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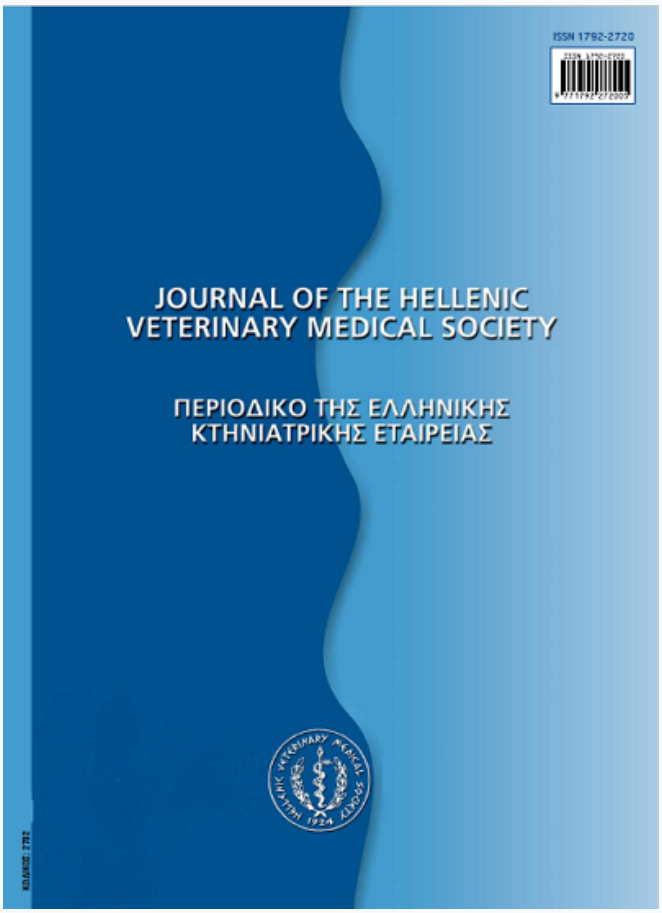

Comparative study of hematological and blood chemistry of Persian sturgeon (Asipencer persicus) exposed to two common anticoagulants

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Research article
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Comparative study of hematological and blood chemistry of Persian sturgeon *(Asipencer persicus)* exposed to two common anticoagulants

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ABSTRACT. The effects of heparin and ethylenediaminetetraacetic acid (EDTA) on plasma biochemistry and hematological parameters in *Asipencer persicus* were studied. Differences in ALT and ALP activity were found between serum and heparinized plasma ($P<0.05$). AST activity in EDTA treated samples increased ($P<0.05$) compared to its values in serum. On the contrary, ALP activity showed a significant decrease compared to its serum level ($P<0.05$). Samples collected with EDTA showed an increase in haematocrit, mean corpuscular volume (MCV) and white blood cells (WBC) values ($P<0.05$) when compared to those collected in heparin. The other measured biochemical parameters did not show any variation. The present study suggests the use of heparin as a preferred anticoagulant for routine haematological analyses in the Persian sturgeon. However, both of these anticoagulants inflicted changes in selected biochemical parameters.

Keywords: Heparin, EDTA, Persian sturgeon, hematology, serum biochemistry

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INTRODUCTION

Obtaining accurate results from routine hematological analysis is imperative for the interpretation of findings, which is crucial in assessing animal health status. However, interpretation of fish haematological data is quite complicated regarding to several variations such as blood sampling, laboratory techniques, storage and handling of blood samples between the time of blood collection and the time of analysis in the laboratory (Clark et al., 2011). In addition, hematologic assessment of blood samples is routinely suggested to be carried out promptly after blood collection or, if this is not possible, after a short term refrigeration. The anticoagulants used have also a determinative role in the results assessment.

Anticoagulant use is almost unavoidable due to quick clotting process of fish blood samples. Consequently, by using anticoagulants the concentration of measured substances may change before the analytical process (Guder, 2001). Heparin and EDTA salts are the most commonly used anticoagulants in fish haematology (Walencik and Witeska 2007). Most of researchers prefer heparin as anticoagulant (Svobodova et al., 1991; Ishikawa et al 2010; Clark et al 2011). However, some consider EDTA salts as suitable anticoagulant for fish blood analyses (Blaxhall and Daisley 1973; Blaxhall 1972; Sala-Rabanal et al 2003). Various anticoagulants have different reactions to blood cells of different animal species (Witeska and Wargocka 2011).

To our knowledge, there is no information on the selection of a proper anticoagulant with little effect on blood results assessment in *Asipencer persicus*. The present study was conducted with an aim to determine and compare how these two commonly used anticoagulants may affect the results of routine haematology and biochemical parameters in the Persian sturgeon.

MATER ALS AND METHODS

Experimental fish

Ninety Juvenile Persian sturgeon (weigh; 1±0.2kg) in apparent good health were recruited from Shahid Marjani sturgeon Propagation and Rearing Center, Golestan, Iran.

The adaptation period was one week. To minimize stress, fish were anesthetized with 25 ppm clove oil then carefully netted and positioned with their ventral side up for blood sampling from the caudal vein using a 2- ml syringe without any anticoagulant. The samples were immediately transferred to blood collecting vials (0.5 ml in each tube) containing the respective anticoagulants (heparin rinsed tube and EDTA rinsed tube). The rest of blood samples were transferred to the tubes without any anticoagulant for serum separation and were then taken (in an ice bath) to Fish Health Unit laboratory at Gonbad Kavous University, Golestan, Iran.

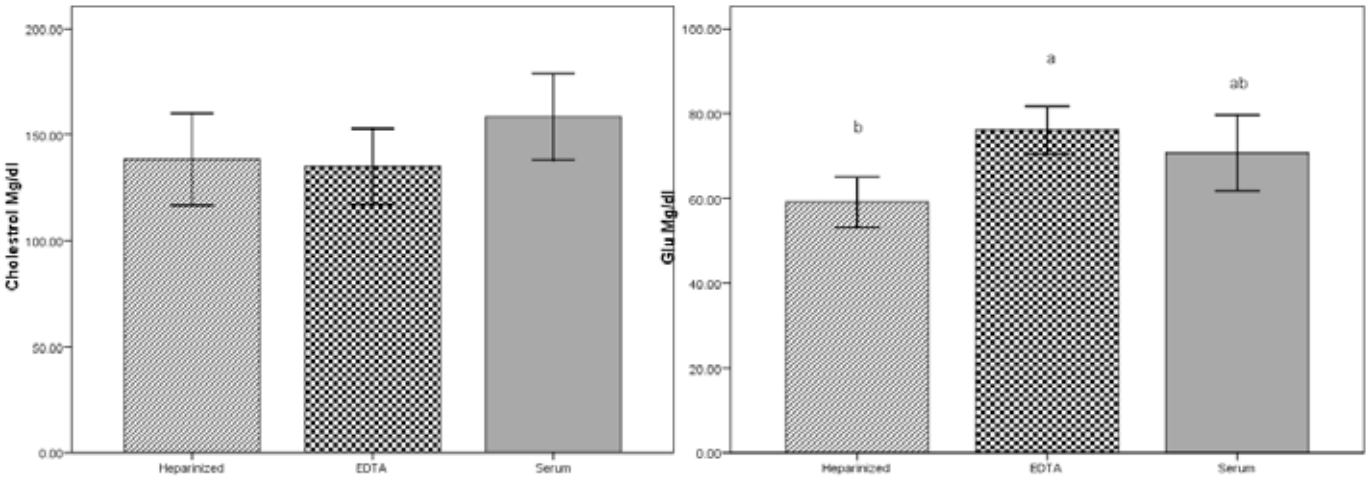
Blood sample collection, hematological and biochemical assay

All the hematological analysis was immediately after transferring to the laboratory. Total erythrocyte (RBC: 103 mm2), and leucocyte counts (WBC: 103 mm2) were done with a Neubauer haemocytometer (Marienfeld-Superior, Lauda-Königshofen,Germany) by using Natt Herrick's (Natt and Herrick 1952) diluent with a ratio of 1:200. Haemoglobin content (Hb: g dl-1), of blood was estimated spectrophotometrically by the method of Drabkin (Drabkin 1946). Heparinized as well as EDTA treated blood (50µl) was taken in micro haematocrit capillaries and centrifuged in a micro centrifuge spun in at 12,000 rpm for 5 min to obtain haematocrit value (HCT: %). Blood indices including mean corpuscular volume (MCV: Fl), mean corpuscular hemoglobin (MCH: pg) and mean corpuscular haemoglobin concentration (MCHC %) were calculated according to Dacie and Lewis (1991).

Serum and plasma samples obtained by centrifugation of blood at 3000 g for 15 min, were stored at -20ºC until analysis. Aspartate aminotrasferase (AST) (Reitman and Frankel 1957) and alkaline phosphatase (ALP) by Hempars® kit, alanine aminotransferase (ALT) (Reitman and Frankel 1957) and lactate dehydrogenase (LDH) (Henry 1974) was determined colorimetrically using kits supplied by Ziestchemistry Diagnostics Tehran, Iran. Total protein (TP) (Henry 1974), albumin (Alb) (Fazlolahzadeh et al., 2011), cholesterol (CHOL) (Zlatki et al 1953), Creatinine (CREA) (Owen et al 1954), glucose (GLU) (Trinder 1969), triglycerides (TG) (Foster and Dunn 1973), Urea (Chaney and Merbach 1962) by Parsazmoon® kits.

Table 1. Mean ± SE of cholestrol in serum and plasma samples in *Asipencer persicus*, n=30.

Table 2. Mean ± SE of Glu in serum and plasma samples in *Asipencer persicus*. Different superscript shows significant difference at p< 0.05, n=30.



Statistical analysis:

The data analyzed using the software program (SPSS 16). One- way analysis of variance (ANOVA) followed by Bonferroni's test was used for comparison between biochemical parameters of serum and plasma (EDTA and heparin). The hematology parameters between heparin and EDTA blood samples were statistically analyzed using the Student's t-test. The results expressed as means ± SE and $P < 0.05$ considered statistically significant.

RESULTS

The metabolite and enzyme values are shown in Figs 1–11, respectively. Similar values with no significant difference were found between heparin and serum in all parameters tested with the exception of increase in ALT and ALP ($p<0.05$). Serum and EDTA plasma samples yielded broadly similar values for enzymes tested except significant increase in AST and decrease in ALP in EDTA treated samples ($p<0.05$). Comparison of haematological parameters of the blood samples treated with heparin and EDTA is presented in Table 1. A significant increase in the haematocrit values, MCV and WBC counts was observed in all the EDTA treated samples compared to those of heparin treated samples ($P<0.05$). Significant decreases were also found in haemoglobin concentration and total RBC counts in blood samples obtained with EDTA compared to those in heparin group ($P<0.05$).

DISCUSSION

It is well known that blood parameters are likely influenced by anticoagulants as independent variables, therefore the choice of anticoagulant is important in diagnostic haematology (Faggio et al., 2012 & 2014). In the present study the values of some haematological parameters showed significant differences among the blood samples collected with EDTA and heparin. EDTA elevated haematocrit, WBC count and MCV values. In contrary, it has decreased haemoglobin concentration and RBC counts.

The elevation in haematocrit levels might be due acidification and an increase in p CO2 in EDTA treated samples (Smith et al., 1977). Similar phenomenon was observed by Blaxhall (1973) , Hattingh (1975) and Korcock et al. (1988). Hattingh (1975) compared the effects of heparin and EDTA on haematocrit of five species of fish and found that EDTA had a tendency to increase haematocrit in fish, and in some species induce haemolysis, while heparin produced very little change in erythrocyte volume and haematocrit values.

In the present study, EDTA treated samples showed a decrease in RBC count, which could be due to the haemolytic action of EDTA. Erythrocyte haemolysis caused by EDTA have been reported in common carp (Walencik and Witeska 2007), *Salmo gairdneri* (Korcock et al. 1988) and in *Cyprinus carpio* and *Oncorhynchus mykiss* (Lataretu 2013) which lead to decrease in RBC counts and haemoglobin

Table 3. Mean ± SE of Albumin in serum and plasma samples in *Asipencer persicus*. Different superscript shows significant difference at p< 0.05, n=30.

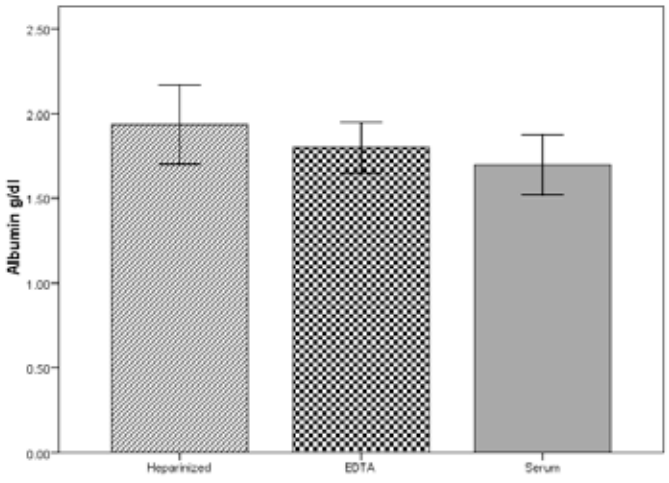
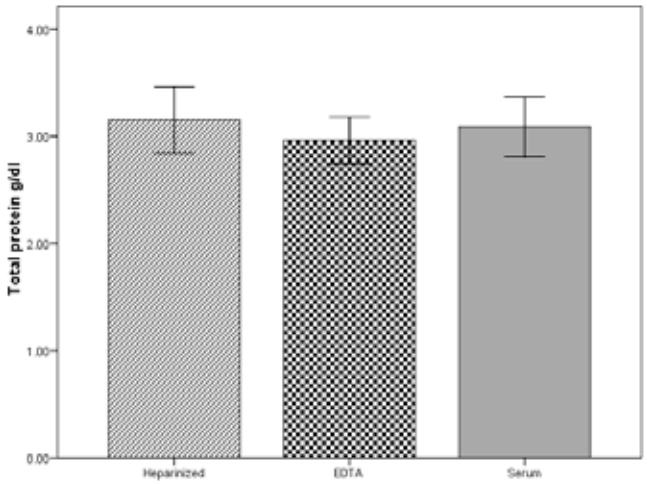


Table 4. Mean ± SE of total protein in serum and plasma samples in *Asipencer persicus*., n=30.



concentration. EDTA increases the osmotic fragility of erythrocytes, making them susceptible to lysis, which ultimately lead to a decrease in the RBC count. In addition, Jain (1993) showed that EDTA sequesters Ca 2+ions are responsible for the activation of Na + and K + ions in the cell membrane allowing the free entry of water into the cell which promote swelling and its consequent lysis. Anticoagulant concentration is also responsible for hemolysis. Imanpour et al (2012) showed that increasing in EDTA concentrations cause to decrease in RBC count and HCT level when compared to heparin treated samples in Huso huso. Moreover, the increase in MCV level in EDTA treated samples may be attributed to the swelling of the erythrocytes resulting in macrocytic anemia.

The use of serum or plasma in clinical pathology remains controversial. However, serum is preferred by many laboratories since it avoids the addition of anticoagulant that can interfere with some analytical methods or change the concentration of parameters being measured (Ceron et al. 2004). On the other hand plasma has three advantages; first it saves times since there is no need to wait for coagulation, secondly, there is 15-20% increase in plasma level than serum obtained from the same blood volume, thirdly, with plasma there is no coagulation induced changes or interferences (Guder 2001).

The present result of glucose and AST levels in EDTA treated samples, increased while ALP level decreased. Furthermore, heparin treated blood showed significant decrease in ALT and ALP. Heparin has generally recommended as more suitable anticoagulant for plasma collection (Burtis and Ashwood 2001). It should be noted that, due to scarcity in articles or related information concerning to the effects of different anticoagulants on plasma biochemistry of fishes and especially sturgeon the authors used available data from other animal species for comparison. In our study serum and heparinized plasma yielded similar results for most of parameters with exception of decrease in ALT and ALP level.

Use of anticoagulant can decrease ALP level in plasma compared to its serum level (Weatherby and Scott 2002). In present research, EDTA induced significant decrease in ALP level. Decrease in ALP level has been described in human, dogs and ruminants (Burtis & Ashwood 2007; Myers & Piersce 1972). The chelating properties of EDTA may have influenced results.

Since ALP is zinc–dependent, magnesium-activated enzyme (Jones 1985; Guder 2001). In addition, influence of EDTA on increase of AST level is described by Jones (1985), AST is present in tissue of high metabolic activity such as liver, brain, heart, skeletal muscle, kidney and red blood cell. Therefore, gross hemolysis of RBC contributes to falsely high AST level (Fischbach and Barnett 2004).

In conclusion, the results of the present study

indicate that heparin should be used as the preferable anticoagulant for hematological analysis in *A. persicus* as it imparts minimum changes to haematological parameters. However, plasma biochemical profile (metabolite and enzymes) of *A.persicus* is influenced by both EDTA and heparin.

The results of the present study apply only to the samples from normal Persian sturgeon and additional information for this species with abnormal results is necessary.

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