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Silk Protein, Sericin Effect on Morphometric and Behavioural Aspects in Alzheimers Disease Induced Rat Model

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ABSTRACT

In the present study we investigate the cognitive effects of Silk protein, Sericin on Morphometric and behavioural memory impairments in Alzheimer's disease induced Rat. The silk cocoons contain two type of proteins Sericin and Fibroin. Sericin has been removed during silk manufacturing process, it is worthwhile the removed Sericin recovered. In the present study eight weeks old rats were exposure to D-Galactose to induce Alzheimer's and they were treated with Sericin to observe the changes in Morphometric aspects such as body weights and to evaluate the learning and memory efficiencies through Morris Water Maze test. From the results, D-Gal administrated rats showed gradual decrease in body weights (90th day - 112.60 grams). When compare with control, Sericin treated rats showing recovery tendency in water maze experiment and reached to normal level at end of the experiment. Finally concluded that, Sericin could effectively reverse the D-Gal induced changes in rat.

KEYWORDS: *Alzheimer's Disease, Rat Model, Sericin, Morris Water Maze, Behavioral aspects.*

INTRODUCTION

Alzheimer's disease is a most common neurodegenerative diseases, clinically manifested by progressive loss of specific population of nerve cells in the brain, spinal cord or less frequently, peripheral nerves or muscles, irreversible deterioration of essential cell and tissue components of the nervous system. AD is most common in older people aged above 60years.According to WHO report AD will grow to nearly 34 million by 2025 and more than 106 million by 2050, and much of this increases will be in the developing countries. Hence there is an urgent need to tackle this life threatening and economically costly disease at the earlist. Two distinct histological changes in the nerve cells of Alzheirmer's brain are the formation of senile plaques, made up of aggregated amyloid β-protein (Aβ) fibrils and Neurofibrillary tangles (NFTs) comprised of hyperphosphorylated collections of the microtubule-associated tau protein which lead to neurotoxicity. The main Causes of Alzheimer's are both genetic and non-genetic, include Age, family history, Heriditary factors, Down's syndrome, Whiplash or Head injuries, Aluminium, Poor education, Consumption of high fat, high calorific diet, previous serious trauma to the head, Smoking, Cardiovascular disorders, Hyper cholesterolemia, Diabetes mellitus, Menopause and sedentary life style. The most accepted mode of therapy for Alzheimer's Disease is to create the most potent reversible cholinesterase inhibitors to restore the acetylcholine levels in AD patient. On the other hand, treatment of AD with single drug is probably not a realistic option due to the complicated nature of disease. Therefore it is worthwhile to identify the new and selective AChE inhibitors from natural source. In this context, a combination therapy of AChE inhibitor along with antioxidants is felt necessary to treat AD effectively [1, 2].

The silkworm, Bombyx *morii* is an important economic insect that are emerging as an ideal molecular genetics resource for solving a broad range of biological problems.The silkworm, B.*morii* produces massive amount of silk proteins during the final stage of larval development. Silk cocoons contains 20 -30% of Sericin and remain Fibroin. Sericin having the value added properties like gelling ability, moisture retention capacity, skin adhesion and a wide variety of applications in medical, pharmaceutical and cosmetic industries. Sericin is resistant to several proteases, there are 18 kinds of amino acids present in Sericin, among these, serine and aspartate have the highest content. The amino acid, methionine present in sericin play an important role in collagen synthesis and induces proliferation of mammalian cells. The silk proteins, sericin and fibroin were proven as potential compound for wound healing drug, post-surgical trauma, anti-oxidative, bio-adhesive and bio-active prospective. Recently, silkworm is being used as bio-factory for production of useful protein through its silk gland and thus silk became a valuable biomaterial for diverse pragmatic applications.

Though out the world, more than 20 countries are producing the silk, the major producers from Asia and several sericulture industries are established in Brazil, Bulgaria, Egypt and Madagascar. About one million workers are employed in silk sector in China and it provides income for 7,00,000 households in India and 20,000 weaving families in Thailand. Though, China produces about 70% of the world's silk, rest was produced by India, Brazil and Thailand. The Sericin has been removed from cocoons during the silk manufacturing process to make silk lustrous and the removed Sericin goes as waste material. It is estimated that out of about 1 million tons of cocoons produced worldwide, approximately 4,00,000 tons of dry cocoons are generated, that have 50,000 tons of recoverable Sericin. Indian production of 1,600 tons of silk can be the source of about 250 to 300 tons of Sericin per year [3]. If this Sericin protein is recovered and recycled, it would be a significant economic and social benefit to produce many value-added compounds from Seri-waste products. It is possible to develop a multipurpose Sericin protein for wide applications.

In view of this, the present study was taken under to assess the potential bioactivity of Natural Silk Protein, Sericin was used as memory enhancer in Alzheimer's induced rat. Until now, several chemical compounds such as amyloid beta, aluminium-maltolate, D-Galactose and Sodium nitrite have been used to induce AD in animal model. But in the present study, D-Galactose used to induce AD rat. Long-term D-Galactose injected rats successfully exhibit a higher degree of cognitive and memory impairments which

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mimic Alzheimer disease [4-8]. Further we are also investigating neuroprotecting, antioxidant properties of Sericin in our laboratory.

MATERIALS AND METHODS

Maintenance of animals:

Eight weeks old Male albino Rat (*Rattus norvegicus)* weighing 140 ± 20 grams, obtained from Sri Venkateswara Enterprises, Bangalore was used as the experimental model. The rats were maintained at laboratory conditions in the animal house at 25± 2°C with a photoperiod of 12hrs light and 12hrs darkness throughout the course of the present study. The rats were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water *ad libitum*. As per the ethical guidelines for animal protection and welfare bearing the Resolution No. **04/(i)/a/CPCSEA/ IAEC/ SVU/ KY- KPR / Dt. 28-03-2011.** The total animals were divided as per the experimental protocol. The dose response studies were conducted for 60 days after completion of treatment time.

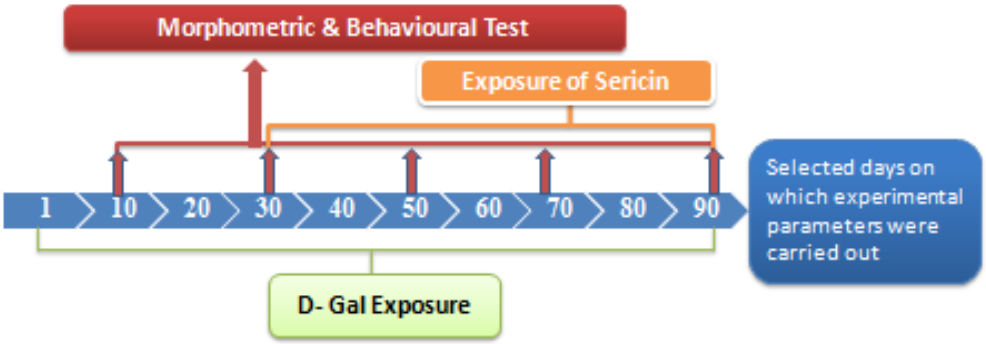


Fig. 1: Schematic representation of Experimental design

Grouping of Animals:

Rats were acclimatized to the laboratory conditions for 10 days before the experimentation, they were randomly divided in to four groups of six each and was housed in separate cages. These different groups of rats were administered with the D- Gal and Sericin as given below. All doses were given once in the morning hours between 8 to 9 A.M. keeping in view the altered activity of rat during the nights compared to day time.

Group I **Control**

Group II Rat, Intraperitoneally(IP) administered with **D-Galactose** (120 mg/kg body weight) continuously once in a day upto the end of the experiment. **(AD)**

Group III Rat, orally administered with Sericin (200 mg/kg body weight) up to 60 days continuously once in a day up to the end of the experiment. **(SP-S)**

Group IV Rat, Intraperitoneally injected with **D-Galactose** (120 mg/kg body weight) once daily and simultaneously administered (oral) with Sericin (200 mg/kg body weight) continuously once in a day up to the end of the experiment. **(AD+SP-S)**

Extraction of Sericin from Cocoon of Silk Worm:

Raw Silk cocoons were purchased from Chittoor local market. After through check for any sticking impurities, the cocoons were placed in the boiling water for about 1hour and the resulting solution was cooled and filtered. The filtrate was concentrated by using rota evaporator and then ethanol (95%) was added to precipitate Sericin. Then Sericin was collected by filtration, dried at 40°C, powdered and finally preserved in clean container for further use.

Administration of Sericin:

1 ml of Silk Protein, Sericin extract (200 mg/kg body weight) dissolved in distilled water and given to rat, through gavage tube to deliver the substances by oral route, which is clinically valid route of administration.

Morphometric Aspects:

The basic Morphometric aspects such as size and total body weight of control and experimental groups were recorded using a digital balance at selected time intervals that is 10th, 30th, 50th, 70th and 90th day. The data thus obtained was analyzed and used to correlate with the behavioural aspects.

Behavioural Aspects:

Behavioral experiments were performed on selected time intervals by using the water maze [9] **(Morris, 1984)** which was originally designed to test the learning and memory ability in rodents. The apparatus consisted of a circular tank, 100 cm in diameter and 50 cm in depth. The tank was filled with water (21-26°C) up to a height of 30cm and the transparent escape platform made of plexiglass, 10cm in diameter and 29 cm in height was hidden 1.5 cm below the surface of water in a fixed location. Water was made opaque with powdered non-fat milk. The platform was not visible from just above the water level and transfer trials have indicated that escape on to the platform was not achieved by visual or other proximal cues [10] **(Morris, 1981).** The time spent by the animal to reach the hidden platform was used as the index of memory.

Statistical Analysis:

Values of the measured in different parameters were expressed as Mean ± SEM, Standard deviation and Analysis of variance (ANOVA) with Dunnett's post-hoc test for multiple comparisons using standard statistical software, SPSS (version 16 for windows) software. The results were presented with the F-value and p-value. In all cases F-value was found to be significant with p-value less than 0.01. This indicates that the effects of factors are statistically significant.

RESULTS

Morphometric Aspects: (Table 1 & Fig. 1)

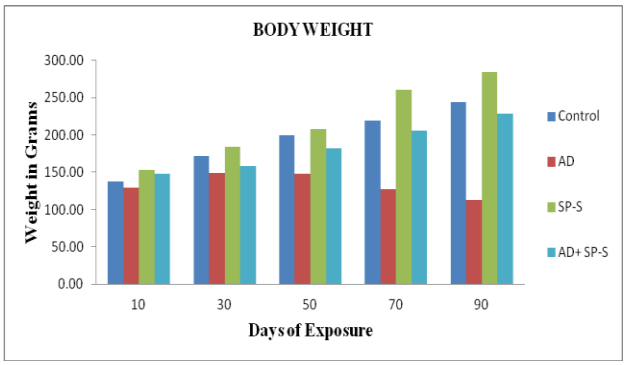
The total body weights of control and experimental groups of rats were recorded using a digital balance at selected time intervals. The results revealed that all groups of rats showed a gradual increase in their body weights from 10th day to 90th day. When results were compared to the control ones, Group-2 (AD induced) rats showed increase in body weight upto 30 day (171.83 grams) and after observed gradual decrease in body weights and getting weaker day by day (90th day - 112.60 grams) throughout the period of experiment they mimics like aged animals. Group-3 & Group-4 rats gained body weights throughout the period of experiment when compare with Group - 4, Group- 3 rats gained increased body weights. Group- 4 (AD induced rats treated with SP-S) showing recovery tendency from 30th day and gained body weight (90th day- 228 grams), it indicates that SP-S could effectively revert the AD induced changes gradually and reached normal level at end of the treatment.

Table No. 1: Differences in the body weights (Grams) of Control and Experimental groups of rats treated with D-Galactose, SP-S, D-Gal+ SP-S at selected time intervals.

Group	Days				
	10th day	30th day	50th day	70th day	90th day
Control	138.00±4.25	171.83±6.74	199.83±3.20	219.33±4.46	243.83±4.09
AD	129.60±1.84 (6.09)	148.60±2.17 (13.52)	147.40±4.89 (26.24)	127.00±1.37 (42.10)	112.60±3.31 (53.82)
SP-S	153.50±2.70 (-11.23)	184.00±3.44 (-7.08)	207.83±2.95 (-4.00)	260.33±6.21 (-18.69)	284.50±5.40 (-16.68)
AD+ SP-S	148.17±4.29 (-7.37)	158.50±1.89 (7.76)	181.83±5.52 (9.01)	205.83±5.49 (6.16)	228.00±5.42 (6.49)

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats; Values in parentheses are percent change from control; Values are significantly different from control at $p < 0.01$

Fig. 1: Graphical representation of differences in the body weights of Control and Experimental groups of rats treated with D-Galactose, SP-S, & D-Gal + SP-S at selected time intervals.



Behavioural Aspects (Morris water maze test): (Table 2 & Fig. 2)

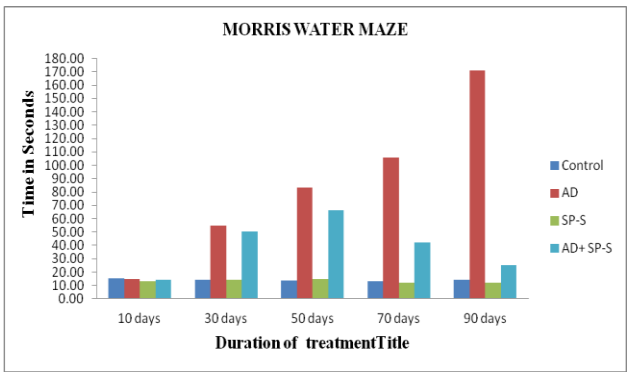
In the present study, the Morris water maze task was performed to assess the spatial learning and memory ability in rat. The results indicated that, compare to control ones, SP-S treated rats showed equal escape latency (time taken to reach the hidden platform). In case of Group-2 (AD induced) rats escape latency was gradually increase from 30th day (54 Sec) to 90th day (171 Sec) and Sometimes they spent more time to reach the hidden platform this indicates that loss of learning and memory impairments in rat. Whereas in Group-4 (AD+SP-S treated) escape latency time was decreased and the rats took less time to reach the hidden platform (70th day - 42 seconds to 90th day - 25 seconds). It indicates that oral administration of SP-S significantly reversed the platform finding impairments in AD induced rat.

Table No. 2: Morris Water Maze test results (in seconds) of learning and memory in Control and Experimental groups of rats treated with D-Galactose, SP-S & D-Gal + SP-S at selected time intervals.

Groups	Days				
	10th days	30th days	50th days	70th days	90th days
Control	15.40±0.83	14.30±0.53	13.43±0.18	13.18±0.22	14.05±0.46
AD	14.70±0.40 (4.55)	54.55±1.18 (-281.47)	83.57±3.54 (-670.97)	105.67±19.00 (-701.52)	171.00±4.40 (-1117.08)
SP-S	13.30±0.22 (13.64)	14.15±0.38 (1.05)	14.98±0.71 (-11.54)	12.20±0.46 (7.46)	12.25±0.31 (12.81)
AD+ SP-S	14.21±0.11 (7.71)	50.50±2.48 (-253.15)	66.42±2.06 (-394.42)	42.00±0.41 (-218.58)	25.27±1.42 (-79.86)

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats; Values in parentheses are percent change from control; Values are significantly different from control at $p < 0.01$

Fig. 2: Graphical representation of Morris Water Maze test results of Control and Experimental groups of rats treated with D-Galactose, SP-S, & D-Gal + SP-S at selected time intervals.



DISCUSSION

The results of the present study demonstrated that administration of Silk Protein, Sericin reduces the loss in body weight and simultaneously enhanced the learning and memory skills in AD induced rat. Morphometric data is an important sources of information to understand many biological phenomena such as Phylogenetic relationships, evolution, reconstruction of history and structure of past populations, sexual dimorphism, fluctuating asymmetry, ecomorphology, bodycondition, growth, heritability [11-19] etc., Morphometrics refers to the quantitative analysis of form, a concept that encompasses size and shape which are commonly performed on organisms and are useful in analyzing their fossil record, the impact of mutants on shape, developmental changes in form, covariances between ecological factors and shape, as well as estimating quantitative-genetic parameters of shape [20].

Learning or acquisition, a highly specialized function of the brain, is a process of acquiring knowledge about the environment around the organism, while memory is the storage or retention of this learnt knowledge which can be retrieved later. In the process of learning, activation of neurons occurs in specific areas of the brain concerned with the processing of the specific modality of sensory information [21]. Physiologically, memories are caused by changes in the capacity of synapses to transmit activity from one neuron to another in a neural circuit as a result of previous neural activity. These changes in turn establish new pathways which, called memory traces which are important because once established, they can be activated by thinking process to reproduce memories whenever required. The intellectual ability of an individual is dependent on memories to which one is adding constantly. The hippocampus and amygdale are concerned with the storage of recent memory and emotional behavior. The structural organization of these areas has been reported to be highly plastic, particularly in hippocampus [22-23]. In rodents, spatial learning and memory are closely related to the function of the dorsal hippocampus, to which cholinergic neurotransmission contributes significantly [24]. Although especially prominent in AD, cholinergic deficits in the cortex and hippocampus occur during normal human ageing [25] and

smaller numbers of neurons and atropy of surviving cholinergic neurons in the basal forebrain were shown in aged animals with impaired learning and memory.

Brain aging is a risk factor of neurodegenerative diseases such as Alzheimer's disease. In the present study, it has been observed that the impaired cognitive functions induced by D-Galactose were restored to almost normal by administering Sericin. It has been reported that long-term injection of D-Galactose inhibited antioxidant enzyme activity leading to decline of immune response, neurodegeneration and behavioural impairment [26-30]. Since these changes are similar to characters of normal aging process. Smilarly, It has been well established that water maze performance abilities decline with aging and thus it is a very sensitive method for assessing the impairment of spatial learning and memory. In this present study, the impaired spatial learning and memory abilities caused by D-Galactose were reverted back to normally by simultaneous administration of AD induced rat which further proved that long treatment of serecin effectively improve the impaired learning and memory performance in diseased rats.

Memory is the natural counterpart of learning, it is necessary condition for the behavioral change to be permanent[31]. Silk Protein, Sericin (SP-S) is a natural protein and having the wide range application in Pharmaceutical and food industry [32-33]. The hydrolysis of silk proteins gives different size of peptides and these peptides exerting diverse bioactivities including anti-diabetic, hypocholesterolemic and antioxidative actions [34-39]. Silk amino acids (SAA) enhanced physical stamina by preventing the tissues from oxidative injures [40-41]. Recently it was reported that, silkworm extract inhibited Monoamine Oxidase-B (MAO-B) a doamine–degrading enzyme [42-43], Silk amino acids improved Parkinson disease (PD) via., dopaminergic neuroprotection [44]. Brain factor-7 (BF-7), a peptide obtained by enzymatic degradation of silk proteins, was found to increase cognitive function in both animal and human [45-46]. Our present research observations on Silk Protein, Sericin provide strong evidence that, natural silk protein, Sericin can prevent the learning and memory deficits effectively, thus paving a way for discovery of novel anti-Alzheimer's drug in future.

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