

The Investigation of Hyaluronidase Activity in Muscle of Medicine Leech (*Hirudo medicinalis*)

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Abstract

The aim study, it was determined the level of hyaluronidase that having a broad range of applications in medicine and pharmacology. Therefore the level and monthly changes of hyaluronidase were spectrophotometrically investigated in the muscle of medicine leech (*Hirudo medicinalis*) reared in Cip Fish Reproduction Unit of Fırat University Fisheries Faculty. During the study, the muscle samples from both anterior and posterior parts of total 168 of leeches were dissected and then they were extracted for determination of hyaluronidase levels. While the highest hyaluronidase level was found as $1692.69 \pm 40.65 \mu\text{mol/L}$ (1190.49-2270.07) in march, the lowest hyaluronidase activities was found as $322.98 \pm 13.41 \mu\text{mol/L}$ (207.31-824.21) in september. In conclusion, it was determined that the monthly changes of hyaluronidase level in the muscle of medicine leech (*Hirudo medicinalis*) were found statistically significant ($P \leq 0.05$). As a result after hibernation, gathered leeches are more appropriate than the other periods as did in point of obtaining hyaluronidase from *H. medicinalis*.

Key Words: Medicine Leech, *Hirudo medicinalis*, Hyaluronidase

Hirudo medicinalis (Tıbbi Sülük)'in Kas Dokusunda Hyaluronidaz Aktivitesinin Araştırılması

Özet

Çalışmanın amacı tıpta ve farmakolojide geniş bir kullanım alanına sahip olan hyaluronidazın seviyesini belirlemektir. Bu nedenle, Fırat Üniversitesi Su Ürünleri Fakültesi Cip Balık Üretim ve Araştırma tesisindeki sülük üretim ve yetiştirme havuzlarında yetiştirilen Tıbbi sülük *Hirudo medicinalis*'in kas dokusunda hyaluronidaz aktivitesi ve aylara göre değişimi spektrofotometrik yöntemle araştırıldı. Çalışmada, bir yıl boyunca toplam 168 adet sülüğün anterior ve posterior bölgelerindeki kas dokularının ekstraksiyonu yapılarak belirtilen dokularda hyaluronidaz miktarı saptandı. En yüksek hyaluronidaz aktivitesi Mart ayında ortalama $1692,69 \pm 40,65 \mu\text{mol/L}$ (1190,49 – 2270,07), en düşük hyaluronidaz aktivitesi ise eylül ayında $322,98 \pm 13,41 \mu\text{mol/L}$ (207,31– 824,21) olduğu tespit edildi. Tıbbi sülük *H. medicinalis*'in kas dokusundaki hyaluronidaz aktivitelerinin aylara göre dağılımları arasındaki farkın istatistiksel olarak önemli olduğu ($p \leq 0,05$) belirlendi. Sonuç olarak, *Hirudo medicinalis*'ten hyaluronidazın elde edilmesi düşünüldüğünde kış uykusundan çıkan sülüklerin toplanarak değerlendirilmesi hyaluronidazın elde edilmesi açısından uygun olacaktır.

Anahtar Kelimeler: Sülük, *Hirudo medicinalis*, Hyaluronidaz

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1. Introduction

Both leeches and enzymes, hormones to obtain from them were replaced drugs sought in treatment of various diseases. Leeches contains many enzymes and hormones in the structure. Hyaluronidase in one of these enzymes. Hyaluronidase which is endoenzyme catalyzes to depolymerization of short chain oligosaccharides. Hyaluronic acid having a cohesive property of the follicular cells. It is reported that the rise of hyaluronic acid levels in serum during infection and this increase will be clinically useful. Hyaluronidase have an important role in balancing the hyaluronic acid. Hyaluronidase enzyme that is formed by specific secretory cells of leech. Likewise, hyaluronidase enzyme exists in specific secretory cells of medicinal leech. In this study, it is aimed to determine by means monthly change and seasonal activity of hyaluronidase enzyme in the muscle tissue of medicinal leech (*H. medicinalis*).

2. Material and Methods

2.1. Material

Having the average weight of 2.19 ± 0.23 g and length in 12.31 ± 0.96 cm medical leeches were used 168 units. Leeches was regularly caught through clamp mouth without November, December and January from Cip Fish Reproduction Unit of Firat University Fisheries Faculty. Then taking 50 mg from anterior and posterior regions of muscle tissue of leeches were placed in aluminum foil envelopes and until making hyaluronidase activity analyze was stored at -20 °C.

2.2. Metod

Standart curve was plotted to determine the enzyme activity of hyaluronidase in which *H. medicinalis* using hyaluronidase standard [28-31]. 50, 100, 200 mg / L the prepared solution of N-asetilglukozamin were used to determine the standart curve. Leech tissue in the freezer with the help of scissors and scalpel were dissected. Segmented tissue was homogenized by glass-glass homogenizer stirring with NaCl (0.45

Hirudo medicinalis). This enzyme helps to penetrate rapidly sperm toward egg [1-2]. Hyaluronidase also used to raise the effect in the body of some chemotherapeutic. Because of this feature, hyaluronidase enzyme is added in the structure of antibiotics for improvement effect of it and absorpsion enhance of it [22]. Hyaluronidase has a therapeutic property and wide spread application in the medical field. It has been reported that hyaluronidase which is obtained from leeches can be used treatment of glaucoma which is an eye disease [14,22]. It is expressed that particularly, hyaluronidase has an important role in terms defence formation against viruses and tumors, egg and sperm activity, proliferation [8,28].

mol/L and diluted 1/5). Obtained extract, was transferred into eppendrof tubes (2 ml) and refrigerated centrifuge (Nüve) was centrifuged at 10,000 rpm for 30 minutes. Taking a pipette supernatant obtained after centrifugation was transferred in to centrifuge tube (15 ml) and centrifuge tube was added 0.1 ml of acetate buffer (0.3 mol/L, pH 3.8) and 0.1 ml of hyaluronic acid. Supernatants in centrifuge tubes were incubated at oven (37 ° C for 24 hours) after centrifuge tubes were tightly closed. After expiry of the incubation time, Supernatants in centrifuge tubes were added 60 ul of potasyumtetraborat (pH of 10 with 0.8 mol / L) and tubes were closed. The tubes are placed into the boiling pot 100 °C for 5 minutes and then the sample was cooled in ice-water. Hydrochloric acid (% 12,5 v/v) was added into glacial acetic acid. dimethylaminobenzaldehyde (DMAB) (10 w/v) was added into this mixture and so the stock solution was composed. When used stock solution, it was diluted with glacial acetic acid (1/10). Taking 2 ml of diluted DMAB was added onto ice-water cooled sample and tubes were incubated for 20 minutes at 37 °C in an oven. After the incubation, the samples were centrifuged again at 1500 rpm for 10 minutes and then UV – spectrophotometer at 582 nm was read the absorbance of samples to determine the hyaluronidase activity. This study was carried out and calculation was used average of the three replications.

2.3. Statistical Analysis

The standard deviation and the average level of hyaluronidase detected in the anterior and posterior of leeches collected from the study area and used this survey were performed using computer programme (Minitab for Windows 10.1 software).

3. Results

According to months, exchange of hyaluronidase activity of leeches used in the study were examined (Table 1). The highest the result of hyaluronidase activity was the value measured in March (1692.68 ± 288.32 mmol/L). In this period, hyaluronidase activity was determined 1696.22 ± 317.37 $\mu\text{mol/L}$ in the anterior part and 1689.15 ± 273.39 mmol/L in the posterior part of the leeches. During this month, average value 1692.68 ± 288.32 mmol / Between the months, outcome of september was the smallest value. There aren't differences between October, June and February months with April, July and August months ($p > 0.05$). In addition, both there were no statistical differences of May and March months and

According to months, the importance of distribution in activities hyaluranidaz was determined by the Kruskal-Wallis test. The difference found in the parameters applying the Mann-Whitney U test, the difference significance was determined between which months.

L was detected. According to months, amount of hyaluronidase was determined as $1070,32 \pm 326.73$ $\mu\text{mol/L}$ in May, 752.90 ± 489.03 $\mu\text{mol/L}$ in August, 749.50 ± 298.45 $\mu\text{mol/L}$ in July, 601.05 ± 322.27 $\mu\text{mol/L}$ in October, 454.21 ± 71.76 $\mu\text{mol/L}$ in April, 445.60 ± 79.67 $\mu\text{mol/L}$ in February, 442.27 ± 11.14 $\mu\text{mol/L}$ June, 318.73 ± 69.54 $\mu\text{mol/L}$ in September. There are statistical differenc between the months at hyaluronidase activity ($p \leq 0.05$). hyaluronidase activity measured during these months was determined much higher than other months. According to months, the statistical significance of the differences at the hyaluronidase activity is given in Table 1.

Table 1. The amount of hyaluronidase in anterior, posterior, and total of *H. medicinalis* amount of hyaluronidase

Months	Amount of hyaluronidase ($\mu\text{mol/L}$)		
	Anterior	Posterior	Ortalama \pm SD
February	460.06 \pm 77.13	431.14 \pm 96.40	445.60 \pm 79.67 ^b
March	1696.22 \pm 317.37	1689.15 \pm 273.39	1692.68 \pm 288.32 ^c
April	602.50 \pm 339.48	599.61 \pm 322.49	601.05 \pm 322.27 ^c
May	1239.65 \pm 293.14	900.99 \pm 275.18	1070.32 \pm 326.73 ^d
June	446.22 \pm 155.02	438.31 \pm 148.68	442.27 \pm 144.14 ^b
July	761.86 \pm 333.13	737.13 \pm 293.42	749.50 \pm 298.45 ^c
August	770.94 \pm 454.97	734.86 \pm 545.12	752.90 \pm 489.03 ^c
September	322.61 \pm 84.09	298.69 \pm 36.39	318.73 \pm 69.54 ^a
October	421.08 \pm 72.27	487.34 \pm 56.64	454.21 \pm 71.76 ^b

Monthly changes are shown with different letters ($p \leq 0,05$)

In this study, leeches samples weren't found in November, December and January

months that lowest water temperature in year. Leeches samples were obtain little or no in September, October and also February months.

Table 2. Changes according to month of temperatures in the study area

Aylar	Ocak	Şubat	Mart	Nisan	Mayıs	Haz.	Tem.	Ağus.	Eylül	Ekim	Kasım	Aralık
Sıcaklık(°C)	4	5	8	12	14	20	24	26	18	14	10	3

In terms of seasonal, when hyaluronidase activity level was analyzed, hyaluronidase activity of leeches was found highest as 1121.307 ± 550.32 mmol/L in Spring time. Afterward, these values

were detected the ($645,18 \pm 175,82$ $\mu\text{mol/L}$) in Summer, (445.60 ± 119.56 $\mu\text{mol/L}$) in Winter and (388.74 ± 65.76 $\mu\text{mol/L}$) in Autumn, respectively (Table 2).

Table 2. Changes in hyaluronidase activity as seasonal

Seasonal	Hyaluronidase level ($\mu\text{mol/L}$)
Spring	388.74 ± 65.76^a
Summer	445.60 ± 119.56^a
Autumn	1121.307 ± 550.32^c
Winter	645.18 ± 175.82^b

Seasonal changes are shown with different letters ($p \leq 0,05$). The statistical significance of differences in hyaluronidase activity at the Spring and Summer was determined as different

from the other seasons ($P < 0.001$). According to the seasons, the statistical significance of differences in hyaluronidase activity was showed in Table 2.

4. Discussion and Conclusion

Hyaluronidase can be obtained from mammals, the liver of mice, bacteria, human placenta, fibroblasts of chicks, snakes, shrimp, the leech tissue, bulls, and sperm of some other mammals [2,8,11,12,14,18,22,26,28]. Scientists have used different methods for the determination of hyaluronidase activity in different animal groups. Various methods like colorimetric, fluorimetric, viscosimetrically and High Performance Liquid Chromatography (HPLC) have been used for the determination of hyaluronidase activity in many studies in the world and in our country [8,10-12,14,16,17,19,27]. Spectrophotometric method is quite common in terms of both economic and practicality of use. This method has been used in many studies conducted in our country and in the world [1,2,14,29,31]. Spectrophotometric method was also successfully used in this study for the determination of change monthly of hyaluronidase activity. Leeches placed in the slots by opening under the soil, when the water cooled in winter while stimulated by an increase in temperature it left the egg in slots in spring [7,9,21,30]. In this study, leeches samples

were not found in November, December and January months. The cause of this condition could be placed under ground of leeches due to the winter sleep. At the same time leeches samples were obtain little or no in September, October and also February months is in parallel with data from other researchers. After the expiry of this period in both vertebrates and invertebrates seen in hibernation and estivation, hyaluronidase activity in the blood and body structure was stated to be more growth [3]. In this study is in parallel with Bayom et al. in terms found to be much higher than other months of hyaluronidase activity in samples taken from leeches began to emerge from the depths of the soil with the start of the increase in temperature at March, in Spring.

As a result, the hyaluronidase activity in muscle tissue of medical leech *H. medicinalis* which has an important market in the world and in our country were determined monthly and seasonal in this study. After hibernation, gathered leeches are more appropriate than the other periods as did in point of obtaining hyaluronidase from *H. medicinalis*

5. References

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