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The effects of hydro-alcohol extract of follower of marshmallow (*Althaea officinalis* L.) on some biochemical and hematological parameters in common carp (*Cyprinus carpio* L.)

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ABSTRACT

Background & Aim: Marshmallow (*Althaea officinalis*) is one of the most popular medicinal plants used in traditional medicine with antibacterial and antioxidant properties. This study was conducted to evaluate the effects of marshmallow extract (*Althaea officinalis* L.) administration on hematological and biochemical parameters in common carp.

Experimental: 150 carp (weighing 37.65 ± 4.40 g) were fed with diets containing 0.0 (control diet), 2.5, 5, and 10 g marshmallow extract for 60 days. Then, the hematological and blood biochemical parameters were measured on days 30 and 60 of the experiment.

Results & Discussion: No significant difference was found ($p>0.05$) in red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCH), number of neutrophils, basophils, and eosinophil in fish fed with different concentrations of marshmallow extract on day 60. Administration of marshmallow extract (5 g) significantly increased ($p<0.05$) white blood cells (WBC) and lymphocytes on day 60. On the other hand, administration with 5 and 10 g of marshmallow extract decreased monocytes ($p<0.05$) on day 30. Administrations of *A. officinalis* extract to the fishes for 60 days did not have any significant effect on alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatine phosphokinase (CPK) activities and creatinine levels. Plasma aspartate aminotransferase (AST), lactate dehydrogenase (LDH) activities and total protein and albumin were significantly high in fishes that fed *A. officinalis* at doses of 10 g/kg ($P < 0.05$) compare to the control group. Plasma cholesterol and triglyceride levels in the treated group were also significantly decreased at the 5 and 10 g/kg dose levels of *A. officinalis* extract. The significant changes was observed in hematological and biochemical parameters in fish fed with 10 g of marshmallow extract may be attributed to cytotoxicity, however 2.5 and 5 g of extract did not have adverse effects on the hematological and biochemical parameters of common carp in this study.

Industrial and practical recommendations: According to the results, it was concluded that preclinical administration of certain concentrations of marshmallow extract (2.5 and 5 g) was beneficial for carp.

1. Introduction

Recent developments in pharmacology and pharmacotherapy in veterinary, especially in aquaculture indicate dramatic changes, so that diseases that were once fatal in aquatic animals are now easily preventable and treatable, thanks to new medicines. In recent decades, many attempts have been directed toward introducing more effective drugs with less severe side effects. Natural resources including medicinal plants, with their good reputation in traditional medicine are one of the ways to develop new drugs with fewer side effects (Banaee, 2010; Asadi *et al.*, 2012).

Using medicinal plants in aquaculture industry goes back to last few decades. However, preclinical evaluation of herbal compounds and the influence of plant derivatives on the health of aquatic animals have not gained much attention and many researchers deal with clinical evaluation of medicinal plants and their effects on the prevention and treatment of diseases (Yin *et al.*, 2006; Divyagnaneswari *et al.*, 2007; Ardó *et al.*, 2008; Yin *et al.*, 2009). That is why in some cases, administration of medicinal plants has not been effective enough in control and treatment of diseases. In fact, prior to conducting any clinical tests and based on the principles of pharmacology, new drugs should be evaluated in terms of immunogenicity and toxicity. In a preclinical stage, usually depending on a drug application, the drug is examined to estimate its toxicity, sub-acute and chronic toxicity, and its effects on blood cells, biochemical and physiological factors and other biological aspects such as mutagenicity and probable side effects on target species or biologically similar species. Hematological and biochemical tests are of great importance in preclinical assessment of new drugs. By conducting biochemical studies, we may predict pharmaceutical effects of a drug on target organs and gain fairly comprehensive information on the physiological state of cells and tissues when changes in tissues are not readily observable, for instance in liver. Such information can be beneficial in evaluating pharmacological and toxicological properties of drugs to determine their nontoxic doses (Banaee *et al.*, 2011, Nafisi Bahabadi *et al.*, 2014). Hematology techniques, including red blood cells count, hemoglobin concentration, hematocrit measurement, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH), changes in mean corpuscular hemoglobin concentration (MCHC), and white blood cells and differential count provide valuable information on the health status of fish (Ahmadi *et al.*, 2012).

Marshmallow (*Althaea officinalis*) is one of the most popular medicinal plants used in traditional medicine with antibacterial (Sartoratto *et al.*, 2004; Burt *et al.*, 2005; Astani *et al.*, 2010; Bilia *et al.*, 2014; Kubiça *et al.*, 2014; Abbaszadeh *et al.*, 2014) and antioxidant properties (Jun *et al.*, 2014; Puri *et al.*, 2014; Ramos *et al.*, 2014) because of having mucilage, various polysaccharides, antioxidants, flavonoids, terpenes, terpenoids, plant sterols, saturated and unsaturated fatty acids, and phenolic and volatile compounds (Elmastas *et al.*, 2004; Wynn and Fougére, 2007; Sadighara *et al.*, 2012; Tešević *et al.*, 2012). Therefore, marshmallow can be used as a medicinal plant in veterinary medicine, particularly in aquaculture. So, to gather information on the probable effects of marshmallow extract on certain physiological aspects of fish, this study investigated preclinical and dietary administration of marshmallow extract at different concentrations on blood cells and some of the blood biochemical parameters in common carp (*Cyprinus carpio*) to determine potential adverse effects of this plant, if any, on fish.

2. Materials and Methods

2.1. Fish

One hundred eighty common carps (with the average weight and length of 37.65 ± 4.40 g and 14.15 ± 0.8 cm) were purchased from a private farm (Carp Farm, Shush, Khuzestan province, Iran) and then were transferred to the aquaculture laboratory of Aquaculture Department at Behbahan Khatam Alanbia University of Technology. Then, 15 fish were randomly distributed in 12 closed water-recirculating systems (2000 L). Fish were acclimated to the experimental conditions for 2 weeks (24 ± 2° C; pH: 7.4 ± 0.2; 16 L/8D; 40% water exchange rate/day). During acclimation, the fish were fed a commercial diet by Beyza Feed Mill, Shiraz, Iran twice a day and no more than 2% of their body weight (Table 1).

2.2. Marshmallow (Althaea officinalis) extract preparation

The powder of dried flower *A. officinalis* was mixed with distillated water and ethanol (1:1), and put on the shaker for 24 hours at room temperature. The resulting hydroalcoholic extract was filtered through Whatman

filter paper and evaporated to dryness on a rotary evaporator until it became creamy, and was then dried in an oven (50 °C) to finally 7gave 8 g (8 % of initial amount) dried powder. The concentration used in the experiment was based on the dry weight of the extract.

2.3. Fish diet preparation

The formulated fish food was prepared in the laboratory using powder of commercial food obtained from Beyza Feed Mill, Shiraz, Iran. To enrich the normal diet, the 2.5, 5 and 10 g of *A. officinalis* extracts were mixed with 1 kg powder feed. Each supplemented diet was mixed with distilled water (1mL/g) until obtaining a homogenous mixture. This mixture was passed through a meat grinder, producing extruded string shapes, which were dried in an oven at 55°C for 12 h and then broken to produce pellets approximately 10 mm long. The pellets were packed and stored at -20°C in a freezer until be used. The control diet was prepared by the same process, although no supplement was added.

2.4. The final experiment

The final experiment was done in a completely randomized design with 3 treatments and a control sample. The fish were fed twice a day with commercial diet (no more than 2% of their body weight) enriched with 2.5, 5 and 10 g of the hydro-alcoholic extract of marshmallow. After 30 and 60 days, 9 fish were captured randomly from each group and then anesthetized with clove powder solution (200 mg/L). Then, the fish blood was collected from their tail stem, and stored in sterilized glass vials at 4 °C containing the anticoagulant heparin. The blood was centrifuged for 15 min at 6000 g, 4 °C. Plasma samples were immediately stored at -21 °C until biochemical analysis.

2.5. Haematological parameters

2.5.1. WBC and RBC. The blood was immediately used to determine the number of red blood cells (RBC) and white blood cells (WBC) by means of a haemocytometer slide (Improved Neubauer type) at a magnification of x 400. Thus, blood was diluted to with diluting fluid including sodium citrate, Crystal violet dissolved in Ringer solution, brilliant blue dissolved in Ringer solution at pH 7.2 (Vázquez and Guerrero, 2007; Banaee *et al.*, 2008).

2.5.2. Haematocrit (Hct). Haematocrit (Hct) was determined by the microhematocrit method described by Vázquez and Guerrero (2007).

2.5.3. Haemoglobin (Hb). Haemoglobin (Hb) concentration was calculated by using the cyanohaemoglobin method (Vázquez and Guerrero, 2007). Briefly, 20 µl of whole blood was mixed with 5 ml of Drabkin's solution (Zist-chemistry Kit Co., Iran) in a test tube before allowing standing for at least 15 min at room temperature. The absorbance (A) was measured at 540 nm. The haemoglobin concentration of the blood sample was calculated from a curve prepared from known standards.

2.5.4. RBC indices. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) index were calculated according to following formula:

$$\mathrm{MCH} = \frac{\mathrm{Hb}\times 10}{\mathrm{RBC}};\ \mathrm{MCV} = \frac{\mathrm{Hct}\times 10}{\mathrm{RBC}};\ \mathrm{MCHC} = \frac{\mathrm{Hb}\times 100}{\mathrm{Hct}}$$

2.6. White blood cell differentiation

To differentiate white blood cell type, blood films of samples were prepared on clean microscope slides before fixation for 5 min in 96% methanol and left to air dry. The slides were stained with Giemsa's stain for 20 min according to Vázquez and Guerrero (2007) and Banaee *et al*. (2008) and examined at a magnification of x 400.

2.7. Biochemical parameters

Measuring the biochemical parameters was done using the kits supplied by Pars Azmoon Company and a UV/ VIS spectrophotometer (model UNISCO 2100). Total protein was measured by the Biuret reaction at 540 nm. The albumin assay is based on the dye-binding properties of plasma albumin with a bromocresol green. An increase in the blue-green color was measured at 630 nm. The plasma globulin based on the ratio of albumin versus total protein (Johnson *et al.*, 1999). Plasma glucose was measured by the glucose-oxidase method at 500 nm (Sacks, 1999). Plasma cholesterol levels by the CHOD-PAP enzymatic method at 510 nm, triglyceride levels by GPO-PAP enzymatic method at 546 nm (Rifai *et al.*, 1999) and creatinine by the JAFFE method and at 510 nm (Foster-Swanson *et al*., 1994). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was measured by NADPH consumption and its conversion to NAD^+ at 340 nm, lactate dehydrogenase (LDH) in plasma was measured based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) based on

converting nitro phenol phosphate into nitrophenol and phosphate at 405 nm, creatinine phosphokinase (CK) based on the conversion of creatinine phosphate into creatinine at 340 nm and based on optical density (OD) absorption and the formula presented in the kits' manual (Moss and Henderson, 1999). All biochemical parameters were measured according to the instructions provided in the kit's manual.

2.8. Data analysis

Significant difference in the biochemical parameters of specimens treated with the different concentrations of *A. officinalis* extracts was statistically analyzed by one-way ANOVA in SPSS (IBM, Release 19) software. All data were examined for normality (Kolmogorov-Smirnov test). The mean evaluation was done using Duncan's test at a confidence level of 95%. Data were presented as the mean ± SD.

3. Results and discussion

No fish died during the experiment. The fish readily accepted all diets in the first few minutes, which minimized leaching marshmallow extract from the feed into the water.

Table 1. Composition of commercial diet.

Nutrients	Value
Gross energy (Kcal/Kg)	3500
Crude protein (%)	35-37
Crude lipid (%)	9-11
Crude fiber (%)	5%
Moisture (%)	<10
Ash (%)	<10
TVN (mg/100gr)	<45

TVN: Total volatile nitrogen

The findings of this study indicate that marshmallow administration has no effects on the number of red blood cells (RBC count), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV) and mean content of hemoglobin per red cell (MCH) during the experiment (Table 2). However, using certain immune stimulating compounds may alter red blood cell indices by affecting the performance of hematopoietic organs such as thymus, spleen, and anterior kidney (Ahmadi *et al*., 2012). For instance, administration of garlic has a great effect on stimulating the immune system (Sahu *et al*., 2007; Ndong and Fall, 2007; Nya and Austin, 2009a) and increases hemoglobin, hematocrit, and white blood cells count as well as thrombocytes in Nile tilapia (Shalaby *et al*., 2006). Also, a significant increase was found in hemoglobin as well as red and white blood cells count in *labeo rohita* that were fed diets enriched with mango kernel powder and garlic compared with the control group (Sahu *et al.*, 2007). Moreover, a significant increase was reported in the number of red blood cells, hematocrit, leukocyte and thrombocyte count in fish fed with garlic (Martins *et al.*, 2002). Feeding flanders with a mixture of herbs including *Medicata fermentata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officinale* increased hemoglobin and hematocrit (Ji *et al*., 2007). Although there was not any significant difference in MCHC value between the treated groups and control at day 30, the MCHC value was higher in fish fed for 60 days with 2.5 g marshmallow extract compare to groups fed with control group.

Fish fed with 2.5 g marshmallow extract revealed lower numbers of WBC than control group at day 60. The increase in the number of white blood cells (WBC) on day 60 in fish fed with 5 g marshmallow extract may be attributed to the effects of this extract on the lymphoid tissue of fish and on the reproduction and proliferation of white blood cells. The increase in white blood cells is important in strengthening the immune system of fish. A significant increase was reported in red blood cells count, hematocrit, leukocyte and thrombocyte of fish after being fed with garlic (Martins *et al.*, 2002). In a similar experiment, increased number of leukocytes was found in hybrid tilapia (Ndong and Fall, 2007).

Differential leucocyte counts for fish fed marshmallow extract for 60 days have been presented in Table 3. A significant increase in the lymphocyte of fish fed with 0.5 and 10 g marshmallow extract may indicate the influence of this plant on the increased synthesis of lymphocytes in lymphoid tissues such as the anterior kidney and spleen. This might be effective in strengthening cellular immunity and increasing the efficiency of acquired immunity (Secombes *et al*., 2005). Conversely, the dose of 5 and 10 g extract led to the lowest significant number of monocytes (p<0.05). There were no significant difference in the neutrophil, basophile and eosinophil counts between treated fish and control group.

However, administration of 0.5 and 10 g marshmallow extract significantly decreased monocytes on day 30. Moreover, the results indicate that using

marshmallow extract in diet of fish had no effects on the number of neutrophils, basophils, and eosinophils. Feeding Pacu (*Piaractus mesopotamicus*) with vitamin supplements (E, C) had no effects on the number of leukocytes, monocytes and neutrophils (Garcia *et al.*, 2007). However, using ginger and garlic in diets given to rainbow trout made significant changes in the number of lymphocytes, monocytes, and neutrophils (Nya and Austin, 2009a; 2009b). Rainbow trout immersion in glucan solution increased neutrophils and activated their non-specific immune responses (Gannam and Schrock, 1999).

As shown in Table 4 administrations of *A. officinalis* extract to the fishes for 60 days did not have any significant effect on ALT, ALP and CPK activities. Although, no significant changes were observed in AST activity on day 30, plasma AST were significantly high in fishes that fed *A. officinalis* at doses of 10 g/kg ($P < 0.05$) compare to the AST activity in the control group on day 60. Plasma LDH activity in the treated group were also significantly increased at the 10 g/kg dose level on day 60 ($p < 0.05$). However, no significant changes were observed in LDH activity on day 30.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two of the most important liver enzymes which help transfer amine groups from alpha amino acids to alpha-keto acids. These enzymes play an important role in the final stages of protein breakdown to produce ATP and increased activity of these enzymes has no role in using amino acids in the oxidation process or glycogenesis (Murray *et al.*, 2012). Increased hepatic AST on day 60 of the experiment in fish fed with diets containing 10 g marshmallow extract may illustrate increased rate of protein breakdown in liver to provide enough energy to deal with the cytotoxic effects caused by the presence of phytochemical compounds, such as furfural, in marshmallow extract. After being oxidized by liver, furfural turns into a toxic material, pyromucic acid (Veličković *et al.*, 2011). Therefore, furfural may indirectly cause oxidative stress and damage liver cells in fish. Increased AST and ALT was observed in tilapia (Soltan *et al.*, 2008) and rainbow trout fed with a mixture of herbs and yarrow 1%, respectively (Nafisi Bahabadi *et al.*, 2014). This could be due to the liver dysfunction which is in turn caused by anti-nutritional compounds in plants (Soltan *et al.*, 2008). However, administration of silymarin to rainbow trout (Banaee *et al.*, 2011) or using garlic and onion extract in catfish diet (Al-Salahy, 2002) decreased AST and ALT activities. Alkaline phosphatase (ALP) is an enzyme found in the epithelium of the bile ducts and liver cells. Alkaline phosphatase in liver functions in glycogen metabolism and can deactivate phosphorylase enzymes, hence activating glycogen synthesis in liver. Therefore, inhibiting the activity of this enzyme in liver is associated with glycogen breakdown to supply the energy required under stressful conditions, decreasing phosphorylation or preventing oxidative phosphorylation in the respiratory chain (Murray *et al.*, 2012). The plasma ALP activity did not change significantly compares to the control fishes.

Lactate dehydrogenase (LDH) is one of the glycolytic chief enzymes in liver which is substantially found in other tissues too. The level of this enzyme alters in hypoxia and severe damages to organs. Pathological damages to the liver and blood hypoxic conditions may prevent oxidative phosphorylation in the mitochondria which in turn reduces ATP production. Therefore, inhibiting oxidative phosphorylation is followed by fatal consequences. In such circumstances reoxidation of NADH with oxygen is not possible via the respiratory chain. During a biochemical reaction catalyzed by lactate dehydrogenase, pyruvate is converted to lactate by NADH. Thus by reoxidation of NADH by lactate, glycolysis continues in the absence of oxygen (Murray *et al.*, 2012).). Increased plasma LDH on day 60 in fish fed 10 g of marshmallow extract may suggest a cellular toxicity caused by phytochemical compounds in this plant (Ebrahimi, 2005). Decreased LDH was reported in rainbow trout treated with silymarin and marrow (Nafisi Bahabadi *et al.*, 2014; Banaee *et al.*, 2011).

Creatine phosphokinase (CPK) activity appears almost completely unaffected by the *A. officinalis* extract; apparently, no changes were observed when compared with that of the control. The effects of marshmallow extract on the activity of hepatic enzymes is comparable to the effects of other herbs such as *Curcuma longa* (Abdelwahab *et al.*, 2012), and *Cyperus rotundus* (Suresh Kumar and Mishra, 2005). Absence of alterations in the level of hepatic enzymes in fish fed with different concentrations of marshmallow extract could be attributed to the influence of flavonoids and antioxidants on the physiological function of cell membrane in liver tissue, increasing the antioxidant capacity of cells and improving the cell membrane stability (Sadighara *et al.*, 2012).

Table 2. Changes in the haematological parameters of fish fed different concentrations of marshmallow extract.

Hematology Parameters	Treatments	Experimental course	
		30th day	60th day
RBC (10^6 cells)	Control	1.74±0.64[a]	1.50±0.38[a]
	2.5 g MAR	1.43±0.50[a]	1.60±0.37[a]
	5 g MAR	1.28±0.39[a]	1.32±0.39[a]
	10 g MAR	1.25±0.55[a]	1.42±0.59[a]
WBC (10^4 cells)	Control	11.13±3.16[ab]	10.61±2.47[a]
	2.5 g MAR	10.51±6.07[ab]	14.18±4.58[ab]
	5 g MAR	7.73±2.67[a]	14.84±4.54[b]
	10 g MAR	12.38±4.98[b]	12.85±2.59[ab]
Hct (%)	Control	33.23±8.44[a]	35.69±6.63[ab]
	2.5 g MAR	27.73±4.72[a]	32.29±3.58[a]
	5 g MAR	29.09±6.39[a]	38.48±5.03[b]
	10 g MAR	32.18±1.60[a]	33.62±2.78[a]
Hb (g/100 mL)	Control	7.53±1.61[a]	6.64±2.09[a]
	2.5 g MAR	6.96±1.89[a]	8.00±2.69[a]
	5 g MAR	7.74±1.77[a]	6.55±1.57[a]
	10 g MAR	7.05±1.32[a]	6.88±1.52[a]
MCV (10^{-4} mm^3)	Control	2.17±1.04[a]	2.57±0.91[a]
	2.5 g MAR	2.26±1.17[a]	2.19±0.80[a]
	5 g MAR	2.43±0.78[a]	3.09±0.79[a]
	10 g MAR	3.03±1.30[a]	2.89±1.54[a]
MCH (10^{-5} pg)	Control	5.11±2.78[a]	4.63±1.48[a]
	2.5 g MAR	5.34±1.97[a]	5.27±2.15[a]
	5 g MAR	6.56±2.47[a]	5.41±2.20[a]
	10 g MAR	6.53±2.57[a]	5.80±3.04[a]
MCHC (%)	Control	23.30±4.59[a]	18.59±4.45[a]
	2.5 g MAR	25.91±8.37[a]	25.42±10.09[b]
	5 g MAR	28.02±9.73[a]	17.40±4.84[a]
	10 g MAR	22.03±4.63[a]	20.78±5.69[ab]

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on haematological parameters determined in common carp after 30 and 60 days. Effects of different concentrations of *Althaea officinalis* extract on haematological parameters were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (p<0.05). Values represent mean ± S.D.

Table 3. Changes in the white blood cell differentiation of fish fed different concentrations of marshmallow extract**.**

WBC differentiation	Treatment	Experimental course	
		30th day	60th day
Lymphocyte	Control	66.07±1.08[a]	66.24±1.07[a]
	2.5 g MAR	67.61±1.08[a]	69.02±1.07[ab]
	5 g MAR	71.90±1.05[b]	71.90±1.05[b]
	10 g MAR	72.81±1.08[b]	73.57±1.07[b]
Neutrophils	Control	19.30±1.23[a]	20.05±1.23[a]
	2.5 g MAR	16.44±1.28[a]	17.42±1.28[a]
	5 g MAR	17.92±1.22[a]	17.29±1.20[a]
	10 g MAR	16.76±1.18[a]	16.85±1.16[a]

Monocyte	Control	11.48±1.45[b]	10.88±1.42[ab]
	2.5 g MAR	13.43±1.32[b]	11.64±1.29[b]
	5 g MAR	8.13±1.27[a]	9.07±1.20[a]
	10 g MAR	8.25±1.48[a]	8.25±1.41[a]
Basophils	Control	1.13±1.45[a]	1.08±1.26[a]
	2.5 g MAR	1.07±1.24[a]	1.07±1.24[a]
	5 g MAR	1.08±1.26[a]	1.08±1.26[a]
	10 g MAR	0.11±0.47[a]	0.11±0.47[a]
Eosinophil	Control	1.82±1.62[a]	1.82±1.92[a]
	2.5 g MAR	1.37±1.81[a]	1.37±1.81[a]
	5 g MAR	1.58±1.83[a]	1.32±1.54[a]
	10 g MAR	1.54±1.73[a]	1.29±1.75[a]

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on white blood cell differentiation determined in common carp after 30 and 60 days. Effects of different concentrations of *Althaea officinalis* extract on white blood cell differentiation were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (p<0.05). Values represent mean ± S.D.

Table 4. Changes in the plasma enzyme activities of fish fed different concentrations of marshmallow extract.

Biochemical Parameters	**Treatments**	**Experimental course**	
		30th day	**60th day**
AST (U/L)	Control	49±16[a]	44±10[a]
	2.5 g MAR	48±4[a]	47±17[a]
	5 g MAR	46±9[a]	57±13[ab]
	10 g MAR	44±10[a]	62±13[b]
ALT (U/L)	Control	14±3[a]	14±4[a]
	2.5 g MAR	13±1[a]	11±3[a]
	5 g MAR	14±4[a]	11±2[a]
	10 g MAR	16±3[a]	12±4[a]
LDH (U/L)	Control	198±46[a]	225±35[a]
	2.5 g MAR	232±72[a]	201±40[a]
	5 g MAR	243±120[a]	203±49[a]
	10 g MAR	261±111[a]	314±23[b]
ALP (U/L)	Control	67±17[a]	57±14[ab]
	2.5 g MAR	48±22[a]	61±19[ab]
	5 g MAR	56±30[a]	50±22[a]
	10 g MAR	55±22[a]	72±16[b]
CPK (U/L)	Control	856±179[a]	1090±318[a]
	2.5 g MAR	1093±422[a]	1060±284[a]
	5 g MAR	1103±360[a]	1200±227[a]
	10 g MAR	934±286[a]	951±237[a]

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on the plasma enzyme activities determined in common carp after 30 and 60 days. Effects of different concentrations of *Althaea officinalis* extract on the plasma enzyme activities were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (p<0.05). Values represent mean ± S.D.

Table 5. Changes in the some biochemical parameters of fish fed different concentrations of marshmallow extract.

Biochemical Parameters	Treatments	Experimental course	
		30th day	60th day
Glucose (mg/dL)	Control	61.57±11^{a}	67.33±12.40^{ab}
	2.5 g MAR	62.08±15^{a}	58.57±9.83^{a}
	5 g MAR	60.81±7.30^{a}	73.47±19.50^{ab}
	10 g MAR	72.43±20^{a}	84.69±21^{b}
Protein (g/dL)	Control	3.22±0.81^{ab}	3.66±0.77^{a}
	2.5 g MAR	2.69±0.60^{a}	5.08±0.75^{c}
	5 g MAR	3.43±0.55^{ab}	4.11±1.03^{ab}
	10 g MAR	3.33±0.52^{ab}	4.65±0.50^{bc}
Albumin (g/dL)	Control	2.12±0.26^{b}	1.87±0.70^{a}
	2.5 g MAR	1.35±0.23^{a}	2.01±0.91^{a}
	5 g MAR	1.76±0.30^{b}	2.58±0.31^{ab}
	10 g MAR	2.08±0.56^{b}	2.89±0.86^{b}
Globulins (g/dL)	Control	1.10±0.62^{a}	1.79±1.26^{a}
	2.5 g MAR	1.34±0.48^{a}	3.07±1.03^{b}
	5 g MAR	1.67±0.70^{a}	1.53±1.18^{a}
	10 g MAR	1.25±0.52^{a}	1.76±0.90^{a}
Cholesterol (mg/dL)	Control	70.53±13.17^{b}	79.29±13^{ab}
	2.5 g MAR	69.20±21.37^{ab}	71.45±10.56^{a}
	5 g MAR	50.11±18.23^{a}	70.14±13.10^{a}
	10 g MAR	50.11±17^{a}	92.60±18.60^{b}
Triglycerides (mg/dL)	Control	225.00±50.34^{ab}	249.19±35^{b}
	2.5 g MAR	184.04±38.48^{a}	191.37±48^{a}
	5 g MAR	230.99±59.40^{ab}	208.60±31^{ab}
	10 g MAR	244.25±47.24^{b}	244.41±48^{b}
Creatinine (mg/dL)	Control	0.45±0.210^{a}	0.35±0.10^{a}
	2.5 g MAR	0.44±0.19^{a}	0.37±0.07^{a}
	5 g MAR	0.39±0.12^{a}	0.38±0.10^{a}
	10 g MAR	0.51±0.21^{a}	0.33±0.08^{a}

Effects of different concentrations of *Althaea officinalis* extract as supplement (0, 0.25, 0.5 and 1 % per 1 kg food) on some blood biochemical parameters determined in common carp after 30 and 60 days. Effects of different concentrations of *Althaea officinalis* extract on some blood biochemical parameters were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (p<0.05). Values represent mean ± S.D.

Plasma total protein and albumin as shown in table 5 significantly increased in fishes treated with *A. officinalis* at doses of 10 g/kg ($P < 0.05$) compared to the control group which received only normal diet on day 60. The significant increase in the level of these parameters is an induction that the *A. officinalis* extract stimulated their synthesis in the liver. The result indicates that *A. officinalis* extract administration caused a significant increase in globulin levels in fishes in the 2.5 g/kg dose on day 60 ($P < 0.05$).

Plasma cholesterol in the treated group was also significantly decreased at the 5 and 10 g/kg dose levels of *A. officinalis* extract. Plasma triglyceride levels were affected by the *A. officinalis* extract (at 2.5 g/kg) administration for 30 days as shown in table 5. Diminishing effect of *A. officinalis* extract on plasma cholesterol and triglyceride in this study is an indication

of the involvement of the flower extract in mechanisms involved in elimination of the lipids from the body, thus confirming its potential hypolipidaemic effects.

The levels of creatinine in the treated fishes did not show any significant difference with respect to the control levels, an induction that flower extract of *A. officinalis* is not nephrotoxic at the dose levels used in this study.

4. Conclusion

Measuring blood biochemical changes and hematological indices in carp treated with different concentration of marshmallow extract proves the safety of this drug at concentrations of 2.5 and 5 g in preclinical stages, although the concentration of 10 g may lead to cytotoxicity.

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