



Research Article

THE PROTECTIVE EFFECT OF ACEROLA FRUIT JUICE AGAINST LEAD TOXICITY ON RED BLOOD CELL SIZE IN MALE ALBINO MICE

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Received: February 05, 2025; Revised: April 21, 2025; Accepted: May 02, 2025

ABSTRACT

This study evaluated the protective effects of acerola fruit juice against lead toxicity by examining the size of red blood cells in male albino mice. The mice were divided into 6 groups, including a control group, a lead-exposed group (70 mg/kg body weight), a vitamin C group (30 mg/kg body weight), and three groups co-exposed to lead with acerola fruit juice at doses of 20, 30, and 40 mg/kg body weight, respectively. The results revealed that while lead exposure over four and eight weeks significantly reduced mouse red blood cells, co-administration of acerola fruit juice, particularly at 40 mg/kg, preserved normal red blood cell morphology and counteracted the hematotoxic effects of lead. These findings highlight the potential of acerola fruit juice as a supportive agent against lead-induced toxicity and warrant further investigation into its underlying mechanisms and therapeutic applications.

Keywords: acerola fruit juice; albino mice; lead toxicity; red blood cell size; vitamin C

1. Introduction

Lead is recognized as one of the most toxic heavy metals, significantly impacting human and animal health, particularly the hematopoietic system (Andjelkovic et al., 2019; Balali-Mood et al., 2021; Ekanem et al., 2015; Sani & Amanabo, 2021; Thuong et al., 2023). According to the Institute for Health Metrics and Evaluation (IHME), the incidence of lead poisoning is significantly increasing worldwide, with estimates suggesting over 1.5 million deaths annually related to lead exposure (World Health Organization, 2024). Among the various toxic effects of lead, its impact on the hematopoietic system is considered one of the most critical manifestations, evidenced by alterations in the quantity, morphology, and function of blood cells, particularly red blood cell size (Mani et al., 2020).

Cite this article as: Truong, V. T., Nguyen, T. T. D., Lu, H. V., & Nguyen, T. T. H. (2025). The protective effect of acerola fruit juice against lead toxicity on red blood cell size in male albino mice. *Ho Chi Minh City University of Education Journal of Science*, 22(9), 1576-1587. [https://doi.org/10.54607/hcmue.js.22.9.4711\(2025\)](https://doi.org/10.54607/hcmue.js.22.9.4711(2025))

Recent studies have highlighted the potential of various natural substances in mitigating oxidative stress and promoting the health of erythrocytes, particularly in mouse models influenced by heavy metals. Several studies have investigated the efficacy of plant-derived extracts in erythrocyte protection. For instance, olive leaf extract has been reported to exhibit significant antioxidant activity, preventing oxidative damage in erythrocytes (Lins et al., 2018). Similarly, chlorogenic acid, prominent in many dietary sources, has demonstrated the ability to improve erythrocytes membrane stability and reduce morphological alterations induced by nitrite exposure, which is often associated with oxidative stress and metal toxicity (Cheng et al., 2020). The protective mechanisms are largely attributed to the antioxidants present in these extracts, which scavenge free radicals and prevent membrane lipid peroxidation. Research has underscored the protective effects of compounds like piperine, which is known for its lipophilic properties that help protect the erythrocyte membrane against oxidative damage (Tupe et al., 2021). Additionally, resveratrol present in various plant sources has been noted for its role in mitochondrial function and enhancing ATP production in erythrocytes, suggesting its role in maintaining energy homeostasis and functionality of RBCs under stress conditions (Schulte et al., 2021).

The normal size of mouse red blood cells ranges from 5.5 to 6.1 μm (Moran, 2001-2005). When red blood cell size is smaller than normal, it can lead to microcytic anemias, which are often characterized as hypochromic microcytic anemias due to iron deficiency, thalassemia, lead poisoning, copper deficiency, zinc excess, alcohol use, or drug abuse (Chaudhry & Kasarla, 2023; Healthline, 2019, October 24). Conversely, if the size of red blood cells is larger than normal, it can result in macrocytic anemia, with hemoglobin levels falling below 12 g/dL (Moore & Adil, 2022). Thus, abnormal erythrocyte sizes pose significant health risks to the body.

Currently, there is an increasing interest in identifying natural, safe, and effective strategies for the prevention and mitigation of lead toxicity. Vitamin C, a well-established antioxidant, has been shown to protect against free radical formation and oxidative stress induced by heavy metal exposure (National Institutes of Health, 2021, October 7; Notariale et al., 2021; Ojeka et al., 2024; Pehlivan, 2017; Sharaf et al., 2017). In addition, a growing body of evidence highlights the potential of plant-derived bioactive compounds in alleviating lead-induced toxicity (Abou-Zeid et al., 2018; El-Tantawy, 2016; Garcia et al., 2020; Prakash & Baskaran, 2018; Ziamajidi et al., 2023). Against this background, the investigation of natural remedies, such as acerola fruit juice, not only for advancing our understanding of lead detoxification mechanisms but also for integrating traditional knowledge with contemporary scientific approaches to develop safe and effective therapeutic interventions.

Acerola (*Malpighia emarginata* DC.) is a fruit renowned for its exceptionally high vitamin C content, reaching levels up to 1677.6 mg/100 g of fruit flesh (NatureClaim, 2024,

September 4; The Natural Food Hub, 2018, January 16). Furthermore, it is a rich source of bioactive compounds, including flavonoids and other antioxidants (Bowen-Forbes et al., 2010; Vilvert et al., 2024). A study has demonstrated that acerola juice possesses anti-mutagenic properties, leading to a significant reduction in micronucleus mean values in bone marrow and offering protection against DNA damage induced by iron (Horta et al., 2016). Additional studies have indicated that acerola juice can prevent weight gain and dyslipidemia (Batista et al., 2023; Dias et al., 2014; Laurindo et al., 2024). Despite these established benefits, research investigating the protective effects of acerola juice against lead-induced toxicity, particularly concerning the hematopoietic system, remains limited. Therefore, this study aims to evaluate the lead toxicity mitigation effects of sour acerola juice by investigating the size of erythrocytes in male albino mice, thereby providing scientific evidence for the use of acerola as a natural supplement in the prevention of lead toxicity.

2. Materials and methods

2.1. Chemicals

Chemicals used in this study were sourced as follows: lead nitrate (L/1500/50, Merck, Germany); L-Ascorbic acid (AC05150250, Scharlab S.L. Spain); sodium sulfate (034825, Merck, Germany); sodium chloride (SO02270500, Scharlab S.L. Spain); hydrochloric acid (HC27864260, Merck, Germany); acid acetic (AC03522500, Scharlab S.L. Spain); magnesium sulfate (MA00850500, Scharlab S.L. Spain); kali dihydrogen phosphate (1.04873.1000, Merck, Germany); and di-sodium hydrogen phosphate (1.06586.0500, Merck, Germany); formalin (CAS:50-00-0, XiLONG, China); and Giemsa power (CAS:51811-82-6, China). All reagents were provided by the Laboratory of Human and Animal Anatomy Physiology - Ho Chi Minh City University of Education.

2.2. Experimental animals

Swiss male mice (n= 54, 4 weeks old, 14-16 g) were obtained from the Pasteur Institute, Ho Chi Minh City. Upon arrival, we were housed in groups of 4-5 within transparent glass cages (30×19×19 cm³) containing a straw layer and covered with stainless inox lids. A 10-hour light/14-hour dark cycle was maintained, with temperature ranging from 27-28°C and relative humidity between 76-80%. These environmental parameters were continuously monitored using a calibrated electronic thermometer. The mice were fed a commercially available rodent diet specifically formulated for laboratory mice, sourced from the Tropical Biology Institute, Ho Chi Minh City. Water was provided ad libitum. Food and water were replenished twice daily at 06:30 and 17:00 hours. The straw layer was changed every 2-3 days. This study was approved by the Animal Ethics Committee under the code number 1121/KHTN-ACUCUS at the University of Natural Sciences, Vietnam, on December 11, 2023.

2.3. Experimental design

Fifty-four mice were divided into six groups (9 mice in each group), and lasted for eight weeks. Group I, control mice (Ctr), were provided with water ad libitum; group II (Pb),

were provided with lead from nitrate doses of 70 mg/kg body weight (b.w) (Thuong et al., 2023); group III (PbVC) were provided with 30 mg/kg BW ascorbic acid; group IV (PbSR20), group V (PbSR30) and group VI (PbSR40) were administered lead from lead nitrate doses of 70 mg/kg BW and 20, 30 and 40 mg/kg BW acerola juice (Autifi et al., 2018; Sharaf et al., 2017), respectively, every other day for 8 weeks).

2.4. Methods

Preparing sour acerola juice: Ripe Brazilian sour acerola fruits, which were still pink and ripe, were obtained from Go Cong, Tien Giang province (10°22'18.1"N 106°41'34.3"E). Upon receipt, the fruits were washed, drained, and stored at -20°C to maintain consistency throughout the experimental process. Juice extraction was conducted daily as follows: the cherries were placed in a refrigerator at 4°C for 30 minutes to facilitate thawing. The pulp, including the peel but with seeds removed, was then pressed to obtain the juice. The extracted juice was utilized to assess vitamin C concentration at the Case Testing Center by high-performance liquid chromatography (HPLC) and through laboratory titration methods. The volume of juice given to the mice was calculated for three distinct treatment groups. Additionally, a daily record of the individual body weights of the mice was kept to adjust the volume of juice administered accordingly.

Delivery of lead, acerola fruit juice, and pure vitamin C to mice: Lead solution (70 mg/kg body weight) was prepared by dissolving lead nitrate in distilled water. A separate solution of pure vitamin C (30 mg/kg body weight) was prepared by dissolving the compound in distilled water. Lead solution, vitamin C solution, and sour acerola juice were administered orally via gavage using a size 8 dosing needle with a rounded tip attached to a 1 mL syringe. The administered volume was determined according to the body weight of each mouse and the tested concentration, ensuring that the volume per administration did not exceed 0.5 mL. Following administration, mice were observed for 30 minutes for changes in general appearance. Lead solution was administered at 07:00 AM, 30 minutes before feeding. Sour acerola juice and vitamin C solution were administered daily between 10:00 and 10:30 AM. This procedure was carried out daily and sustained for a period of 8 weeks.

Preparation of mouse blood smears: mouse blood samples were collected at three distinct time points: week 0 (baseline), week 4 (mid-experimental period), and week 8 (semi-chronic exposure to lead toxicity). Before blood collection, the mice were fasted overnight to ensure that they had not consumed food on the day of sampling. Blood was drawn from the tail vein for smear preparation. Each blood sample was mixed with a drop of 14% magnesium sulfate (MgSO₄) solution, spread onto a glass slide, and allowed to air dry. The smears were subsequently fixed with methyl alcohol to preserve the cellular morphology and further dried. Following fixation, the smears were stained with Giemsa solution for 15 to 20 minutes. Post-staining, the slides were washed with distilled water until the effluent was

clear and devoid of discoloration, and then allowed to air dry. The prepared smears were subsequently used for the evaluation of erythrocyte size (Nguyen & Vo, 2019).

Determining mouse erythrocyte size: measurements were conducted using a microscope (40x objective) equipped with S-EYE software connected to a laptop. In each experiment, 300 erythrocytes were randomly selected and measured. The radius and perimeter of each erythrocyte were recorded and exported into an Excel file.

Statistical Analysis: all collected data were analyzed using GraphPad Prism 9.2 software. The results are presented as mean $\pm$ standard deviation (SD). One-way and two-way analysis of variance (ANOVA) with Tukey's test was used to determine statistical significance. A p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Results

- **Baseline erythrocyte size**

This study investigated the effects of lead exposure and acerola juice on red blood cell (erythrocyte) size in mice. At baseline, erythrocyte size was uniform across all experimental groups. The measurements of erythrocyte radius and perimeter were randomly distributed, ranging from 2.81-2.82 μm and 17.64-17.74 μm , respectively ($p > 0.05$) (Figures 1A and 1B). These values were consistent with the reference range for the diameter of mouse erythrocytes on blood smears (5.5-6.1 μm) (Moran, 2001-2005). Thereby confirming the reliability of the initial conditions before experimental interventions.

- **Changes in erythrocyte size at week 4**

At the fourth week of the study, the control group exhibited a slight increase in erythrocyte size compared to baseline values. This increase was maintained through the eight-week assessment, with statistical analysis revealing no significant difference ($p > 0.05$). Conversely, the lead-exposed group (Pb) exhibited a marked reduction in erythrocyte size ($p < 0.001$). Notably, mice supplemented with acerola juice (PbSR20, PbSR30, PbSR40) or pure vitamin C (PbVC) showed a clear protective effect against lead-induced reduction in erythrocyte size. The degree of decrease in erythrocyte size was significantly smaller in the supplemented groups compared to the Pb group. Importantly, the highest concentrations of acerola juice (PbSR40) and pure vitamin C (PbVC) produced protective effects that were comparable to those observed in the control group, suggesting a potential role of these supplements in maintaining erythrocyte size instead of the size reduction caused by lead.

- **Changes in erythrocyte size at week 8**

At the eighth week, the protective efficacy of acerola juice was more markedly pronounced. Specifically, the groups administered PbSR40 and PbVC exhibited results that were nearly indistinguishable from those of the control group, with observed radii measuring 2.83, 2.84, and 2.85 μm , and perimeters of 17.77, 17.81, and 17.92 μm , respectively ($p >$

0.05) (Figures 1 and 2). The comparative effectiveness of the various treatment groups followed a gradient, ranked as follows: PbSR20 < PbSR30 < PbSR40 $\approx$ PbVC $\approx$ control.

• Two-way ANOVA analysis

A two-way analysis of variance (ANOVA) was performed to assess the effects of treatment and experimental duration on erythrocyte size. Both factors exerted a statistically significant influence on erythrocyte dimensions ($p < 0.001$). Furthermore, a significant interaction effect between treatment and duration was identified ($p < 0.001$) (Supplementary tables S1 and S2).

These findings indicate that prolonged lead exposure exacerbates erythrocyte shrinkage, whereas supplementation with acerola juice or pure vitamin C attenuates the extent of damage in a time-dependent manner.

Overall, this study demonstrates the deleterious impact of lead exposure (70 mg/kg body weight) on the erythrocyte morphology in mice, manifested as a marked reduction in cell size. Supplementation with acerola juice, particularly at 40 mg/kg body weight, effectively preserved erythrocyte dimensions at levels comparable to pure vitamin C (30 mg/kg body weight). These results highlight the therapeutic potential of acerola juice as a natural antioxidant source in mitigating lead-induced hematological alterations.

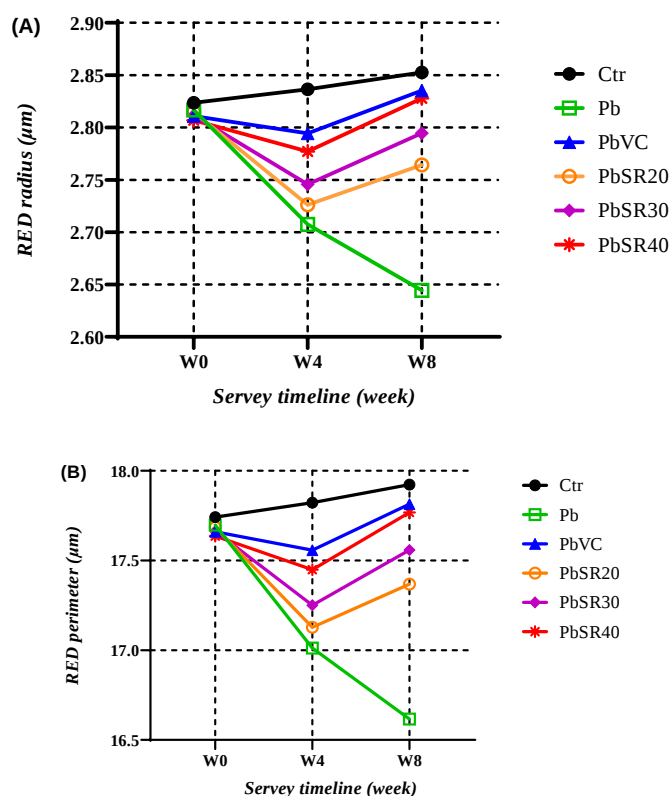


Figure 1. Graph illustrating the average erythrocyte size in mice across treatment groups over four weeks

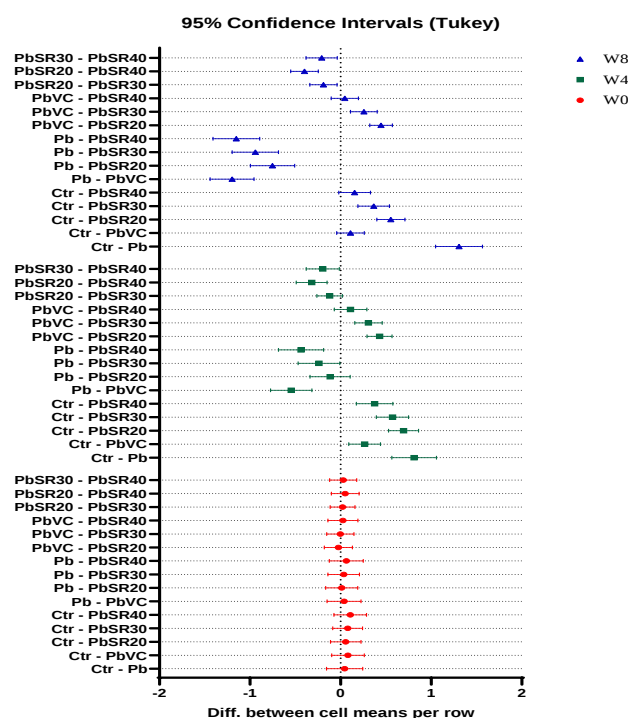


Figure 2. Comparison of red blood cell size among the treatments (Tukey test).

A difference is significant when the 95% confidence interval lies entirely to the left or right of zero

3.2. Discussion

This study demonstrates that lead exposure significantly reduces the size of red blood cells (both radius and circumference) in male albino mice after 4 and 8 weeks of exposure. These findings align with previous studies on the toxic effects of lead on the hematopoietic system, particularly the notable decrease in red blood cell counts and hemoglobin levels in the blood of mice (Ibrahim et al., 2012). Additionally, elevated blood lead levels and decreased activity of δ -aminolevulinic acid dehydratase (ALAD) were observed, alongside reductions in red blood cell counts, hemoglobin concentration, and hematocrit levels compared with control groups (Mani et al., 2020). These results underscore the role of lead in impairing erythrocytes and affecting erythropoiesis in the bone marrow. The mechanism of lead toxicity may involve the inhibition of proliferation and differentiation of erythroid progenitor cells, disrupting normal erythrocyte development (Peters et al., 2021). Furthermore, lead is known to interfere with heme synthesis in the bone marrow by inhibiting δ -aminolevulinic acid dehydratase (δ -ALAD), leading to anemia (Bergeson, 2008; Sani & Amanabo, 2021). Oxidative stress induced by lead contributes to cellular damage, including membranes and proteins (Mani et al., 2020; Mohanty et al., 2014), which can result in alterations in the shape and size of erythrocytes. Lead may compromise the integrity and fluidity of erythrocyte membranes, potentially causing cells to swell or change shape, resulting in size alterations (Mohanty et al., 2014). Such changes can lead to the formation of microcytic anemia, characterized by smaller-than-normal red blood cells (Chaudhry & Kasarla, 2023; Healthline, 2019, October 24). Consequently, these alterations may adversely impact the overall health of the exposed mice.

Vitamin C is a potent antioxidant (National Institutes of Health, 2021, October 7) and plays a crucial role in counteracting the toxicity of heavy metals, including lead (Garcia et al., 2020; Notariale et al., 2021; Ojeka et al., 2024; Pehlivan, 2017; Sharaf et al., 2017). Acerola juice is a source of vitamin C, antioxidants, 55 types of flavonoids, and 21 non-flavonoids (Vilvert et al., 2024), as well as various vitamins (NatureClaim, 2024, September 4; Prakash & Baskaran, 2018). These compounds help reduce lead toxicity by neutralizing free radicals, protecting red blood cell membranes, and maintaining cell integrity and size (Bourekoua et al., 2021). Flavonoids found in acerola juice exhibit anti-inflammatory effects (Bowen-Forbes et al., 2010), which may aid in protecting erythrocytes from lead-induced damage. Studies indicate that vitamin C supplementation, whether pure or from acerola juice, can enhance the activity of antioxidant enzymes such as superoxide dismutase, thereby reducing cellular damage caused by oxidative stress (Bourekoua et al., 2021; Ojeka et al., 2024). This suggests that both natural and pure vitamin C not only act as antioxidants but also contribute to various physiological processes, thereby helping to maintain normal erythrocyte size in lead-exposed conditions and reducing the risk of anemia and related health problems caused by lead toxicity.

4. Conclusion

This study found that lead exposure (70 mg/kg body weight) significantly decreased red blood cell size in mice. However, acerola juice, especially at 40 mg/kg body weight, provided protection, helping maintain normal red blood cell size despite lead's toxicity. These results suggest that acerola juice may serve as a supportive intervention against lead poisoning. Further studies are needed to understand how acerola juice and vitamin C provide this protection, and to explore other supportive therapies for lead exposure.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

❖ **Acknowledgments:** This research is funded by Ho Chi Minh City University of Education Foundation for Science and Technology under grant CS.2022.19.4TĐ.

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KHẢ NĂNG KHÁNG ĐỘC TÍNH CHÌ CỦA DỊCH ÉP QUẢ SƠ RI QUA KÍCH THƯỚC HỒNG CẦU CHUỘT NHẮT TRẮNG ĐỤC

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Ngày nhận bài: 05-02-2025; Ngày nhận bài sửa: 21-4-2025; Ngày duyệt đăng: 02-5-2025

TÓM TẮT

Nghiên cứu này đánh giá tác dụng kháng độc tính chì của dịch ép sơ ri thông qua việc khảo sát kích thước hồng cầu chuột nhắt trắng đục. Chuột được chia thành 6 nhóm, bao gồm nhóm đối chứng, nhóm nhiễm chì (70 mg/kg thể trọng), nhóm dùng vitamin C (30 mg/kg thể trọng) và ba nhóm kết hợp nhiễm chì với dịch ép sơ ri ở liều 20, 30, và 40 mg/kg thể trọng. Sau 4 và 8 tuần, kết quả cho thấy chì làm giảm kích thước hồng cầu chuột, trong khi dịch ép sơ ri, đặc biệt ở liều 40 mg/kg thể trọng, giúp duy trì kích thước hồng cầu ở mức bình thường trước tác động độc hại của chì. Những phát hiện này làm nổi bật tiềm năng của dịch ép sơ ri như một tác nhân hỗ trợ chống lại độc tính do chì gây ra, đồng thời khuyến nghị các nghiên cứu sâu hơn để làm rõ cơ chế và khám phá các ứng dụng tiềm năng của nó.

Từ khoá: dịch ép sơ ri; chuột nhắt trắng; nhiễm độc chì; kích thước hồng cầu; vitamin C

SUPPLEMENTARY DATA

Table S1. The average erythrocyte radius
of mice in the experimental groups after 4 and 8 weeks of treatment

Groups	Ctr		Pb		PbVC		PbSR20		PbSR30		PbSR40	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocyte radius (μm)												
Week 0	2.82	0.13	2.82	0.14	2.81	0.11	2.81	0.10	2.81	0.09	2.81	0.11
Weed 4	2.84	0.14	2.71	0.20	2.79	0.10	2.73	0.09	2.75	0.11	2.78	0.14
Weed 8	2.85	0.12	2.64	0.22	2.84	0.08	2.76	0.09	2.79	0.12	2.83	0.12
ANOVA												
Effect		F ratio and degrees of freedom						P value		Eta Sq (%)		
Experimental duration *			F (10, 3588) = 30.99***						p<0.0001		4.964	
treatment			F (1,979, 3551) = 68.75***						p<0.0001		2.202	
Experimental duration			F (5, 1794) = 90.82***						p<0.0001		7.144	
treatment												
Note: N = 1800; ANOVA: Analysis Of Variance; *** p<0.001; Eta Sq: effect size.												

Table S2. Average erythrocyte perimeter of mice
in the experimental groups after 4 and 8 weeks treatment

Groups	Ctr		Pb		PbVC		PbSR20		PbSR30		PbSR40	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocyte perimeter (μm)												
Week 0	17.74	0.82	17.70	0.88	17.66	0.72	17.69	0.61	17.66	0.57	17.64	0.71
Weed 4	17.82	0.85	17.01	1.23	17.56	0.64	17.13	0.54	17.25	0.66	17.45	0.88
Weed 8	17.92	0.76	16.62	1.37	17.81	0.52	17.37	0.55	17.56	0.73	17.77	0.74
ANOVA												
Effect	F ratio ratio and degrees of freedom								P value		Eta Sq (%)	
Experimental duration * treatment	F (10, 3588) = 30.98***								p<0.0001		4.962	
Experimental duration treatment	F (1,979, 3551) = 68.63***								p<0.0001		2.199	
	F (5, 1794) = 90.85***								p<0.0001		7.145	
Note: N = 1800; ANOVA: Analysis Of Variance; *** p<0.001; Eta Sq: effect size.												