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THE INFLUENCE OF METHYLENE BLUE AND MALACHITE GREEN ON COMMON CARP (*CYPRINUS CARPIO*) BLOOD PARAMETERS

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SUMMARY

Methylene blue (MB) and malachite green (MG) are pharmaceuticals which were used in aquaculture to treat fish diseases for many years. Nowadays, they are prohibited in many countries, but illegal use is still reported. The aim of the present study was to evaluate the effects of these agents on blood parameters of juvenile common carp (*Cyprinus carpio* L.). The fish were exposed for 7 or 14 days to therapeutic concentrations of the drugs (2 mg/l in the case of MB and 0.2 mg/l for MG). The results indicate that fish treated with MB or MG showed inflammation and an anaemic response (minor in the case of MG). Moreover, increased erythropoiesis was observed in MB-treated fish. The results of the study indicate that these pharmaceuticals at therapeutic concentrations are toxic to juvenile common carp. MB proved to be more toxic than MG. Therefore, we advise against the use of these pharmaceuticals at the concentrations tested (even if permitted by the legislation of a given country). In our opinion, a ban on their use in fish intended for human consumption is justified.

KEW WORDS: **methylene blue, malachite green, fish, toxicity**

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INTRODUCTION

Methylene blue (MB) was the first fully synthetic drug used in human medicine (Schirmer et al., 2011). Its clinical efficacy in the treatment of malaria was reported in 1891 by Guttmann and Ehrlich (Wainwright and Amaral, 2005). In fish culture, it was used to treat diseases caused by protozoa and in combating fungal infections (Supriyadi and Rukyani, 2000). However, the use of MB in aquaculture is currently prohibited in many countries, including the USA, the European Union, and Japan (Zhang et al., 2021). Nevertheless, our own observations show that MB is still occasionally used in Polish fish farms despite the restrictions. To our knowledge, both short-term and long-term (probably less common) treatments are used. Thus, the question of the risks associated with the use of this pharmaceutical remains open. The scientific data on the toxicity of methylene blue to fish is scarce and insufficient. Soltanian et al. (2021) demonstrated that 21-day exposure of goldfish (*Carassius auratus*) to a therapeutic concentration of methylene blue (2 mg/l) led to various pathophysiological changes. On the other hand, fish previously exposed to methylene blue exhibited significantly lower mortality in *Aeromonas hydrophila* challenge (Soltanian et al., 2021). MB is stable in various environmental conditions, and therefore its presence in water or fish tissues may pose a threat to human health. MB can have various health consequences in humans, including digestive disorders, mental disorders, methemoglobinemia, tissue necrosis, and increased heart rate (Khan et al., 2022).

Malachite green (MG) was used in aquaculture to control fungal, bacterial and protozoal infections, but has never been registered as a veterinary drug for treatment of food fish (Mitrowska and Posyniak, 2005; Sudova et al., 2007). Its use is now prohibited in the European Union and many other countries (except for aquarium fish treatment) (Mitrowska and Posyniak, 2005). However, it is sometimes used illegally as a disinfectant, to treat diseases caused by fungi (*Achlya*, *Aphanomyces*, or *Saprolegnia*), to treat bacterial infections caused by *Flexibacter* spp., and to control parasitic protozoa (*Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Trichodina* spp., *Trichodinella* spp., or *Chilodonella* spp.). Both short-term and long-term treatments are applied (Mitrowska and Posyniak, 2005). MG is considered to be a toxic chemical. Li et al. (2019) demonstrated that it affected the gut microbiota and immune status of goldfish (*C. auratus*). Moreover, various pathological changes and processes, i.e. carcinogenesis, mutagenesis, chromosomal fractures, teratogenesis, histopathological changes, and haematological changes, have been observed in fish exposed to MG (Srivastava et al., 2004). Sinha and Jindal (2020) showed that *Cyprinus carpio* treated with a low concentration (0.087 mg/l) of MG exhibited behavioural changes, oxidative stress, and histopathological changes. MG is readily absorbed by fish tissues, where it undergoes biotransformation to leucomalachite green (LMG), which is lipophilic and remains in fatty tissue for long periods of time and can enter the human food chain and accumulate in human tissues (Gharavi-Nakhjavani et al., 2023). Residues of MG and its reduced form (LMG) have been detected in serum, liver, kidney, muscles and other fish tissues, as well as in eggs and fry. For mammals, MG is mutagenic and carcinogenic and can cause organ damage and developmental abnormalities (Srivastava et al., 2004).

Despite the fact that both MB and MG were used in fish farming for many years, we hypothesise that data on the toxicity of these drugs to fish are often out of date. Analysis of blood parameters is known to be an important tool in assessment of the toxic effects of pharmaceuticals and other xenobiotics on fish (Burgos-Aceves et al., 2019; Bojarski and Witeska, 2020; Bojarski et al., 2020). Thus, we decided to evaluate the effects of methylene blue and malachite green applied at therapeutic

concentrations, selected after consulting ichthyologists and fish pathologists (2 mg/l MB and 0.2 mg/l MG), on the haematological parameters (including the erythrogram – erythrocyte morphology based on a blood smear) and selected blood biochemical parameters of common carp (*Cyprinus carpio* L.). We chose common carp because it is a model organism commonly used in toxicological research (e.g. Kondera et al., 2020; Kondera et al., 2021; Bojarski et al., 2022). Moreover, it is an important aquaculture species in many Asian and some European countries (such as Poland) (Rahman 2015).

MATERIALS AND METHODS

Animals and experimental conditions

The study was approved by the Local Ethics Committee for Animal Testing at the Medical University of Silesia in Katowice (Resolution No. 32/2022 of 30.06.2022). Clinically healthy sexually immature common carp of the R3R8 laboratory line were used. The weight and length of the fish were 64.73 ± 6.75 g (mean ± SD) and 15.96 ± 0.71 cm (mean ± SD), respectively. The animals were kept in six 300-litre tanks filled with water to 200 litres each (10 fish per tank). Before exposure to the drugs they underwent acclimation (14 days). Next, three equal groups were established: the methylene blue (MB) group, malachite green (MG) group, and control (C) group. Each group consisted of two tanks (10 fish per tank). The fish were continuously exposed to the chemical applied at a therapeutic concentration, i.e. 2 mg/l of methylene blue or 0.2 mg/l of malachite green. After 7 and 14 days of treatment, blood was sampled from 10 fish from each group. Water quality parameters were measured twice a week during both acclimation and exposure. The analysis was conducted using colorimetric kits produced by Zoolek (Poland) and a WTW oximeter. The water parameters were as follows: temperature 20.6–21.5°C; O_2 5.12–5.77 mg/l; pH 7.04–7.45; NH_3 0.01–0.02 mg/l; NO_2^- 0–0.2 mg/l; NO_3^- 5–10 mg/l; total hardness 3–5 °n; carbonate hardness 3–4 °n.

Haematological analysis

After blood collection, haematological analysis was performed. Red blood cell (RBC) count and white blood cell (WBC) count were estimated using a Bürker chamber and a standard optical microscope. Erythrocytes were counted in an area of 0.2 mm^2, while leukocytes were counted in an area of 4 mm^2. Before counting, the blood was diluted 100 times with Hayem's solution. Haematocrit (Ht) was determined by the microhaematocrit method (16,000 g, 5 min), and haemoglobin (Hb) concentration by the cyanmethaemoglobin method; the absorbance was read at 540 nm. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulas (Bomski, 1983). Blood smears were made and stained with the HemKolor kit (Stamar, Poland) for analysis of the leukogram (differential leukocyte count) and erythrogram (red blood cell morphology determined on the basis of a stained blood smear). The following types of erythrocytes were differentiated in the erythrogram analysis: mature normal erythrocytes, immature normal erythrocytes – erythroblasts (Fig. 1), haemolysed erythrocytes (Fig. 2), erythrocytes showing cytoplasm vacuolization (Fig. 3), erythrocytes with an abnormal nucleus shape (Fig. 4), and erythrocytes with an abnormal cell shape (Fig. 5).

Biochemical analysis

A portion of the blood was centrifuged (3,000 g, 20 min) to obtain plasma for biochemical analysis. The plasma was stored at −80°C prior to the measurements. It was then used to determine the concentrations of glucose (GLU), total protein (TP), triglycerides (TG), and cholesterol (TCH),

as well as alanine aminotransferase (ALT) activity. All the biochemical parameters listed above were measured using BioSystems assays according to the manufacturer's instructions (BioSystems S.A., Barcelona, Spain). For determination of GLU, TP, TG, and TCH concentrations, the EPOCH microplate reader (BioTek Instruments, USA) was used. ALT activity was analysed using the V-730 UV-visible spectrophotometer (Jasco).

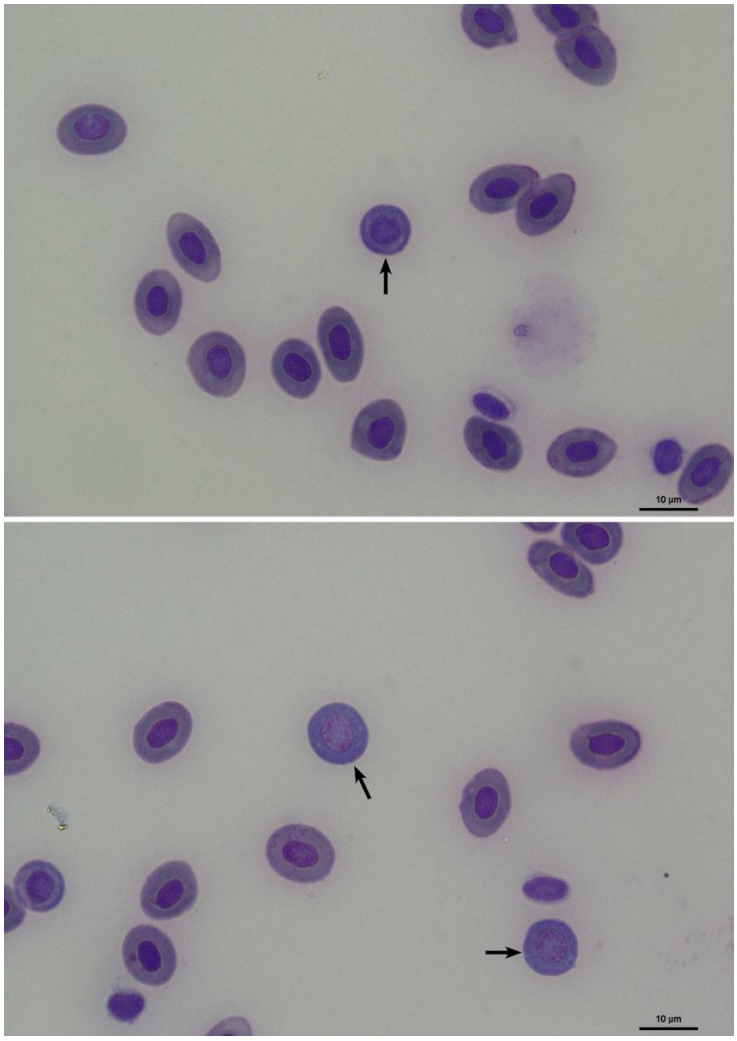

Figure 1. Immature common carp red blood cells – erythroblasts (marked with arrows)

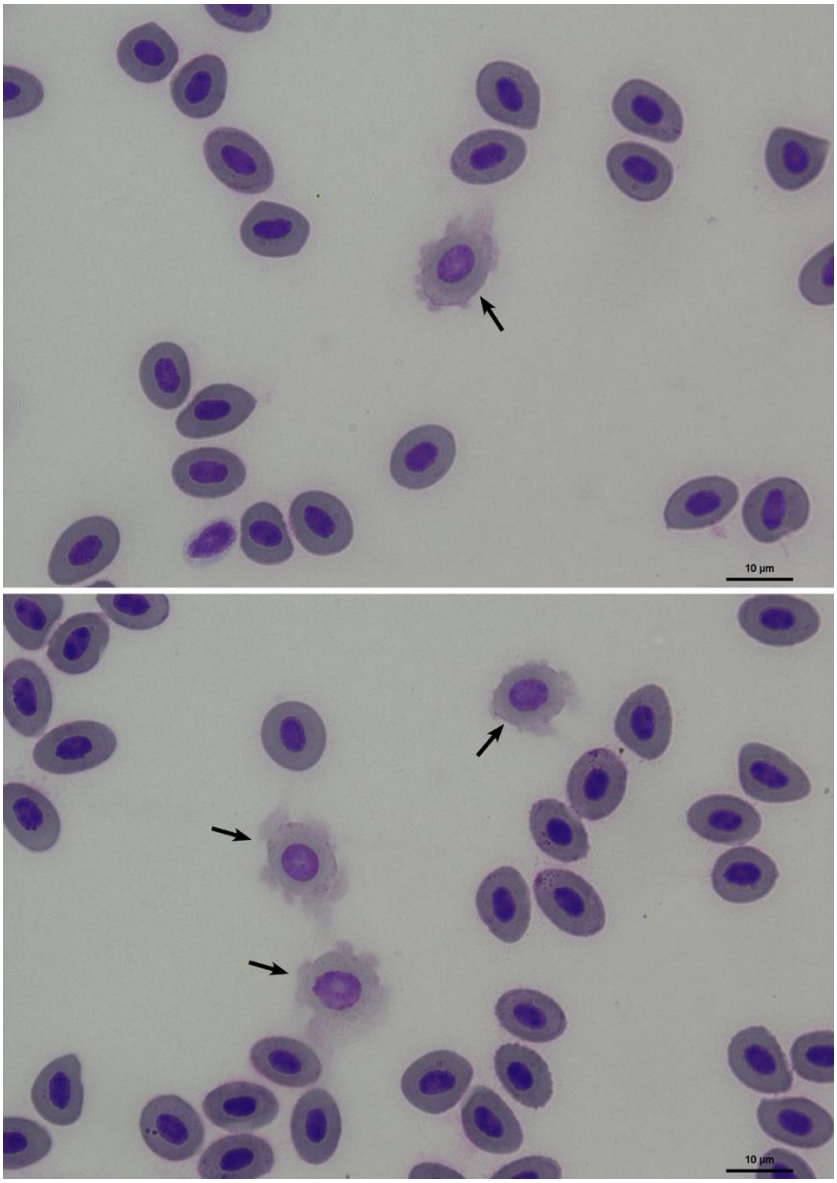

Figure 2. Haemolysis of common carp red blood cells (haemolysed erythrocytes are indicated with arrows)

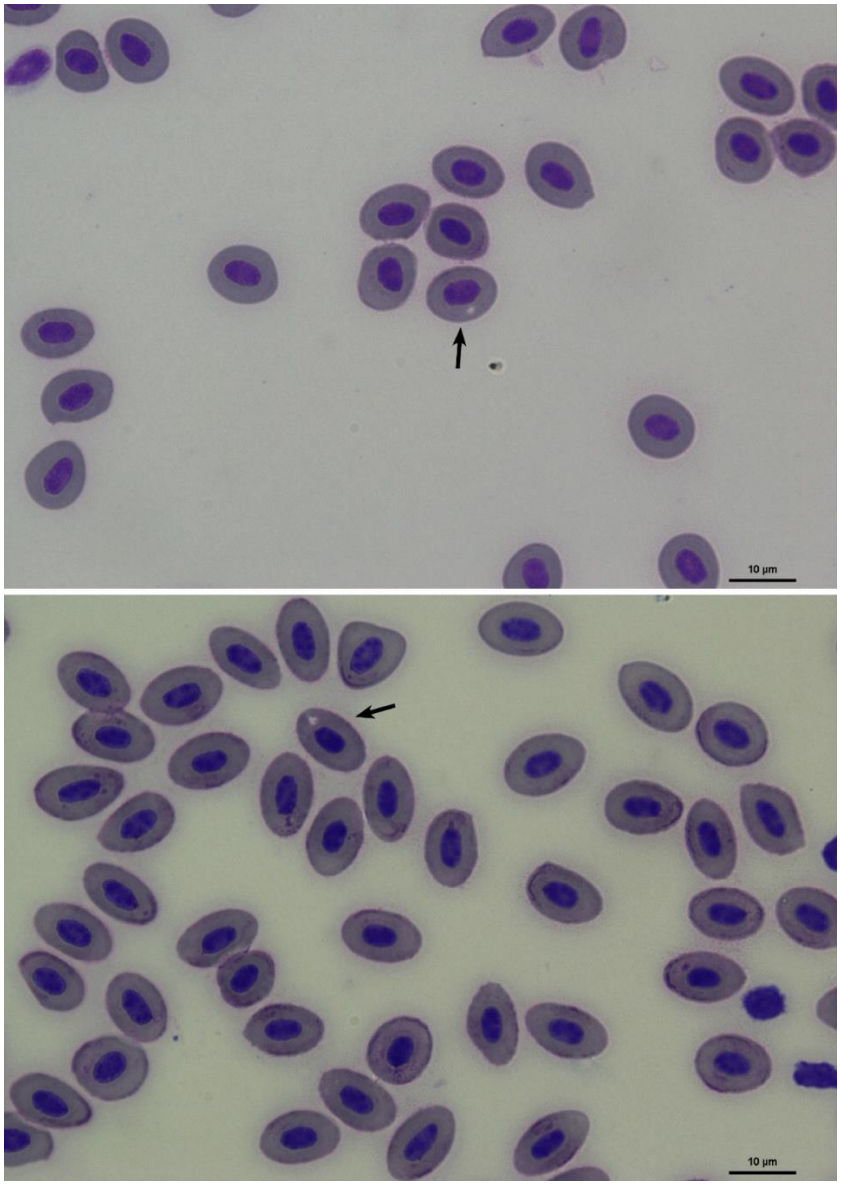

Figure 3. Vacuolization of common carp red blood cells (vacuolated erythrocytes are indicated with arrows)

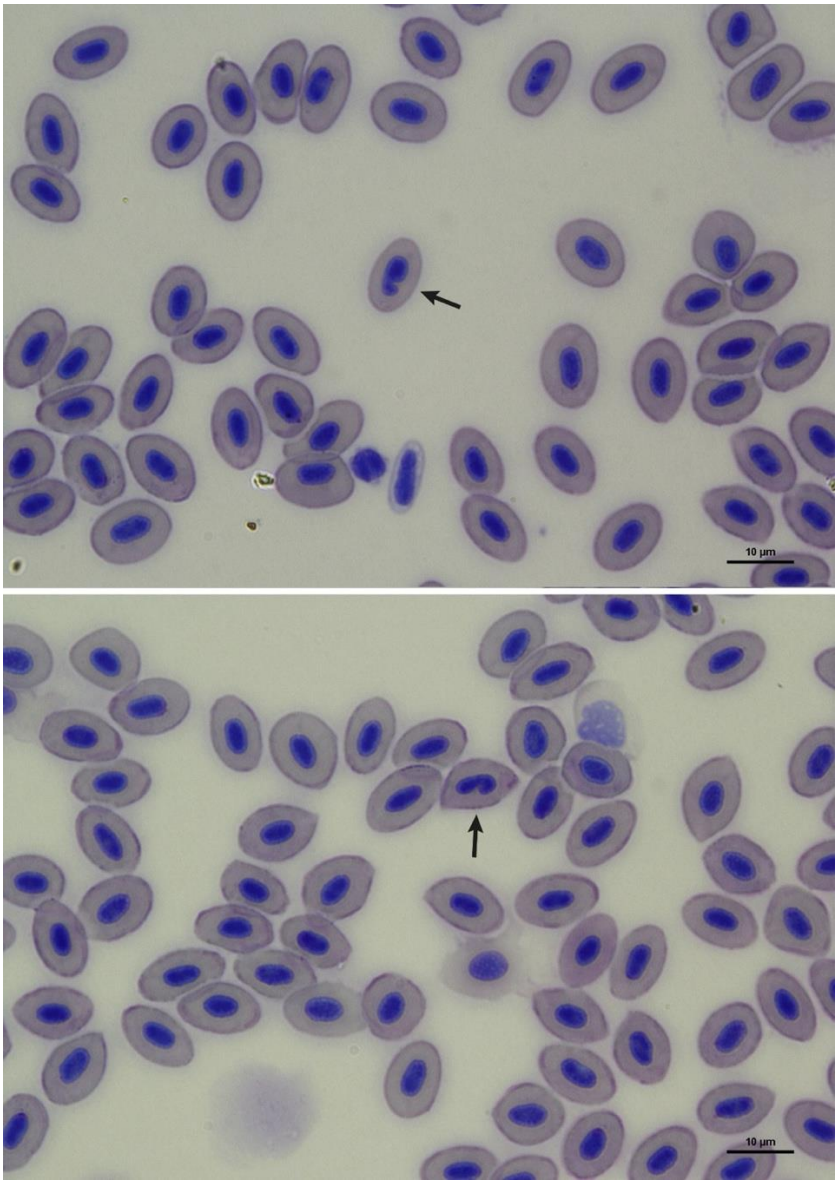

Figure 4. Abnormal nucleus shape of common carp red blood cells (erythrocytes with an abnormal nucleus shape are indicated with arrows)

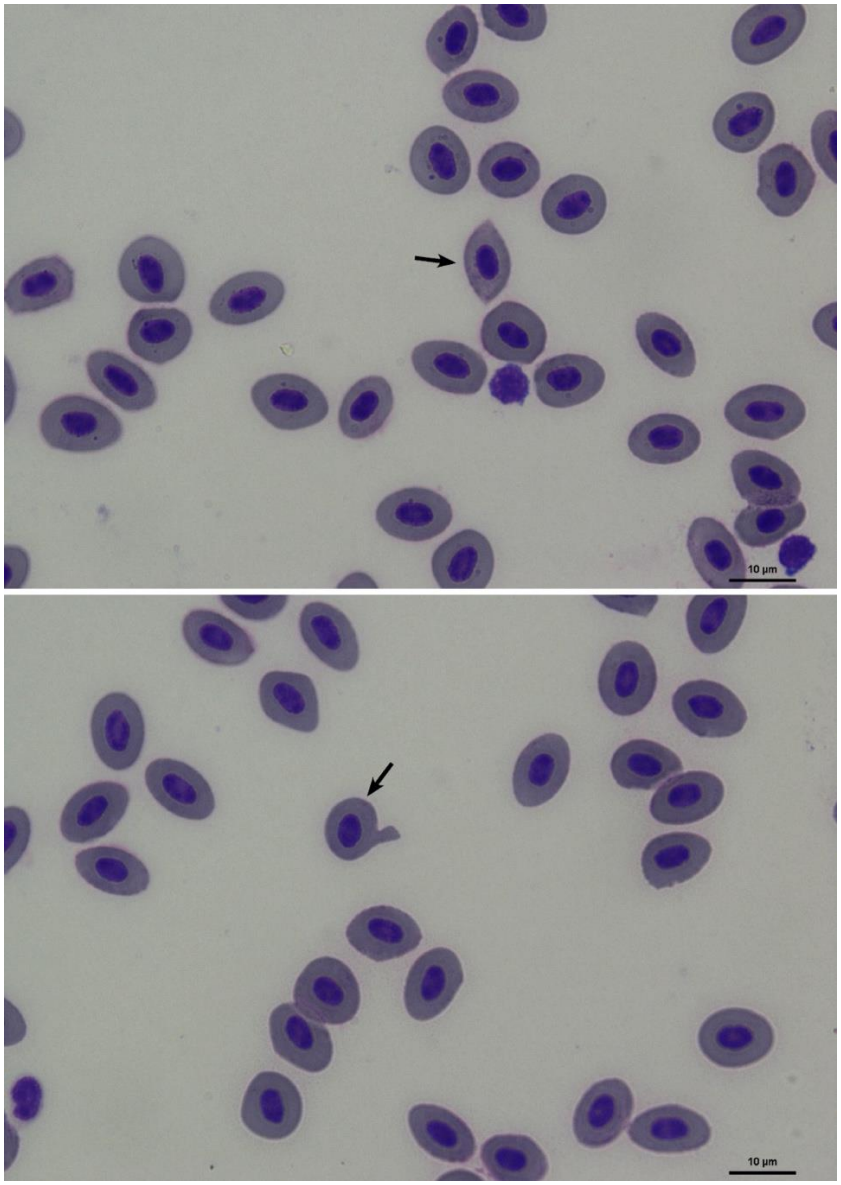

Figure 5. Abnormal cell shape of common carp red blood cells (erythrocytes with an abnormal cell shape are indicated with arrows)

Statistical analysis

In the statistical analysis, the choice between the parametric model (one-way ANOVA, Tukey HSD) and the non-parametric model (Kruskal-Wallis, Dunn) depended on the results of verification of one-way ANOVA model assumptions. The assumption of compliance of the data with the normal distribution was verified using the Shapiro–Wilk test, and the assumption of the homogeneity of variances was verified using the Levene test. The significance level of the tests was set at 0.05.

For the haematological parameters (except the erythrogram and leukogram) obtained after 7 and 14 days of exposure, in 8 of 14 cases the Shapiro–Wilk test did not reject the assumption of compliance of the data with the normal distribution, while the Levene test did not reject the assumption of homogeneity of variances in any of the 14 cases. For the biochemical parameters obtained after 7 and 14 days of exposure, in 8 of 10 cases the Shapiro–Wilk test did not reject the assumption of compliance of the data with the normal distribution, while in 9 of 10 cases the Levene test did not reject the assumption of homogeneity of variances. Thus one-way ANOVA was applied, and (where $p < 0.05$) the Tukey HSD test was used for post-hoc analysis.

For the erythrogram results obtained after 7 and 14 days of exposure, in 3 of 12 cases the Shapiro–Wilk test did not reject the assumption of compliance of the data with the normal distribution, while in 10 of 12 cases the Levene test did not reject the assumption of homogeneity of variances. For the leukogram results obtained after 7 and 14 days of exposure, in 2 of 16 cases the Shapiro–Wilk test did not reject the assumption of compliance of the data with the normal distribution (in 3 cases the Shapiro–Wilk test could not be used due to the data structure), while the Levene test did not reject the assumption of homogeneity of variances in 14 of 16 cases. Therefore the Kruskal–Wallis test was applied, and (where $p < 0.05$) the Dunn test with Bonferroni correction for three comparisons was used for post-hoc analysis. The p-values presented were adjusted when corrections were applied. Each significance level was set at 0.05.

The statistical analysis was carried out using R free software (R Foundation for Statistical Computing, version 4.2.2).

RESULTS AND DISCUSSION

The fish exposed to methylene blue showed lower Ht ($p < 0.001$), Hb concentration ($p < 0.001$) and MCV ($p < 0.01$) after 7 days of exposure. A lower count of mature red blood cells ($p < 0.01$) was accompanied by a higher percentage of immature forms ($p < 0.05$), WBC count and metamyelocyte percentage were increased ($p < 0.05$ and $p < 0.01$, respectively), while the monocyte percentage was lower ($p < 0.05$) than in the control group (Table 1). Longer exposure (14 days) resulted in a reduction in the RBC count ($p < 0.01$), Ht level ($p < 0.001$), Hb concentration ($p < 0.001$) and MCHC ($p < 0.001$). The percentage of mature erythrocytes was also decreased ($p < 0.05$), while the ratio of immature forms was increased ($p < 0.01$). A decrease in the percentage of haemolysed red blood cells ($p < 0.05$) and an increase in the percentage of vacuolated erythrocytes ($p < 0.05$) were observed as well. Moreover, the percentage of lymphocytes was lower in comparison to the control value ($p < 0.05$), while the ratio of segmented neutrophils was increased ($p < 0.05$) (Table 2).

Table 1.
Haematological changes in common carp (*Cyprinus carpio*) after 7 days of exposure to methylene blue (MB; 2 mg/l) or malachite green (MG; 0.2 mg/l)

Parameter	Control group		Group MB		Group MG	
	mean	SD	mean	SD	mean	SD
RBC [10^6/µl]	1.64^{ab}	0.44	1.56^{a}	0.60	2.23^{b}	0.59
Ht [%]	27.68^{a}	5.21	17.63^{b}	3.10	28.48^{a}	2.43
Hb [g/dl]	6.81^{a}	0.98	4.64^{b}	0.83	7.21^{a}	0.67
MCV [fl]	173.50^{a}	34.46	122.59^{b}	33.33	136.36^{ab}	36.59
MCH [pg]	43.29^{a}	9.39	32.37^{a}	9.51	35.02^{a}	11.71
MCHC [g/dl]	25.20^{a}	5.07	26.36^{a}	1.63	25.36^{a}	1.77
MNE [%]	95.40^{a}	1.51	88.23^{b}	6.31	89.53^{b}	5.38
INE [%]	2.20^{a}	1.22	6.53^{b}	6.15	3.53^{ab}	1.88
HE [%]	0.87^{a}	0.80	1.57^{a}	0.69	2.70^{a}	2.77
ECV [%]	0.77^{a}	0.50	1.83^{a}	1.33	1.13^{a}	0.53
ECN [%]	0.40^{a}	0.31	0.63^{a}	0.29	0.77^{a}	0.50
ECS [%]	0.37^{a}	0.37	1.20^{ab}	0.55	2.33^{b}	0.87
WBC [10^3/µl]	17.40^{a}	5.86	28.78^{b}	9.37	30.73^{b}	9.13
Lym [%]	89.90^{a}	3.87	85.30^{a}	3.43	89.60^{a}	3.84
Seg [%]	2.00^{a}	2.11	1.70^{a}	1.16	1.50^{a}	0.97
Band [%]	1.00^{a}	1.33	1.70^{a}	1.06	1.50^{a}	0.85
Meta [%]	1.80^{a}	1.62	5.40^{b}	2.41	2.60^{ab}	0.97
Myelo [%]	1.90^{a}	1.97	3.70^{a}	2.11	2.90^{a}	2.02
Baso [%]	0.40^{ab}	0.97	1.00^{a}	0.82	0.00^{b}	0.00
Eos [%]	0.20^{a}	0.63	0.20^{a}	0.42	0.00^{a}	0.00
Mono [%]	2.80^{a}	1.93	1.00^{b}	0.94	1.90^{ab}	1.20

a, b – values in the same row with different superscripts are significantly different ($P < 0.05$).

MNE = mature normal erythrocytes; INE = immature normal erythrocytes; HE = haemolysed erythrocytes; ECV = erythrocytes showing cytoplasm vacuolization; ECN = erythrocytes showing abnormal nucleus shape; ECS = erythrocytes showing abnormal cell shape; Lym = lymphocytes; Seg = segmented neutrophils; Band = band neutrophils; Meta = metamyelocytes; Myelo = myelocytes; Baso = basophils; Eos = eosinophils; Mono = monocytes

Table 2.
Haematological changes in common carp (*Cyprinus carpio*) after 14 days of exposure to methylene blue (MB; 2 mg/l) or malachite green (MG; 0.2 mg/l)

Parameter	Control group		Group MB		Group MG	
	mean	SD	mean	SD	mean	SD
RBC [10^6/µl]	2.30^a	0.49	1.58^b	0.34	1.99^{ab}	0.41
Ht [%]	28.70^a	1.03	23.25^b	2.19	25.00^b	1.67
Hb [g/dl]	6.87^a	0.44	4.51^b	0.49	6.71^a	0.48
MCV [fl]	129.87^a	25.76	153.13^a	36.53	130.29^a	29.09
MCH [pg]	31.16^a	6.86	29.80^a	7.87	34.66^a	5.84
MCHC [g/dl]	23.98^a	1.94	19.41^b	1.23	26.87^c	1.55
MNE [%]	86.80^a	7.70	70.37^b	11.28	90.90^a	3.39
INE [%]	4.53^a	2.59	24.80^b	9.55	3.03^a	1.27
HE [%]	5.00^a	4.88	0.73^b	1.35	1.67^{ab}	1.95
ECV [%]	1.03^a	0.48	1.90^b	0.89	1.47^{ab}	0.88
ECN [%]	0.83^a	0.77	0.93^a	0.86	1.53^a	1.48
ECS [%]	1.80^a	0.80	1.27^a	1.23	1.40^a	0.94
WBC [10^3/µl]	49.45^a	9.42	38.98^a	8.72	39.00^a	11.92
Lym [%]	95.20^a	1.81	88.50^b	9.08	88.80^b	6.32
Seg [%]	0.30^a	0.67	2.20^b	2.25	2.40^b	1.43
Band [%]	0.80^a	1.14	2.00^a	2.31	1.20^a	1.03
Meta [%]	0.80^a	0.79	2.20^a	2.94	2.30^a	2.67
Myelo [%]	1.30^a	1.34	2.50^a	2.27	2.20^a	1.69
Baso [%]	0.30^a	0.48	1.10^a	1.73	0.70^a	0.82
Eos [%]	0.00^a	0.00	0.00^a	0.00	0.10^a	0.32
Mono [%]	1.30^a	1.25	1.50^a	1.08	2.30^a	1.64

a, b – values in the same row with different superscripts are significantly different ($P < 0.05$). Abbreviations are given under Table 1.

The results of the haematological analysis indicate that exposure of common carp to methylene blue resulted in an anaemic response and inflammation (irrespective of exposure time). The increase in the percentage of juvenile erythrocytes (detected after 7 and 14 days of treatment), which indicates increased erythropoiesis, appears to be a compensatory response associated with anaemia.

The fish exposed to malachite green for 7 days had fewer mature erythrocytes ($p < 0.01$), a higher percentage of erythrocytes with an abnormal shape ($p < 0.001$), and a higher WBC count ($p < 0.01$) than the control individuals (Table 1). Longer exposure (14 days) led to a decrease in Ht ($p < 0.001$) and an increase in MCHC ($p < 0.01$). The percentage of lymphocytes was decreased ($p < 0.05$), while the percentage of segmented neutrophils was higher than in the control group ($p < 0.01$) (Table 2).

The increased level of red blood cells with an abnormal shape observed after 7 days of exposure to MG may be a result of cell damage caused by the agent. The results also suggest inflammation (after 7 and 14 days of exposure) and a slight anaemic response (after 14 days of treatment).

The results of the biochemical analysis are presented in Tables 3 and 4. The only statistically significant ($p < 0.05$) change in the biochemical parameters tested in the study was an increase in TP concentration in fish exposed to methylene blue after 14 days of treatment (Table 4). This change may be associated with the inflammation mentioned above. No statistically significant changes in the blood biochemical parameters were noted after 7 days of exposure to the pharmaceuticals (Table 3), which suggests that the processes observed are time-dependent.

Table 3.
Biochemical changes in common carp (*Cyprinus carpio*) after 7 days of exposure to methylene blue (MB; 2 mg/l) or malachite green (MG; 0.2 mg/l)

Parameter	Control group		Group MB		Group MG	
	mean	SD	mean	SD	mean	SD
GLU [mg/dl]	130.10[a]	21.49	113.37[a]	28.30	124.40[a]	23.44
TP [g/l]	24.74[a]	6.05	28.68[a]	4.19	26.91[a]	2.97
TG [mg/dl]	121.86[a]	26.41	142.82[a]	25.59	148.80[a]	23.10
TCH [mg/dl]	155.91[a]	33.13	147.40[a]	31.74	170.10[a]	34.50
ALT [U/l]	64.60[a]	12.21	61.03[a]	13.62	58.39[a]	12.25

a, b – values in the same row with different superscripts are significantly different ($P < 0.05$).
GLU = glucose; TP = total protein; TG = triglycerides; TCH = cholesterol; ALT = alanine aminotransferase

Table 4.
Biochemical changes in common carp (*Cyprinus carpio*) after 14 days of exposure to methylene blue (MB; 2 mg/l) or malachite green (MG; 0.2 mg/l)

Parameter	Control group		Group MB		Group MG	
	mean	SD	mean	SD	mean	SD
GLU [mg/dl]	89.05[a]	13.56	88.03[a]	24.84	93.01[a]	27.16
TP [g/l]	24.48[a]	2.20	27.33[b]	1.93	26.79[ab]	3.10
TG [mg/dl]	141.54[a]	21.58	163.54[a]	17.75	146.24[a]	22.98
TCH [mg/dl]	159.76[a]	32.33	167.26[a]	23.05	147.02[a]	29.28
ALT [U/l]	49.50[a]	8.54	56.51[a]	13.96	60.55[a]	11.13

a, b – values in the same row with different superscripts are significantly different ($P < 0.05$). Abbreviations are given under Table 3.

Interestingly, exposure to malachite green in the present study induced considerably fewer pathophysiological changes in comparison to methylene blue, suggesting that it is less toxic to common carp. However, it should be noted that the concentration of malachite green was ten times lower than that of methylene blue.

Changes in the haematological profile (red or white blood cell indices) in fish have been reported in the context of various pharmaceutical agents used in aquaculture. The evident anaemic state demonstrated in the present study in fish from the methylene blue-treated group was previously observed in goldfish (*C. auratus*) by Soltanian et al. (2021). The authors demonstrated a marked reduction in RBC count, Ht value, Hb concentration and MCV after 14 and 21 days of exposure (2 mg/l). This may indicate that the pathophysiological changes occurring in fish as a result of MB

exposure are not species-specific. Svobodova et al. (1997) exposed common carp to MG (0.5 mg/l) for 6 days and observed that the Ht value and MCV were decreased, while MCHC was increased. Similar changes (decreased Ht and increased MCHC) were noted in the present study after 14 days of exposure to the same chemical applied at a lower concentration (0.2 mg/l). This suggests that the toxicological effects of malachite green in common carp are dose- and time-dependent. Omoregie and Oyebanji (2002) revealed reductions in the RBC count, Ht value, and Hb level in Nile tilapia (*Oreochromis niloticus*) treated with oxytetracycline. According to the authors, the anaemia reported in their study may be a result of destruction of erythrocytes, inhibition of erythrocyte production, or poor utilization of the nutrients contained in the medicated feed. In the present study, an increase in the percentage of deformed red blood cells was detected after 7 days of treatment with MG. Similarly, an increased ratio of abnormal erythrocytes in common carp blood after short-term exposure (1 hour, 4 times) was observed by Witeska et al. (2013). This may confirm the conclusion by Omoregie and Oyebanji (2002) that some pharmaceuticals appear to damage red blood cells. Therefore, it can be assumed that exposure to drugs may lead to hypoxia in fish. This is confirmed by observations reported by Svobodova et al. (1997), who demonstrated that common carp exposed to MG exhibited histopathological changes in the gills.

Changes in white blood cell parameters have also been reported in the context of exposure of fish to pharmaceutical agents. For example, research by Saglam et al. (2003) showed that rainbow trout (*Oncorhynchus mykiss*) subjected to a therapeutic bath in MG (5 mg/l for 15 min or 66.67 mg/l for 30 sec; 5 times) exhibited a decreased WBC count, which may indicate immune suppression. Similarly, a lower leukocyte count was detected in common carp exposed to the same agent (Witeska et al., 2013). On the other hand, oxytetracycline enhanced the non-specific immune response in sea bream (*Sparus aurata*) (Serezli et al., 2005). Sahan (2020) observed an increased WBC count by in trichlorphon-exposed and in formalin-exposed common carp, possibly due to inflammation generated by the toxic action of the drugs. On the other hand, Witeska et al. (2013) found that formalin had no effect on the number of white blood cells in fish of the same species. Interestingly, exposure of *Cirrhinus mrigala* to sulfamethazine may lead to leucocytosis or leukopenia (Ramesh et al., 2018). In the present study, haematological changes suggesting an inflammatory response (i.e. increased WBC count and neutrophil percentage) were detected in fish exposed to methylene blue as well as in those treated with malachite green.

CONCLUSIONS

As mentioned above, the use of the substances tested in the present study is prohibited in fish intended for human consumption in the European Union and many other countries. Our results confirm their toxic effect on fish. Methylene blue (2 mg/l) proved to be more toxic to juvenile common carp than malachite green (0.2 mg/l). We advise against the use of MG and MB at these concentrations, even where permitted by the legislation of a given country.

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