Research Article

Effect of Squalene in Morphometric and Cognitive Aspects in D-Galactose induced Alzheimers Disease in Male Albino Rats

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ABSTRACT

Alzheimers disease is a type of dementia, even though extensive research work has been carried out since decades till now, there is no specific therapeutic drug available for the permanent cure of the Alzheimer's Disease. Now a days the marine bioactive compound squalene plays a therapeutic role morphometric and cognitive aspects in D-Galactose induced alzheimers disease in male albino rats. The morphometric aspects which refer to the quantitative analysis of a concept that encompasses size, shape and other physical parameters is useful for assessing the impact of mutants on shape, their fossil records, developmental changes in the form between ecological factors and as well as evaluating the quantitative genetic aspects of shape. To evaluate the spatial or place learning in experimental rat, the most popularly known water maze test is Morris Water Maze (MWM) experiment. The most important utilization of the Morris Water Maze includes that its high reliability across a wide range of tank configurations and testing procedures, its cross-species utility in several chosen experimental animals such as mice and rats including humans in a virtual maze. The marine bioactive compound Squalene plays a major impact on brain function and behaviour by enhancing the learning and memory in the AD- Model rats.

Keywords: Alzheimers Disease (AD), Marine bioactive compound Squalene, Morris Water Maze (MWM) and D-Galactose.

INTRODUCTION

Alzheimers disease (AD) is the most common single cause of dementia in our ageing society. AD is estimated to account for between 50 and 60% of dementia cases in persons over 65 years of age [1-3] and is progressive, neurodegenerative disease that primarily affects the elderly population. The symptoms associated with AD involve decline in cognitive dysfunction, primarily memory loss [4,5] and in the later stages of the disease language deficits, depression, agitation, mood disturbances and psychosis are often seen [6].

It is a multifactorial illness, mainly related to oxidative stress leading to the inequity between production and detoxification of Reactive Oxygen Species (ROS), a chief issue for developments of neurodegenerative diseases [7]. Oceans account for 71% of the earth's surface and are the largest remaining reservoirs of bioactive compounds [8]. Consequently, through the past decade, the Neuro protective properties of diverse compounds from Squalene have been investigated in neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) [9].

Morphometric Aspects

Morphometric[10] studies play a significant role in understanding many biological functions. Further, the major advantages of morphological measurements have wide applications in creating phylogenetic relationships [11], sexual dimorphism [12], evolution [13], body condition [14], Eco morphology [15], heritability [16] and growth [17]. Thus, the major objective of the marphometrics is to statistically test hypothesis about the factors that affect shape. In the wider sense, it is also used to locate precisely certain areas of featureless organs such as brain and is used in describing the shape of other organs.

The traditional morphometric information is nevertheless useful when either absolute or relative sizes are of particular interest such as in studies of growth. Besides this, it is also useful for measurement of size of theoretical importance such as body weight and limb cross-sectional area and length in studies of functional morphology. However, all these measurements have one important limitation i.e. they contain little information about the spatial distribution of shape changes across the organism. Several studies conducted by researchers highlighted the importance of these studies: For example the study conducted by [18] to determine variability in body morphometric measurements in goats of Uganda, concluded that live body weight could be predicted with accuracy from linear body measurements especially in height at wither, heart girth, corpus length and ramp height. The body, carcass weights and chest circle would be determined more efficiently by measuring the width of long bones in Morkara man sheep [19-20]. It is also been

reported that there isa positive correlation between body weight and Linear Body Measurements in Chinchilla rabbits implying that a particular Linear Body Measurements or a combination of it can be used to determine the body weight.

Cognitive Aspects

The practice of Morris Water Maze in assessing learning and memory has been reviewed [21-22] as the relationship between performance in the Morris Water Maze and both neurotramitter systems and drug effects [23]. The Morris Water Maze concert has been linked to Long- Term Potentiation and NMDA receptor function [24], making it a key technique in the investigation of hippocampal circuitry.

Hence, the locomotory activity is considered as an index of alertness and a decrease in the activity would indicate the sedative state of the animal. The locomotor activity is one of the most widely used behavioral paradigms to determine the effects of various experimental manipulations ranging from genetic changes, knockout mice to pharmacological challenges. Thisextensive range of applications is based on the fact that unconditioned motor activity probes a variety of behaviours that can be recorded automatically and quickly generate and effect profile [25].The multivariate characterizations of rat locomotory activity, including measures of crossings, time in the centre vs. the periphery, distance travelled, rearing and sometimes whole pokes, having many advantages over univariate assessments are limited to measures of theamount of activity [26]. However, studies in rats also clearly confirmed the additional utility of assessments of sequential patterns of locomotor activity in pharmacological and neurobiological studies. The maximum drugs acting on the Central Nervous System in general and the depressant drugs such as barbiturates and alcohol influence the locomotor activities by reducing the motor activity, though the stimulants such as caffeine and amphetamines increase the activity in man and animals.

The laboratory rats have proved as valuable animal models in psychological studies such as learning and other mental processes. The ancient significance of this species to scientific research is reflected by the amount of literature on it, unevenly 50% more than that on other animals [27]. It has been well established that the water maze performance declines with ageing of animals and it is a very sensitive method for evaluating the impairment of spatial learning and memory. The water maze studies help to reveal general principles about learning that can be useful to many species, including humans. Water mazes are also used to determine whether different treatments or conditions affect memory and learning in rats. The rats having the maze running capability comes from their inheritance through evolution, as rats are small burrowing rodents that have spent millennia in digging and finding their way in and around underground tunnels. The maze apparatus are useful for assessment of memory with little

effort. The T-maze tests have been used to assess the spatial learning behaviour of animals. Some previous studies have confirmed that spatial and non-spatial cognition are two important aspects of cognitive function [28]. The locomotor abnormalities are associated with several human diseases like Parkinson's, Huntington's and Hyperactivity syndrome. The marine bioactive compound like shark liver oil, Squalene plays a major impact on brain function and behaviour by mainly controlling the pre-squalene and post-squalene pathway in the brain lipid molecules.

MATERIALS AND METHODS

Healthy male albino rats (48) of wistar strain were divided into four groups viz., Group-I: control rats received saline, Group-II: rats received intraperitoneal injection of D-Galactose (120 mg/kg body weight) throughout experiment, Group-III: initially animals were subjected to D-Galactose injection for six weeks, then followed by simultaneously received both D-Galactose and Squalene (150 mg/kg body weight) for 90 days and Group-IV: rats which were orally administered only with Squalene for 90 days. During the experimentation, both morphometric and behavioural aspects were studied in selected brain regions of all experimental groups of rats on selected days of experimentation (60th and 90th days).

Statistical analysis

All the experiments were carried out in triplicates and values of the measured parameters were expressed as Mean ± SEM. Two way ANOVA was used to test the significance of difference among nine different groups followed by Dunnet's Multiple Range Test (DMRT). Statistical analysis was performed by using Statistical Program of Social Sciences (SPSS) for windows (Version 16; SPSS Inc., Chicago, 1L, USA). The data was regarded as significantly different at $P<0.05$.

RESULTS

Morphological features

The morphological topographies such as Body weight, general appearance, and organ indexwere recorded in all groups of rats during the total experimentation period. From these results, it was observed that when compared to Control group rats, AD model rats showed gradual loss of hair, elasticity of the skin which later became stiff, thin and saggy. Besides these, significant andgradual reduction in the body weight by 18.4% on 60th day and 26.73% on 90th day was also recorded with reference to the control group. The control group, and Squalene alone treated groups have exhibited a similar trend. On the contrary, the protective group of rats (ADI+SQT), which were treated with Squalene for 60 and 90 days exhibited increase in their body weights when compared to Alzheimer`s Diseased model group (318.21 ±0.88 vs. 261.84 ± 18.44 and 331.12 ± 3.13 vs. 235.22 ± 26.73) respectively. In parallel to these parameters, the organ index of selected organs such as Brain, Liver, Kidney and Spleen, also showed

significant weakening in the AD-model group when compared to that of squalene treated group. However, interestingly, the organ index in protective group exhibited better gain in organ weight than that of AD- model group.

Cognitive behaviour

The modulatory effects of squalene on spatial memory (wherein the escape latency was taken as an index of impairment of cognitive function) were studied by Morris water maze experiment. The results showed that,

Squalene alone treated rats have showed better escape latency on selected days of experiment, when compared to control rats. On the contrary, the rats treated with D-Galactose alone showed a significant enhancement in the escape latency time on 60th day (70.97%) and 90th day (133.48%). Interestingly, the protective group of rats (ADI+SQT), showed better recovery tendency with reduced latency on 60th day (8.89%) and 90th day (-10.85%) and it was almost very close to that of the control group rats.

Table 1: Changes in Organ index for selected organs from different groups of rats on 60th and 90th days of Experimentation.

Name of organ	Brain		Kidney		Liver		Spleen	
Selected days	60th	90th	60th	90th	60th	90th	60th	90th
Group								
Control	1.90 ±0.4	2.03 ±0.10	2.01 ±0.22	2.05 ±0.11	9.41 ±0.63	10.06 ±1.25	1.32 ±0.17	1.27 ±0.27
SQ-T	1.83 ±0.05	1.92 ±0.19	4.07 ±0.31	5.35 ±0.75	8.66 ±0.68	9.02 ±0.97	1.45 ±0.27	1.24 ±0.25
AD-I	1.78 ±0.16	1.84 ±0.18	2.98 ±0.80	4.52 ±0.25	8.26 ±0.82	9.57 ±3.09	0.92 ±0.19	1.28 ±0.31
Protective Group (ADI+SQT)	2.05 ±0.25	1.95 ±0.30	1.90 ±0.18	2.46 ±0.37	8.67 ±0.69	10.32 ±1.69	1.46 ±0.27	1.69 ±0.60

(Note: The above values are expressed in grams)

Table 2: Differences in total body weights (Grams) of Control and Experimental groups of rats on selected days of experimentation.

Group	60th day	90th day
Control	321.04±28.90	340.31± 68.24
SQ-T	292.23±16.71 (-8.97)	332.12±64.00 (3.45)
AD-I	261.84±21.53 (-18.44)	235.22±16.78 (-26.73)
ADI+SQT	318.21±60.32 (-0.88)	331.12±58.17 (3.13)

Values are Mean ± SEM of six observations pooled from 6 rats.; Values in parentheses are percent changes from control (Except *); *Values in parentheses are percent changes from AD-induced rats;

Values are significantly different from control at $p < 0.05$

Table 3: ANOVA

Body weight		Sum of squares	Df	Mean Square	F	Sig.
On 60th day	Between Group	13679.054	3	4559.685	11.569	0.000
	Within group	7882.859	20	394.143		
	Total	21561.914	23			
On 90th day	Between Group	44678.966	3	14892.989	34.870	0.000
	Within group	8542.118	20	427.106		
	Total	53221.084	23			

Graph-1: Effect of squalene on total body weight of control and experimental groups of rats on selected days of experimentation. Values are mean ± SEM (n=6). *p<0.01 vs. Control; #p<0.05 vs. AD model group.
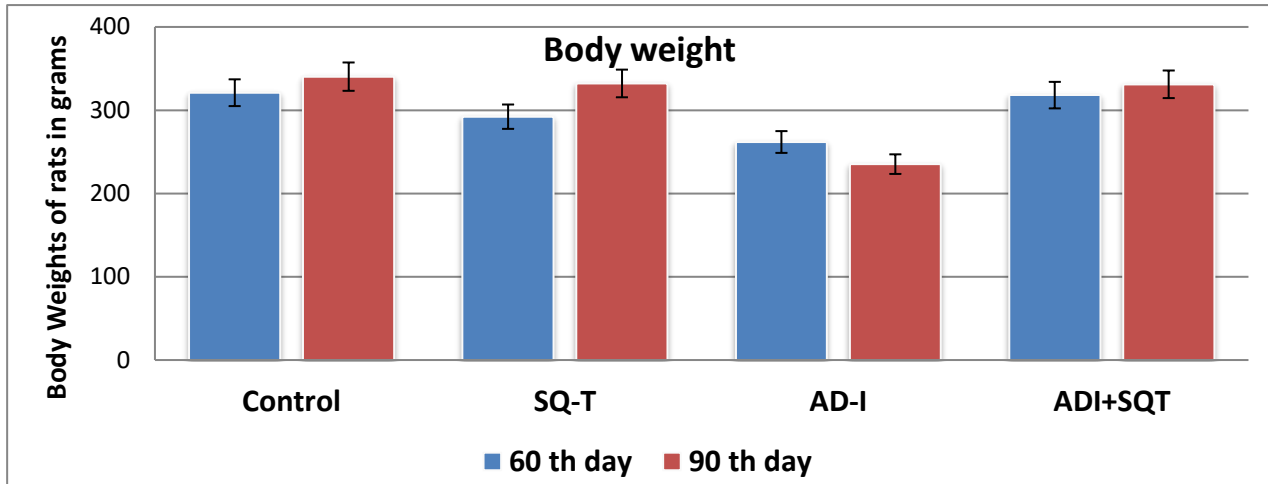
Table 4: Alterations in the Cognitive performance of Control and Experimental group of rats on selected days of experimentation.

| Group	60th day	90th day
Control	13.16±1.34	12.99±0.57
SQ-T	12.33±0.74 (-6.30)	10.58±0.60 (-18.55)
AD-I	22.5±3.09 (70.97)	30.33±1.88 (133.48)
ADI + SQT	14.33±0.47 (8.89)	11.58±0.73 (-10.85)

Values are Mean ± SEM of six observations pooled from 6 rats.; Values in parentheses are percent changes from control (Except *); *Values in parentheses are percent changes from AD- rats;

Values are significantly different from control at p < 0.05

Table 5: ANOVA

Morris Maze Test		Sum of squares	Df	Mean Square	F	Sig.
On 60th day	Between Group	394.833	3	131.611	36.058	0.000
	Within group	73.000	20	3.650		
	Total	467.833	23			
On 90th day	Between Group	1565.833	3	521.944	347.963	0.000
	Within group	30.000	20	1.500		
	Total	1595.833	23			

Graph 2: Effect of squalene on cognitive performance in different groups of experimental rats against the Control on selected days. Values are mean ±SEM (n=6). *p<0.05vs. Control; #p<0.05 vs. AD model group.
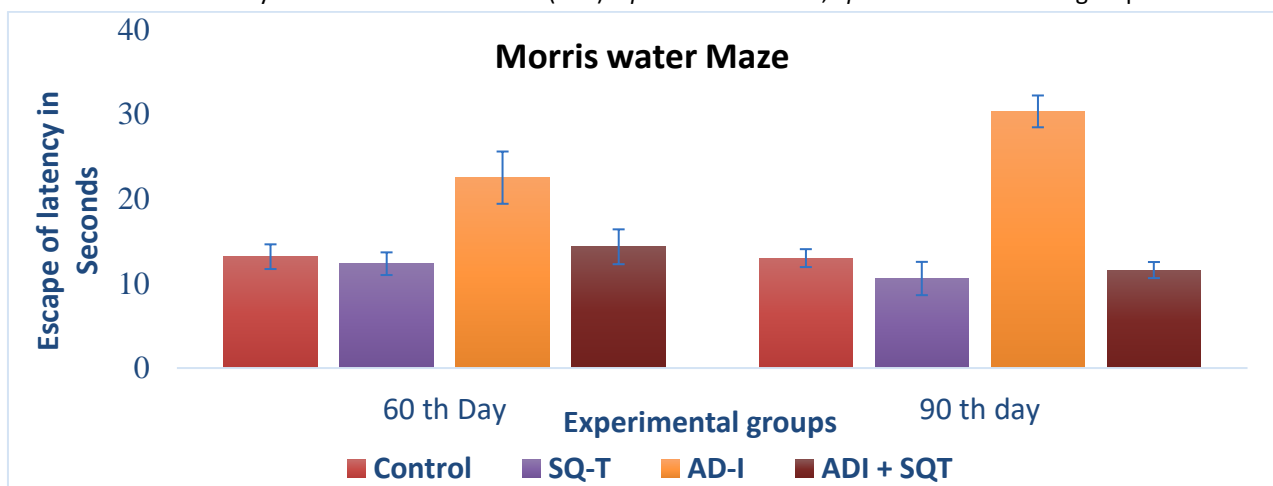
DISCUSSION

The present findings in my study clearly indicated that the marine compound, squalene showed positive modulatory effects against the experimentally induced changes due to Alzheimer`s Disease which are reflected in behavioraland morphometric aspects. Throughout the period of experimentation, the AD-model rats show a significant decline in their organ index, cognitive skills, total body weight, loss of hair and elasticity of the skin. Besides these, Alzheimer`s Disease-induced rats showed deficiency in memory and learning. However, oral administration of Squalene significantly reversed not only the morphological parameters but also the memory impairment caused by AD-induction, indicating that Squalene acted as Anti-Alzheimer compound.

Squalene is an intermediate compound in the cholesterol biosynthesis having antioxidant and membrane stabilizing property. The marine bioactive compounds play important roles in development of functional foods and drugs based on their Antitumor, Antimicrobial, Antioxidant and other properties. The Pharmacological studies with marine compounds involve areas of neuropharmacology, such as those of stimulation of neurogenesis, modulation of receptors and voltage-dependent ion channels and enzymes inhibition [29], that correct several neurological disorders. Therefore, several research studies have emerged which could explain that the potential mechanism of action and the direct stimulation of cytokine and chemokine production by granulocytes and macrophages [30]. In rat model, oral administration of squalene was absorbed by lymphatic vessels like cholesterol with only 20% becoming sterols during transmit through the small intestine. Its consumption also reduce cholesterol, LDL cholesterol and triglyceride levels after 20 weeks with Squalene treatment. Other *in vivo* studies, have demonstrated that administration of a dose of 2% of squalene for 45 days could significantly reduced the levels of triglycerides, cholesterol and fatty acids in plasma and heart tissues thus confirming the cardio protective effects of Squalene has a nonpolar nature that allows to function as a purifier of xenobiotic substances in the human body.

The observation that squalene has reversed the AD-induced effects with reference to the locomotor behaviour can be justified with a supporting mechanism explaining the role of Calcium and its associated enzyme viz. Ca^{2+} ATPase. The transport of Ca^{2+} across the Sarcoplasmic reticulum membrane by Sarco-Endoplasmic Reticulum Ca^{2+} ATPases (SERCA) can be controlled by the regulatory protein, phospholamban (PLB). In normal condition the SERCA is inhibited by PLB and is closely associated with it. The increased β-adrenergic stimulation reduces the association between SERCA and PLB by Protein kinase. When Phospholamban is associated with Sarco-Endoplasmic Reticulum Ca^{2+} ATPases, it leads to reduction of Ca^{2+} movement whereas dissociation of phospholamban with SERCA, results in increased Ca^{2+} movement. The sarcoplasmic reticulum has a much higher concentration of Ca^{2+} inside when compared to the cytoplasmic Ca^{2+} concentration. In view of the above results, it is understood further that the effect of squalene can be comparable with several compounds of plant origin which lend strong support to our findings.To cite a few: Chronic injection of D-Galactose induced memory impairment, oxidative damage in mice followed by neurodegeneration [31], Chronic administration of D-Galactose caused significant decline in reduced gross behavioral activity and spatial memory suggesting impairment of memory [32], It was demonstrated that ethanol extract of *Bacopa monniera* has effectively reversed the D-Galactose-induced memory impairment and learning.

The comparative studies conducted in the present study on the above aspects on 60th and 90th day treated rats, it was obvious that chronic administration of Squalene for 60 days and 90th day showed significant improvement and recovery from Alzheimer`s Disease effects which was dependent on the duration of Alzheimer`s Disease induction and also treatment of AD-induced rats with marine bioactive compound Squalene. As the treatment days of rats with Squalene increased, the amount of memory gaining was also improved. Our research finding further proved that oral administration of squalene extended positive effect on the memory aspects ofrat. The squalene has the ability to produce neurochemicals that are exactly analogues in structure those produced by the host nervous system and act as vehicles for neurotransmitters and influence the mood and behaviour [33].

From these results, it is observed that when compared to Control group rats, AD model ratsshowed gradual loss of hair, elasticity of the skin which later became stiff, thin and saggy. Besides these, significant and gradual reduction in the body weight on 60th day and 90th day was also recorded with reference to the control group. The Squalene alone treated rats showed significant effect on increased body weights on selected days of experiment when compare to control group. On the contrary, the protective group rats (ADI+SQT), which were treated with squalene for 60 and 90 days exhibited improved body weights when compared to Alzheimer`s Diseased model group.

In parallel to these parameters, the organ index of selected organs such as Brain, Liver, Kidney and Spleen, also showed significant weakening in the AD-model group when compared to thatof squalene alone treated group. The modulatory effects of squalene on spatial memory (wherein the escape latency was taken as an index of impairment of cognitive function) were studied by Morris water maze experiment. The results shows that, Squalene alone treated rats have showed better escape latency on selected days of experiment, when compared to control rats. On the contrary, the rats treated with alone D-Galactose showed a significant enhancement in the escape latency time on 60th day and 90th day. Interestingly, the protective group of rats (ADI+SQT), showed better recovery tendency with reduced latency on 60th day and 90th day treated rats and it was almost enhanced than that of the control group rats.

Based on these findings along with the previous research support, it can be suggested that the marine bioactive compound squalene selected for present study, play an important role in treatment of one of the cognitive disorders viz. Alzheimer`s Disease. From my observations as detailed above, it was concluded that Squalene showed **positive effects** on total body weight, Learning and Memory (Cognitive skills) in AD-induced rats.

CONCLUSION

My observations on protective group revealed significant differences between the control and AD-model rats in Morris water maze test, suggesting that oral administration of squalene might ameliorate memory impairment and behavioral changes in AD model rats. Our research results derive strong support from earlier reports where significant improvement in memory loss in mouse caused by scopolamine and D- Galactose. From the results obtained in the present investigation, it was obvious that the squalene showed positive effects on morphological and behavioral aspects wherein D-Galactose injection impaired the learning and memory performance, while though oral administration of squalene significantly reversed the memory impairments and behavioral activity in AD induced rats.

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