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**Comparative study of Nucleic Acid anabolism pathway in
Medical Leech *Hirudo medicinalis* and Earthworm
*Aporrectoda trapezoides***

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Abstract: The present study included a study of the amount of nucleic acids (Deoxyribonucleic acid DNA and Ribonucleic acid RNA) and their configuration pathway for the two types of Annelida parasitic and non-parasitic medical leech *Hirudo medicinalis* and earthworm *Aporrectoda trapezoides* respectively, Has been collecting from the Khurmala village in the province of Sulaymaniyah governorate, while earthworm samples were collected from certain areas in the province of Erbil governorate, was estimated the amount of nucleic acids and calculated the specific activity of the enzymes responsible for the formation of nucleic acids enzyme Thymidylate synthase and thymidine phosphorase and their role in the metabolism of nucleic acids, in tissue extract of medical leeches and earthworms.

The total amount of DNA and RNA for the suspended medical leeches ($523 \pm 1.52 \mu\text{g}/\text{cm}^3$, $139 \pm 22.5 \mu\text{g}/\text{cm}^3$) and for earthworms ($12933 \pm 2.6 \mu\text{g}/\text{cm}^3$, $58.58 \pm 0.89 \mu\text{g}/\text{cm}^3$).



Results showed that the specific activity of Thymidylate synthase enzyme in the medical leeches and the earthworms is $(178.2 \pm 6.55, 291.95 \pm 9.82)$ nanomol / min / mg protein respectively, while the specific activity of Thymidine phosphorylase enzyme is $(0.0 \pm 0.0, 1235.82 \pm 157.3)$ nanomol /min / mg protein respectively. It was also concluded from the study that the formation of nucleic acids and the multiplication of the DNA in medical leeches depend on the Salvage pathway only while earthworms rely on the Salvage pathway and the Denovo pathway.

Keywords: medical leeches, earthworms, nucleic acids, enzyme Thymidylate synthase ,and thymidine phosphorylase.

دراسة مقارنة لمسار بناء الأحماض النووية في العلق الطبي *Hirudo medicinalis* ودودة الأرض *Aporrectodea trapeziodes*

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Introduction

Annelida of Oligochaeta class is widely occupying in the biomass weight of invertebrates, including a lot of species among them *Aporrectodea trapezoids* that were found out in 1982 in soil tunnels and then named Intestine of earth. The earth worm is very important in agriculture which acts to fixing azotic compounds and nutritional materials in soil (Ruess, 2005).

The current study has covered another type of Annelida which is *Hirudo medicinalis*, so for medical importance which is considered an external parasite on vertebrate blood like fishes and frogs. leeches have been used by humans since a long time ago for healing by sucking the patient blood, and for this reason, it is named or called medical leech (Brown, 2005).



Studies on earthworms and leeches were varied and included biochemical and physiological studies (Kutschera and Ebermann, 2007). which analyzed the DNA that has an important role in transporting and storing inherited information from generation to another (Perez *et al.*, 2009).

The organisms have two paths of DNA synthesis. Denovo pathway, where nuclear acids are built from primary elements in which several enzymes participate mainly to synthesize nucleic acids i.e. thymidine synthase. And the second path, which is Salvage pathway which depends on previously available compounds in the medium and involving a group of enzymes like thymidine phosphorylase, (Upadnye, 2012 ; Al- helaly and Ahmed 2010).

This study has come to know the path of nucleic acids synthesis in the two Annelida species as a deference indicator for the class itself, that through the estimation of DNA and RNA amounts and the evolution of some enzymes activities such as thymidine synthesise (ThS) and thymidine phosphorlase (ThP).

Materials and methods

Sample collection:

Samples of earthworms are weekly collected in physiological solution during August until the end of September of 2012. The samples are took out from different locations of Erbil province like Birzin, Kardrash, Bena and qorq vilages. So leeches also gathered in the same time period from Khormal village of Sulamania province.

Preparation of cell extract and analyzing biochemical variations:

Annelida worms were washed several times with phosphate buffer saline (PBS), then put off in between two filter papers to dry. 0.5 gm of worms were homogenized at 1500 rpm in 5cm³ of triss-sucrose buffer that consists of 20 mg sucrose, 650 mg of triss in 20 cm³ distel water and adjusted PH at 7.2 and the volume complete to 100cm³ (Al- Tikrity, 2006).

The homogenization was performed in the cold water path to prevent warming, the homogenization was completed by ultra sonication at 12000 wave/min for 30 seconds, this process was repeated four times with rest time of 5 minutes to keep the extraction solution cold.



Estimation of DNA and RNA amounts.

The amount of DNA and RNA were measured due to (Shneider, 1957).

Detection of Thymidin synthase activity.

Crusbery route for THS activity detection was used (Crusbery *et al*, 1970).

Detection of thymidine phosphorylase activity.

Specific activity of THP according Jankeir (1999) was estimated.

RESULTS OF DISCUSSION

Results of the current study has revealed that the amount of DNA in earthworms was $22.5 \pm 1.52 \text{ mg/cm}^3$ and $53 \pm 1.52 \text{ mg/cm}^3$ for leeches, and there was significant difference at the probability level $p \leq 0.01$ figure(1). As it is known, that the function of the DNA is saving heredity information necessary to form most of the proteins and nucleic acids for all species (Perkins, 2001).

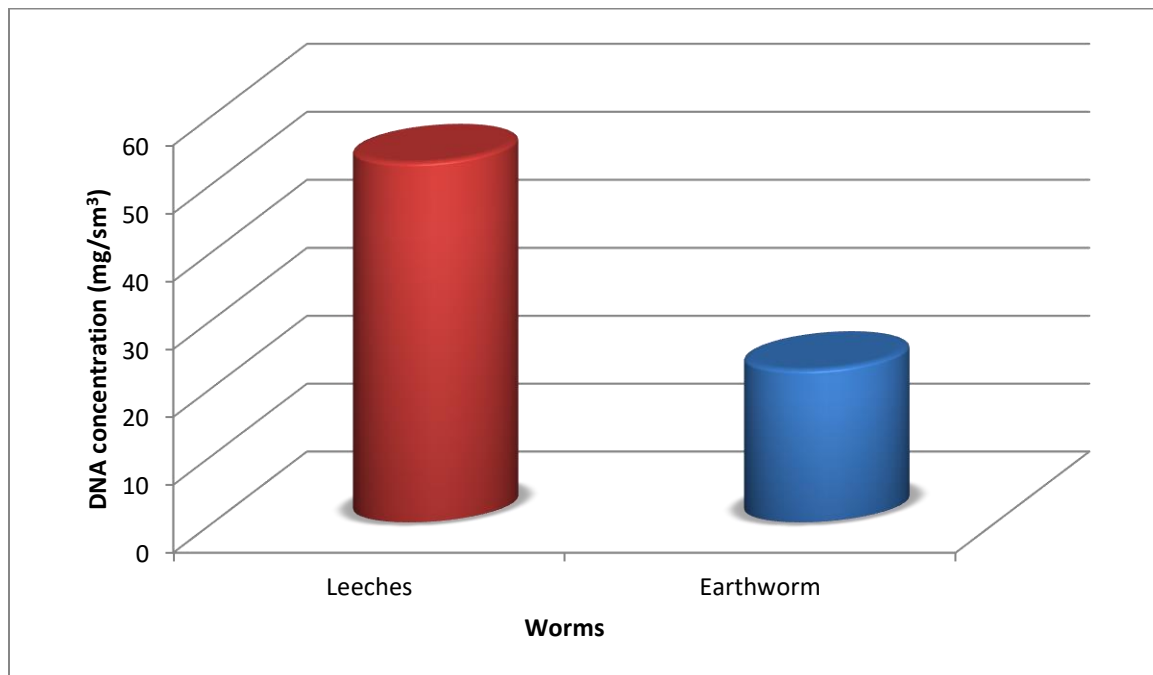


Figure (1):



As for the amount of RNA was found out from figure(2) that it reached $58.58 \pm 0.8 \text{ mg/cm}^3$ in earthworms and $129.33 \pm 2.6 \text{ mg/cm}^3$ on leeches.

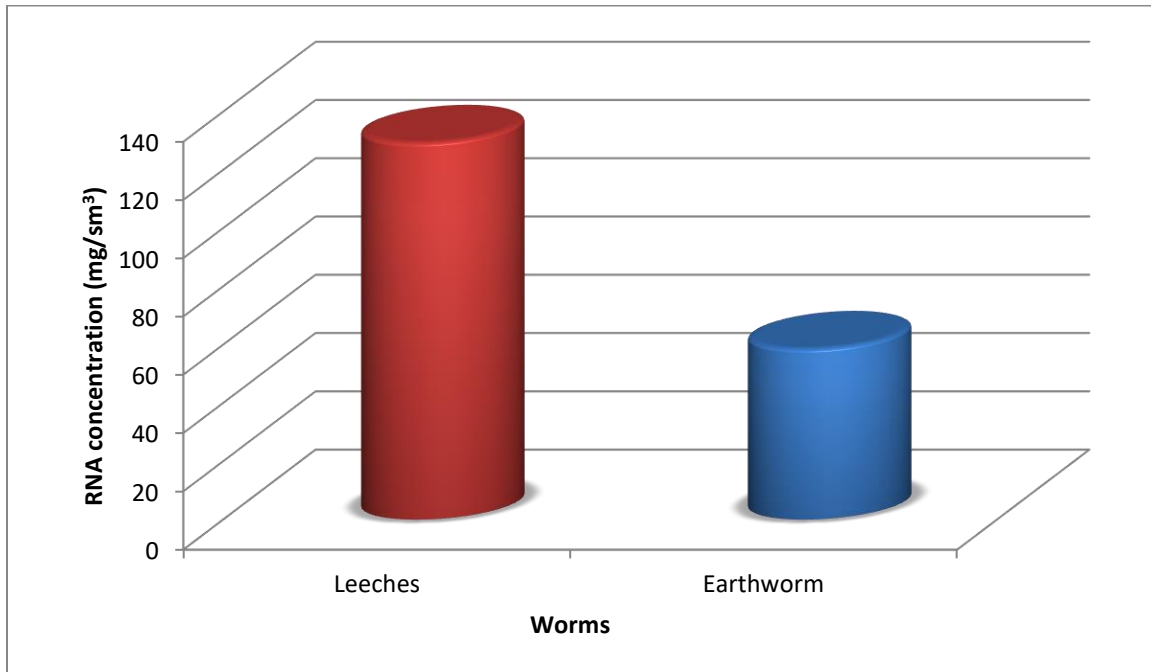


Figure (2):

This difference in the amounts of DNA and RNA between earthworms and leeches maybe caused by hereditical variations between the worms. Or the high amounts of DNA in leech may return to the nutritional nature of the host where it sucks suitable compounds for synthesise nucleic acids, also the changes in RNA amounts are consistent with the changes in the DNA amounts, so that the synthesis of RNA depend on DNA which is affected by the adaptational changes in the parasites in varying hosts or environment. The RNA-DNA ratio being larger due to the type of organism, current results comes in consistency with Al- Naftaji (2006) which has referred to the presence of vary differences in the amounts of DNA and RNA in different species of Cestoda worms that parasites of varying hosts, and it was caused by the varying conditions of organisms'



environment or the nature of nutrition. Also, the researcher, found that the change of RNA amounts comes consistent with changes in DNA amounts that was (4.7-2.113).

Current study results has showed that highest specific activity of thymidine synthase in earthworms is 291.55 ± 9.82 nanomol/min/mg protein figure(3), whereas, decreased in leech 178.2 ± 6.55 nanomol/min/mg.

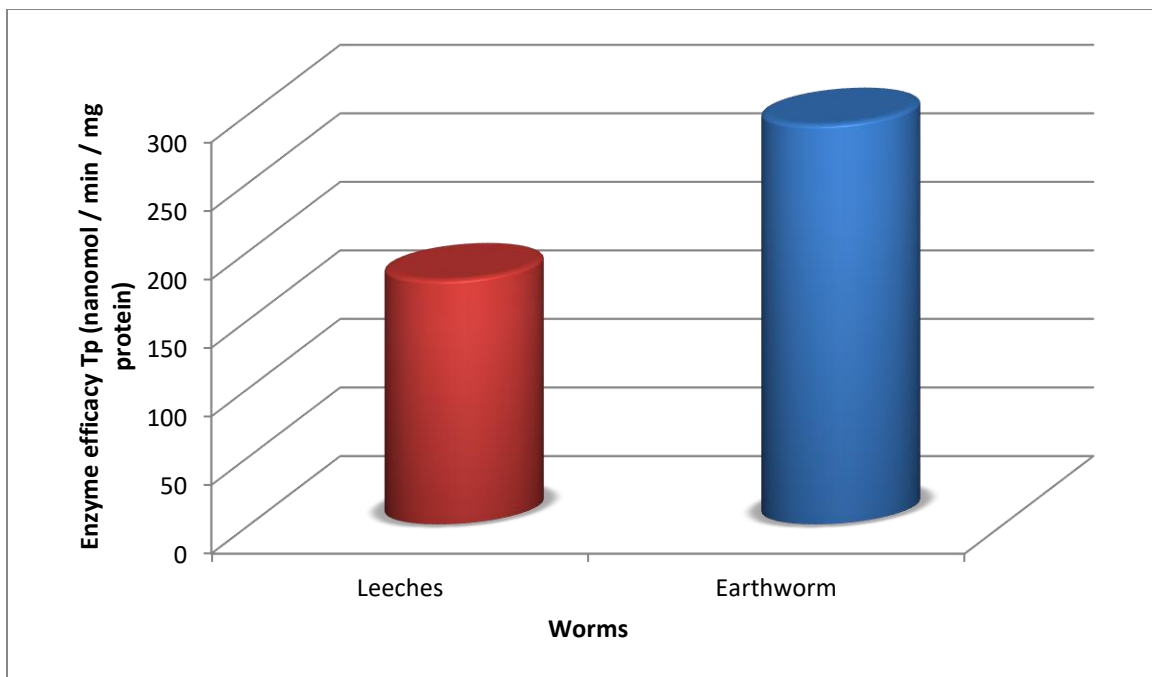


Figure (3):

Also, results showed high specific activity for thymidine phosphorylase figure(4), which is recorded 1235 ± 157.8 nanomol/min/mg protein. while, TP did not reveal specific activity in leeches.

From these results we concluded that earth worm has both paths Salvage and Denovo paths for nucleic synthesis, because there are specific activity of ThP and ThS. This was ensured by (Upadyne, 2012) and (Al- Helaly and Ahmed, 2010), with the knowledge that both enzymes participate to build nucleic acids by Salvage and Denovo paths respectively.



And or also the specific activity in leeches was low, that's why leeches lack the Salvage path for nucleic acid synthesise. But it depends on Denovo path solely. These differences between earthworms and leeches comes in consistent with Arteen (1982) had conducted, where he mentioned that nematode *Caenorhabditis* depends on Denovo path because it lacks to specific activity of ThP.

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