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Histological Aspects of Cerebrum and Cerebellum in Adult Male and Female Mongoose (*Herpestes edwardsii*)

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Abstract

BACKGROUND: Mongooses are small carnivores residing in different regions from Africa to southeast Asia. The Indian gray mongoose (*Herpestes Edwardsii*) is one of the mongoose species that is mostly found in southern Asia particularly India and the south of Iran. Anatomical studies on the animal's brain have always been of interest to the scientist due to its high importance in various branches of biology, such as zoology, veterinary medicine and ethology.

OBJECTIVES: Our study aimed to enrich the current data pool by providing a histomorphologic description of mongoose's brain. The present research was conducted to investigate the histomorphologic and histomorphometric aspects of cerebrum and cerebellum in the Indian grey mongoose.

METHODS: For this purpose, eight adult mongooses were considered, which were in an end-stage disease or the status of approaching death. In the following, after removing the skull, the brain structure was accurately dissected and placed in 10% buffered formalin. The samples were embedded in paraffin, cut into serial sections and stained using standard Hematoxylin and Eosin protocol.

RESULTS: In this study, the thickness of the white matter and cortex layers in the cerebrum and cerebellum, the number of neurons and neuroglia cells per unit area, and morphological features of tissue of the organs were measured and recorded. The results of cell count showed that the cell density of neurons in female and neuroglia in male were higher, significantly ($P \leq 0.05$). Also in the cerebellum, the Purkinje cells were oval to round and were very close to the granular layer.

CONCLUSIONS: Despite minor differences, it could be concluded that the general morphologic and morphometric characteristics of cerebrum and cerebellum for mongoose are similar to the other animals. Although the study of these features has not been done in a wild carnivore so far, these features can be considered as an intermediate between rodents and human.

KEYWORDS: Cerebellum, Cerebrum, Histomorphology, Histomorphometry, Mongoose

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Introduction

Mongooses are carnivores occupying various regions from Africa to Southeast Asia. The genus Herpestes contains ten species and is considered the oldest genus in Carnivora's order, dating back to approximately 30 million years (Shil *et al*., 2012). The Indian gray mongoose (Herpestes edwardsii) is a mongoose species principally found in southern Asia and the South of Iran. Also, males' body mass is considerably more than females (Rasouli *et al*., 2015)

The brain is made of three main parts: cerebral, cerebellum, and medulla oblongata. The cerebrum is the largest part of the brain and involves reasoning, learning, sensory perception, and emotional responses. On the other hand, the cerebellum is responsible for the maintenance of equilibrium of the body by coordinating somatic motor activity and by regulating muscle tone. This part is more important in active vertebrate genera since it is intimately involved in the control and maintenance of muscle tones and thereby motor coordination (Dyce *et al*., 2017). Brain study in animals is important for animal's welfare and knowledge about the nervous system function, physiology, behavior habits, and developmental anatomy.

Histological studies on cerebrum and cerebellum have been of interest to the anatomical scientists in the last decade. The microanatomy of the cerebrum has been studied and described in human, mouse (Treuting and Dintzis, 2012), rabbit, rat (Ibegbu *et al*., 2014), and guinea pig (Musa *et al*., 2016). Moreover, (Pal *et al*., 2003; Irimescu *et al*., 2015; Treuting and Dintzis, 2012; Danmaigoro *et al*., 2016; Sur *et al*., 2011; Musa *et al.,* 2016; Beheiry, 2015) provide valuable information about the cerebellum histological aspects in the fowl, chinchilla, mouse, catfish, birds, guinea pig, and camel, respectively. Also, Jardim-Messeder *et al*. (2017) carried out extensive comparative studies on the number of brain neurons and their brain-size in various carnivores including one of the mongoose species.

Comparative morphology has been served as evidence for evolution and indicates that various animals share a common ancestor. This allows scientists to classify animals based on similar characteristics or diversity of their body structures. There is a dearth of information on the comparative histological organization of the cerebrum and cerebellum in the carnivores. In addition, this study may be helpful for a better understanding of the brain structural variations related to behavioral habitats and functional anatomy of this species.

Our study aimed to enrich the current data pool by providing a comparative histological description of the mongoose's cerebrum and cerebellum.

Materials and methods

Eight adult mongooses (4 male and 4 female), which have been found in Shiraz's surrounding areas, were used for this study over the last two years. The age of the animals was estimated to be more than one-year-old concerning the teeth inspection. These fresh specimens in an end-stage disease or the status of approaching death (severe hypothermia; 36○c body temperature) were admitted to anatomy laboratory in veterinary faculty, Shiraz University, Shiraz, Iran. All these activities were done in coordination with the environmental organization of Fars province. Therefore, the license was obtained from this organization with the number 5599/700 / 93 / p.

The animals were euthanized with Ketamine 10% and Xylazine 2%. Then 10% buffered formalin solution was injected into the lateral ventricles by creating a small pore in the frontal bone. In this way, the fixative material was well distributed in the cerebral ventricles and the subarachnoid space and the nervous tissue became more firm. These methods were performed based on the study of Musa *et al*. (2016).

The specimens were then transferred into the big containers of phosphate buffered formalin solution, 24 h later, the skull was removed and the brain was exposed. Then, the tissue samples were collected from parietal and frontal lobes of the right cerebral hemisphere and right cerebellar hemisphere (Figure 1). These samples were embedded in paraffin, cut into serial sections, and stained using a standard Hematoxylin and Eosin protocol (H&E stain). In this study, the thickness of the white matter and cortex layers in the cerebrum and cerebellum, the number of neurons and neuroglia cells per unit area, and morphological features of the tissue of the organs were measured and recorded by Image Pro 7 plus software (Pal *et al*., 2003). In the following, a comparison is made between the results regarding the histological aspects of cerebrum and cerebellum and those of literature data about the other species.

Statistcal analysis

Analysis of morphometrical data was carried out with Student's T-test using the SPSS software (Version 23) at the significance level $P \leq 0.05$.

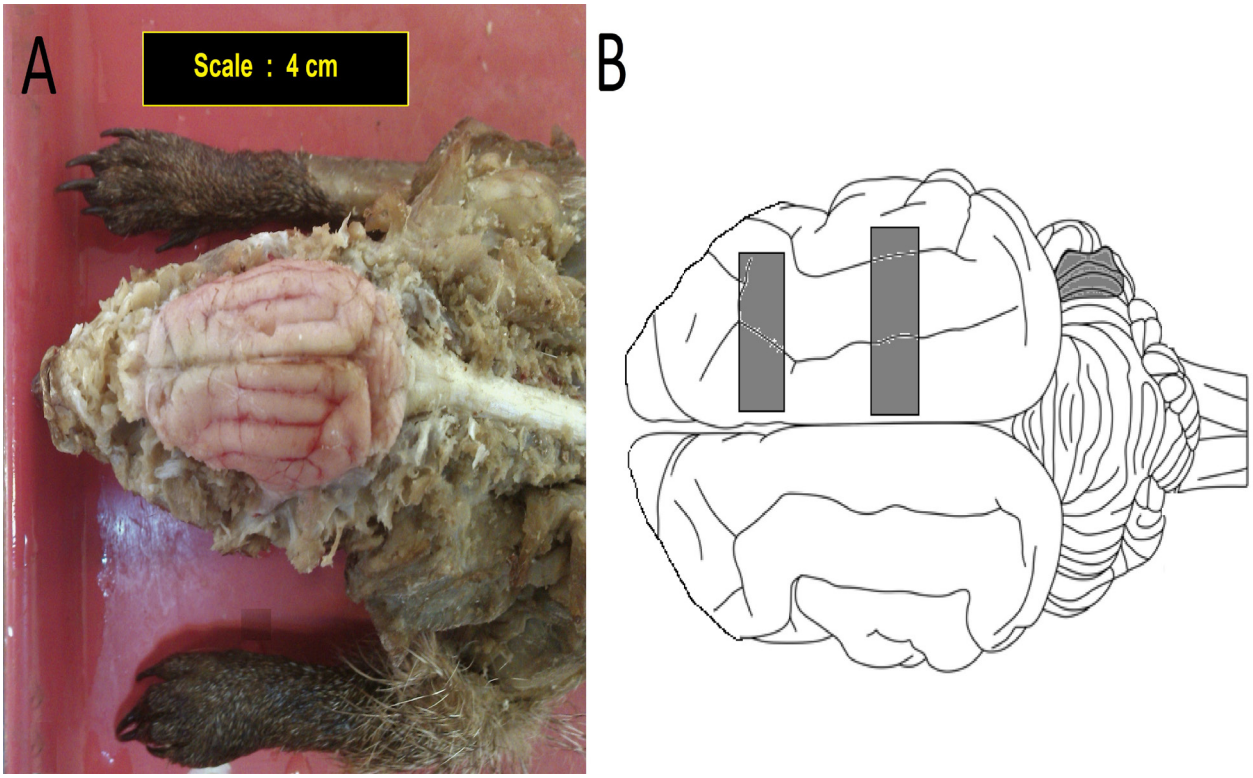

Figure 1. A: Structure of the brain after removing the skull in an adult mongoose (Dorsal view). B: Areas for tissue sampling of the cerebrum and cerebellum

Results

The mongoose's cerebral histological section consists of two distinguished materials: gray matter and white matter. The gray matter or cerebral cortex is situated externally while the white matter is situated internally (Figure 2). The results of the studies on thickness measurements of the white and gray matter layers, as well as the ratio between them in the frontal and parietal lobes, are summarized in Table 1. Further, the results of studies on the cellular density of neurons per unit area of gray matter, and also the neuroglia cells per unit surface area of white matter are reflected in Table 1. All the results and significant differences were performed considering $P \leq 0.05$.

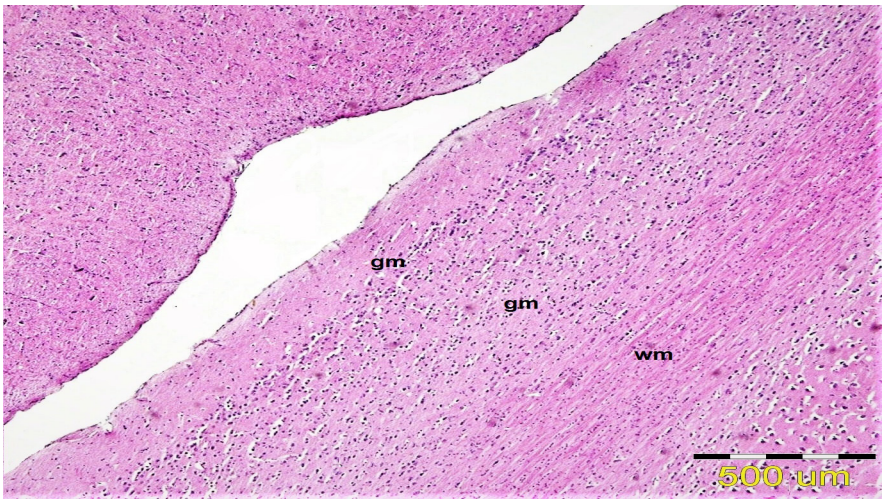

Figure 2. Photomicrograph of the frontal lobe of the cerebrum in the male mongoose. Stain: H&E. White matter (wm) and Grey matter (gm)

Table 1. Thickness of the gray matter and white matter of the cerebrum, their ratio, and cellular density per unit area (Mean ± SD) both in male and female mongoose.

	Frontal lobe		Parietal lobe	
	Male(n=4)	Female(n=4)	Male(n=4)	Female(n=4)
Gray matter thickness (mm)	0.994 ± 0.084	0.803 ± 0.025*	1.245 ± 0.164	0.723 ± 0.02*
White matter thickness (mm)	0.717 ± 0.15	0.63 ± 0.29	0.92 ± 0.042	0.613 ± 0.07*
Ratio of gray matter to white matter	1.38 ± 0.11	1.27± 0.12	1.35 ± 0.1	1.19 ± 0.14
Number of neurons per unit area of gray matter (n/mm^2)	17430.80 ± 1029	19140 1610.28 ±	18565.45 ± 2566.88	19120.43 ± 1475.5
Number of neuroglia cells per unit area of white matter (n/mm^2)	11577.55 ± 892.28	8625.32 ± 532.12*	10370 ± 480	7665.67 ± 989.44*

(* significant difference $P \leq 0.05$).

The observations of the present study showed that the layers of the frontal lobe in cerebrum cortex are relatively recognizable so that the molecular layer of the cortex was a marked layer with very few cells. The outer granular and pyramid layers are close and distinguishable from each other. The internal granular and pyramidal layers are highly interconnected. However, as the outside moves to the medullary area, the pyramidal cells (Betz cells) becomes larger. The polymorphic layer also has high cell density and cellular forms. The boundary between the gray matter and the white matter is also clearly evident (Figure 3A). The mentioned features are also seen in the parietal lobe. The difference is that six layers appear more clear and distinct from each other (Figure 3B).

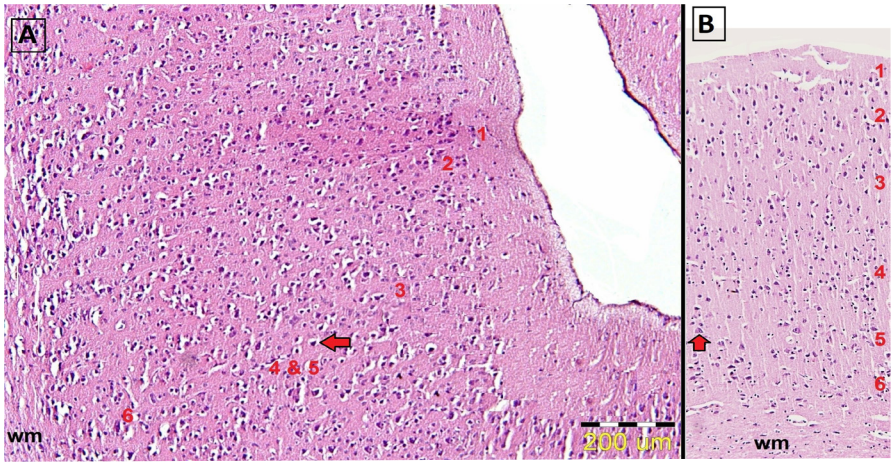

Figure 3. Photomicrograph of cortex layers in the parietal lobe (A) and frontal lobe (B) in the mongoose's cerebrum. Stain: H&E.
White matter (wm), Betz cell (red arrow), Molecular layer (1), External granular layer (2), External pyramidal layer (3), Internal granular layer (4), Internal pyramidal layer (5), Polymorphic layer (6).

Histologically, the cerebellum also has white and gray matter. The cerebellar white matter was observed to be featureless. Moreover, its function as a germinal zone is controversial. Microscopic examination of the mongoose cerebellum samples represented the usual structure of the cerebellar cortex or grey matter in all animals: the outer, molecular, the middle layers are composed of a single row of purkinje cells and an inner granular layer (Figure 4).

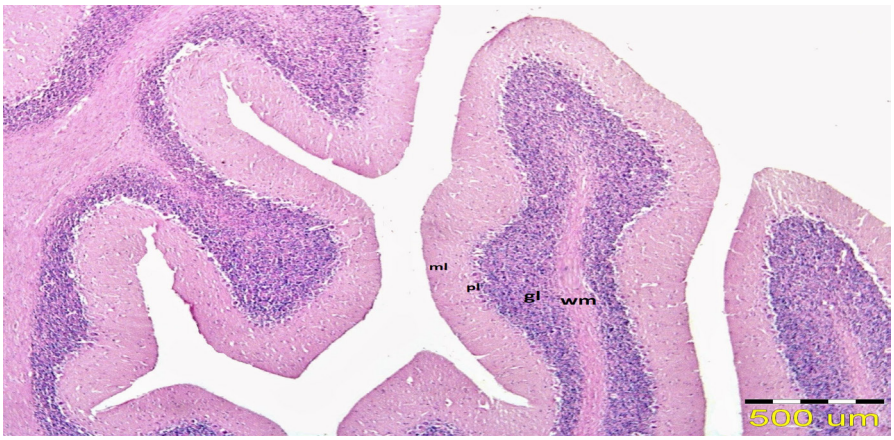

Figure 4. Photomicrograph of mongoose's cerebellum. Stain: H&E.
White matter (wm), Molecular layer (ml), Purkinje cells layer (pl), Granular layer (gl)

The results of the thickness measurements of white matter, gray matter, and also various layers of gray matter are summarized in Table 2, considering $P \leq 0.05$. Further, the results from studies on density measurements of cells per unit area (n/mm^2) for different layers of gray matter are presented in Table 2. It should be noted that because of the low thickness of the purkinje cell layer, the density of these cells has been measured in unit length (n/mm).

Table 2. Thickness of the cerebellum layers and their cellular density per unit area (Mean ± SD) in the male and female mongoose

	Male (n=4)	Female (n=4)
Thickness of white matter (mm)	0.15 ± 0.69	0.136 ± 0.023
Thickness of gray matter (mm)	0.425 ± 0.02	0.362 ± 0.07
Thickness of molecular layer (mm)	0.21 ± 0.4	0.205 ± 0.029
Thickness of granular layer (mm)	0.193 ± 0.13	0.143 ± 0.42
Thickness of Purkinje cell layer (mm)	0.026 ± 0.00	0.023 ± 0.005
Cellular density in the molecular layer of the cerebellum (n/mm^2)	290.00± 15.05	362.5 ± 10.15*
Cellular density in the granular layer of the cerebellum (n/mm^2)	6540.49 ± 532.12	8249.35 ± 602.04*
Cellular density in the Purkinje cell layer (n/mm)	16.34 ± 2.91	14.48 ± 1.05 *

(* significant difference $P \leq 0.05$).

In the molecular layer, we can see the stellate and basket cells and numerous rich dendritic branches from the Purkinje cells, oriented eccentrically towards the cortex's external surface. In this layer, the basket cells are placed next to the Purkinje cells and the stellate near the outer surface. The middle layer includes the characteristic of Purkinje cells. Our photomicrographs indicate that their soma is circular or oval-shaped, the length is about 20 micrometers, and the width of about 15 micrometers, in both sexes. The granular layer presented a high concentration of heterochromatin granular cells and few Golgi cells with a pale appearance in between. Among the granular cells, there were small spaces called cerebellar islands appearing as irregular light areas (Figure. 5).

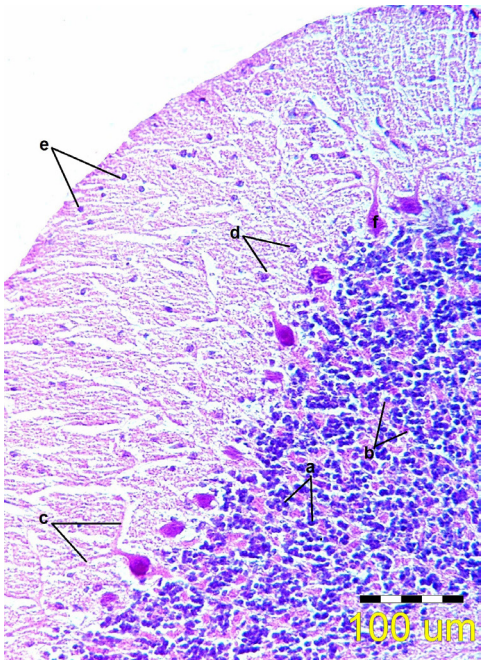

Figure 5. Photomicrograph of cortex layers of the mongoose's cerebellum. Stain: H&E. Granular cells of the granular layer (a), Golgi cells of the granular layer (b), Dendritic branches of Purkinje cells (c), Basket cells of the molecular layer (d), Stellate cells of the molecular layer (d), Soma of Purkinje cell (f)

Discussion

The present study showed that the gray matter and white matter in the cerebrum are placed in the cortex and center, respectively, which is similar to other animals (Dellman and Eurell, 1998). In the molecular layer, cell density was very low, due to the presence of dendritic fibers of the neurons in the underlying layers, which helps to distinguish this layer from other layers (Figure 3). This layer is thicker in Indian guinea pigs than that of laboratory mouse and rats (Treuting and Dintzis, 2012). In addition, the highest proportion of gray matter to white matter was found in the frontal lobe of male mongoose. This proportion is lower in the female mongooses in two lobes unclearly (Table 1).

Under it, external granular cells are observed and below it, there is a row of small to medium pyramidal cells that are related to the outer pyramidal layer. In the frontal lobe, the inner granular layer is combined with the inner pyramidal layer of the large pyramidal cells and the polymorphic layer was thick with high cell density. This is while these two layers in the parietal lobe are separated and the thickness of the polymorphic layer is less (Figure 3). Studies on guinea pigs showed that there is no external pyramidal layer in this animal, but the rest of the layers exist (Musa *et al*., 2016). Also, in the laboratory mouse, the second and third layers are combined, but other layers are separated. In humans, all six layers are distinct and clear (Treuting and Dintzis, 2012).

The remarkable point in these layers is the high thickness of the polymorphic layer and the increased betz cells in the cortex, as compared to studies on rats, guinea pigs and rabbits (Ibegbu *et al*., 2014). This may be the reason why carnivores like mongoose have more sense of understanding and intelligence than rodents (Kelly and Stick, 2003). Therefore, the agility and intelligence of this animal in nature are related to these characteristics.

In this study, it was determined that all layers of the cerebellar cortex, as well as white matter in male, are more than female. However, the difference is not significant (Table 2). It is necessary to note that molecular and granular layers in the cerebellar folia summits are thicker and have a lower thickness in the folia fissures. This is while the white matter thickness is constant in all areas of the cerebellum (Figure 4). However, studies on human, camel, chicken, and pigeon show that in the cerebellum folia fissure only the granular layer has a lower thickness (Pal *et al*., 2003; Sur *et al*., 2011; Beheiry, 2015).

In cellular density measurements of the cerebral tissue, it was found that the cell density of the neurons (n/mm^2) in the female mongoose is higher than male, while the density of neuroglia cells was higher in males (Table 2). These results are in good agreement with studies conducted on human (Witelson *et al*., 1995). The performed research shows that the density of cortical neurons in banded mongoose is lower than that of other carnivores and is not related to the domestication or body mass (Jardim-Messeder *et al*., 2017). Given the smaller size of females, these findings are consistent with the present study.

Pearson (1972) stated that throughout all vertebrate genera the organization of cells and axons within the cerebellum had a similar and distinctive appearance. A number of small star-shaped cells found in a scattered manner close to the periphery of the cerebellar molecular layer represented stellate cells (Figure 5). This is also in agreement with studies performed on fowl, camel, catfish, and chinchilla (Irimescu *et al*., 2015; Dan-

maigoro *et al*., 2016).

The present study stated that most of the purkinje cells had a circular or oval shape, and their nucleus is large and has a specific nucleolus. In humans, camel, and fowl purkinje cells are reported to have a circular and cylindrical shape and are flask-shaped (Pal *et al*., 2003). In the laboratory mouse and guinea pig, these cells were less and lacking in regular form and had less cytoplasm, thus, the purkinje cells appear to be more similar to human. However, in humans, these cells are more spaced than granular layers (Treuting and Dintzis, 2012). This study showed that unlike humans, granular layer cells are found between purkinje cells, which was consistent with the studies performed on the other animals (Beheiry, 2015; Bacha, 2000).

This study showed that the cell density in the 1mm length of the purkinje cell layer in male and female mongoose was 16.34 and 14.48, respectively. Studies performed on other animals showed that this number is generally higher in birds, for example, in pigeon it is 27.39, in duck it is 20.98, in domestic chicken it is 18.9, and in humans it is 6.6 (Sur *et al*., 2011; Pal *et al*., 2003).

Purkinje cells are considered as the most important cells of the cerebellum according to their functional capabilities, as well as the most emphasized cells in behavioral and cognitive studies (Pal *et al*., 2003). This study and previous studies have shown that the form and amount of cytoplasmic content of purkinje cells, in proportion to their number, has more to do with these capabilities.

The granular layer was the innermost layer of the cortex and was situated between the purkinje cell layer and a medullary layer of white matter. The appearance of the nucleus of the granular cells was due to the bulk of the nuclei, as well as to the lymphocytes (Figure 5). In studies done on humans and chickens, it was observed that golgi cells were interspersed with granular layers and the purkinje layer had a large size. This was different from our observations on camel and goat (Pal *et al*., 2003; Beheiry, 2015).

According to our studies, it could be concluded that the general morphologic and morphometric characteristics of cerebrum and cerebellum of mongoose are considered as an intermediate between rodents and humans. In addition, therange of the evolution of these structures in the mongoose is consistent with the perceptual and functional capabilities of the animal compared to the other animals. This work could probably be the first histological study on the brain of a wild carnivore. Our results provide an incipient description in this direction and we consider that further stereotaxic studies and other kinds of staining are needed to provide more accurate cerebral and cerebellar histological landmarks.

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Conflict of interest

The authors declared that there is no conflict of interest.

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مطالعه بافت شناسی مخ و مخ چه در خدنگ نر و ماده بالغ *(Herpestes edwardsii)*

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چکیده

زمینه مطالعه: خدنگیان، گوشتخوارانی کوچک می باشند که در نواحی وسیعی از قاره آسیا و آفریقا یافت می شوند. خدنگ هندی خاکستری گونه ای از این خانواده است که در هند و نوار جنوبی ایران دیده شده است. مشخصات آناتومیکی مغز به دلیل اهمیت این ساختار در شاخه های مختلف بیولوژی مانند جانورشناسی، دامپزشکی و رفتار شناسی همواره مورد توجه متخصصین امر بوده است.

هدف: مطالعه ی حاضر بیان‌کننده توصیفات بافت شناسی از ساختمان مخ و مخچه خدنگ می باشد تا بدین ترتیب کمبود های اطلاعاتی که در این زمینه وجود دارد تا حدی جبران گردد.

روش کار: به منظور رسیدن به این هدف تعداد ۸ قلاده خدنگ که به علت حوادث طبیعی در مراحل پایانی زندگی یافت شدند، استفاده شد. پس از برداشت جمجه و نمونه‌گیری از اندام های مذکور، نمونه‌ها در ماده فیکساتیو قرار گرفت. در ادامه مقاطع بافتی به صورت سریالی اخذ شد و از روش رنگ آمیزی هماتوکسیلین – ائوزین به منظور ارزیابی‌های هیستولوژی استفاده شد.

نتایج: در این بررسی ضخامت لایه های مختلف در مخ و مخ چه و هم چنین تراکم سلولی در این لایه ها اندازه گیری و شمارش شد. در مخ شمارش سلولی نشان داد که تراکم نورون ها در جنس ماده و تراکم سلول های نوروگلیا در جنس نر، به صورت معنی داری بیشتر است ($P\leq ۰/۰۵$). در مخچه سلول های پورکنژ ظاهر بیضی یا گرد داشته و با فاصله ی کمی از لایه گرانولار دیده می شدند.

نتیجه گیری نهایی: به نظر می‌رسد که خصوصیات کلی مورفولوژیک و مورفومتریک مخ و مخچه در خدنگ از همان چهارچوب موجود در سایر حیوانات تبعیت می کند. اگرچه اطلاعات ما پیرامون جنبه های هیستولوژی مغز در گوشتخواران وحشی ناچیز است، با این حال می توان این ویژگی ها را بینابین انسان و جوندگان دانست.

واژه‌های کلیدی:

هیستومورفولوژی، هیستو مورفومتری ،خدنگ ،مخ ،مخچه.

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Amelioration of Lipid Peroxidation and Antioxidant Enzymes Status in the Serum and Erythrocytes of Phenylhydrazine-Induced Anemic Male Rats: The Protective Role of Artichoke Extract (*Cynara scolymus* L.)

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Abstract

BACKGROUND: Hemolytic anemia is a disorder characterized by the premature erythrocytes destruction. Phenylhydrazine (PHZ) induces oxidative stress and reactive oxygen species (ROS) formation, which causes hemolytic anemia. *Cynara scolymus* due to its antioxidant compounds, has been used for various therapeutic purposes in traditional medicine.

OBJECTIVES: The present study was designed to evaluate the effects of *Cynara scolymus* extract on PHZ -induced anemia in male rats.

METHODS: Hemolytic anemia was induced by intraperitoneal injection of PHZ (40 mg/kg) for 2 days. Thirty male Wistar rats were divided into five groups. Group 1 (normal control). Group 2 (anemic control) received only PHZ. The groups 3 to 5 were injected with 100, 200, 400 mg/kg of the *Cynara scolymus* by gavage, respectively, daily from day 2 to day 15 after PHZ administration. At the end of the treatment period, blood samples were collected to assess hematological parameters, malondialdehyde (MDA) level and antioxidant enzymes activity, including superoxide dismutase (SOD) and total antioxidant capacity (TAC) in the serum and erythrocytes.

RESULTS: In anemic rats, serum and erythrocytes MDA level increased, but SOD and TAC activity decreased significantly when compared with control group ($P \leq 0.05$). These changes were ameliorated by treatment with *Cynara scolymus* at different doses ($P \leq 0.05$). Also, improvement in several hematological parameters was observed in anemic rats after administration of *Cynara scolymus* ($P \leq 0.05$).

CONCLUSIONS: *Cynara scolymus* extract exhibits protective property against PHZ-induced oxidative stress presumably due to antioxidative activity.

KEYWORDS: *Cynara scolymus,* Hemolytic Anemia, Oxidative Stress, Phenylhydrazine, Rat

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Introduction

Anemia is defined as a condition in which the number of red blood cells (RBCs) or their oxygen-carrying capacity is inadequate to meet the physiological needs of the body (Prasad *et al*., 2018). There are several kinds of anemia classified by a variety of underlying causes. Hemolytic anemia is the most frequent form of anemia (Lee *et al*., 2012). Hemolytic anemia is categorized as acquired or hereditary. Common acquired causes of hemolytic anemia are autoimmunity, microangiopathy, infection and drug (Dhaliwai *et al*., 2004; Paul *et al*., 2018).

Phenylhydrazine (PHZ) is a strong oxidant agent which has toxicity on various tissues at various levels. It is known to shorten life-span of erythrocytes resulting in hemolytic anemia, increased erythropoietic activity, enhanced iron absorption and tissue iron overload (Luangaram *et al*,. 2007). PHZ has been used experimentally for the induction of hemolytic anemia in animal models. The auto-oxidation of PHZ leads to generation of ROS and PHZ-derived radicals, which causes a wide variety of deleterious cellular response including hemolytic anemia (Sung *et al.*, 2013). The observed haematotoxicity is a result of the reaction of PHZ with oxygenated hemoglobin to form oxygen radicals and methemoglobin (Shetlar and Hill, 1985).

Medicinal plants and natural products have been used as traditional treatments for numerous diseases particularly in developing countries because of several reasons including therapeutic effects, affordability, accessibility and fewer side effects (Asase *et al*., 2008). Several studies have demonstrated that natural medicinal plants with potent antioxidant activity and their potential protective effects can alleviate the damage of oxidative stress-associated diseases through inhibition of ROS generation and improvement of antioxidant defense mechanisms (Forni *et al*., 2019; Banerjee *et al*., 2018).

Cynara scolymus L. (Artichoke), a member of Asteraceae family, is an ancient herbaceous perennial plant, originating from the Mediterranean area, which today is widely cultivated all over the world because of its nutritional benefits and medicinal purposes (Salekzamani *et al*., 2019). Phytochemicals analysis of *Cynara scolymus* has been found to contain powerful polyphenolic compounds which have therapeutic options including remarkable antioxidant activity against ROS and preventing the formation of free radicals (El-Boshy *et al*., 2017). Previous studies have reported that artichoke extract has important activities such as hepatoprotective (Gebhardt and Fausel, 1997), hypoglycemic (Salem *et al*., 2017), antibacterial (Shimoda *et al*. 2003), antioxidant (Salekzamani *et al*., 2019), anti-inflammatory and immunomodulatory (El-Boshy *et al.*, 2017).

There is little evidence to indicate artichoke is useful to alleviate hemolytic anemia. Therefore, this study was undertaken to evaluate the putative antioxidant action of *Cynara scolymus* extract in an experimental model of PHZ-induced toxicity in Wistar rats.

Materials and methods

Chemicals and preparation of extract

The ethanolic extract of artichoke that was used in this study was purchased from Dineh Iran Industries Complex (Pharmaceutical Company, Tehran, Iran). Phenylhydrazine (PHZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals housing conditions

This study was carried out on 30 mature male Wistar rats, weighing approximate-

ly 210-220 gr that were obtained from the Animal Care Unit of Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. The rats were acclimatized for approximately one week before use. The animals were housed in stainless steel cages under controlled environmental conditions with temperature (22±2 °C), relative humidity of 55±5% and lighting (12-h light/12-h dark cycle). All rats were fed with a standard laboratory pelleted chow diet and fresh water *ad libitum*. Animal experiments used in this study were approved by the Animal Ethics Committee of Razi University following the Guidelines for the Care and Use of Laboratory Animals in Research (Animal Ethical Approval Number: 397-2-008).

Experimental procedure

After one week of acclimatization, the rats were randomly assigned into five groups with six animals per group. In group 1, animals were orally and daily injected with normal saline and served as the normal control. In groups 2 to 5, anemia was induced by Intraperioneal (IP) injection of PHZ at 40 mg/kg for 2 consecutive days. Rats that developed anemia with hemoglobin (Hb) concentration lower than 14 g/dl were used for the study. The anemic rats were randomly divided into four groups and treated as follows: In group 2, rats were given normal saline orally and served as the anemic control. Groups 3, 4 and 5, anemic rats daily received the hydroalcoholic extract of C. *scolymus* at doses of 100, 200, 400 mg/kg body weight by oral gavage, respectively. The normal saline and hydroalcoholic extract of *C. scolymus* was administered from days 2 to 15 after PHZ injection. The experimental period was 14 days. In this study, dose of PHZ to induce anemia and *C. scolymus* doses were determined according to previous studies (Diallo *et al*., 2008; Lee *et al*., 2012; Salem *et al*., 2017).

Sample collection

At the end of the study period, all rats were weighed and then anesthetized using diethyl ether. Blood samples were taken from the heart to determine the hematological and antioxidant parameters. For erythrocyte preparation, the erythrocytes were sedimented by centrifuge at 500 g for 10 min at 4 °C. The erythrocytes were washed three times (5 ml, each) with cold isotonic saline then the buffy coat was discarded. RBCs and sera were stored at -20 °C until assayed.

Determination of hematological parameters

For hematological analyses, blood was examined using an automatic hematology analyzer (Celltac, Alpha Vet MEK-6550; Nihon Kohden Co, Tokyo, Japan).

Measurement of total antioxidant capacity (TAC) levels in serum and erythrocytes

Spectrophotometer analysis with the aid of colorimetric assay kit (Naxifer™, Navandsalamat Co., Iran) was used to estimate the concentrations of TAC in RBCs and serum by the ferric reducing ability (FRAP) method. This procedure is based on the ability of serum or RBC lysis to reduce iron III (Fe^{3+}) to iron II (Fe^{2+}) in the presence of 2,4,6-Tripyridyl-S-triazine (TPTZ). A complex with blue color and maximum absorbance appeared at 593 nm with reaction of Fe^{2+} and TPTZ. The serum level of TAC was expressed in nanomoles per milliliter (nmol/ml) and nmol per gr of hemoglobin (nmol/grHb) for RBC lysis.

Measurement of malondialdehyde (MDA) levels in serum and erythrocytes

The levels of lipid peroxidation in the serum and RBCs lysis were measured as TBARS using a Nalondi™ assay kit (Navandsalamat Co., Iran). Sera were assayed directly using the kit. The RBCs were first Lysed in deionized water containing butylated hydroxytoluene (BHT) provided in the kit,