

# $\beta$ -aescinate Improved the Learning and Memory Abilities and Decreased $\beta$ A4 Deposition through the Anti-Inflammatory Pathway in the Rat Amyloid Model

Tao Zhang<sup>1</sup>, Jianying Zhang<sup>2</sup>, Xiaoping Ma<sup>3</sup>, Yanping Zang<sup>4</sup>, Huanmin Gao<sup>2\*</sup>

**Received date:** 04-Jul-2022, Manuscript No. NPY-22-68416;

**Editor assigned date:** 06-Jul-2022, PreQC No. NPY-22-68416(PQ);

**Reviewed date:** 18-Jul-2022, QC No NPY-22-68416;

**Revised date:** 29-Jul-2022, Manuscript No. NPY-22-68416(R);

**Published date:** 06-Aug-2022, DOI: 10.37532/1758-2008.2022.12(7).637

## ABSTRACT

$\beta$ -aescinate has a wide variety of pharmacological properties, including anti-inflammatory, analgesic, and antipyretic activities. The inflammatory pathways in the brain have been closely related to pathological development of the Alzheimer's Disease (AD). However, the anti-inflammatory mechanism of  $\beta$ -aescinate on AD has not been scrutinized. Thus the present study was designed to investigate whether  $\beta$ -aescinate improve the abilities such as learning and memory through the anti-inflammatory pathway in the rat amyloid model.  $A\beta_{1-40}$  was microdialyzed into the lateral ventricle. Learning and memory were evaluated by Morris Water Maze (MWM) test. Pathological changes in the hippocampus were estimated by H&E stained. The quantity of neuroglia and cytokine were investigated by immunohistochemistry. BSI-B<sub>4</sub>, GFAP,  $\beta$ A<sub>4</sub> and TNF- $\alpha$  positive cells in  $A\beta$  group were higher than that in the sham group ( $P < 0.01$ , respectively), but the latent period was lengthen in  $A\beta$  group compared with sham group significantly ( $P < 0.05$ ); BSI-B<sub>4</sub>, GFAP,  $\beta$ A<sub>4</sub> and TNF- $\alpha$  positive cells were lower than that in  $A\beta + \beta$ -aescinate group significantly ( $P < 0.01$ ), and the latent period reduced in  $A\beta + \beta$ -Aescine group compared with  $A\beta + \text{saline}$  group significantly ( $P < 0.01$ ). These results suggested that  $\beta$ -aescinate decreased the activation of neuroglia, reduced inflammatory cytokines and decreased  $\beta$ A<sub>4</sub> deposition, and improved the abilities of learning and memory through the anti-inflammatory pathway in the rat amyloid model

**Keywords:**  $A\beta_{1-40}$ , microdialysis, inflammation,  $\beta$ -aescinate,  $\beta$ A<sub>4</sub>, GFAP

<sup>1</sup>Department of Neurology, Baoan Central Hospital of Shenzhen, Guangdong, China

<sup>2</sup>Department of Neurology, The Affiliated Hospital of Qingdao Binhai University, Haiya Road, Qingdao, China

<sup>3</sup>Department of Neurology, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, Ningxia Hui Autonomous Region, China

<sup>4</sup>Qingdao Eighth People's Hospital, The Affiliated Hospital of Weifang Medical College, Qingdao, Shandong Province, China

\***Author for Correspondence:** Huanmin Gao, Department of Neurology, The Affiliated Hospital of Qingdao Binhai University, Haiya Road, Qingdao, China. email: gaohuanmin@126.com

## Introduction

Inflammatory mechanisms have been recognized as one of the most important pathways in the genesis and development of Alzheimer's Disease (AD). Pathogenesis like  $\beta$ -Amyloid ( $A\beta$ ) and neurofibrillary tangles have certain antigenicity to the central nervous system, stimulate the body's immune system to produce excessive inflammatory chain reactions in the brain, and eventually produce degeneration injuries in the brain aggravate the pathological progress of AD [1-5].

Aescine (escin) is derived from the seeds of horse chestnut (*Aesculus hippocastanum* L.) with a natural mixture of triterpenesaponins exhibiting a wide variety of pharmacological properties, including anti-inflammatory, analgesic, and antipyretic activities [6]. Clinically Sodium  $\beta$ -Aescine with 7 hydroxy in its molecular structure, is commonly used as dehydrating agent with hormone-like effects, effectively eliminate free radicals produced in the tissues, so as to reduce the lipid over oxidation; It can also regulate the secretion of adrenocorticotrophic hormone and prostaglandin, and play anti-inflammatory effects in multiple levels of central nervous system [7-9]. The anti-inflammatory property of Aescine like adrenocorticotrophic hormone provided clues to us that Aescine might have therapeutic effects in the treatment of AD. However, the relationship between Aescine and AD has not been known. Therefore, the present study was to observe whether the anti-inflammatory effects of Sodium  $\beta$ -Aescine would inhibit the inflammatory lesions and improve the abilities of learning and memory in this AD rat model.

## Methods

### Animal and group

24 male Wistar rats, body weighing 250g-300g, were selected by Morris Water Maze (MWM), purchased from the Chinese Academy of Medical Sciences, Beijing, China). Rats were randomly divided into 4 groups: Sham operation control group,  $A\beta$  group,  $A\beta$ + $\beta$ -Aescine group, and  $A\beta$ +saline group. 6 rats were in each group.

The protocol had been approved by Qingdao Central Hospital, Qingdao Municipal Hospital, Qingdao Eighth People's Hospital, and Qingdao Songqiao Hospital.

### The main reagent

$A\beta$ 1-40 was purchased from Sigma-Aldrich Corporation (China Branch, Shanghai, China). Di-

luted into  $A\beta$ 1-40 into 1g/L by sterilized Normal Saline before use, and incubated for 1 week, then stirred with care to make it "a aggregate" [10,11]. Plant lectins (BSI-B4), from Sigma-Aldrich Corporation (China Branch, Shanghai, China);  $A\beta$  antibody, TNF $\alpha$  antibodies, from Tianjin HaoyangCompany (Tianjin, China); Glial Fibrillary Acidic Protein (GFAP) antibody, from Beijing Boaosincompany (Boaosin, Beijing, China); Immunohistochemical kit, from Maixin reagent company (Maixin, Beijing, China); Sodium  $\beta$ -Aescine, from Yantai Green Leave Pharmaceutical Co., Ltd (Yantai, Shandong, China).

### AD rat model

This  $A\beta$  lateral ventricle microdialysis rat model has been widely used, and in this study, we selected parameters as A 0.3mm, R1.3mm, H3.6mm based on Giovannelli and colleagues [10], Yang and colleagues [11]. This amyloid model could be useful to investigate AD pathogenic mechanisms and evaluate the drug effects.

$A\beta$ 1-40 was incubated with sterile 0.9% sodium chloride at the concentration of 10 $\mu$ g/10 $\mu$ L for 7 days in 37 $^{\circ}$ C thermostat, and then  $A\beta$  was in the status of aggregation.

The rat was fixed on the stereotaxic instrument (stereotaxic apparatus, SN-3, Narishige, Tokyo, Japan) after anesthetized by 10% Chloral Hydrate (0.3 to 0.35 ml/100g, intraperitoneal injection, ip), skin disinfection, skin incision at the center of the parietal, bone drilled, microdialysis into the right side of the ventricle through the microdialysis pump for consecutive 14 days.

Sham operation control group also microdialysis 0.9% sodium chloride as vehicle.

### Drug intervention group

$A\beta$ + $\beta$ -Aescine group: Sodium  $\beta$ -Aescine solution intraperitoneal injection (5mg/kg, diluted with 0.9% Sodium Chloride to 2ml), at 8 a.m., daily, for 14 consecutive days.

$A\beta$ + saline group: As the control group of  $A\beta$ + $\beta$ -Aescine, 2 ml 0.9% Sodium Chloride intraperitoneal injection at 8 a.m., daily, for 14 consecutive days.

### Water maze test

The pool of the labyrinth was divided into four quadrants. All preoperative rats were trained swimming in the water maze 3 times daily, for 4 consecutive days. Every time training rats would head toward the wall according to the different order randomly into three quadrants to swim, until they found the platform. Recorded the time of the rat found the platform (known as latent period), let the rat stranded on the platform for 10s in order to achieve improved memory effect; if the rat

did not arrive at the platform within 180s, the latent period would be recorded as 180s. After 4 days 24 rats were chosen based on arriving at the platform within 10s three consecutive times, grouped according to random number table. 14 days after surgery the memory retrieval abilities were tested 3 times, and the average was calculated.

#### Histopathological study

The rat was perfused with 100 ml 0.9% Normal Saline, then 100ml 4% paraformaldehyde via the left ventricle with the descending aorta clipped. The brain was carefully removed and suspended in 4% buffered paraformaldehyde+20% sucrose for two days at 4°C, then transferred into 30% sucrose perfusion fixative for two days at 4°C. The brain gradient desiccation was made. Paraffin brain section was produced after dehydration. Serial sections were 5 microns thick, then:

- HE staining
- Immunohistochemical study

Immunohistochemical staining was used to detect microglia labeled by BSI-B4 and astrocytes labeled by GFAP,  $\beta$ A4 and TNF $\alpha$ . Three slices were chosen from each rat brain sections, and observed under optical microscope. 4 visual fields ( $\times$ 400) were randomly selected from each section of the left side of the hippocampus CA1 region. The numbers of positive cells per visual field were counted; the means and standard deviation of four visual fields were calculated.

#### Statistical analysis

Data were summarized using standard descriptive statistics: frequency and percentage for categorical variables; and mean, Standard Deviation (SD) for continuous variables. All calculated p-values were two-sided and p-values less than 0.05 were considered statistically significant. Statistical analyses were performed using the SAS version 9.2 software package (SAS Institute, Inc., Cary, NC).

## Results

### Comparison of learning and memory ability

Comparison of learning and memory ability of the rat in water maze performance showed no difference between preoperative groups; Postoperative test were shown in Table 1.

\*P<0.01, A $\beta$ group compared with sham control; or with A $\beta$ + $\beta$ - aescine group.

\*P<0.01, A $\beta$ + $\beta$ - aescine group compared with A $\beta$ +saline group.

\*P>0.05, A $\beta$ group vs. A $\beta$ +saline group.

The latent period was lengthen in A $\beta$  group compared with A $\beta$ +saline group, but the difference had no statistical significance (P>0.05); The latent period was longer in A $\beta$  group compared with Sham control statistically significant (P<0.05); This result showed that A $\beta$  microdialysis into the ventricle damaged the learning and memory abilities as AD in rats.

The latent period reduced in A $\beta$ + $\beta$ -Aescine group compared with A $\beta$ +saline group significantly (P<0.01).

This result implied that Sodium  $\beta$ -Aescine improved the learning and memory abilities in this AD rat model.

### The inflammatory reaction in hippocampus CA1 area and effect of $\beta$ -aescine

A large number of glial cell proliferation, aggregation, glial cells volume increased, and deepen dyeing were observed at the left hippocampus CA1 area in A $\beta$  group under the optical microscope. Cone cells stripes were scattered, and cell shrinkage, nucleus pycnosis, dyeing to deepen in the CA1 area. The glial cells lose, sparse distribution, shallow and small cell dyeing was observed in the Sham control group. This result showed that A $\beta$  microdialysis yield more brain injuries in this region (Figure 1).

The glial cell hyperplasia reactivity and the neuron lose were reduced significantly in A $\beta$ + $\beta$ -Aescine group compared with A $\beta$  group (Table 2). As shown in Figure 1-B, compared with Figure 1-A (A $\beta$  model), A $\beta$ + $\beta$ -Aescine decreased the IL-1 $\beta$  expression, showed that  $\beta$ -Aescine treatment decreased IL-1 $\beta$  expression.

This result showed that Sodium  $\beta$ -Aescine reduced the glial cell hyperplasia reactivity and the neuron lose in this rat AD model.

### Changes of glial cells and cytokines in left hippocampus CA1 in this AD rat model

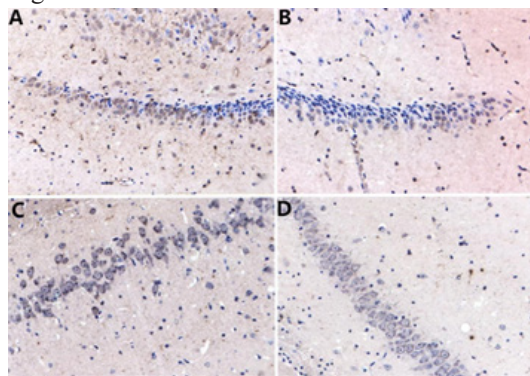
Under the optical microscopy, BSI-B4 labeled glial, GFAP labeled astrocytes,  $\beta$ A4 and TNF $\alpha$  expressed

Table 1: Comparison of learning and memory between groups of AD rats by water maze test ( $x \pm s, s$ ).

Group	n	Latent period
Sham	6	28.9 $\pm$ 5.0
A $\beta$	6	56.2 $\pm$ 7.7*
A $\beta$ + $\beta$ -Aescine	6	29.1 $\pm$ 9.2**
A $\beta$ +saline	6	53.4 $\pm$ 9.4

positively with the cytoplasm full of tan color, number of positive cells was shown in Table 2. As shown in Figure 1-C,D, Positiveness cells increased in the left hippocampus Aβ group (Figure 1-C), while Aβ+β-Aescine decreased the TNFα expression (Figure 1-D), showed that β-Aescine treatment decreased TNFα expression in the left hippocampus Aβ model.

Figure



**Figure 1:** β-Aescine treatment decreased the inflammatory expressions in the Aβ rat model  
A:Aβ model showed increased IL-1β expression;

B: Aβ+β-Aescine decreased the IL-1β expression, showed that β-Aescine treatment decreased IL-1β expression.

C: TNFα positive cells increased in the left hippocampus Aβ group.

D: Aβ+β-Aescine decreased the TNFα expression, showed that β-Aescine treatment decreased TNFα expression in the left hippocampus Aβ model.

Table 2

\*P<0.01, Aβgroup vs. sham;

△P<0.05, Aβgroup vs.β+β- aescine group;

※P<0.01, Aβgroup vs. 与Aβ+β-Aescine.

Hippocampal CA1 area glial cells, βA4, and TNFα positive cells were significantly increased in Aβ group compared with Sham operation control.

This result showed inflammatory reaction was active in the Aβ group. Hippocampal CA1 area glial cells, βA4, and TNFα positive cells were significantly decreased in Aβ+β-Aescine group compared with Aβ group. This result showed Sodium β-Aescine inhibited the inflammatory reaction in the AD rat model.

Hippocampus CA1 area glial cells, βA4, and

Table 2: Effect of β-Aescine on hippocampal microglia, radial glial cells and IL-1β, TNFα in AD rat model (x ± s).					
Group	n	Number of positive cells			
		BSI-B4	GFAP	IL-1β	TNFα
Sham	6	14.8 ± 3.4*	19.6 ± 4.2*	22.1 ± 4.5*	31.1 ± 5.3*
Aβ	6	30.3 ± 4.1	59.4 ± 5.8	49.7 ± 5.7	60.3 ± 11.4
Aβ+β- aescine	6	20.8 ± 6.5*	33.8 ± 6.5*	25.5 ± 3.9*	33.8 ± 6.8*
Aβ+saline	6	26.4 ± 4.9△*	52.5 ± 6.0※	41.7 ± 8.9※	56.3 ± 11.1※※

TNFα positive cells were significantly decreased in Aβ+β-Aescine group compared with Aβ+saline group.

This result showed Sodium β-Aescine inhibited the inflammatory reaction compared with the normal saline as the vehicles control in the AD rat model.

These results suggested that Aβ activated the glial cells and astrocytes proliferation in hippocampus and launched its immune inflammatory response; Sodium β-Aescine had certain inhibitory effect on the hyperplasia of activation of glial cells and astrocytes in the hippocampus and decreased the expression of βA4, TNFα, blocking the inflammatory interaction, therefore improved the learning and memory in this rats AD model [12, 13].

### Discussion

Along with the aging accelerating, much attention has been paid on the problems of the cognitive impairment. In recent years the mechanisms of

Alzheimer’s disease have been focused on the abnormal deposition Aβ in the brain, that may be a motivating factor in the inflammatory response of the AD genesis and development, continuous activation of inflammatory stimulations will change the normally acute reaction into chronic inflammatory lesions and interfere with the homeostasis of the central nervous system [1-5, 14]. Literature showed that Aβ deposition in the senile plaques in the inflammatory activation of microglia caused inflammation, which is an important pathological mechanism of AD [15]. The activation of microglia and astrocytes and subsequent inflammation is very important in the AD pathological damage, has become one of research hotspots in recent years [16]. So, the present study also based on inflammatory mechanisms observed the anti-inflammatory effects of Sodium β-Aescine on learning and memory ability in this AD rats.

The structure of β-Aescine is ((2S,3S,4S,5R,6R)-6-(((3S,4S,4aR,6aR,

6bS,8R,8aR,9R,10R,12aS,14aR,14bR)-9-acetoxy-8-hydroxy-4,8a-bis (hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-(2-methyl-1-oxobut-2-enoxy)-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl)oxy}-4-hydroxy-3,5-bis(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-tetrahydropyranyl)oxy)}-2-tetrahydropyrancarboxylic acid. The unique structure with seven hydroxyls would capture the free radicals effectively.

This model has been widely recognized internationally, and in this study we based on Giovannelli and colleagues [10], Yang and colleagues [11]. This model mimic the natural aging progress of mankind, including cognitive dysfunction, immune function decline, and degenerative changes in the brain neurons.

This study showed that the latent period was significantly prolonged, and the memory retrieval reduced in A $\beta$  group, while after Sodium  $\beta$ -Aescine treatment the latent period was shortened obviously. These results suggested that Sodium  $\beta$ -Aescine improved the learning and memory abilities of the AD rats.

This study also showed that Sodium  $\beta$ -Aescine inhibited the hyperplasia of activation of glial and astrocytes in the hippocampus by immunohistochemical staining, and inhibited the expressions of  $\beta$ A4, TNF $\alpha$ , thus to some extent, blocked the inflammatory injury of the brain in AD rats, so as to improve the learning and memory ability of AD rats.

Aescine appears to produce effects through a wide range of mechanisms at different levels. It induces endothelial nitric oxide synthesis by making endothelial cells more permeable to calcium ions, and also induces release of prostaglandin F $2\alpha$  [17]. Other possible mechanisms include serotonin antagonism and histamine antagonism and reduced catabolism of tissue mucopolysaccharides [18].

This study also suggested that Sodium  $\beta$ -Aescine inhibited the hyperplasia of glial cell induced by A $\beta$  and suppressed the expression of inflammatory cytokines, reduced the brain damage, thus improved learning and memory ability of AD rats. The possible mechanism was the anti-inflammatory role of Sodium  $\beta$ -Aescine through the following four ways: (1) Adrenocorticotrophic hormone-like effects: Aescine can increase the concentration of plasma adrenocorticotrophic hormone and corticosterone, those pharmacological effects are similar with dexamethasone, but hormone kind effects of Sodium  $\beta$ -Aescine is much weaker compared with dexa-

methasone, therefore Sodium  $\beta$ -Aescine can avoid the disadvantage of adrenocorticotrophic hormone.

(2) Regulate the secretion of prostaglandin: Sodium  $\beta$ -Aescine can promote PGF $2\alpha$  secretion, increase PGF $2\alpha$ /PGE1 ratio, have the effect of inhibition of PGE1, therefore, can reduce inflammatory exudation of the tissue.

(3) Antioxygen free radical activity: Sodium  $\beta$ -Aescine with 7 hydroxy in molecular structure can effectively capture free radicals produced in the body, reduce lipid peroxidation. In addition, it can effectively reduce the activation of nuclear factor  $\kappa$ B, block a series of chain reaction induced by  $\kappa$ B, and reduce the expression of cytokines.

(4) Reduced  $\beta$ A4 deposition in the brain tissue. Aggregation/misfolding of  $\alpha$ -synuclein and  $\beta$ A4 proteins cause neuronal cell death (NCD) associated with Parkinson's and Alzheimer's disease [19]. This study showed that Sodium  $\beta$ -Aescine decreased the expression of  $\beta$ A4, blocked the inflammatory interaction, and therefore improved the learning and memory in this rat's amyloid model.

The limitations of this study was short term observations, as we know, AD has a long time span in degeneration, so the effects of Aescine on AD needed further long term study.

In summary, this study contributed to the research on Sodium  $\beta$ -Aescine improved the ability of learning and memory by inhibited glial cell proliferation, activation and cytokine production, reduced inflammatory lesions and reduced  $\beta$ A4 deposition in the brain tissue, in the rat amyloid model.

#### Author Contributions

Conceptualization: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao. Data curation: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao. Formal analysis: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao. Investigation: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao. Methodology: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao. Project administration: HuanminGao. Resources: HuanminGao. Writing-original draft: HuanminGao. Writing-review & editing: Tao Zhang, Jianying Zhang, Xiaoping Ma, YanpingZang, HuanminGao.

#### Acknowledgements

We thank Deng Pan and Cuixia Bi for technique assistance.

References

1. Miners S, Moulding H, De Silva R, et al. Reduced vascular endothelial growth factor and capillary density in the occipital cortex in dementia with lewy bodies. *Brain. Pathol* 24(4), 334-443 (2014).
2. Barker R, Ashby EL, Wellington D, et al. Pathophysiology of white matter perfusion in Alzheimer's disease and vascular dementia. *Brain* 137(5), 1524-1532 (2014).
3. Laurén J. Cellular prion protein as a therapeutic target in Alzheimer's disease. *J. Alzheimers. Dis* 38(2), 227-244 (2014).
4. Bradley-Whitman MA, Timmons MD, Beckett TL, et al. Nucleic acid oxidation: An early feature of Alzheimer's disease. *J. Neurochem* 128(2), 294-304 (2014).
5. Ferreira ST, Clarke JR, Bomfim TR, et al. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. *Alzheimers. Dement* 10(1), S76-S83 (2014).
6. Franiczek R, Gleńsk M, Krzyżanowska B, et al. β-Aescin at subinhibitory concentration (sub-MIC) enhances susceptibility of *Candida glabrata* clinical isolates to nystatin. *Med. Mycol* 53(8), 845-851 (2015).
7. Wang K, Jiang Y, Wang W, et al. Escin activates AKT-Nrf2 signaling to protect retinal pigment epithelium cells from oxidative stress. *Biochem. Biophys. Res. Commun* 468(4), 541-547 (2015).
8. Çiftçi GA, Işcan A, Kutlu M. Escin reduces cell proliferation and induces apoptosis on glioma and lung adenocarcinoma cell lines. *Cytotechnology* 67(5), 893-904 (2015).
9. Vašková J, Fejerčáková A, Mojžišová G, et al. Antioxidant potential of Aesculushippocastanum extract and escin against reactive oxygen and nitrogen species. *Eur. Rev. Med. Pharmacol. Sci* 19(5), 879-886 (2015).
10. Giovannelli L, Casamenti F, Scali C, et al. Differential effects of amyloid peptides beta-(1-40) and beta-(25-35) injections into the rat nucleus basalis. *Neuroscience* 66(4), 781-792 (1995).
11. Yang HY, Qiao HX, Tian X. Proteomic analysis of Alzheimer's disease rats serum. *Int. J. Biomed. Eng* 33(3), 138-141 (2010).
12. Huang GL, Shen DY, Cai CF, et al. β-escin reverses multidrug resistance through inhibition of the GSK3β/β-catenin pathway in cholangiocarcinoma. *World. J. Gastroenterol* 21(4), 1148-1157 (2015).
13. Hollerbach EH, Haas CA, Hildebrandt H, et al. Region-specific activation of microglial cells in the rat septal complex following fimbria-fornix transection. *Comp. Neurol* 390(4), 481-496 (1998).
14. Cotman CW, Tenner AJ, Cummings BJ. Beta-Amyloid converts an acute phase injury response to chronic injury responses. *Neurobiol. Aging* 17(5), 723-731 (1996).
15. Sheng JG, Griffin WS, Royston MC, et al. Distribution of interleukin-1 immunoreactive microglia in cerebral cortical layers: Implications for neuritic plaque formation in Alzheimer's disease. *Neuropathol. Appl. Neurobiol* 24(4), 278-283 (1998).
16. Griffin WS, Sheng JG, Roberts GW, et al. Interleukin-1 expression in different plaque in Alzheimer's disease; significance in plaque evolution. *Neuropathol. Exp. Neurol* 54(2), 276-281 (1995).
17. Sirtori CR. Aescin: Pharmacology, pharmacokinetics and therapeutic profile. *Pharmacol. Res* 44(3), 183-193 (2001).
18. Carrasco OF, Vidrio H. Endothelium protectant and contractile effects of the antivaricose principle escin in rat aorta. *Vascul. Pharmacol* 47(1), 68-73 (2007). [Crossref],
19. McCanney GA, McGrath MA, Otto TD, et al. Low sulfated heparins target multiple proteins for central nervous system repair. *Glia* 67(4), 668-687 (2019).