Evaluation of pre-clinical safety and toxicology of *Althaea officinalis* extracts as naturopathic medicine for common carp (*Cyprinus carpio*)

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Abstract

The current study was done to investigate the preclinical safety and toxicology of *Althaea officinalis* extract as naturopathic medicine in common carp (*Cyprinus carpio*). Specimens were treated with 0 (control), 2.5, 5 and 10 g of marshmallow extract for 45 days. Plasma biochemical parameters were measured after 15 and 45 days. Total protein, albumin and globulin levels ($p<0.05$) were significantly higher in the fish fed with 10 g *A. officinalis* extract than that in control groups on day 45. Although, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities significantly decreased ($p<0.05$) in fish fed with *A. officinalis* extract on day 15. The use of the *A. officinalis* extract (10 g) led to a significant increase in AST, lactate dehydrogenase (LDH) and ALP activities and cholesterol levels on day 45 ($p<0.05$) and a significant decrease in plasma glucose and cholesterol levels on day 15 ($p<0.05$). There was no significant difference in glucose levels and creatine kinase (CK) activity between all treatments and the control group on day 45 ($p>0.05$). During the experimental period, triglyceride levels noticeably decreased in fish fed with 2.5 g of *A. officinalis* extract ($p<0.05$). Although, administration of marshmallow extract up to 5 g per kg of feed did not show any side effect on fishes, the use of the *A. officinalis* extract (10 g) led to cytotoxicity and modifications in blood biochemical parameters of fish. Therefore, we recommend the use of the lower concentrations than 10 g *A. officinalis* extract in prospective clinical studies.

Keywords: Biochemical parameters; Common carp; Marshmallow; Medicinal plants, pre-clinical study

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Introduction

Medical plants are used in folk veterinary medicine for treating or preventing many diseases in domesticated animals (Viegi *et al*., 2003; Suresh Kumar and Mishra, 2004). Herbal products provide an important source of potential medicines from which humans have produced phytomedicines and herbal remedies. Herbal medicines often contain organic chemical compounds with different roles; including chemotherapeutic, immune-stimulant, bacteriostatic, bactericidal, antifungal and anti-parasitical functions (Banaee, 2010; Ahmadi *et al*., 2012; Asadi *et al*., 2012). In the last two decades, many studies have been conducted to determine the feasibility of using herbal medicine in prevention and curing of aquatic animals' diseases (Yin *et a*l., 2006; Divyagnaneswari *et al*., 2007; Ardó *et al*., 2008; Yin *et al*., 2009).

There are several basic methods for the selection of medicinal plants including a random selection of plant species, a choice based on plants' therapeutic properties and their ethnomedical use, examining literature on phytochemical compounds and pharmacological activities as well as using chemotoxonomic approaches.

Traditionally, it is being thought that plants and plant products are non-toxic and do not have any adverse side effects, however; phytochemical ingredients of plant extracts are chemicals that are similar to those found in synthetic drugs with the same potential to cause serious adverse effects. Therefore, choosing plant species is the first step in evaluating medicinal properties of plants and the assessment of a crude medicine is an essential part of establishing its correct identity. Prior to including any crude medicine in herbal pharmacopoeia; pharmacognostical parameters must be established. Thus, in introducing herbal medicine the pharmacognostical parameters by pre-clinical pharmacology and toxicology studies must be determined. In these studies, evaluating the changes in biochemical parameters of blood is a common clinical procedure in evaluating the medicinal effects on specific organs, especially in the case of existing difficulties in studying the changes in tissues. Measurement of biochemical markers offers comprehensive information on the physiological state of cells and tissues, particularly the liver, as a target issue. This information can be used in the pharmaceutical and toxicological evaluation to determine non-toxic doses of the drug (Banaee *et al*., 2010).

In this study, marshmallow was chosen as a candidate drug to be used in the aquaculture industry. Marshmallow (*A. officinalis*) is a medicinal plant, the roots, leaves and flowers of which are usually used in traditional medicines in many countries all over the world (Wynn and Fougère, 2007). The flowers of marshmallow are in three colors: white, pink, and pinkish red. Sadighara *et al.* (2012) found out that

antioxidant activities and the amount of flavonoids in marshmallow extract are heavily dependent on the color of the flowers. *A. officinalis* L. are rich in mucilage polysaccharides, antioxidants, flavonoids, terpenes, and several terpenoids, sterols, saturated and unsaturated fatty acid, the amino acid asparagines, coumarins, kaempferol, phenolic acids, quercetin, sugars, tannins, and volatile oil (Elmastas *et al*., 2004; Sartoratto *et al*., 2004; Teśević *et al*., 2012; Puri *et al.,* 2014).They could be found in some other medicinal plants, too. Some of these compounds such as carvacrol, thymol, linalool, β-bisabolene, terpinene, and 8,1- cineole, p-cymene, eugenol, furfural and camphor have antibacterial, antifungal and antiviral properties (Sartoratto *et al*., 2004; Burt *et al*., 2005; Jung *et al*., 2007; Astani *et al*., 2010; Abbaszadeh *et al*., 2014; Bilia *et al*., 2014; Kubiça *et al*., 2014) or demonstrate antioxidant activity (Jun *et al*., 2014; Puri *et al*., 2014; Ramos *et al*., 2014). Aristatile *et al.* (2009a;b) verified the liver-protective properties of carvacrol against the toxicity of D-glucosamine in rats. Similarly, Ezhumalai *et al.* (2014) reported that the activity of aminotransferase which had increased because of a diet rich in fats, decreased to a normal level after carvacrol administration. Considering the aforementioned properties, marshmallow may be a good alternative in the prevention and cure of bacterial, viral and fungal infections and oxidative stress.

Nevertheless, marshmallow's ingredients may have adverse side effects on the physiology and biology of fishes. Therefore, preclinical evaluation and safety evaluation of the marshmallow for fish are necessary. Hence, this study was done to collect basic information on the effects of marshmallow extract on biochemical parameters of blood in common carp.

Specimens of common carp, *C. carpio* were selected for the present study based on the following criteria: The family Cyprinidae is well represented amongst the fish inhabiting the freshwaters of Iran. This species can be found in abundant quantities throughout the year, and is of great commercial importance to people in developing countries. This species is voraciously omnivorous and is easily adapted to artificial diets. Also, this fish is hardy in nature for handling and transport. Therefore, common carp is a suitable aquatic animal for bioassay testing.

Materials and methods

Fish

Common carp, *C. carpio*, (with the average weight of 37.65 ± 4.40 g and total length of 14.15 ± 0.8 cm), were used in this study according to National Ethical Framework for Animal Research in Iran (Mobasher *et al*., 2008). Fishes were purchased from a commercial farm in Shush, Khuzestan Province, Iran, and they were transferred to the aquaculture laboratory of Natural Resources Faculty,

Behbahan Khatam Alanbia University of Technology. Fishes were randomly distributed in 12 fiberglass tanks (200 L) and acclimatized in aerated freshwater (24 ± 2°C; pH, 7.4 ± 0.2; 16 L/8D; 40% water exchange rate/day) for two weeks before use. During the acclimatization period, specimens were fed two times daily with commercial diet from Beyza Feed Mill, Shiraz, Iran.

Phytochemical analysis of Marshmallow (A. officinalis) flower

Essential oil extraction of A. officinalis

Marshmallow (*A. officinalis*) flower was selected from a list of herbs commonly used as medicinal plants in Iran. The hydro-distillation was done using a Clevenger-type apparatus. Dried flowers of *A. officinalis* were purchased from the local market. In the experiment, 100 g of dried flower of *A. officinalis* (as powder) was distilled with 1000 mL of water for 3 hours. The amount of the essential oil obtained was expressed in mL/100 g calculated on the basis of dry matter content.

Characterization of the oil extract by Gas Chromatography (GC-MS)

The analytical GC-MS system used was an Agilent GC-MSD system (Agilent Technologies-5975C-MS, 7890A-GC) with helium as the carrier gas at a constant linear velocity of 1 ml/min. The transfer, source and quadrupole temperatures were 280°C, 230°C and 150°C respectively, operating at ionization energy of 70 eV. A HP-5MS capillary column (30.0 m × 0.25 mm ID × 0.25 µm film thickness) was used and programmed from 60 to 240°C at 8.5/min. Oil samples (20 µL) were diluted with acetone (1000 µL). The injection volume was 0.2 µL, the split ratio was 1:50 and the injector temperature was 280°C. Composition values were recorded as percentage area based on the total ion current chromatogram.

Extract of A. officinalis

The powder of dried flower *A. officinalis* was mixed with distilled water and ethanol (1:1), and the mixture was 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 at 50°C that finally gave 8 g (8% of initial amount) of dried powder. The concentration used in the experiment was based on the dry weight of the extract.

Fish diet preparation

The formulated fish feed was prepared in the laboratory using the powder of a commercial feed obtained from Beyza Feed Mill, Shiraz, Iran. In order to enrich the normal diet, the 2.5, 5 and 1 g of *A. officinalis* extracts were mixed with 1 kg feed powder. Each supplemented diet was mixed with distilled water (1mL/g) until a homogenous mixture was obtained. 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 were broken to produce pellets approximately 10 mm in length. The pellets were packed and stored at -20°C in a freezer until used. Although no supplement was added to the feed, the control diet was prepared using the same process.

The final experiment

The final experiment was done in a completely randomized design with 3 treatments and 1 control. The fishes were fed the commercial diet enriched with the hydroalcoholic extract of flower *A. officinalis* 0.25% (2.5 g), 0.5% (5 g), and 1% (10 g) of the commercial diet and each was repeated 3 times. Common carp were fed a diet supplemented with marshmallow extract for 45 days. During experiments, specimens were monitored for appetite. Fish appetite was evaluated based on the weight of intestinal contents and their feeding behavior.

After 15 and 45 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 at 4 °C in sterilized glass vials with heparin as anticoagulant. The blood was centrifuged for 15 min at 4000 g, at 4 °C. Plasma samples were immediately stored at -21 °C until biochemical analysis.

Biochemical parameters of blood

Determination of the biochemical parameters was done using the kits supplied by Pars Azmoon Company and a UV/ VIS spectrophotometer (model UNICO 2100). Each blood biochemical parameter was determined by a certain method. Total plasma protein was measured at 540 nm by the Biuret reaction. The albumin assay is based on the dye-binding properties of plasma albumin with bromocresol green. An increase in the blue-green color was measured at 630 nm. The plasma globulin was 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 determined by NADPH consumption and its conversion to NAD^+ at 340 nm. Lactate dehydrogenase (LDH) in plasma was determined 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 determined according to the instructions provided in the kit's manual.

Data analysis

A significant difference in the biochemical parameters of specimens treated with the different concentrations of *A. officinalis* extracts was examined using one-way ANOVA. All the data were examined for normality (Shapiro-wilk test). Means were compared by Tukey's test and a $p<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (IBM, 19) software. Data are presented as mean ± SD.

Results

The phytochemical analysis of the marshmallow flower sample used in this study identified 43 compounds, representing 97.823 % of the total oil content (Fig. 1 and Table 1). Table 1 represents major constituents as follows 6,10,14-trimethyl-2-Pentadecanone (47.31%), Carvacrol (17.65%), 2-Pentadecanone (11.22%), Dodecanoic acid (2.322%), n-Tetradecanoic acid (4.917%), n-Tetradecanol (1.978%), n-Nonanoic acid (1.176%), Thymol (1.073%), Methyl hexadecanoate (1.312%).

The plasma AST activities significantly decreased in fish which were fed for 15 days with feeds supplemented with 5 and 10 g of *A. officinalis* extract ($p<0.05$). The highest AST activity was observed in fish fed with 10 g *A. officinalis* extract-supplemented feed for 45 days.

No significant difference was distinguished in AST activity after 15 days of treatment. When fish were fed with diets supplemented with 5 g *A. officinalis* extract for 45 days, the ALT activities significantly decreased ($p<0.05$).

Dietary intake of *A. officinalis* extract had significantly decreased the plasma ALP activity after 15 days of feeding ($p<0.05$). ALP activity decreased significantly in fish fed with diets supplemented with 5 g *A. officinalis* extract for 45 days ($p<0.05$).

In fish that received 2.5 g *A. officinalis* extract-supplemented feeds for 15 days, there was significant difference ($p<0.05$) in the CPK activity. However, after 45 days of feeding with extract supplemented diet, there was no significant alteration in the CPK activity.

In the present study, although no significant change in the LDH activity was observed in the fish that were fed with different doses of *A. officinalis* extract for 15 days, this activity increased significantly in fish which were fed diets supplemented with 10 g marshmallow extract for 45 days ($p<0.05$).

When administered as a diet supplement for 15 days, all the doses of the *A. officinalis* extract tested significantly decreased the plasma glucose levels ($p<0.05$). However, there was no significant difference in glucose levels between the treatments and the control group on day 45 ($p>0.05$).

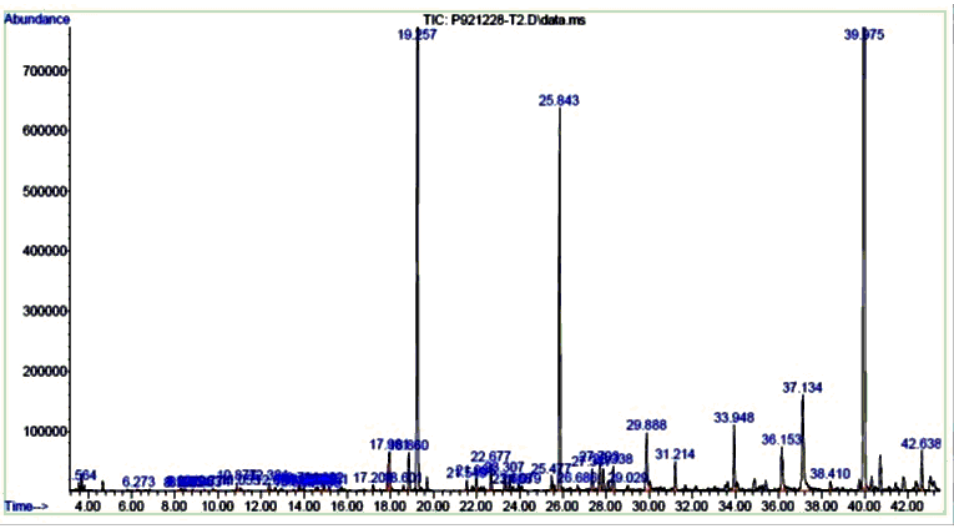

Figure 1: Gas chromatography analysis of essential oil of the marshmallow (*Althaea officinalis*) flower.

No significant changes were observed in the total protein and globulin levels on day 45 and 15 ($p>0.05$). However, after 15 days of feeding on *A. officinalis* extract-supplemented feed, a significant decrease was observed in plasma albumin. Fish that were fed for 45 days with 5 and 10 g of *A. officinalis* extract-supplemented diet exhibited significant increase of albumin ($p<0.05$). The cholesterol significantly decreased in the fish fed for 15 days with feed supplemented with different doses of *A. officinalis* extract ($p<0.05$). However, no significant changes were observed in cholesterol levels in fish after being fed *A. officinalis* extract on day 45 ($p>0.05$).

The triglyceride levels significantly decreased in fish fed with 2.5 g of *A. officinalis* extract-supplemented diet for 15 and 45 days ($p<0.05$). No significant changes were observed in creatinine levels of fish ($p>0.05$).

Table 2: Enzymatic activities in plasma of common carp fed with *Althaea officinalis* extract as supplement.

Biochemical parameters	Concentration of *A. officinalis* extract in diet (g/Kg)	Sampling time 15th day	Sampling time 45th day
AST ($U.L^{-1}$)	Control (0.0 g)	59.89 ± 15.45^{c}	38.15 ± 10.47^{ab}
	2.5 g	48.97 ± 5.04^{bc}	32.45 ± 8.17^{a}
	5.0 g	28.61 ± 5.23^{a}	54.11 ± 12.74^{bc}
	10.0 g	36.06 ± 7.09^{ab}	60.86 ± 17.30^{c}
ALT ($U.L^{-1}$)	Control (0.0 g)	11.69 ± 2.39^{a}	15.55 ± 1.86^{b}
	2.5 g	13.45 ± 1.23^{a}	11.36 ± 3.77^{ab}
	5.0 g	15.44 ± 5.87^{a}	10.15 ± 2.64^{a}
	10.0 g	15.11 ± 4.53^{a}	15.11 ± 2.02^{b}
LDH ($U.L^{-1}$)	Control (0.0 g)	166.54 ± 21.22^{a}	247.77 ± 7.38^{a}
	2.5 g	185.24 ± 21.55^{a}	210.17 ± 46.17^{a}
	5.0 g	164.75 ± 46.76^{a}	224.42 ± 61.20^{a}
	10.0 g	157.63 ± 19.34^{a}	300.12 ± 20.94^{b}
ALP ($U.L^{-1}$)	Control (0.0 g)	56.83 ± 18.79^{b}	48.71 ± 6.18^{b}
	2.5 g	27.88 ± 2.45^{a}	45.80 ± 10.95^{b}
	5.0 g	27.57 ± 1.84^{a}	29.87 ± 8.15^{a}
	10.0 g	33.70 ± 3.11^{a}	58.21 ± 8.35^{b}
CPK ($U.L^{-1}$)	Control (0.0 g)	821.27 ± 244.54^{a}	1206.93 ± 280.03^{a}
	2.5 g	1432.07 ± 269.30^{b}	956.11 ± 330.41^{a}
	5.0 g	1191.33 ± 578.86^{ab}	1200.30 ± 293.98^{a}
	10.0 g	1126.44 ± 261.23^{ab}	972.19 ± 105.10^{a}

Significant differences between data were characterized by alphabet letters when compared with control data in the same test group along time (One way ANOVA, $p<0.05$). Values sharing the same superscript letter indicate no significant differences compared with control value during the same experiment time ($p > 0.05$). Data are expressed as means ± S.D. Enzyme activity is expressed as units per liter ($U.L^{-1}$ or U/L).

Table 3: Biochemical parameters in plasma of common carp fed with *Althaea officinalis* extract as supplement.

Biochemical parameters	Concentration of *A. officinalis* extract in diet (g/Kg)	Sampling time 15th day	Sampling time 45th day
Glucose ($mg.dL^{-1}$)	Control (0.0 g)	95.79 ± 10.79^{b}	68.19 ± 10.05^{a}
	2.5 g	53.65 ± 11.35^{a}	56.64 ± 10.74^{a}
	5.0 g	61.24 ± 4.35^{a}	66.60 ± 19.78^{a}
	10.0 g	59.27 ± 9.74^{a}	66.86 ± 9.34^{a}
Total Protein ($g.dL^{-1}$)	Control (0.0 g)	3.72 ± 0.90^{a}	3.70 ± 0.63^{a}
	2.5 g	3.15 ± 0.16^{a}	4.70 ± 0.64^{a}
	5.0 g	3.41 ± 0.55^{a}	4.10 ± 0.93^{a}
	10.0 g	3.13 ± 0.31^{a}	4.73 ± 0.68^{a}

Continued Table 3:

Biochemical parameters	Concentration of *A. officinalis* extract in diet (g/Kg)	Sampling time	
		15th day	45th day
Albumin ($g.dL^{-1}$)	Control (0.0 g)	2.24 ± 0.31^{b}	1.58 ± 0.58^{a}
	2.5 g	1.47 ± 0.23^{a}	1.43 ± 0.41^{a}
	5.0 g	1.67 ± 0.41^{a}	2.73 ± 0.38^{b}
	10.0 g	1.69 ± 0.31^{a}	3.60 ± 0.49^{c}
Globulin ($g.dL^{-1}$)	Control (0.0 g)	1.48 ± 0.68^{a}	2.12 ± 1.12^{ab}
	2.5 g	1.68 ± 0.26^{a}	3.28 ± 0.30^{b}
	5.0 g	1.74 ± 0.83^{a}	1.37 ± 1.19^{a}
	10.0 g	1.45 ± 0.46^{a}	1.13 ± 0.76^{a}
Cholesterol ($mg.dL^{-1}$)	Control (0.0 g)	59.03 ± 2.37^{c}	73.04 ± 14.35^{ab}
	2.5 g	49.86 ± 4.45^{b}	66.37 ± 9.76^{a}
	5.0 g	36.69 ± 6.69^{a}	67.20 ± 16.57^{a}
	10.0 g	35.52 ± 7.12^{a}	99.05 ± 24.76^{b}
Triglyceride ($mg.dL^{-1}$)	Control (0.0 g)	239.44 ± 59.28^{b}	261.74 ± 25.08^{b}
	2.5 g	153.05 ± 13.05^{a}	180.05 ± 65.39^{a}
	5.0 g	192.49 ± 43.84^{ab}	223.47 ± 35.49^{ab}
	10.0 g	208.45 ± 23.63^{ab}	261.50 ± 63.47^{b}
Creatinine ($mg.dL^{-1}$)	Control (0.0 g)	0.57 ± 0.26^{a}	0.30 ± 0.06^{a}
	2.5 g	0.45 ± 0.22^{a}	0.35 ± 0.05^{a}
	5.0 g	0.38 ± 0.13^{a}	0.30 ± 0.06^{a}
	10.0 g	0.36 ± 0.19^{a}	0.31 ± 0.09^{a}

Significant differences between data were characterized by alphabet letters when compared with control data in the same test group along time (One way ANOVA, $p < 0.05$). Values sharing the same superscript letter indicate no significant differences compared with control value during the same experiment time ($p > 0.05$). Data are expressed as means±S.D.

Discussion

Dodecanoic acid and n-Tetradecanoic acid are the major saturated fatty acid found in the essential oil of *A. officinalis*. Monoterpenes have various biological properties including anticancer (Astani *et al*., 2010), antimicrobial, antifungal, anti-genotoxic, anti-inflammatory, insecticidal and antioxidant (Aydın and Türkez, 2014). *P-Cymene* (1-isopropyl-4-methylbenzene) is a natural hydrocarbon that is a component of essential oils, which are natural products extracted from marshmallow. *P-Cymene* has anti-bacterial properties. Borneol and camphor are bicycle monotrpenes found in marshmallow extract. α-Terpineol (TPN), a volatile monoterpene alcohol, is relatively non-toxic and one of the major components of the essential oils of marshmallow. Benzaldehyde, n-decanal, is mainly used as a food and flavoring additive and can be found in many fruit and plants. Menthol is a cyclic terpene

alcohol detected in marshmallow extract. Therefore, marshmallow has the potential to be used for prevention and treatment of bacterial, viral, and fungal infections.

During the experimental periods, no mortalities or changes in the appetite of the fish were observed. Platel *et al*. (2002) evidenced the favorable effect of medicinal plants on digestion and a stimulating effect on bile secretion and the activity of pancreatic enzymes. Moreover, adding plants extracts to the diet can affect the ability of the fish to find food by stimulating their sense of smell and can encourage them to eat more (Adams, 2005).

In this study, oral administration of the *A. officinalis* extract significantly decreased the plasma ALT activity at a dose of 5 g compared to the control group on day 45 ($p<0.05$). Supplementation of *A. officinalis* extract to fish significantly decreased the activity of alkaline phosphatase on day 15 ($p<0.05$). Treatment of the fish with 2.5 g *A. officinalis* extract decreased activities AST, and LDH in plasma compared to the control group. Although, increased creatine kinase (CK) activity was observed in the plasma of fish fed 2.5 g *A. officinalis* extract on day 15 ($p<0.05$), CK activity returned to the normal levels in fish treated with A. *officinalis* extract at the end of the experimental period (Table 2). More hydrophilic flavonoids interacting at the membrane surface through hydrogen bonding; may reduce the access of oxidants and per-oxidants, thus protecting the structure and function of cellular membranes (Oteiza *et al*., 2005). Oral administration of thymol and carvacrol activated antioxidant enzymes and protected liver cells against severe damages (Hashemipour *et al*., 2013). Carvacrol supplement reduced AST, ALT, and LDH activity in plasma of the fish treated with D-glucosamine (Jayakumar *et al*., 2012). The effects of marshmallow extract on the activity of plasma enzymes can be compared with the effects of other extracts such as *Allium cepa* and *A. sativum* (Al-Salahy, 2002), *Curcuma longa* (Deshpande *et al*., 2003) *Cyperus rotundus* (Suresh Kumar and Mishra, 2004), *Pterocarpus santalinus* (Palanisamy *et al*., 2007), *Aloe vera*, *Clematis hirsute*, *Cucumis prophetarum* (Alqasoumi *et al*., 2008), *Hybanthus enneaspermus* (Premalakshmi and Thenmozhi, 2011). The useful effect of *A. officinalis* in decreasing ALP activity might be due to the presence of minerals, particularly calcium levels in the *A. officinalis* extract. A low concentration of silymarin (Banaee *et al*., 2011), yarrow extract (Nafisi Bahabadi *et al*., 2014) and garlic (Al-Salahy, 2002) in diet of fish are proven to regulate the plasma activities of AST, ALT, ALP, CK and LDH.

The results show that AST and LDH activities increased in plasma of fish fed a diet supplemented with 10 g/Kg of *A. officinalis* extract on day 45 which may be due to leakage of these enzymes from the liver cytosol into blood. Some

monoterpenes such as d-limonene, α-pinene, myrcene and linalool, stylosin could have cytotoxic effects (Aydın and Türkez, 2014). *P-Cymene* is metabolized by the liver and the major *p*-cymene metabolite is cuminyl alcohol which can cause mitochondrial toxicity by affecting the process of energy production in hepatocytes (Custódio *et al.,* 2011). Toxicological studies show that safranal, and furfural, are other toxic compounds found in plants such as marshmallow. Dimethyl glutarate is an ester that may cause olfactory toxicity (Morris *et al.,* 1991). Furfural turns into the toxic pyromucic acid in liver after oxidation. Therefore, furfural may indirectly cause oxidative stress and damage the liver cells (Veićković *et al.*, 2011). Increased activity of AST, ALT and ALP was reported in the rats treated with furfural during 7 days (Veićković *et al*., 2011).

A significant lower concentration of glucose was noted in fish fed a diet enriched with *A. officinalis* extract as compared to control fishes on day 15 ($p<0.05$). The probable mechanism of *A. officinalis* extract may be partly due to the stimulation of insulin secretion or may be attributed to the activation of glycogen synthesis and healthy hepatic function (Ji *et al*., 2007). Moreover, this was probably because of the flavonoids in the plants which reduce glucose uptake in the intestine via the inhibition of sodium-dependent glucose transport (Song *et al*., 2002). Decreased glucose levels in the blood of rainbow trout fed with silymarin extract (Banaee *et al*., 2011), yarrow extract (Nafisi Bahabadi *et al*., 2014) and African catfish fed with onion and garlic extracts are reported (Al-Salahy, 2002).

Since there is a close relationship between the rate of protein synthesis in liver and total protein concentration in plasma (Banaee *et al*., 2011), the increased concentration of total protein in plasma of the fish fed with marshmallow extract may indicate increased protein synthesis rate in the liver. Amino acids present in the *A. officinalis* extract may increase protein synthesis in the liver and other tissues. Although, there was no significant difference in plasma globulin level on days 15, oral administration of 2.5 g *A. officinalis* extract significantly increased plasma globulin level on day 45 ($p<0.05$). Oral administration of marshmallow extract decreased albumin level on day 15, while the albumin level in the fish fed with marshmallow extract (5 and 10 g) increased significantly on day 45 compared with the control group ($p<0.05$). An increased level of total protein, albumin and globulin in plasma of the fish fed with diets enriched with *Echinacea purpurea* and *Silybum marianum* was reported by Bohlouli Oskoii *et al.* (2012) and Banaee *et al*. (2011). No significant changes were reported in the levels of albumin and total protein in plasma of fish fed with diets enriched with onion and garlic extract (Al-Salahy, 2002).

Supplementation of *A. officinalis* extract in the diet significantly reduced

the cholesterol levels in the plasma (p<0.05). Interference with cholesterol absorption from intestine due to administration of *A. officinalis* might have played a role in decreasing plasma cholesterol levels. The beneficial effects of *A. officinalis* on cholesterol might be due to pectin and mucilage (Ezhumalai *et al*., 2014) and phytosterols (Marinangeli *et al*., 2006; Jones *et al*., 2007). Triglycerides or triacylglycerol are neutral fats, major energy reserves for the body stored in the adipose tissue. Therefore, decrease in triglyceride levels in plasma of fish fed with 2.5 g *A. officinalis* extract in diet may be due to increased lipogenesis and decrease lipolysis and ketogenesis. In the lipogenesis process, triglycerides and fatty acids are synthesized and stored in adipose tissue. Decreases in cholesterol and triglyceride levels were also reported in blood of rainbow trout and catfish respectively fed with silymarin extract (Banaee *et al*., 2011), yarrow extracts (Nafisi Bahabadi *et al.,* 2014) and onion and garlic extract (Al-Salahy, 2002).

This work has demonstrated that *A. officinalis* is by far relatively non-toxic (2.5 and 5 g) in terms of biochemical parameters. Other findings obtained in the present study are that *A. officinalis* extract exhibited moderate cytotoxicity in high dose (10 g), while its extract has moderate antioxidant properties at doses of 2.5 and 5 g. Indeed, the potential in the plant to influence several clinically important parameters could be linked to various levels of pharmacological advantages. So, we recommend the use of these dosages in prospective clinical study to further evaluate the efficacy and safety of the *A. officinalis* extract as naturopathic medicine for fishes.

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