rats from the "multiple" treatment group received 1% methylcellulose or chrysin once a day for 7 subsequent days. Each rat was weighed just before the beginning

of the study and each day of the experiment. In the “single” group, animals were not fed for 6 h before the chrysin or vehicle applications and in the “multiple” group before the 7th day of the experiment. Rats from both groups were killed by lethal anaesthesia 30 minutes after the administration of chrysin or the vehicle. Blood samples from each rat were taken by means of cardiac puncture under anaesthesia with isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) and then collected to anticoagulated (K3-EDTA) tubes for haematological evaluation.

#### *Haematological analysis*

The haematological analyses were determined immediately after collecting the blood samples. White blood cells (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined using an ABC Vet Haematology Analyser in the Centre of Experimental Medicine, Medical University in Bialystok, Poland.

#### *Statistical analysis*

The data were analysed using SPSS for Windows software, Version 9.0 using one-way analyses of variance (ANOVA). The differences between groups were determined using Dunnett's test with data exposed as average  $\pm$  standard deviation and p values  $< 0.05$  accepted as statistically significant.

### **Results and Discussion**

The effects of chrysin on the haematological parameters are presented in Table I. As the obtained results show, RBC values in rats exposed to a single dose of 25, 50 and 100 mg/kg bw of chrysin decreased significantly. On the other hand, rats treated for 7 days with all the studied doses of chrysin showed marked increases in RBC values as compared with the control group. Rats exposed once to all the studied doses of chrysin presented a significant decrease ( $p < 0.01$ ) in HGB values; however, chrysin administered for 7 days at the dose of 100 mg/kg bw induced an increase in the value of HGB ( $p < 0.05$ ) as compared with the control group. It was observed that the changes (decreased values) in the haematocrit (HTC) in the blood of rats with two different doses of chrysin administration, 25 and 100 mg/kg bw (given once), were markedly significant. Additionally, HTC values increased when all the doses of chrysin were given for 7 days, as compared with the control group. Finally, there was a statistically significant decrease in the values of MCHC as compared with the control group after 50 mg/kg bw of chrysin given once ( $p < 0.05$ ) or after giving multiple doses of 25 and 50 mg/kg bw ( $p < 0.01$ ) of chrysin. The obtained

data show that that there are important differences between single and multiple administrations of chrysin in haematological parameters in RBC, HGB, and HTC of rats in the tested doses. There were no significant changes in the values of WBC, MCV, and MCH in chrysin treated groups compared to the control groups. These results are not shown.

**Table I**  
Effect of chrysin on haematological parameters in rats

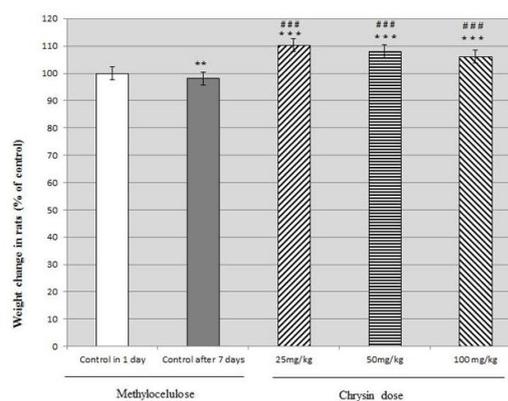
Haematological parameters		Control	Chrysin – dose (mg/kg bw)		
			25	50	100
Single treatment	RBC ( $10^6/\text{mm}^3$ )	7.99±0.63	6.95±0.17**	7.24±0.46*	6.71±0.54***
	HGB (g/dL)	16.06±1.16	14.26±0.40**	13.91±0.86**	13.47±1.37***
	HCT (%)	48.21±3.19	43.52±3.22**	44.93±2.59	39.90±2.4***
	MCHC (g/dL)	33.20±0.35	32.17±1.24	31.00±2.61*	33.24±0.47
Multiple treatment	RBC ( $10^6/\text{mm}^3$ )	7.10±0.20	7.63±0.24*	7.82±0.45**	7.88±0.39**
	HGB (g/dL)	14.99±0.56	15.17±0.32	15.41±0.84	15.83±0.4*#
	HCT (%)	43.59±0.87	46.19±1.23*#	46.52±2.18**	47.27±1.65***###
	MCHC (g/dL)	34.05±0.73	32.81±0.41***	32.96±0.25**	33.54±0.51

RBC - red blood cell count, HGB – haemoglobin, HCT – haematocrit, MCHC – mean corpuscular haemoglobin concentration

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001 vs. control group

# p < 0.05; ### p < 0.01 vs. single dose

In this study, the body weight of rats in the control group decreased as compared to their body weight before the study and at the end of the experiment (Figure 1).



**Figure 1.**

Effect of chrysin on the body weight of rats.

\*\* p < 0.01; \*\*\* p < 0.001 vs. control group treated with methylcellulose as vehicle in single dose (mean weight 145.75 ± 10.36 g)

### p < 0.001 vs. control group treated with methylcellulose as vehicle in multiple doses (7 days).

Chrysin administered in all tested doses during a 7-day period increased significantly (p < 0.001) the body weight of rats. The correlation

between the body weight of rats and the dose of chrysin,  $r = -0.6001$  and  $p = 0.002$  respectively, showed that the gain of body weight decreased along with the increase of the chrysin dose. The daily food intake  $10 \pm 2$  g per rat of the control group was significantly lower ( $p < 0.05$ ) than in chrysin groups ( $15 \pm 3$  g;  $13 \pm 2$  g;  $14 \pm 1$  g, respectively for 25, 50 and 100 mg/kg bw of chrysin). Water intake was similar ( $30 \pm 5$  mL per rat/ day) in both groups of rats (data not shown).

Chrysin is a good candidate for being a health-promoting substance; however, no sufficient work has been done to study its influence on haematological parameters of blood. Haematological data showed that chrysin at various doses exerted a certain influence on some of the studied blood indices. Rats which were exposed once to all of the used doses of chrysin presented a decrease in RBC, HGB, HCT, and MCHC values which may suggest hypochromic anaemia. This kind of anaemia is characteristic to iron and vitamin B12 deficiencies [27]; however, participation of a single dose of chrysin in the absorption of iron and vitamin B12 is unlikely. Similarly to our study, Talas and Gulhan [19] showed that propolis given once at various concentrations (0.02 and 0.03 g/L) for 96 h significantly decreases RBC, HGB and HCT values, in contrast with the increase of the average corpuscular haemoglobin concentration (MCHC) of rainbow trout (*Oncorhynchus mykiss*). Authors suggest that the decrease in haematological parameters at high doses of propolis may also indicate anaemia. Taking our results into account, we speculate that the acute effect of chrysin may be dependent on its proapoptotic activity. However, we do not have any additional results which would consider such an explanation. Although the potential side effects of chrysin have not been studied well, a previous research has demonstrated that chrysin has cytotoxic effects on normal trout liver cells [28]. We have also obtained a strong decline of viability of astroglia cells SVGp12, approximately to 33%, after chrysin application (manuscript under consideration).

We should also take into consideration that blood sampling occurred within a short time period, after *per os (po)* chrysin administration. Chrysin given orally at a dose of 400 mg is very quickly absorbed. The human peak plasma chrysin concentration is between 30 minutes and 60 minutes and the rats' peak plasma chrysin (oral dose 5 mg/kg bw) was similar to that of humans [26]. The results of the study by Brown *et al.* [25] showed anxiolytic effect of chrysin, 30 minutes after intraperitoneal administration at a dose 2 mg/kg bw in rats, and suggested very effective kinetics of this flavonoid. Despite the influence on absorption we can suggest that stress, due to application of chrysin by gastric canula (gavage not implanted), may

change haematological parameters. Oral application by gastric canula can be more stressful than other routes of administration. It has been suggested that intraperitoneal application of propolis provides better absorption of compounds, which can be reflected in the medical results [29, 30]. Also Gugler *et al.* [31] showed a better quercetin resorption after a single intravenous dose, compared to an oral one. Similar data about such route of administration of chrysin is not available. Despite the results concerning the haematological disadvantages of propolis, the study by Cetin *et al.* [15] showed that administration of propolis alone did not cause any significant alteration in the haematological indices. These findings are similar to the data reported by Sforcin *et al.* [16] and Mani *et al.* [17] who indicated that the treatment by means of propolis did not cause any significant changes in haematological parameters such as RBC, HGB, and platelets.

In order to determine the effect of multiple doses of chrysin, the study was conducted on rats after 7 days of treatment. Chrysin administered once a day during a 7-day period in all the studied doses (25, 50 and 100 mg/kg bw) induced an increase in RBC, HGB, and HCT. Fiorani *et al.* [32] suggest that RBC are natural flavonoid reservoirs. The results presented by Cetin *et al.* [33] demonstrated that propolis treatment for 12 weeks (3 g/kg bw) caused a significant increase ( $p < 0.05$ ) of RBC in the value of *White Leghorn* layer hens. Chaudhuri *et al.* [34] showed that chrysin interacts with both lipid and protein components in RBC ghost membranes. Significant antioxidant and antihaemolytic effects of the chrysin are related to the increase in the membrane integrity of the erythrocyte. In addition, this flavonoid was shown as effective regarding the inhibition of membrane peroxidation of erythrocytes. These effects may increase the viability of RBC observed as an increase in its amount. Based on the results provided by Chaudhuri *et al.* [34], chrysin is a promising substance for preventing peroxidation and lysis of erythrocytes induced by free radicals. It was also found that propolis decreases the erythrocytes membrane fragility, which increases under oxidative stress conditions, and the protection effect of propolis is due to its antioxidant properties [35]. The increased values of HGB and HTC confirm the beneficial influence of chrysin of RBC counts. A decrease in the values of MCHC after administration of chrysin in multiple doses 25, 50 mg/kg bw ( $p < 0.01$ ) was unexpected and the qualification of the mechanism of the obtained effect needs additional study. However, there are studies that showed a significant decrease of red blood cell number and concentration of hemoglobin in men after 30 days of propolis supplementation with chrysin [18].

The opposite effects of single and multiple doses of chrysin are difficult to explain without additional studies. We suggest that chrysin administered once may influence erythrocytes directly, probably by increasing the apoptosis. The decreased levels of HGB and HTC are strongly connected with the destruction process of RBC. The multiple doses of chrysin, probably due to an antioxidative activity, increased viability and improved the function of RBC. The decrease of the rats' body weight in the control group at the end of the experiment, as compared to their body weight before the study, results probably from the stress induced by the everyday application of methylcellulose as a vehicle by gastric canula (gavage not implanted) and then influences the absorption of food. The increased body weight of rats after 7 days of chrysin administration is probably a result of an increased absorption of food. Additionally, oral chrysin treatment obviously enhanced the weight loss in mice after experimental induction of colitis due to its anti-inflammatory effects as reported in literature [36]. These results may suggest that multiple administration of chrysin enhances iron and vitamin B<sub>12</sub> absorption or stimulates erythropoiesis. However, the presented effect of chrysin is not useful in the case of obese patients, but is beneficial from a clinical point of view in thin anemic people.

### **Conclusions**

Chrysin may significantly influence haematological parameters and the differences between a single *versus* multiple administrations have strong clinical implications. Single doses may produce an adverse effect, mainly on RBC, HTC, and HGB. Multiple application (7 days) markedly increased the amount of RBC, HTC, and HGB values, thus, suggesting its use in the prevention or treatment of anaemia. In the present study, we underlined that multiple administration of chrysin plays a crucial role in its effectiveness on haematological parameters. This is the first work reporting an evaluation of the chrysin effect on haematological parameters in two regimens of administration: single or multiple. The increased body weight of rats, following the administration of chrysin, may suggest its beneficial effect in anaemia. This study advances our understanding of the health-promoting role of chrysin and its promising application in patients with anaemia.

### **Acknowledgements:**

This work was supported by the Polish Ministry of Science and Higher Education (Grant project No 3-16948F of Medical University in Bialystok).

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*Manuscript received: February 25<sup>th</sup> 2013*