CHAPTER IV
RESULTS

1. Neuropharmacological Profiles of Quercetin With Oral Conventional Delivery System and Nasal Administration of Quercetin Liposomes

In order to determine the neuromapharmacological profiles both of quercertin and quercetin liposomes had been evaluated using the behavioral tests. The tests fulfill this requirement were elevated plus maze test, forced swimming test, spontaneous motor behaviors, object recognition and Morris water maze test.

1.1 Anxiolytic activity

The elevated plus maze test is the most widely used model for the anxiolytic activity assessment of novel substances. Administration of diazepam (DZP, 2 mg/kg BW) significantly ($p < 0.05$) increased the number of opened arms entries and the time spent in opened arms at all treatment duration used in this study, confirming an anxiolytic effect. Animals receiving quercetin (100 mg/kg BW) produced significant ($p < 0.05$) reverse the diminution in these parameters after single and repetitive administration. Single administration of quercetin (200 and 300 mg/kg BW) did not produce behavioral changes on both mentioned parameters. However, repetitive administration of them started to produce significant increases in number of opened arms entries and the time spent in opened arms ($p <.05$). Unfortunately, this study failed to show dose and exposure time dependent studies (Figure 14).

Based on the previous information about the ability to decrease the first pass effect and vesicle carrier system such as liposomes and the advantage of the nasal delivery to overcome the blood brain barrier, the anxiolytic effect of quercetin liposomes via nasal administration on the number of opened arms entries and time spent in the opened arms in elevated plus maze test was also determined.

Quercetin liposomes administered via nasal route produced significant ($p < 0.05$) attenuate the diminution in both number of opened arms entries and time spent in the opened arms both after single and repetitive administration while both free liposomes and vehicle (PEG) failed to show the significant changes on both parameters mentioned above throughout the 28 days of treatment (Figure 15).

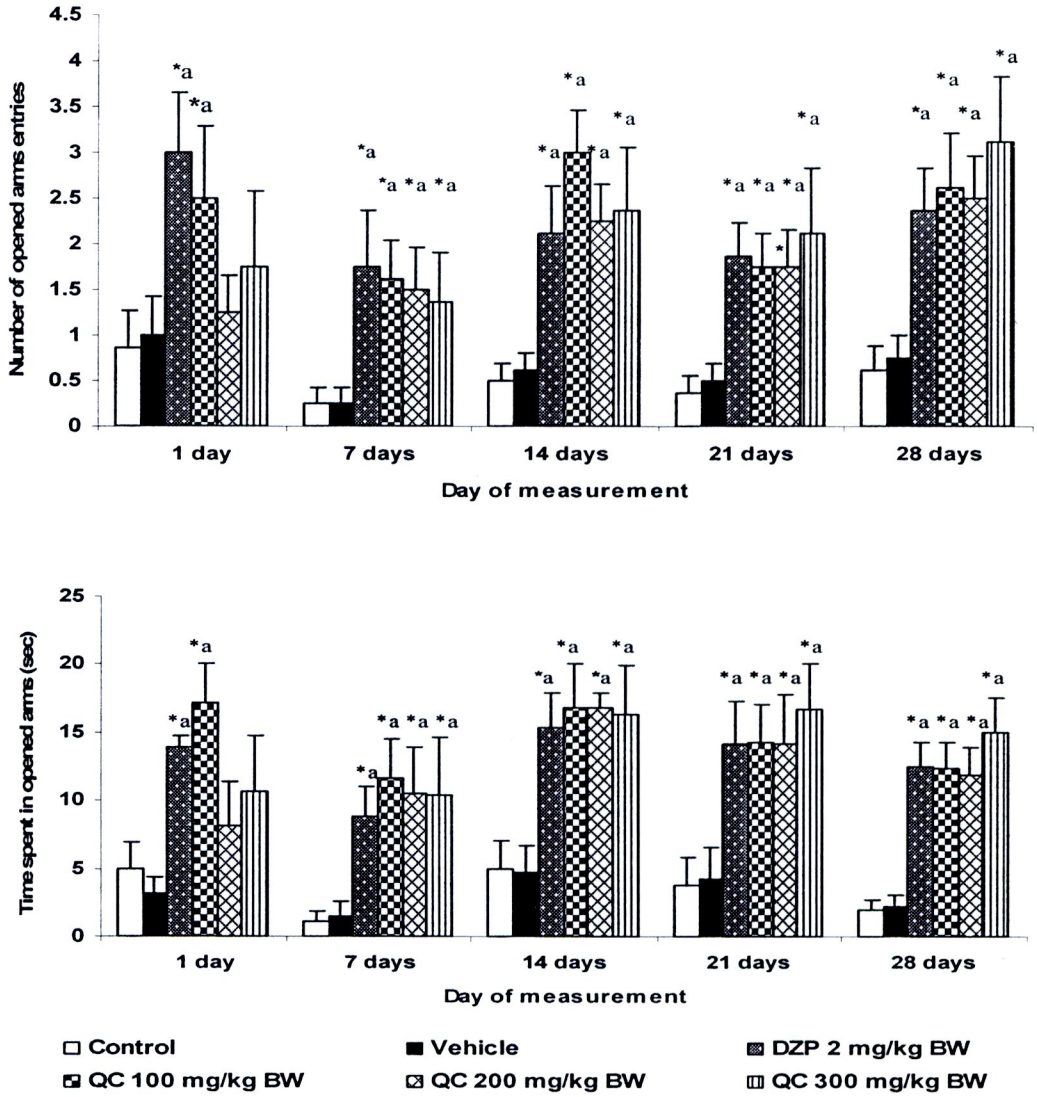

Figure 14 Effect of quercetin via oral administration on anxiolytic activity. Rats were treated with vehicle or diazepam (2 mg/kg BW) or quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the number of opened arms entries and times spent in opened arms in elevated plus maze test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control and $^{a}p < 0.05$ vs. vehicle.

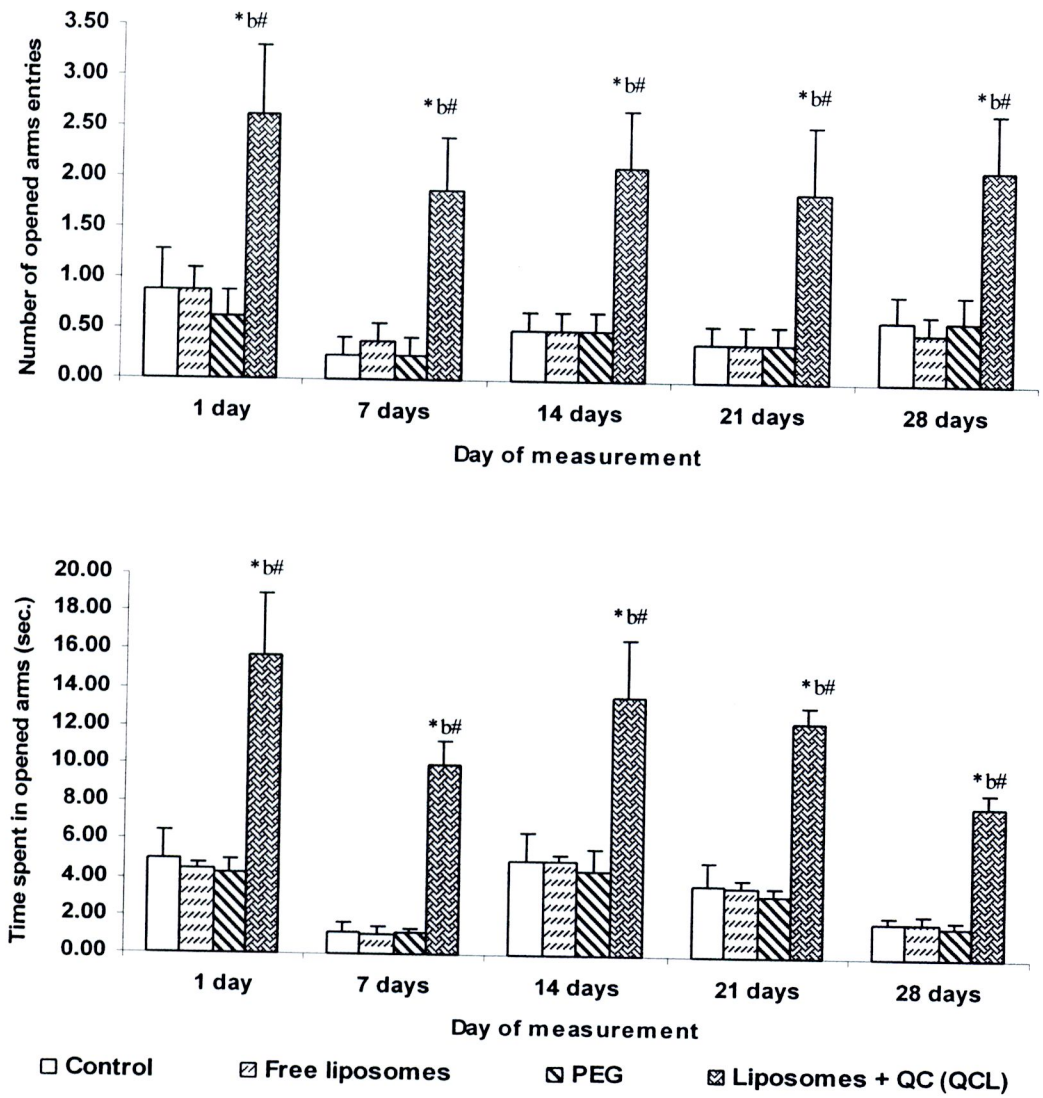

Figure 15 Effect of quercetin liposomes via nasal administration on anxiolytic activity. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the number of opened arms entries and times spent in opened arms of elevated plus maze test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and

$^{\#}p < 0.05$ vs. free liposomes.

1.2 Anti-depression activity

In order to investigate the anti-depressant effect of quercetin via oral administration. It was found that the quercetin at all dosage range used in this study decreased the immobility time in the forced swimming test throughout the experimental period, as compared to the control and vehicle treated groups ($p < 0.05$). Fluoxetine (20 mg/kg BW), used as a positive control, also produced a significant reduction in the immobility time in this test ($p < 0.05$). Again, no dose and exposure time dependent effect were observed in this study (Figure 16).

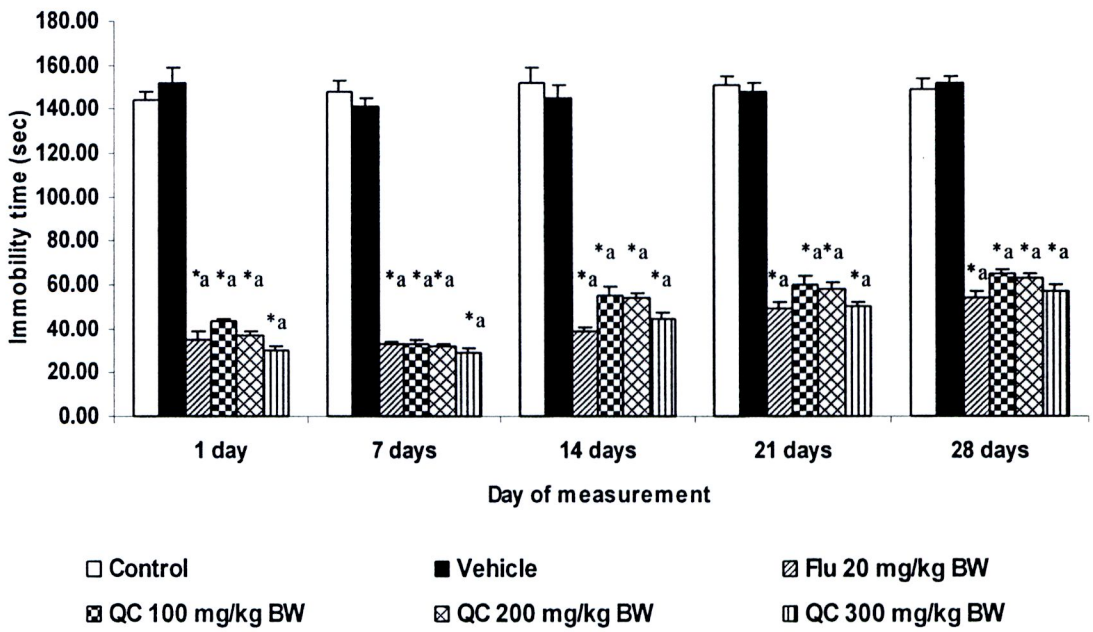

Figure 16 Effect of quercetin via oral administration on anti-depression activity. Rats were treated with vehicle or fluoxetine (20 mg/kg BW) or quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the immobility times in forced swimming test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control and $^{a}p < 0.05$ vs. vehicle.

Figure 17 showed that after single and repetitive administration of quercetin encapsulated liposomes via nasal route significantly decreased the immobility time in the forced swimming test as compared to control, free liposomes and vehicle (PEG) group ($p < 0.05$) whereas, vehicle (PEG) and free liposomes did not produce significantly alter this parameter.

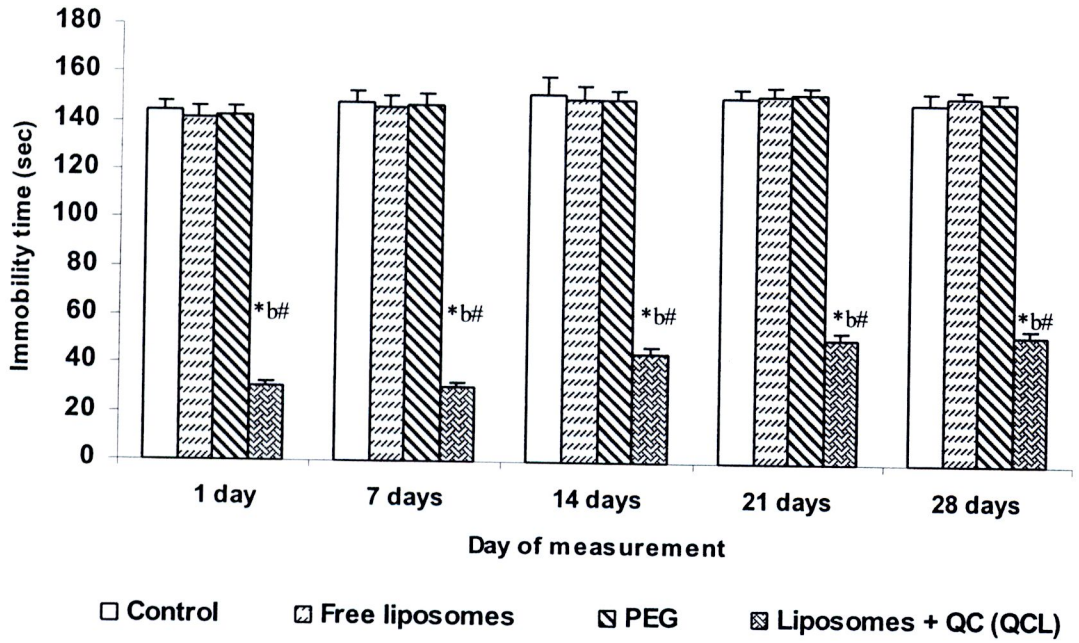

Figure 17 Effect of quercetin liposomes via nasal administration on anti-depression activity. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the immobility time in forced swimming test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and
$^{\#}p < 0.05$ vs. free liposomes.

1.3 Cognitive enhancing effect

Previous studies had demonstrated that different types of memory involved different pathways. Thus, both the spatial and non-spatial memory were assessed using Morris water maze and object recognition test respectively. In this study, donepezil, a cholinesterase inhibitor, had been used as positive control. Moreover, recent evidence had demonstrated that numerous anti-oxidants could improve memory, therefore, vitamin C, a well known antioxidant had been used as positive control too.

1.3.1 Effect of quercetin with oral conventional delivery system and the nasal administration of quercetin liposomes on spatial memory

Hippocampus-dependent spatial memory was assessed with the Morris water maze test. The results showed that vehicle treated rats did not differ from their controls either in the escape latency for finding the platform and the retention time. After 30 min of single administration, Donepezil, a cholinesterase inhibitor, increased the retention time ($p < 0.05$) but no significant change of escape latency was observed. Vitamin C, a well known antioxidant failed to show the significant changes on both escape latency and retention time. Quercetin at a dose of 300 mg/kg per day, significantly enhanced the retention time ($p < 0.05$) whereas, no significant changes were observed after the single administration of quercetin at doses of 100 and 200 mg/kg BW. Repetitive administration of donepezil could significantly decrease escape latency time while increased retention time ($p < 0.05$) throughout the study period. Quercetin at all dosage range used in this study showed continuous decreasing escape latency and increase retention time after 7 days of treatment ($p < 0.05$). In addition, this phenomenon was still observed when the treatment duration was increased to 28 days (Figure 18).

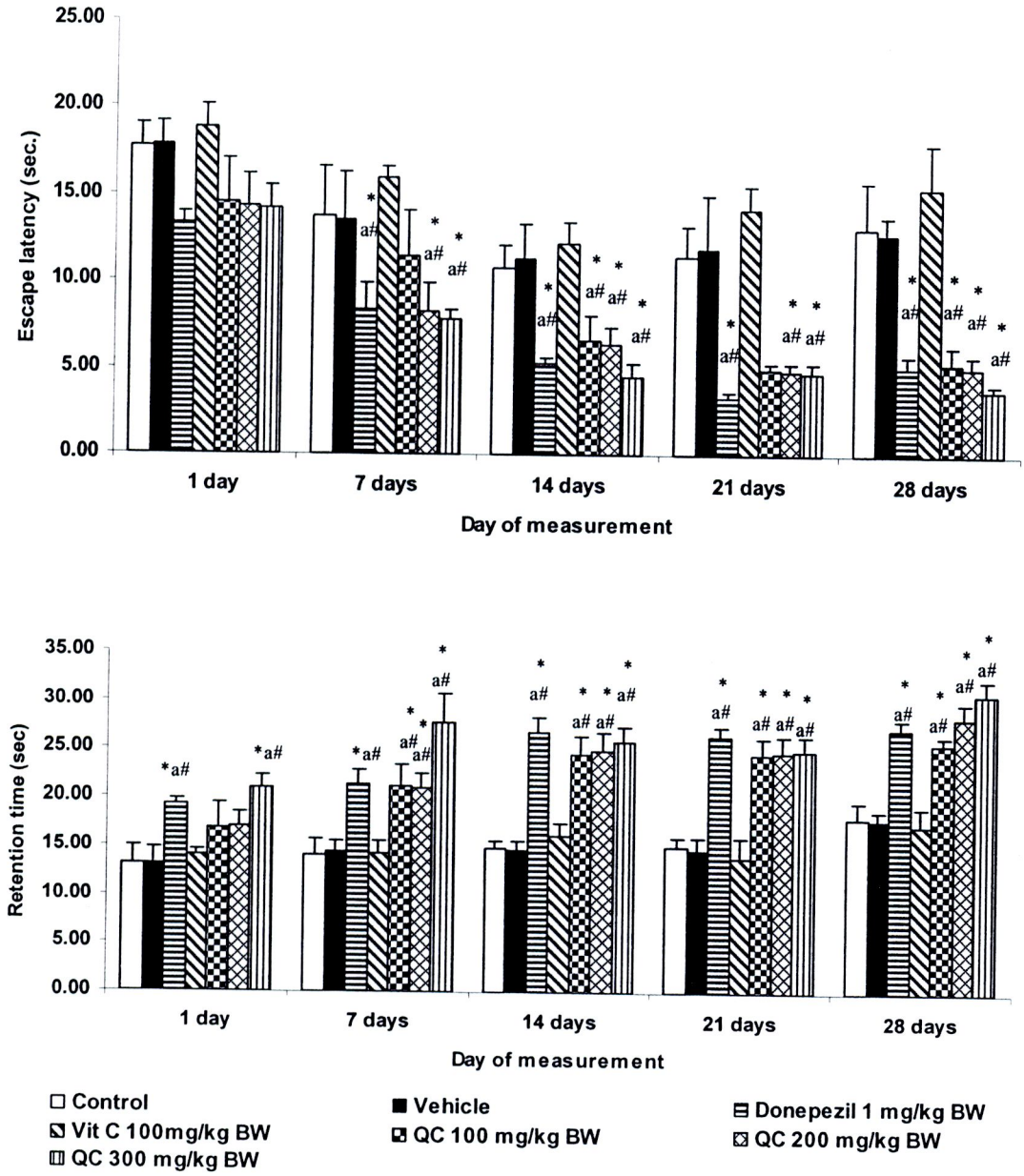

Figure 18 Effect of quercetin via oral administration on spatial memory. Rats were treated with vehicle, Vit C (100 mg/kg BW), donepezil (1 mg/kg BW) or the quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the escape latency and retention time in Morris water maze after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

Figure 19 showed the results of single and repetitive administration of quercetin liposomes on spatial memory. The group treated with quercetin liposomes administered via nasal route showed a significant decreased in escape latency but increased in retention time compared to the control, free liposomes and vehicle (PEG) treated group ($p < 0.05$). No statistically significant difference was observed between control, vehicle (PEG) and free liposomes group rats.

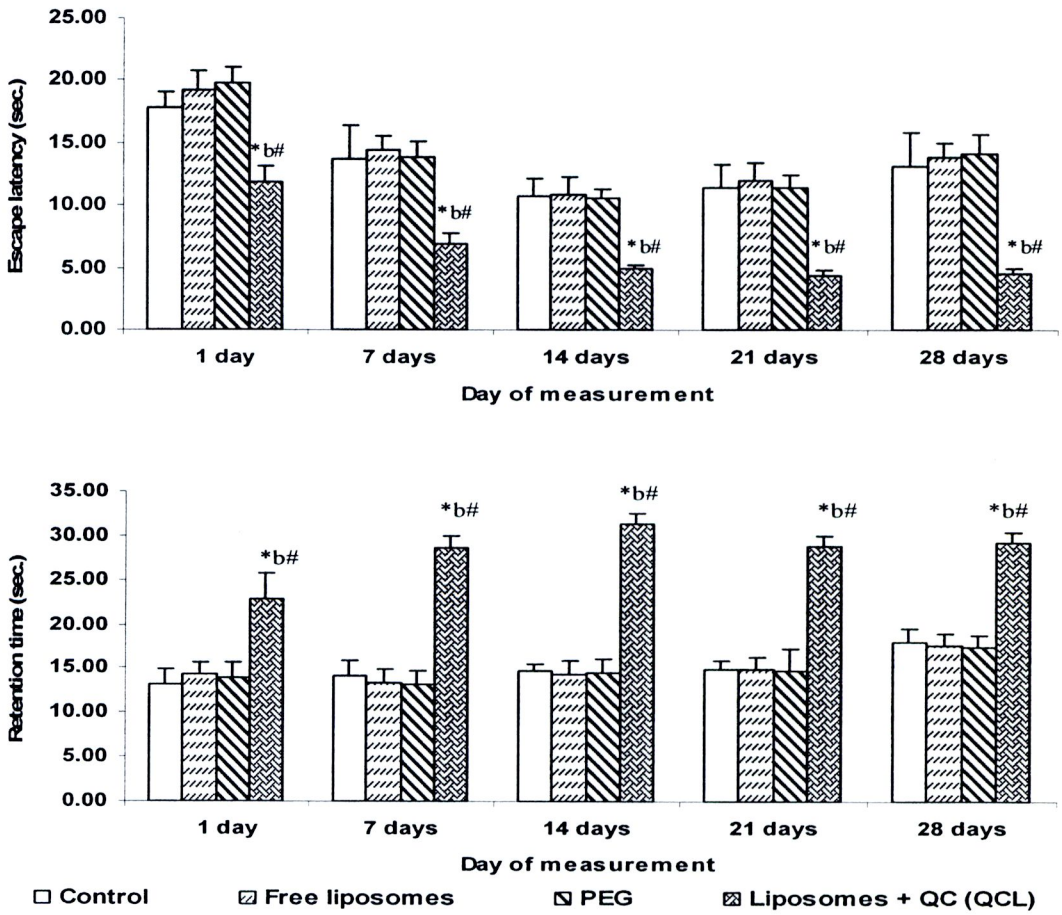

Figure 19 Effect of quercetin liposomes via nasal administration on spatial memory. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the escape latency and retention time in Morris water maze test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and

$^{\#}p < 0.05$ vs. free liposomes.

1.3.2 Effect of quercetin with oral conventional delivery system and nasal administration of quercetin liposomes on non-spatial memory

Figure 20 showed the preference of total exploration time for the novel object exhibited by quercetin at various dosages ranging from 100, 200 and 300 mg/kg BW at 30 minutes after substances administration. This study found no significant difference in exploration time of novel objects in vehicle treated group when compared to control group throughout the experimental period. Both donepezil, and quercetin at all dosage used in this study failed to show the significant changes on total exploration time assessed at 30 minutes. However, after 14 days of treatment, donepezil treated group started to produce the significant increase ($p < 0.05$) in total exploration time at 30 minutes after substance administration when compared to control and vehicle group. In addition, these changes were still observed when the treatment duration was increase to 28 days. Vitamin C did not produce the positive changes on this parameter throughout the study period. However, rats treated with the 300 mg/kg quercetin spent significantly more total exploration time ($p < 0.05$) after 7 days of treatment which was more sooner than donepezil, a positive control. In addition, rats treated with the 100 and 200 mg/kg quercetin could also increase spent more total exploration time ($p < 0.05$) after 21 and 28 days of treatment.

In order to determine, how long the memory can still exist after substance administration. The present study also determined the exploration time at 6 and 24 hr after substance administration.

Figures 21 showed the total exploration time within 6 after the substance administration. It was found that rats treated with donepezil and 300 mg/kg quercetin also showed the same pattern as that determined at 30 min after substance administration. After 21 days of treatment, rats receiving the 200 mg/kg quercetin significantly increased the total exploration time at 6 hr after substance administration. When the treatment duration increased, rats treated with the 100, 200 and 300 mg/kg quercetin enhanced object recognition time at all assessment schedule.

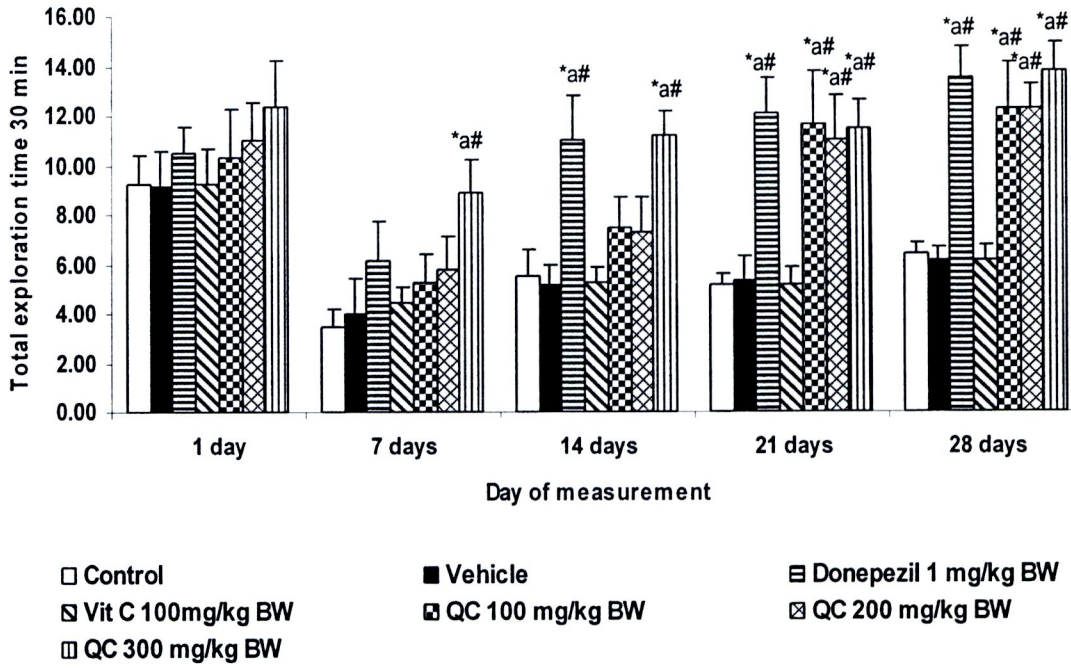

Figure 20 Effect of quercetin via oral administration on non-spatial memory within 30 minutes after substances administration. Rats were treated with vehicle, Vit C (100 mg/kg BW), donepezil (1 mg/kg BW) or the quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the total exploration time in object recognition test at 30 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

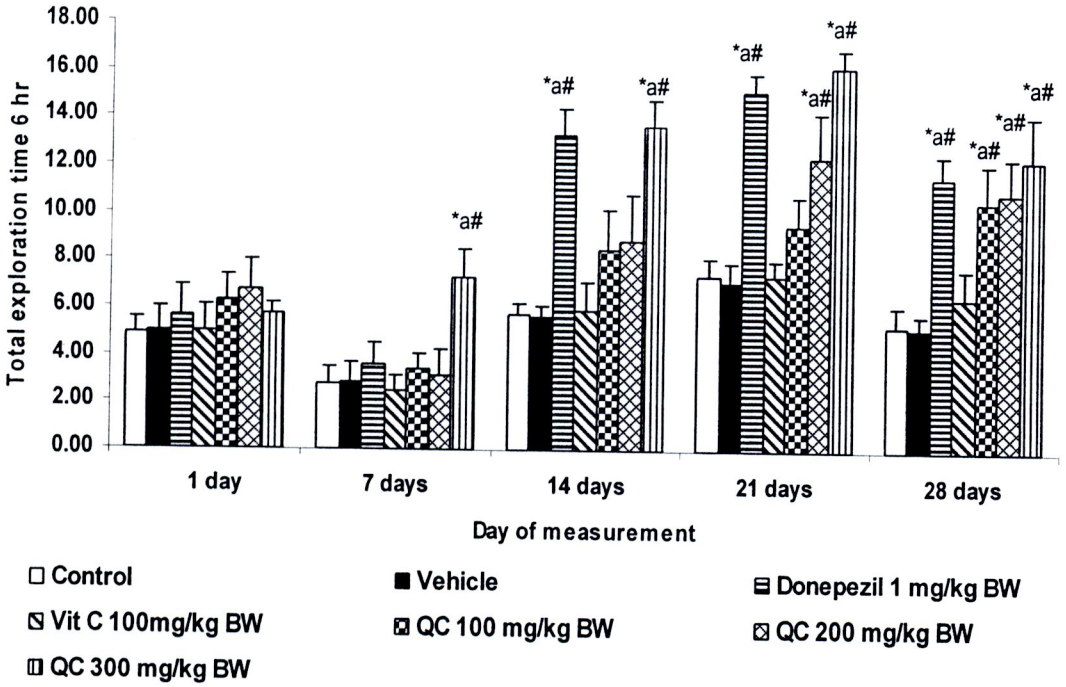

Figure 21 Effect of quercetin via oral administration on non-spatial memory within 6 hours after substances administration. Rats were treated with vehicle, Vit C (100 mg/kg BW), donepezil (1 mg/kg BW) or the quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the total exploration time in object recognition test at 6 hours after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

In addition, the results of total exploration time at 24 hr after substance administration also showed the same pattern as that determined within 6 hr after substance administration. Except that, after 21 days of treatment, quercetin at all dosages range used in this study significantly enhanced the total exploration time at all assessment period (Figure 22).

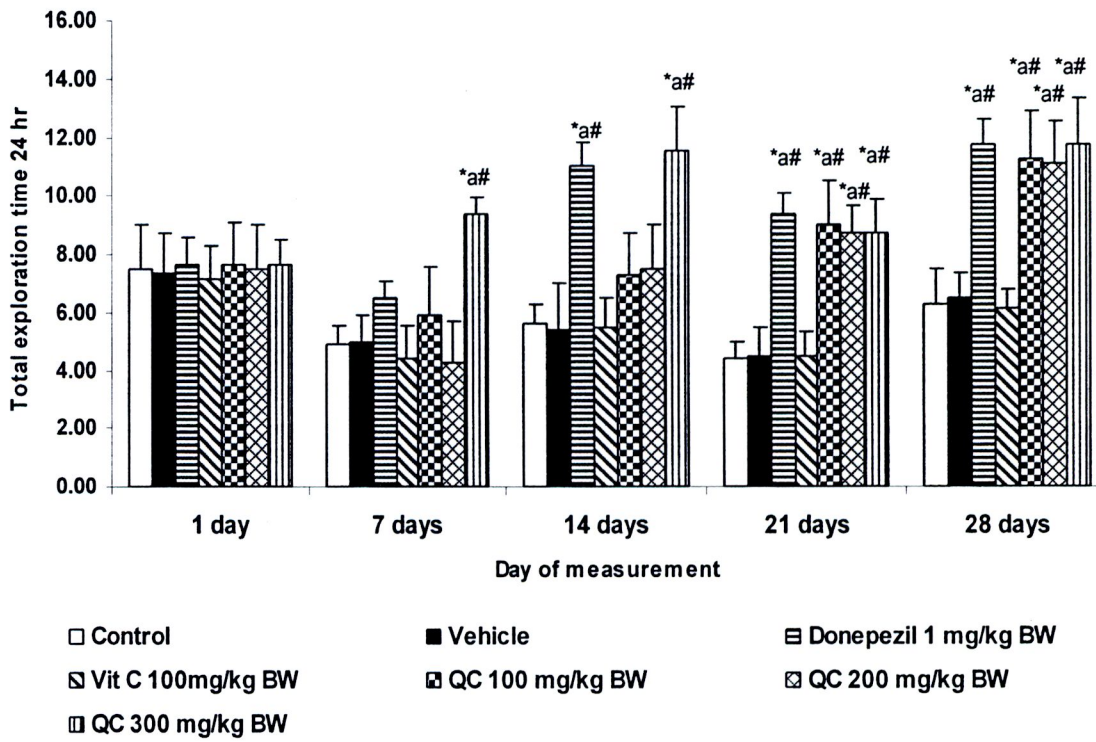

Figure 22 Effect of quercetin via oral administration on non-spatial memory within 24 hours after substances administration. Rats were treated with vehicle, Vit C (100 mg/kg BW), donepezil (1 mg/kg BW) or the quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined the total exploration time in object recognition test at 24 hours after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

Results of quercetin liposomes administered via nasal route on non-spatial memory assessment using the object recognition test (Figure 23) demonstrated that the single and repetitive administration of intranasal quercetin liposomes showed an increase in total exploration time ($p < 0.05$) within 30 minutes after substance administration when compared to control, free liposomes and vehicle (PEG) group.

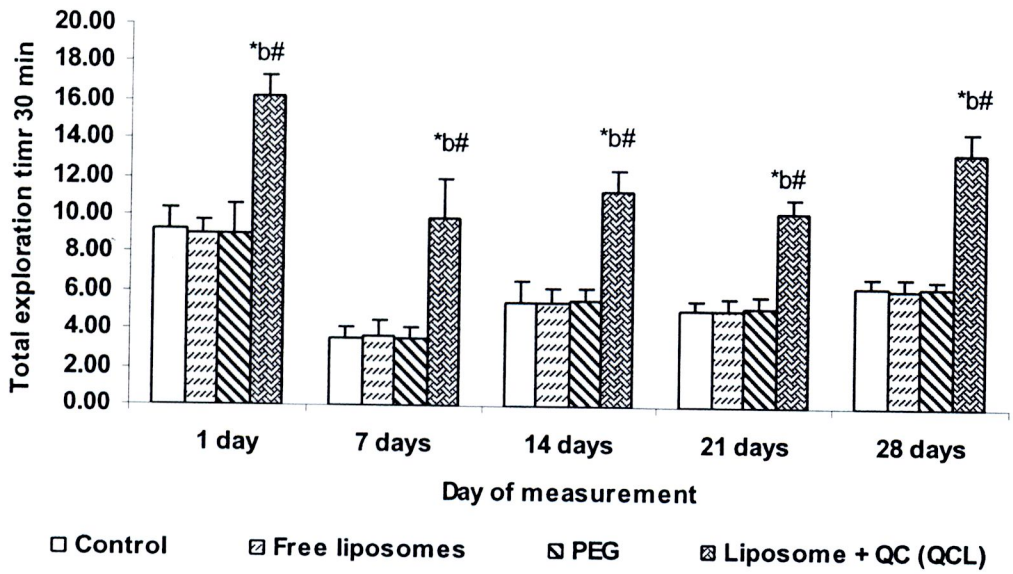

Figure 23 Effect of quercetin liposomes via nasal administration on non-spatial memory at 30 minutes after substances administration. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the total exploration time in object recognition test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

Moreover, the repetitive administration of this strategy started to increase the total exploration time at 6 and 24 hours after substance administration ($p < 0.05$) after 1 week of treatment and these changes were still observed throughout 28 days of study period (Figures 24-25).

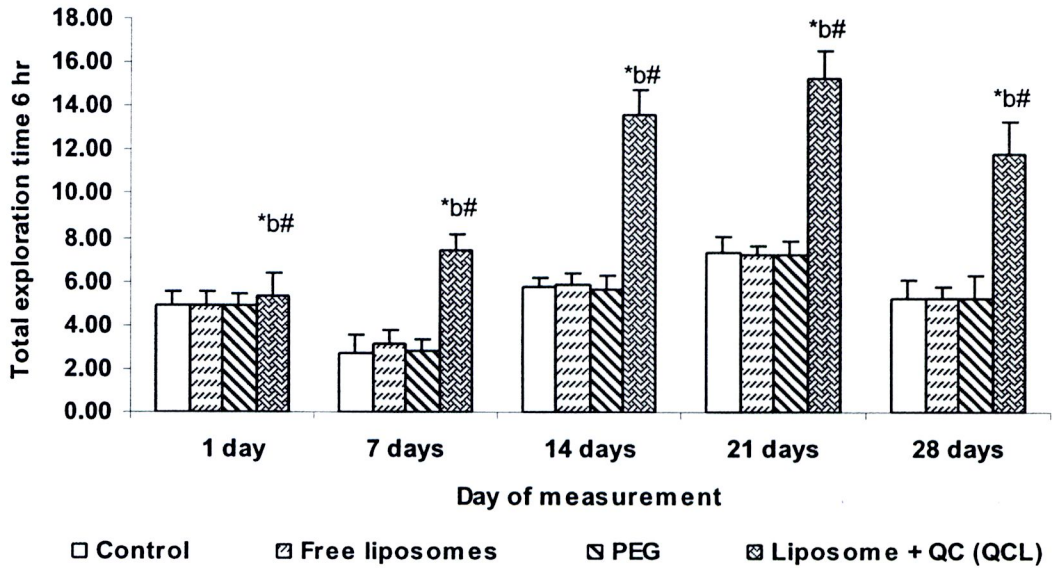

Figure 24 Effect of quercetin liposomes via nasal administration on non-spatial memory at 6 hours after substances administration. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the total exploration time in object recognition test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

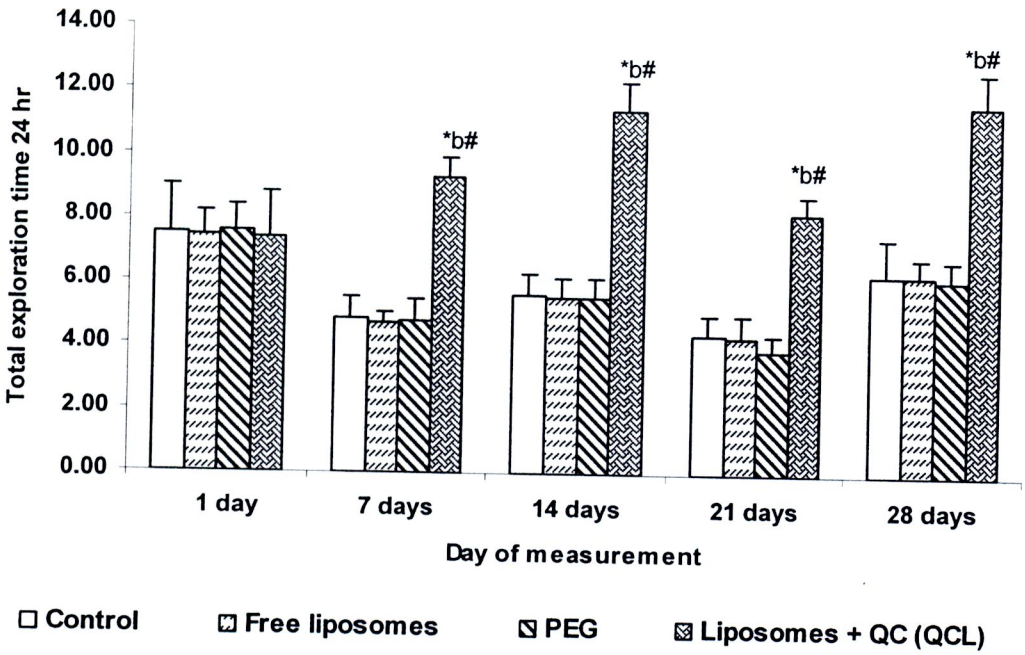

Figure 25 Effect of quercetin liposomes via nasal administration on non-spatial memory at 24 hours after substances administration. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined the total exploration time in object recognition test for 5 minutes after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

1.4 Spontaneous locomotor activities

Spontaneous locomotor activities of animals expressed by grooming, licking and rearing were observed to evaluate drug action on CNS.

The data showed that the spontaneous behavior including grooming, licking and rearing did not differ significantly between the control, vehicle treated group and quercetin at all dosage range used in this study throughout the experimental period (Figure 26).

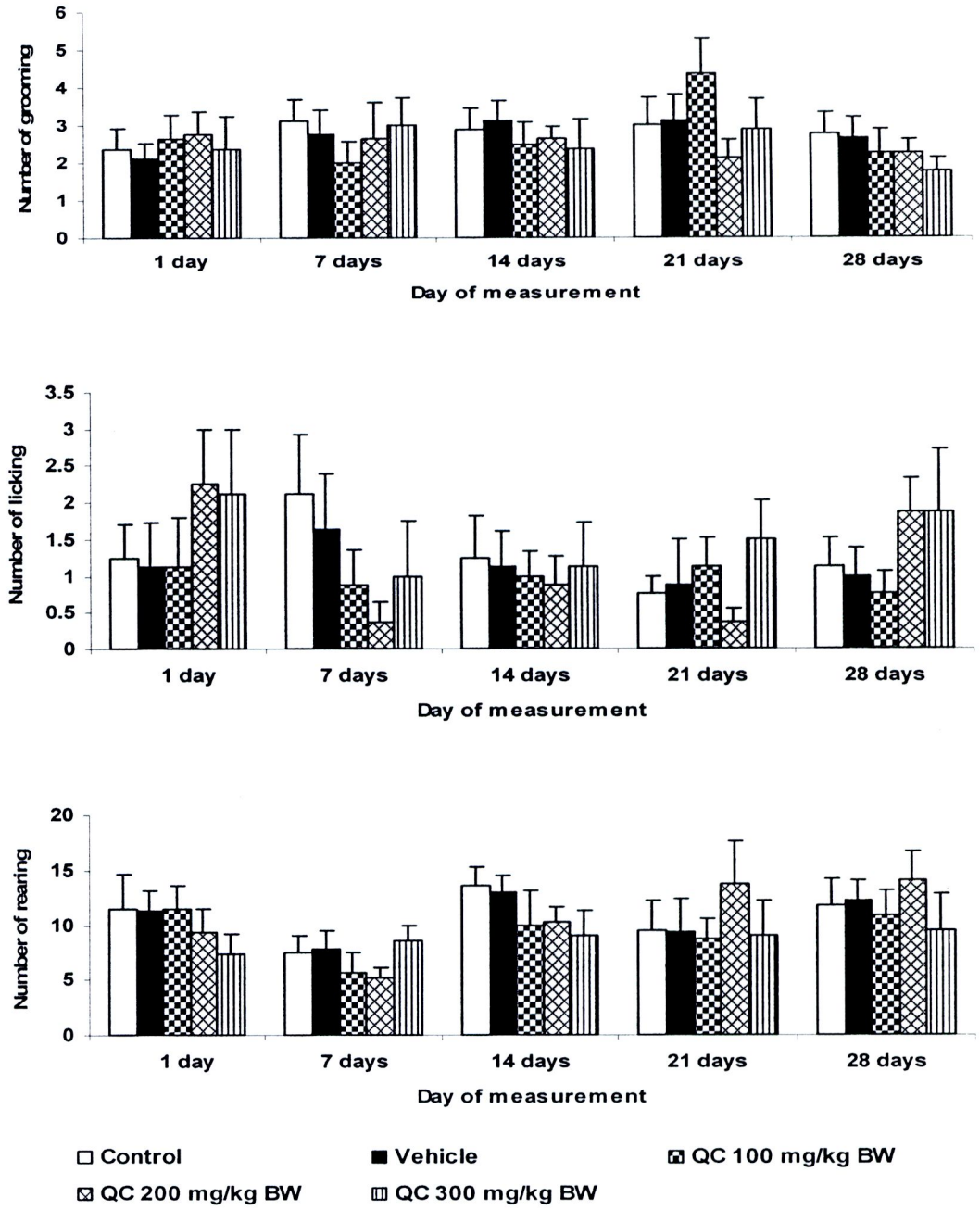

Figure 26 Effect of quercetin via oral administration on spontaneous locomotor activities. Rats were treated with vehicle or the quercetin (100, 200 and 300 mg/kg BW) via intragastric tube for 4 weeks, then they were determined grooming, licking and rearing behaviors after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

In addition, our data showed that nasal administration of quercetin liposomes did not produce the significant changes on these parameters (Figure 27).

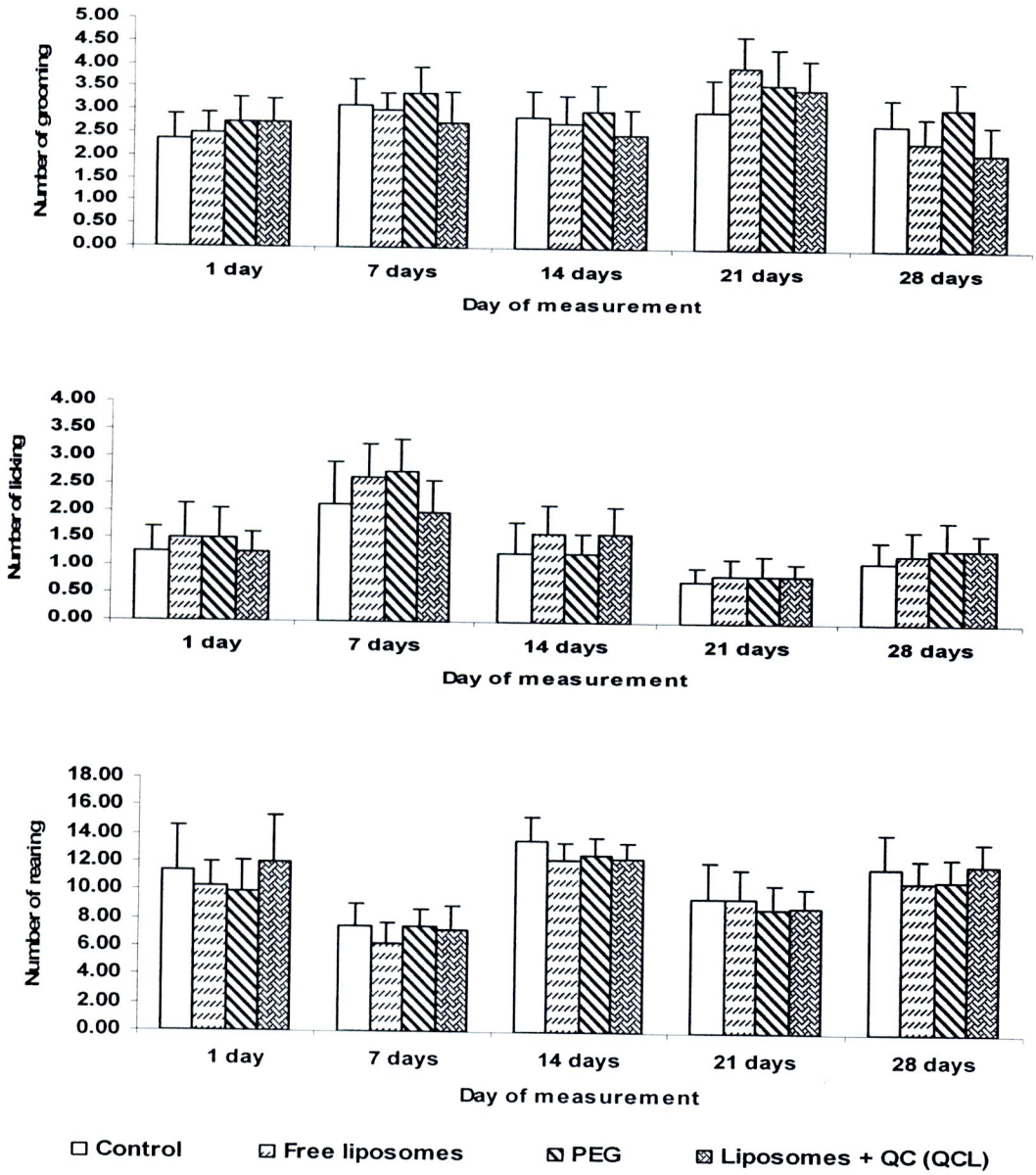

Figure 27 Effect of quercetin liposomes via nasal administration on spontaneous locomotor activities. Rats were treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then they were determined grooming, licking and rearing behaviors after single, 1, 2, 3 and 4 weeks of treatment. Data were presented as mean ± S.E.M. (n=8/group).

2. Neuroprotective Effect of Quercetin Via Oral Administration and Nasal Administration of Quercetin liposomes

Accumulating data demonstrated that learning and memory were tightly associated with the function of various brain areas, which in turn depended on the density of neurons particularly cholinergic neurons (Katzman, 1986). Therefore, this study also determined the effect of oral administration of quercetin and nasal administration of quercetin liposomes on the alteration of the survival neurons and cholinergic neurons density in various brain areas of cerebral cortex and hippocampus in both healthy and cognitive deficit conditions induced by AF64A.

Based on the data obtained from the first part of my study, the effective dose of quercetin on cognitive enhancing effect was 300 mg/kg BW. Therefore, this optimum dose had been selected for further investigated its effect on neurons density.

2.1 Healthy condition

The present results demonstrated that mean ± S.E.M. neurons density in all subregions of cerebral cortex in vehicle group was not significantly different from that of control group while the mean ± S.E.M. neurons density in these areas was significantly ($p<0.05$) higher in both quercetin and donepezil treatment (Figure 28).

In addition, the effect of oral administration of quercetin on the survival neurons density in various subregions of hippocampus was also determined. Mean ± S.E.M. neurons density in various subregions of hippocampus both in donepezil and quercetin were significantly ($p < 0.05$) higher density than that of the control and vehicle treated group (Figure 29).

Rats treated with quercetin liposomes administered via nasal route enhanced the survival neurons density both in cerebral cortex and hippocampus. As shown in Figures 30-31, no statistically significant differences were found between vehicle (PEG), free liposomes and control rats regarding the mean ± S.E.M. neurons density in all subregions of cerebral cortex and hippocampus while the mean ± S.E.M. neurons density in these areas were higher in both quercetin liposomes than in vehicle (PEG), free liposomes and control groups ($p < 0.05$).

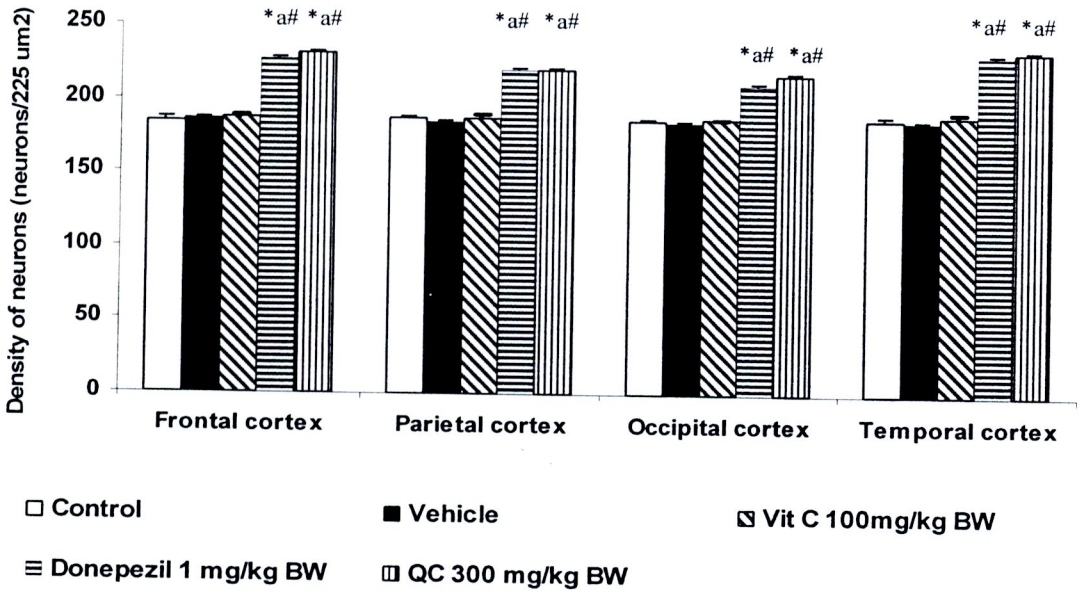

Figure 28 Effect of quercetin via oral administration on the survival neurons density in various subregions of cerebral cortex in healthy condition. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

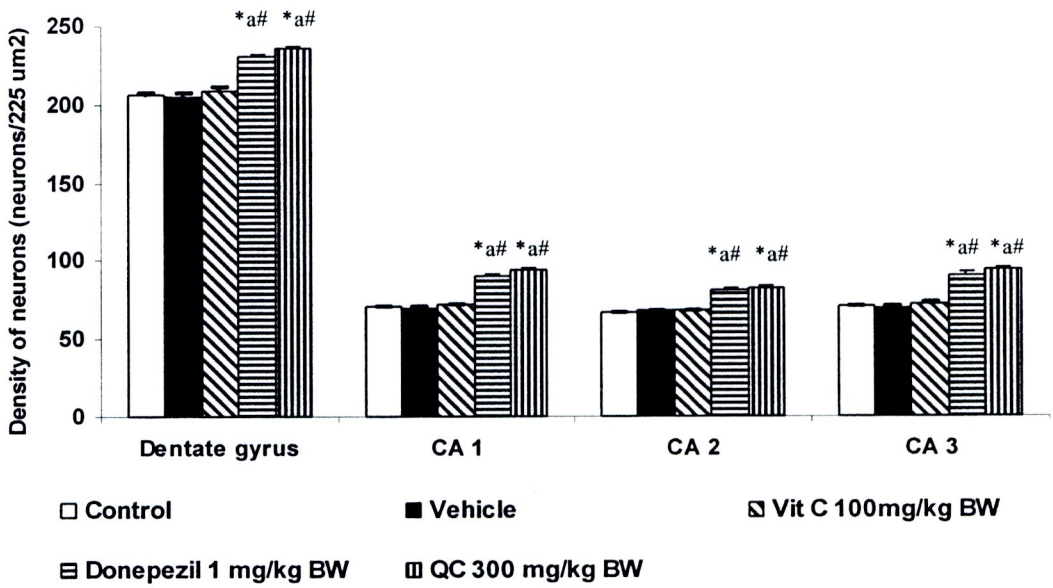

Figure 29 Effect of quercetin via oral administration on the survival neurons density in various subregions of hippocampus in healthy condition. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).
$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

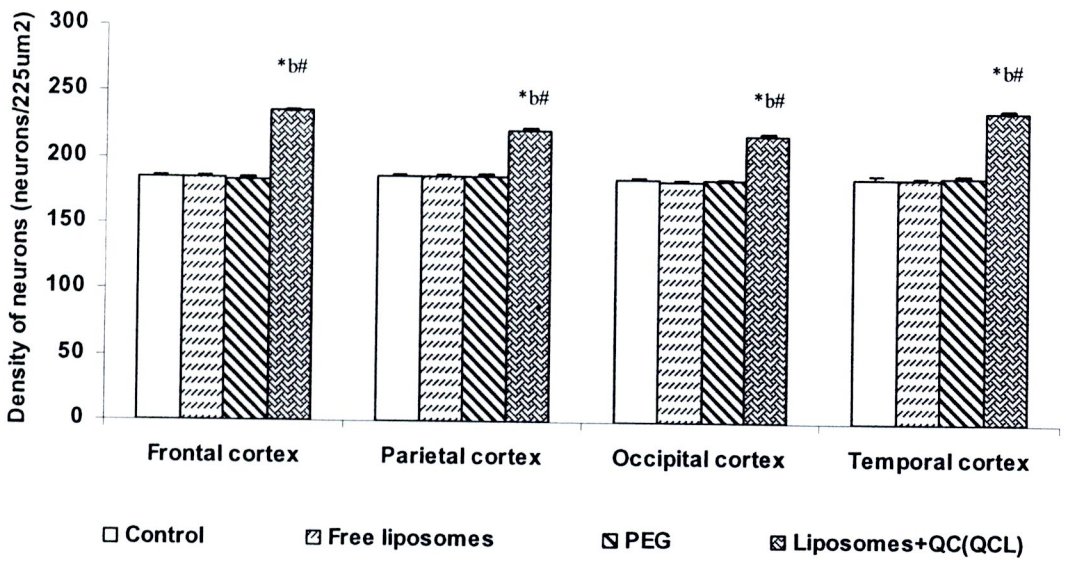

Figure 30 Effect of quercetin liposomes via nasal administration on the survival neurons density in various subregions of cerebral cortex in healthy condition. Each rat had been treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

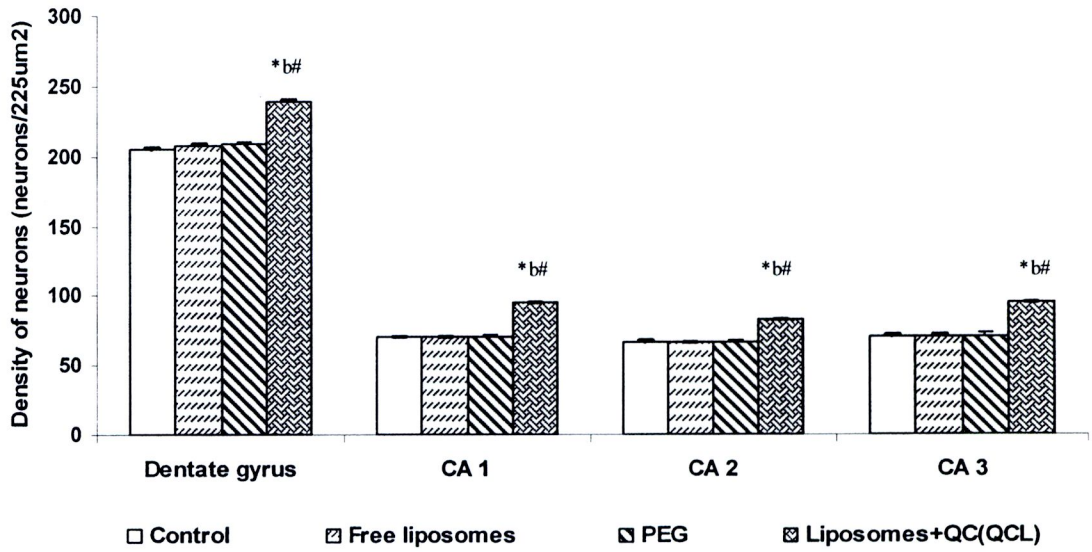

Figure 31 Effect of quercetin liposomes via nasal administration on the survival neurons density in various subregions of hippocampus in healthy condition. Each rat had been treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

Figure 32 showed the example of photomicrographs of coronal sections of CA1 histologically stained with cresyl violet at 40x magnification. The results revealed that subchronic nasal administration of quercetin liposomes significantly increased the density of survival neurons in CA1 of the hippocampus ($P < 0.05$ vs. control, vehicle (PEG) and free liposomes).

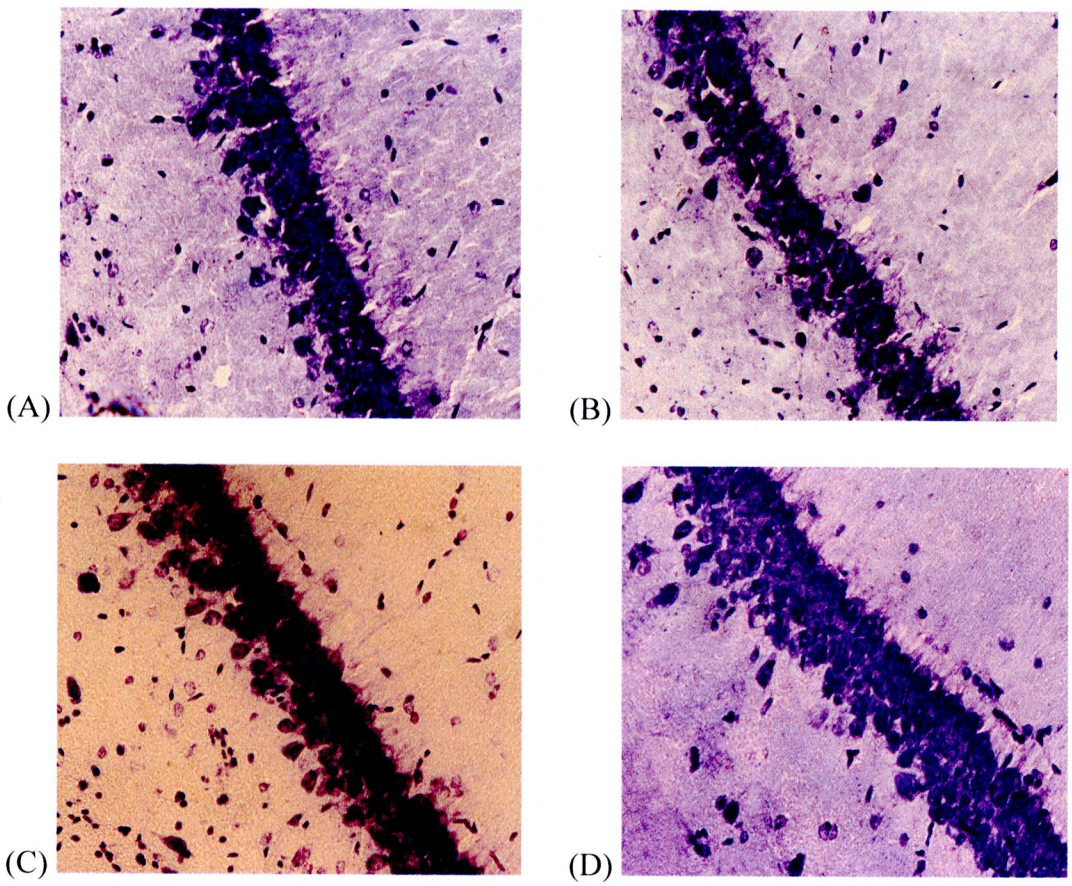

Figure 32 Effect of quercetin liposomes via nasal administration on survival neurons density in CA1 of the hippocampus stained with cresyl violet at 40x magnification. (A) Control; (B): Vehicle (PEG); (C): Free liposomes; (D): Quercetin liposomes. Each rat had been treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. The survival neurons density in CA1 was stained with cresyl violet.

Several lines of experimental evidence have reported that cholinergic neurons play a crucial role in cognitive processes like memory and attention (Baxter and Chiba, 1999; Sarter et al., 2003). Therefore, the effect of quercetin administered via oral route and nasal administration of quercetin liposomes on the density of cholinergic neurons in various subregions of hippocampus, a brain region contributing important role on spatial memory were also determined.

Statistical analysis revealed that there was no significant differences in the number of cholinergic neurons density in various subregions of hippocampus between both control and vehicle treated group. However, subchronic querctin administration at dose of 300 mg/kg via oral route increased the cholinergic neurons density in these areas ($p < 0.05$ vs. control and vehicle, Figure33).

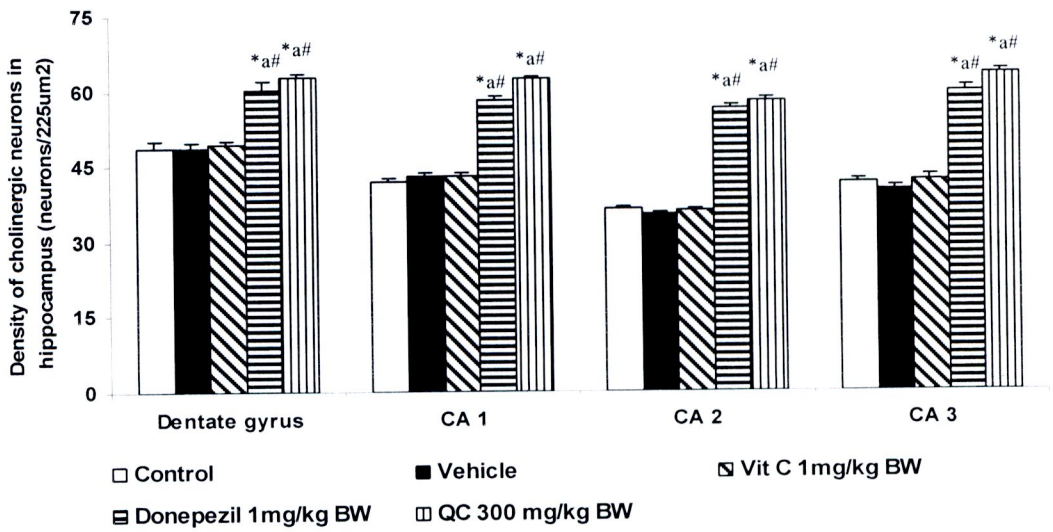

Figure 33 Effect of quercetin via oral administration on the cholinergic neurons density in various subregions of hippocampus in healthy condition. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were immunological stained against choline acetyltransferase (ChAT), a cholinergic marker of cholinergic system. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{a}p < 0.05$ vs. vehicle and $^{\#}p < 0.05$ vs. Vit C.

Figure 34 showed the example of photomicrographs of coronal sections of CA2 histologically stained with monoclonal antibody against ChAT at 40x magnification. The results demonstrated that subchronic quercetin administration at dose of 300 mg/kg via oral administration produced a remarkable increase of cholinergic neurons density in CA2 of the hippocampus ($P < 0.05$ vs. control and vehicle).

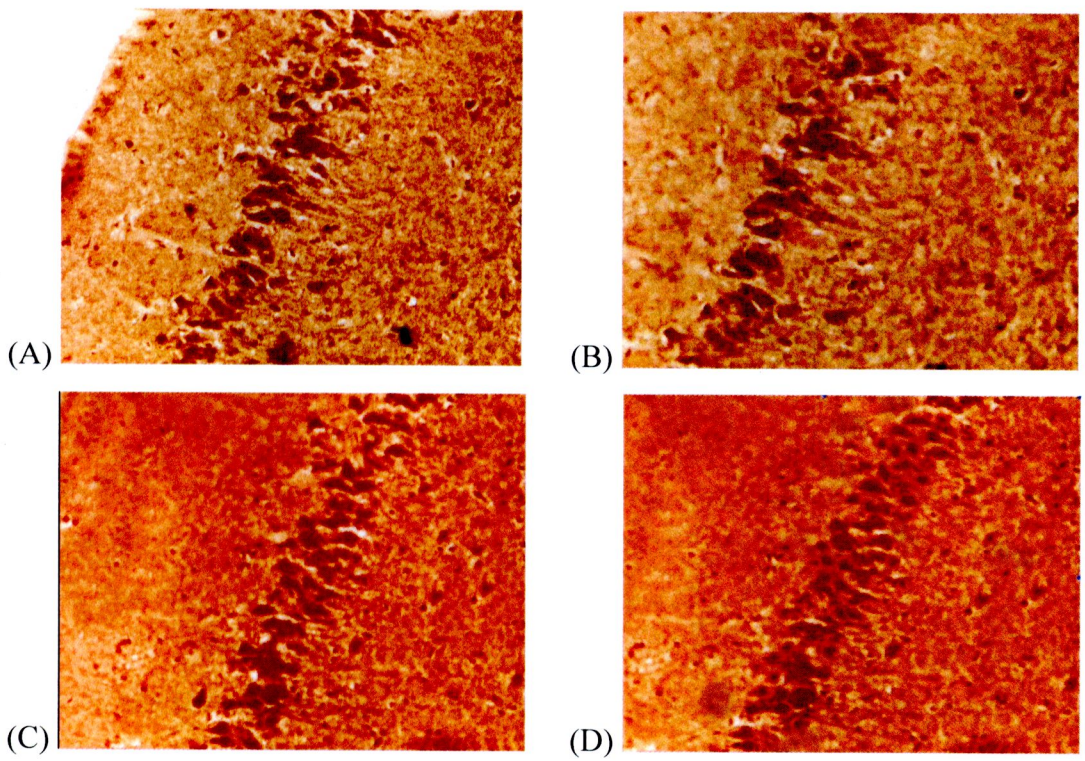

Figure 34 Effect of quercetin via oral administration on ChAT-positive neurons density in CA2 of the hippocampus stained with monoclonal antibody against ChAT at 40x magnification. (A) Control; (B) Vehicle; (C) Donepezil at dose of 1mg/kg BW; (D) Quercetin at dose of 300 mg/kg BW. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. The ChAT-positive neurons density in CA2 was detected by immunohistochemistry.

The effect of quercetin liposomes administered via nasal route on the alteration of cholinergic neurons density in all subregions of hippocampus was shown in Figure 35. The ChAT-positive neurons density of vehicle (PEG) and free liposomes group did not differ from control group while there was a significant induction in ChAT-positive neurons density of quercetin liposomes treated group in areas as mentioned earlier ($p < 0.05$), reflecting the improvement memory via increase in the cholinergic neurons density after nasal administration of quercetin liposomes.

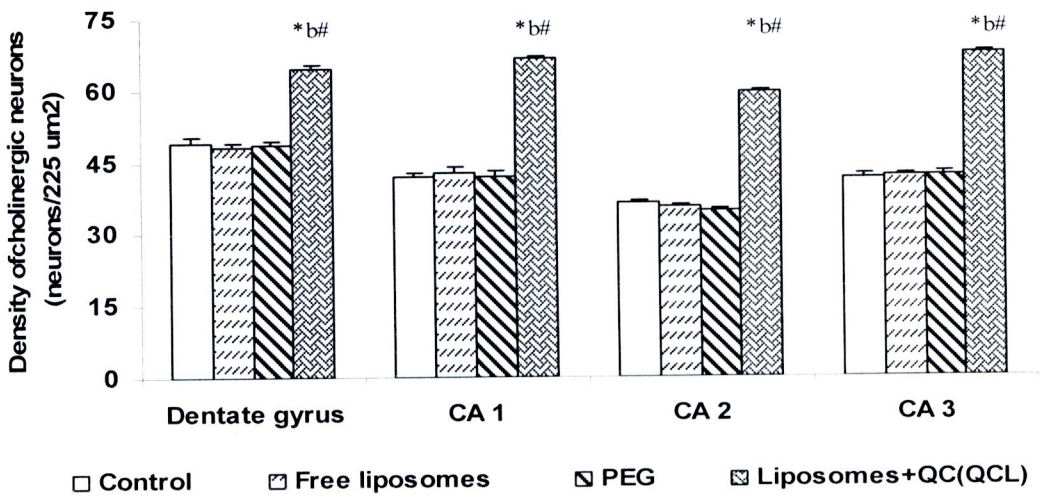

Figure 35 Effect of quercetin liposomes via nasal administration on the cholinergic neurons density in various subregions of hippocampus in healthy condition. Each rat had been treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route for 4 weeks, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were immunological stained against choline acetyltransferase (ChAT), a cholinergic marker of cholinergic system. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. vehicle (PEG) and $^{\#}p < 0.05$ vs. free liposomes.

2.2 Cognitive deficit condition in Alzheimer's disease model

Recently, the evidence for the involvement of the cholinergic system deficit in the pathogenesis of AD was increased (Gallagher and Colombo, 1995; Muir, 1997; Dickinson-Anson et al., 2003; Yan and Feng, 2004; Zhang, 2004).

Alternatively, the use of anti-oxidant intake had been proposed as another new therapeutic approach in the treatment of AD. Therefore, this raised the possibility that oral administration of quercetin and nasal administration of quercetin liposomes could exhibit the neuroprotectant on the survival neurons and cholinergic neurons under cognitive deficit condition induced by AF64A which mimicked the cognitive deficit condition as those presented in Alzheimer's disease.

Statistical analysis revealed that there was no significant change in mean ± S.E.M. of neurons density in subregions of cerebral cortex in vehicle + sham operation treated group. The significant changes in the density of survival neurons in the areas mentioned earlier were observed in the vehicle + AF64A and Vit C + AF64A groups ($p < 0.05$) as compared to the vehicle + sham operation treated group. On the other hand, the mean ± S.E.M. of neurons density in these areas increased significantly in quercetin + AF64A groups ($p < 0.05$) as compare to the vehicle + AF64A treated group (Figure 36).

Interestingly, under cognitive deficit condition, both donepezil and quercetin significantly attenuated the reduction of survival neurons (Figure 37) and cholinergic neurons density induced by AF64A in all areas of hippocampus ($p < 0.05$) as compare to the vehicle + AF64A treated group (Figure 38).

Figure 39 showed the effect of quercetin at dose of 300 mg/kg BW given via oral route on the alteration of survival neurons density in CA3 of the hippocampus stained with cresyl violet at 40 x magnification. The photomicrograph showed that the density of survival neurons in CA3 was decreased by AF64A. Both donepezil (1 mg/kg, p.o.) and quercetin (300 mg/kg, p.o.) treatment reversed these alterations and significantly increased the survival neurons density in this area.

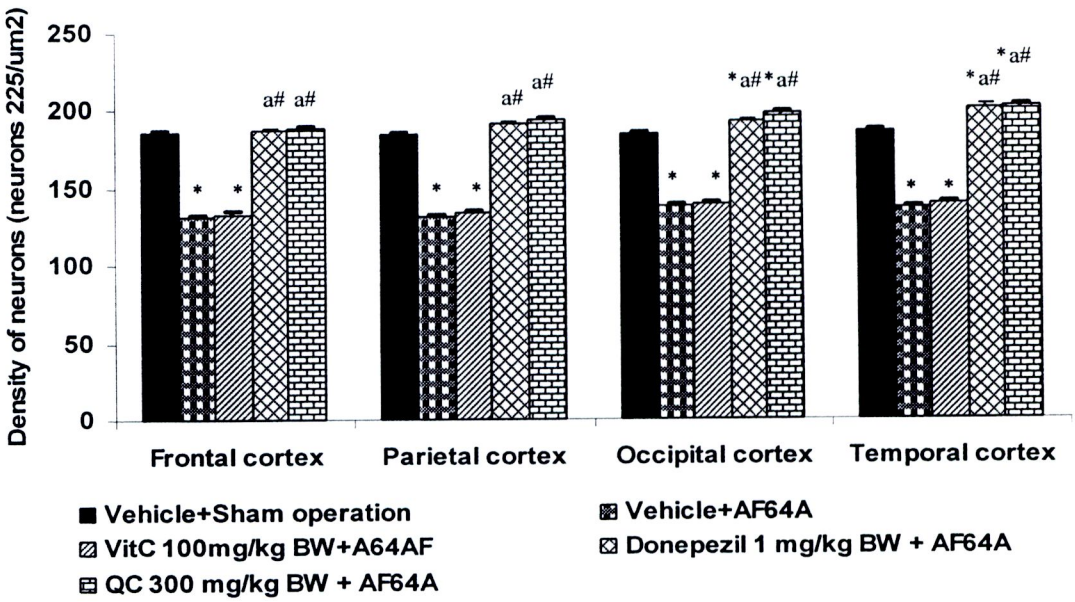

Figure 36 Effect of quercetin via oral administration on the survival neurons density in various subregions of cerebral cortex in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A and $^{\#}p < 0.05$ vs. Vit C + AF64A.

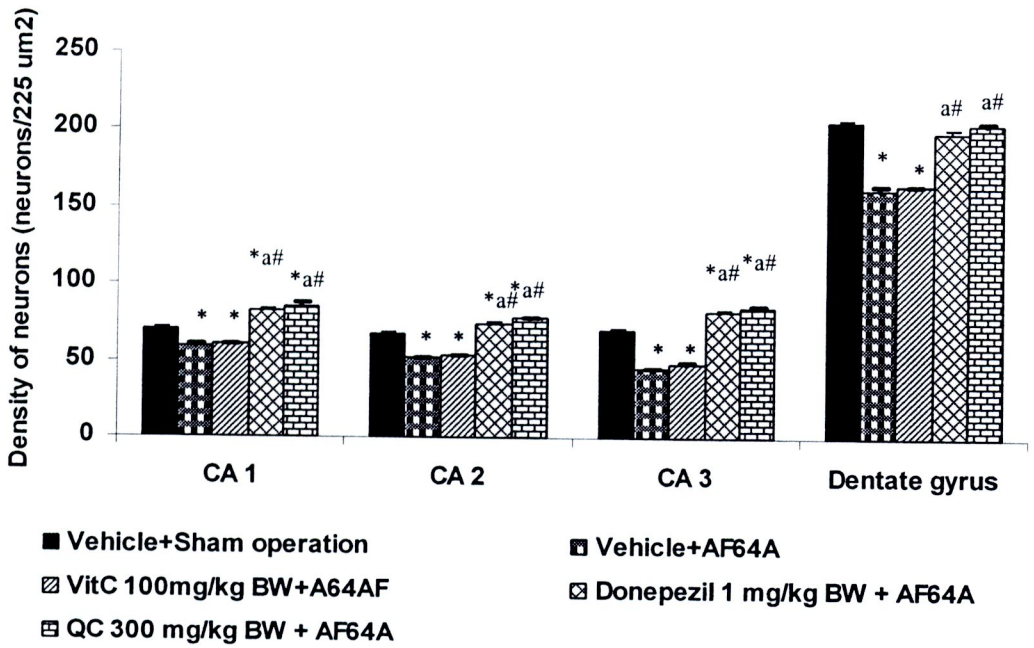

Figure 37 Effect of quercetin via oral administration on the survival neurons density in various subregions of hippocampus in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A and $^{\#}p < 0.05$ vs. Vit C + AF64A.

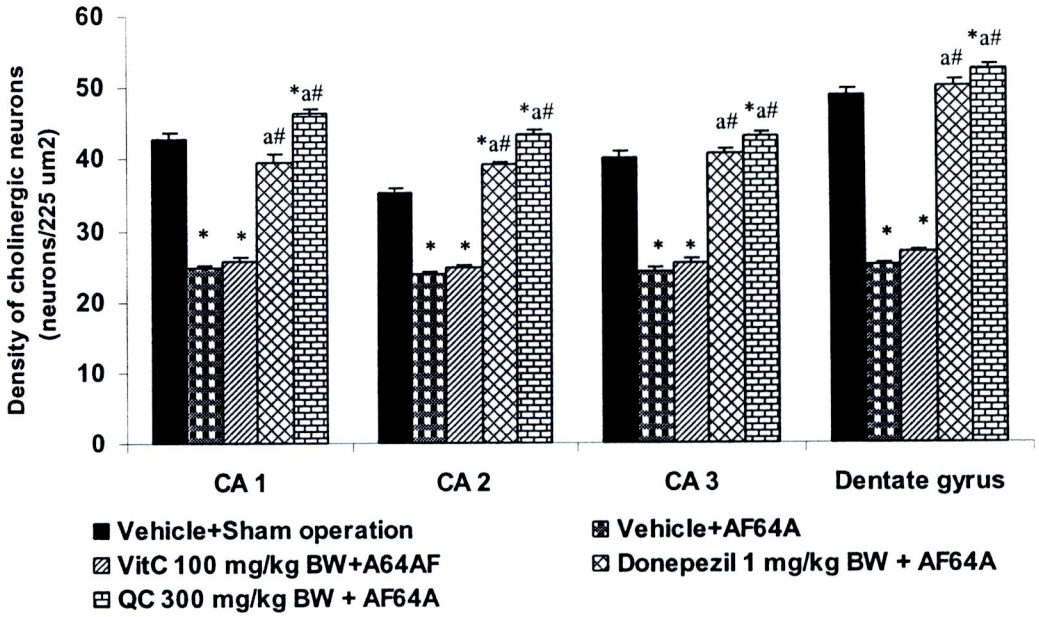

Figure 38 Effect of quercetin via oral administration on the cholinergic neurons density in various subregions of hippocampus in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were immunological stained against choline acetyltransferase (ChAT), a cholinergic marker of cholinergic system. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A and $^{\#}p < 0.05$ vs. Vit C + AF64A.

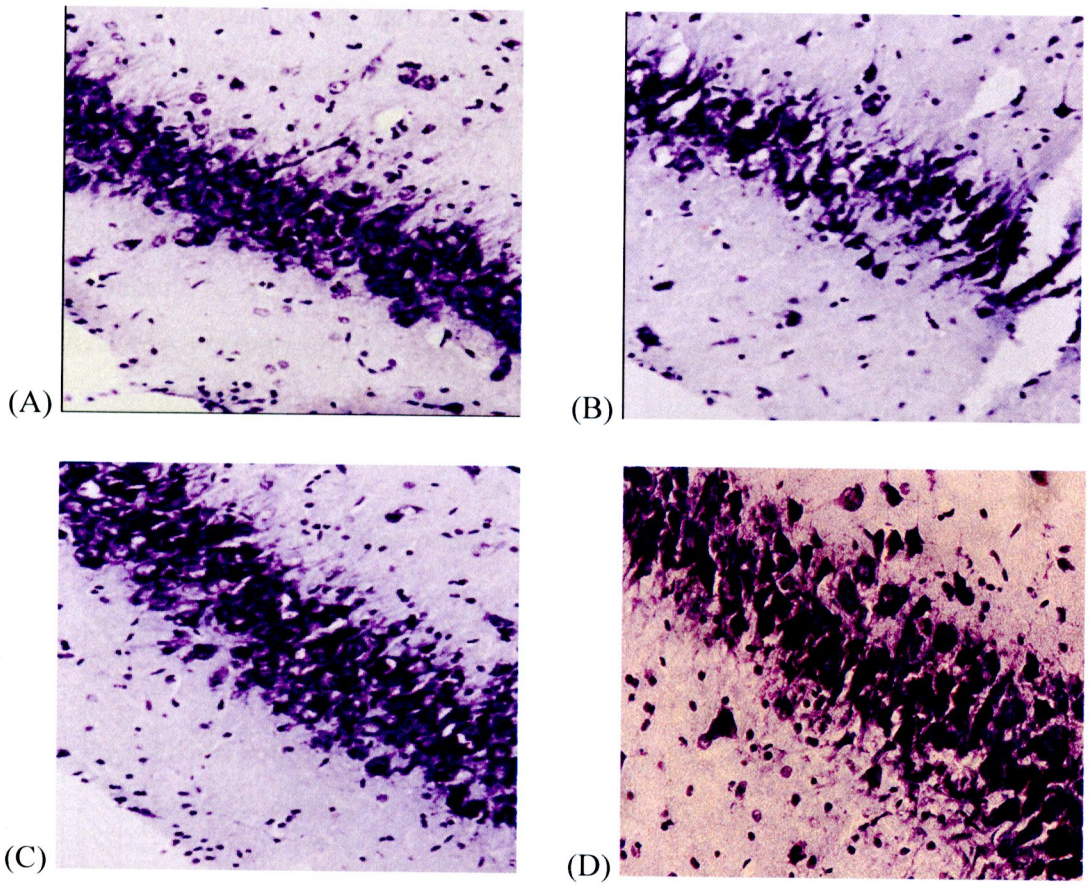

Figure 39 Effect of quercetin via oral administration on the survival neurons density in CA3 of the hippocampus stained with cresyl violet at 40x magnification. (A) Vehicle plus Sham operation; (B): Vehicle plus AF64A; (C): Donepezil at dose of 1 mg/kg BW plus AF64A; (D): Quercetin at dose of 300 mg/kg BW plus AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1 mg/kg BW) or quercetin (300 mg/kg BW via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. The survival neurons density in CA1 was stained with cresyl violet.

In addition, It was also found that the animals which received free liposomes or vehicle (PEG) + AF64A markedly showed the reduction in the density of survival neurons in both cerebral cortex and hippocampus ($p < 0.05$) when compared with free liposomes + sham operation and vehicle (PEG) + sham operation treated groups (Figures 40-41). Quercetin liposomes could attenuate the reduction of survival neurons density induced by AF64A in all areas of cerebral cortex and hippocampus ($p < 0.05$) when compared with free liposomes + AF64A and vehicle (PEG) + AF64A treated group.

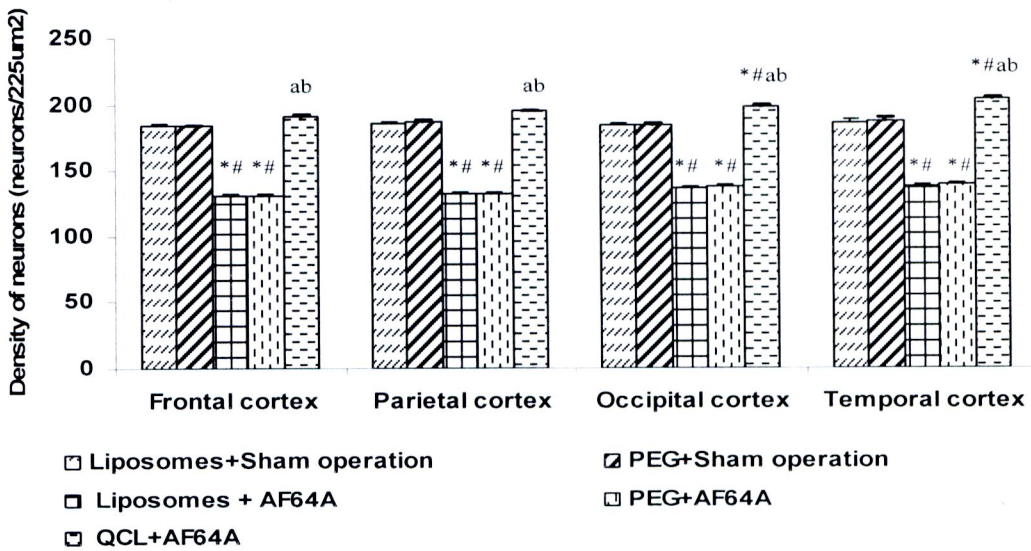

Figure 40 Effect of quercetin liposomes via nasal administration on the survival neurons density in various subregions of cerebral cortex in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

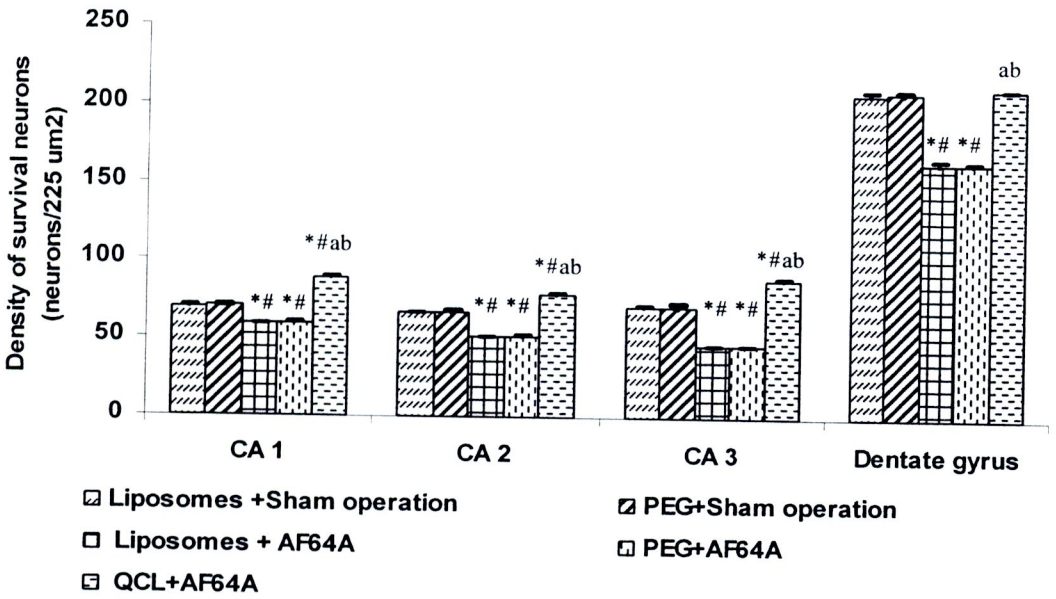

Figure 41 Effect of quercetin liposomes via nasal administration on the survival neurons density in various subregions of hippocampus in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were stained with cresyl violet. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

Moreover, AF64A also decreased the cholinergic neurons density in all areas of hippocampus ($p < 0.05$) when compared with free liposomes + sham operation and vehicle (PEG) + sham operation treated group. There was a significant ($p < 0.05$) rise in the cholinergic neurons density of quercetin liposomes + AF64A as compared to the free liposomes + AF64A and vehicle (PEG) + AF64A treated group (Figure 42).

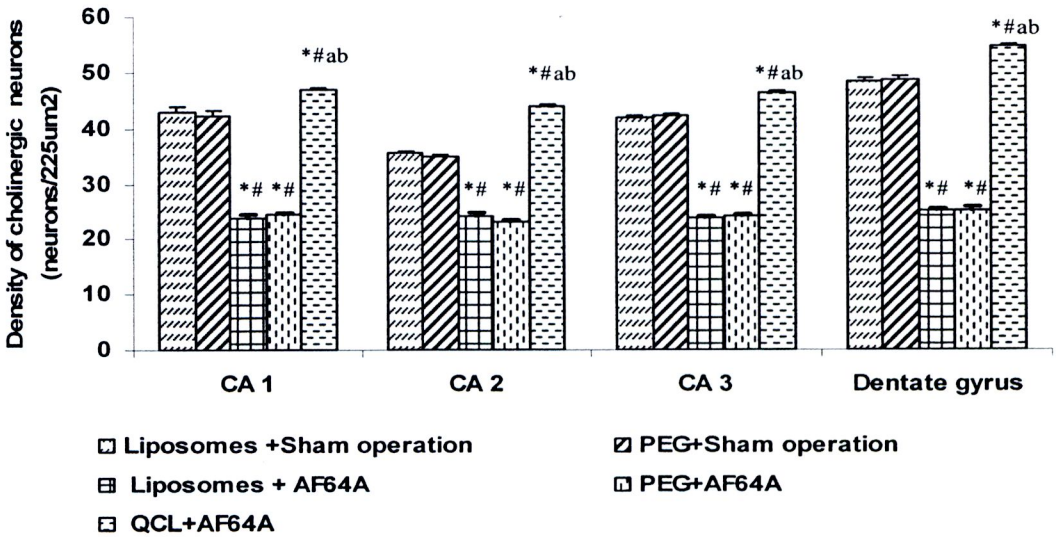

Figure 42 Effect of quercetin liposomes via nasal administration on the cholinergic neurons density in various subregions of hippocampus in cognitive deficit condition of Alzheimer's disease model. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were cut as coronal sections at 25 μm thick. All sections were immunological stained against choline acetyltransferase (ChAT), a cholinergic marker of cholinergic system. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

Figure 43 showed the example of photomicrographs of coronal sections of CA3 histologically stained with monoclonal antibody against ChAT at 40x magnification. The results demonstrated that nasal administration of quercetin liposomes produced a remarkable increase of cholinergic neurons density in CA3 of the hippocampus ($P < 0.05$ vs. free liposomes plus AF64A and vehicle (PEG) plus AF64A treated group).

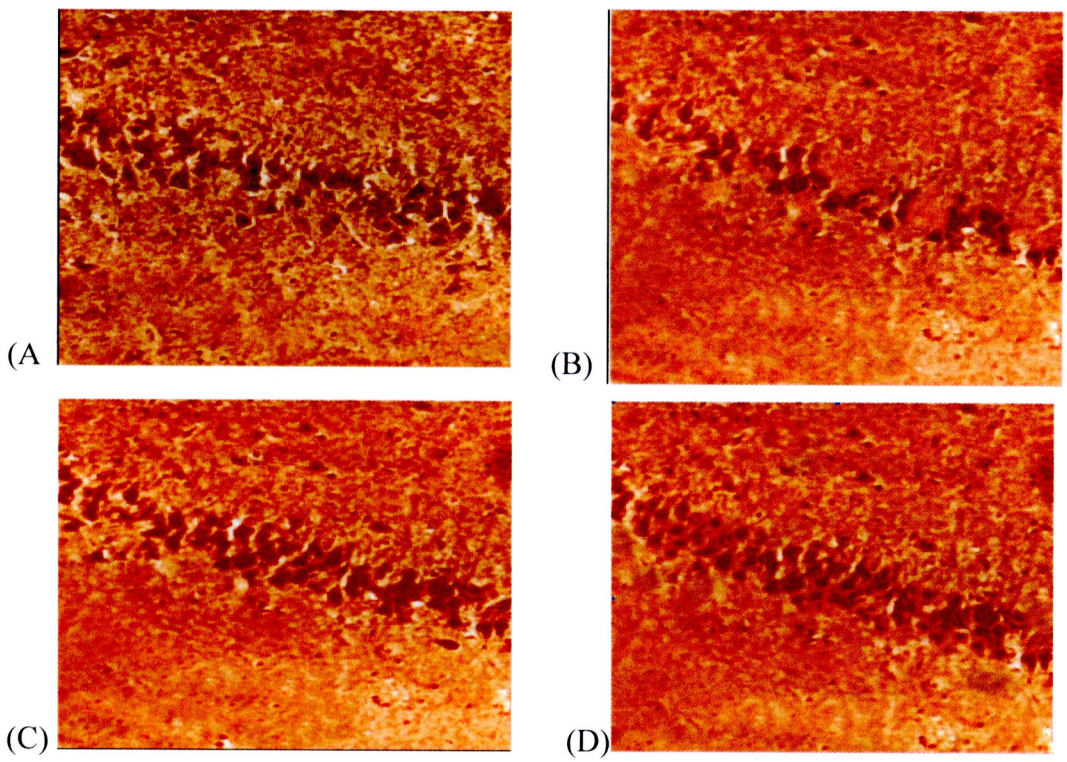

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Figure 43 Effect of quercetin via nasal administration on ChAT-positive neurons density in CA3 of the hippocampus stained with monoclonal antibody against ChAT at 40x magnification. (A) Free liposomes plus AF64A; (B) Vehicle (PEG) plus AF64A; (C) Vehicle (PEG) plus Sham operation; (D) Quercetin liposome plus AF64A. Each rat had been treated with vehicle (PEG), free liposomes or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals was sacrificed and the brains were cut as coronal sections at 25 μm thick. The ChAT-positive neurons density in CA3 was detected by immunohistochemistry.

3. Possible Mechanisms Underlying the Cognitive Enhancement and the Neuroprotective Effect of Quercetin With oral Conventional Delivery System and Nasal Administration of Quercetin Liposomes Against Alzheimer's Disease

Cholinergic neurons and their projections are widely distributed throughout the CNS with an essential role in regulating many vital functions, such as learning, memory, cortical organization of movement and cerebral blood flow regulation (Mesulam et al., 2002). Previous study had demonstrated that one of the most important mechanisms responsible for the magnitude of performance of cholinergic function is performed by acetylcholineaterase (AChE) (Appleyard, 1992). This enzyme hydrolysed the neurotransmitter acetylcholine in the synaptic cleft of cholinergic synapses and neuromuscular junctions (Soreq and Seidman, 2001). In addition to its role on cholinergic neurotransmission, AChE had been implicated in several non-cholinergic actions such as cell proliferation (Appleyard, 1994) and neurite outgrowth (Chacón et al., 2003). Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders, particularly Alzheimer's diseases (Chauhan and Chauhan, 2006). Thus, the effect of oral administration of quecetin and nasal administration of quercetin liposomes on the alteration of AChE activity in animal model of Alzheimer's disease was focused.

The results on AChE activity both in cerebral cortex and hippocampus of quercetin via oral administration were presented in (Figure 44). AChE activity was significantly increased in the vehicle + AF64A and Vit C + AF64A group ($P < 0.05$) compared to vehicle + sham operation group. On the other hand, treatment with 300 mg/kg quercetin and 1 mg/kg donepezil inhibited significantly AChE activity ($p < 0.05$) when compared to vehicle + AF64A group.

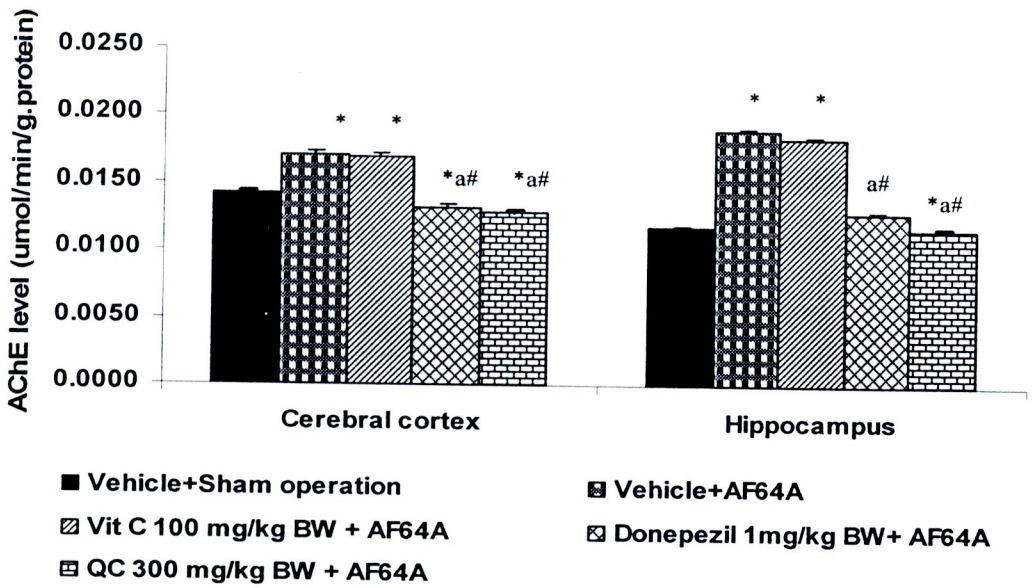

Figure 44 Effect of quercetin via oral administration on the activity of acetylcholinesterase enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of acetylcholinesterase activity both in cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A and $^{\#}p < 0.05$ vs. Vit C + AF64A.

Surprisingly, quercetin liposomes via nasal route could attenuate the elevation of AChE activity induced by AF64A in both areas mentioned above ($p < 0.05$) when compared to vehicle (PEG) + AF64A and free liposomes + AF64A treated groups (Figure 45).

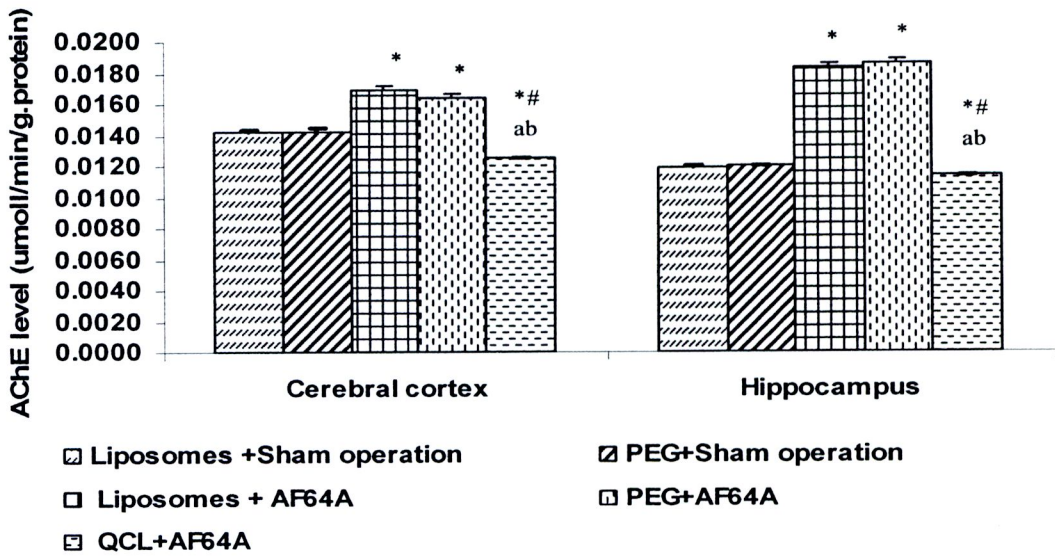

Figure 45 Effect of quercetin liposomes via nasal administration on the activity of acetylcholinesterase enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of acetylcholinesterase activity both in cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

Based on the results of extensive experimental work, free radicals appear to be a major culprit causing cellular damage. In addition, a large number of evidences had demonstrated the linkage of free radical generation and neuronal degeneration which highlighted the importance of antioxidants in the treatment of neurodegenerative disorders like AD. The detoxification of free radicals was especially important for the brain because this organ had a high content of polyunsaturated fatty acid and high utilization of oxygen, but its ability to combat oxidative stress was limited, making it sensitive to free radicals (Halliwell and Gutteridge, 1985; Halliwell, 2001). These radicals could attack lipid (lipid peroxidation), thereby disrupted the structure and functions of the cell membrane. The body used endogenous scavenger enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to neutralize free radicals (Ashok and Ali, 1999). Measurements of these antioxidant enzymes, malondialdehyde (MDA), a marker of lipid peroxidation product, might yield a snapshot of oxidative damage.

Intracerebroventricular administration of AF64A caused marked increase in free radical generation and significant rise in brain MDA, reduced SOD, catalase and GPx levels as compared to the vehicle + sham operation treated group. However, both oral administration of donepezil and quercetin treatment significantly ($p < 0.05$) prevented the increase in MDA both in cerebral cortex and hippocampus as compared to the vehicle + AF64A treated group (Figure 46).

Both donepezil and quercetin treatment caused a significant increase ($p < 0.05$) in the levels of SOD, CAT and GPx both in areas as mentioned earlier. Moreover, quercetin treatment appeared to exert this influence on the activities of SOD, CAT and GPx greater than donepezil ($p < 0.05$) as compared to the donepezil + AF64A treated group (Figures 47-49).

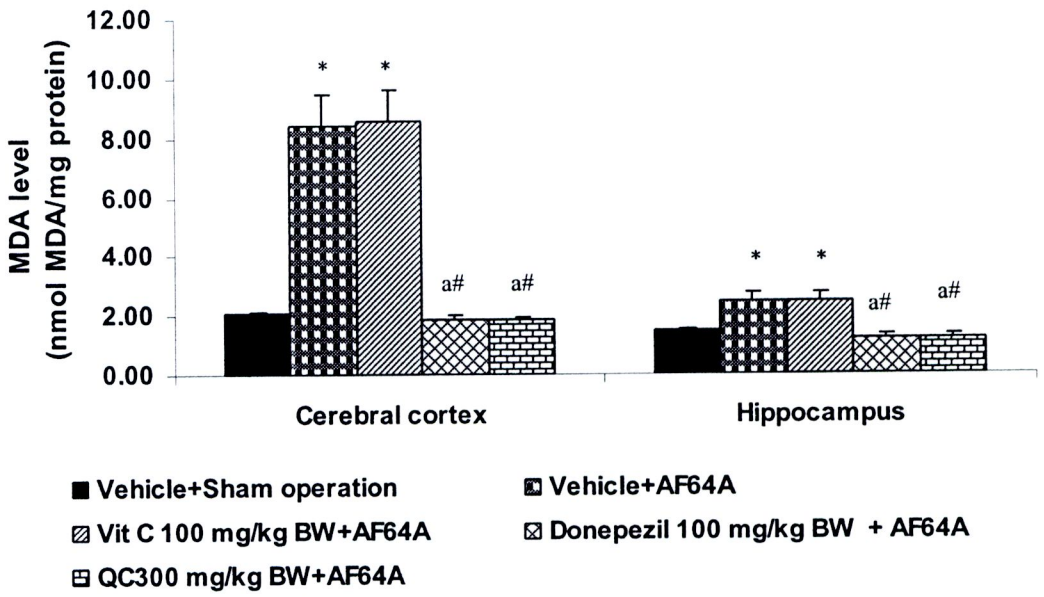

Figure 46 Effect of quercetin via oral administration on the level of malondialdehyde (MDA), a marker of lipid peroxidation product in both cerebral cortex and hipocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administrations, then the animals were sacrificed and the brains were removed in order to determine the level of MDA in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group). $^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A and $^{\#}p < 0.05$ vs. Vit C + AF64A.

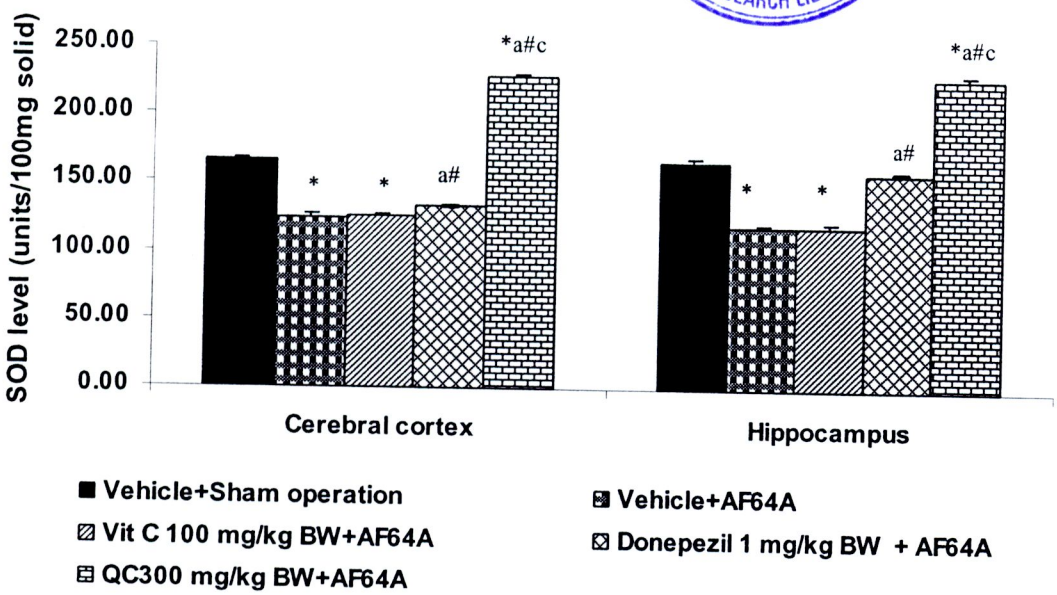

Figure 47 Effect of quercetin via oral administration on the activity of superoxide dismutase (SOD) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of superoxide dismutase (SOD) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A, $^{\#}p < 0.05$ vs. Vit C + AF64A and $^{c}p < 0.05$ vs. donepezil + AF64A.

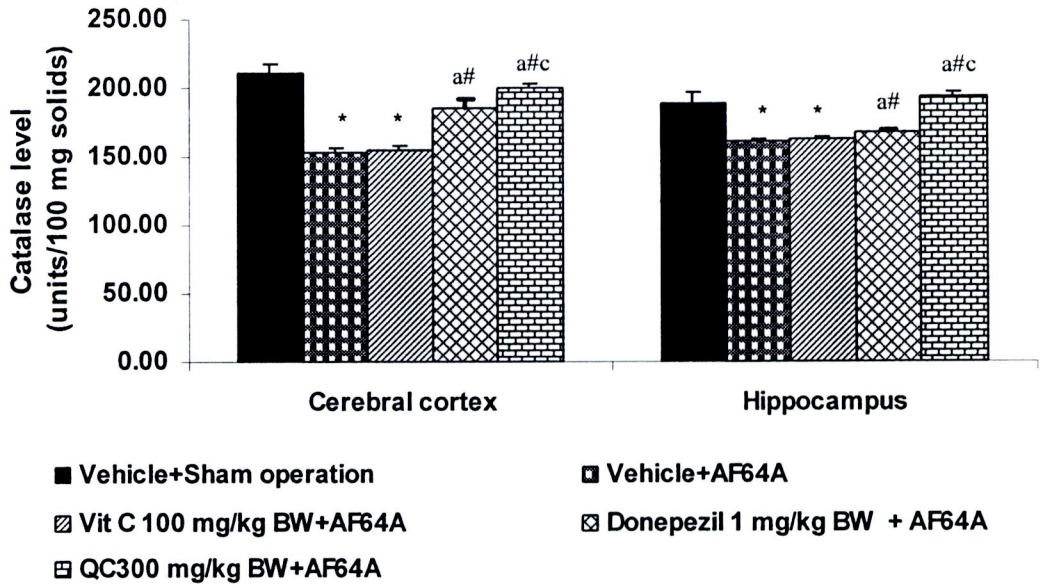

Figure 48 Effect of quercetin via oral administration on the activity of catalase (CAT) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of catalase (CAT) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A, $^{\#}p < 0.05$ vs. Vit C + AF64A and $^{c}p < 0.05$ vs. donepezil + AF64A.

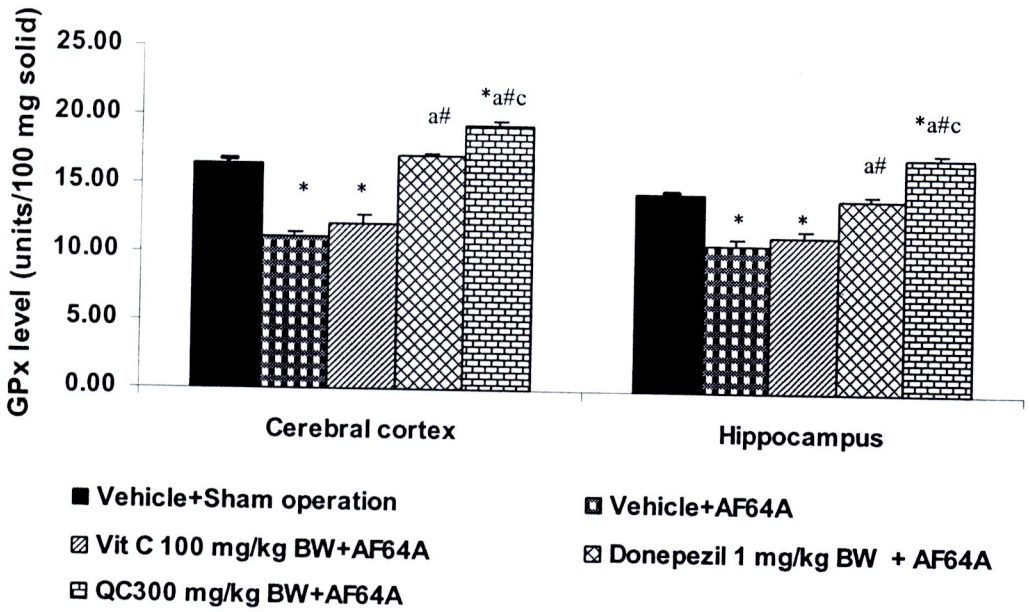

Figure 49 Effect of quercetin via oral administration on the activity of glutathione peroxidase (GPx) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with vehicle, Vitamin C (100 mg/kg BW), donepezil (1mg/kg BW) or quercetin (300 mg/kg BW) via intragastric tube 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of glutathione peroxidase (GPx) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. vehicle + Sham operation, $^{a}p < 0.05$ vs. vehicle + AF64A, $^{\#}p < 0.05$ vs. Vit C + AF64A and $^{c}p < 0.05$ vs. donepezil + AF64A.

In order to determine the possible mechanisms underlying the cognitive enhancement and the neuroprotective effect of quercetin liposomes administered via nasal route against Alzheimer's disease.

The results showed that AF64A treatment caused significant increase in level of MDA in both cerebral cortex and hippocampus, as compared with vehicle (PEG) + sham operation and free liposomes + sham operation ($P < 0.05$). However, the rats treated with nasal administration of quercetin liposomes significantly reduced ($P < 0.05$) the elevation of MDA content induced by AF64A in all areas as mentioned earlier (Figure 50).

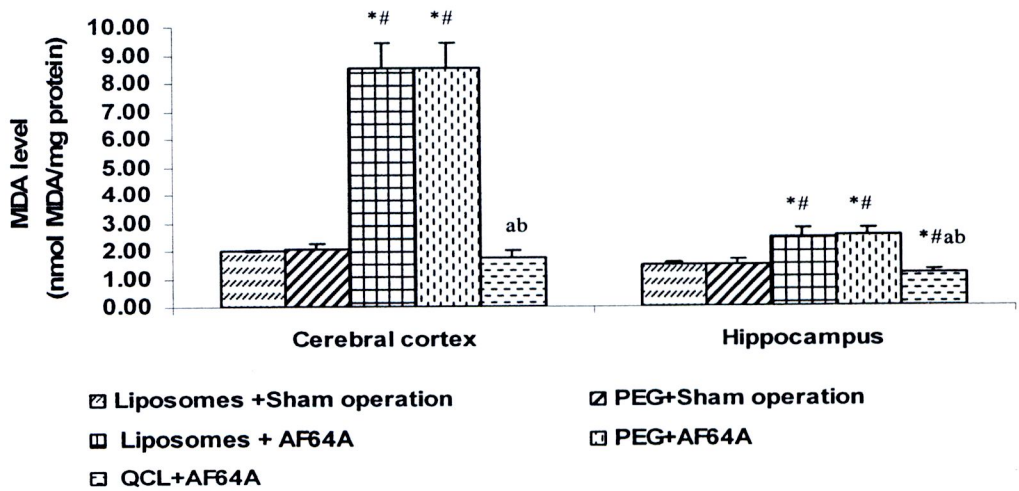

Figure 50 Effect of quercetin via nasal administration on the level of malondialdehyde (MDA) in both cerebral cortex and hipocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and and 1 week after AF64A administrations, then the animals were sacrificed and the brains were removed in order to determine the level of MDA in both cerebral cortex and hipocampus. Data were presented as mean ± S.E.M. (n=8/group). $^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

To determine whether quercetin liposomes could attenuate the increased oxidative damages in the brain of AF64A treated rats, the activities of major antioxidant enzymes, including SOD, CAT and GPx, in both cerebral cortex and hippocampus were evaluated in this study. The present results showed that quercetin liposomes could renew the activities of these antioxidant enzymes in the brain of AF64A treated rats ($p < 0.05$) as compared to the vehicle (PEG) + AF64A and free liposomes + AF64A treated group (Figures 51-53).

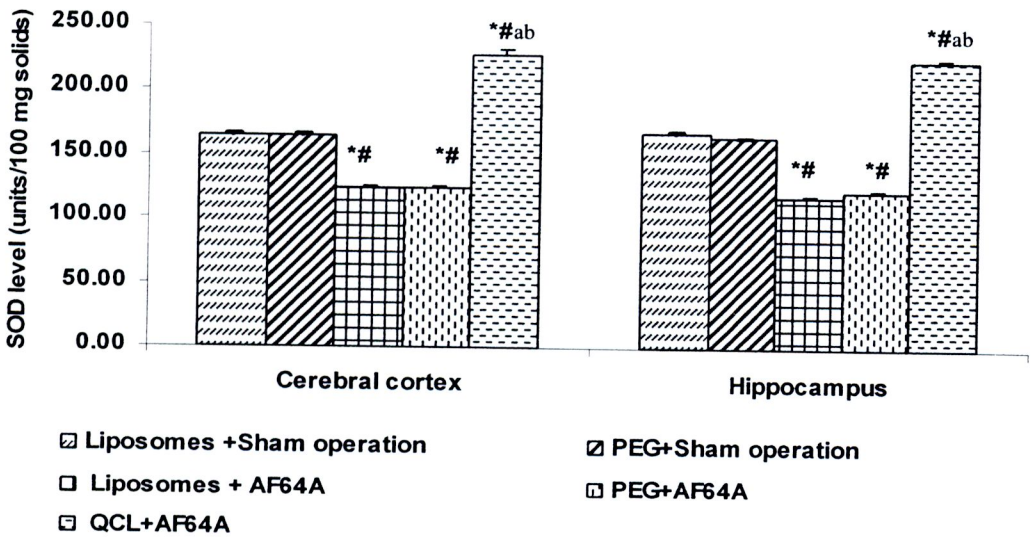

Figure 51 Effect of quercetin via nasal administration on the activity of superoxide dismutase (SOD) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of superoxide dismutase (SOD) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

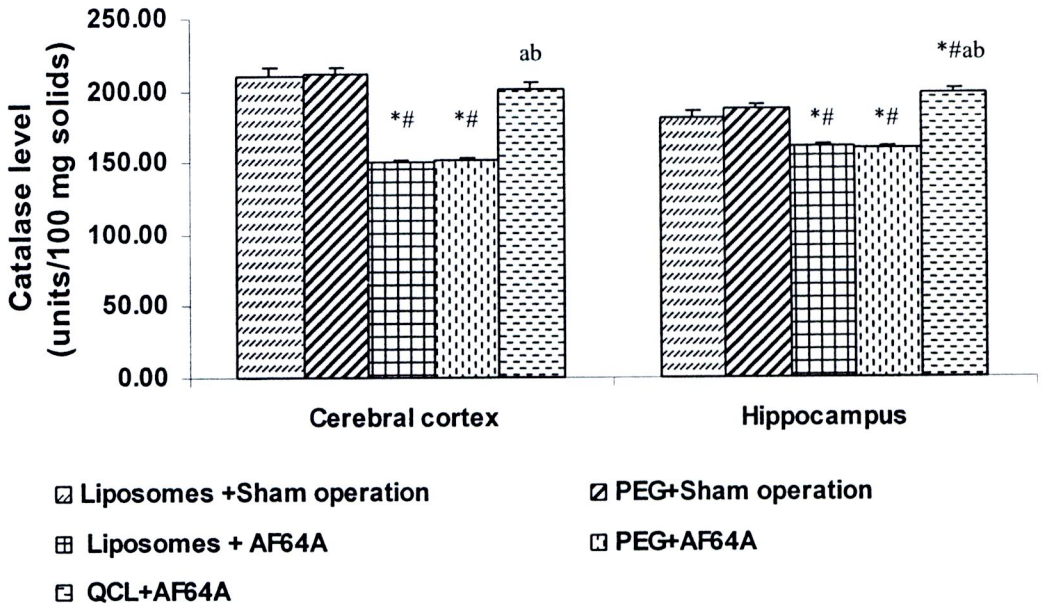

Figure 52 Effect of quercetin via nasal administration on the activity of catalase (CAT) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of catalase (CAT) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group). $^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.

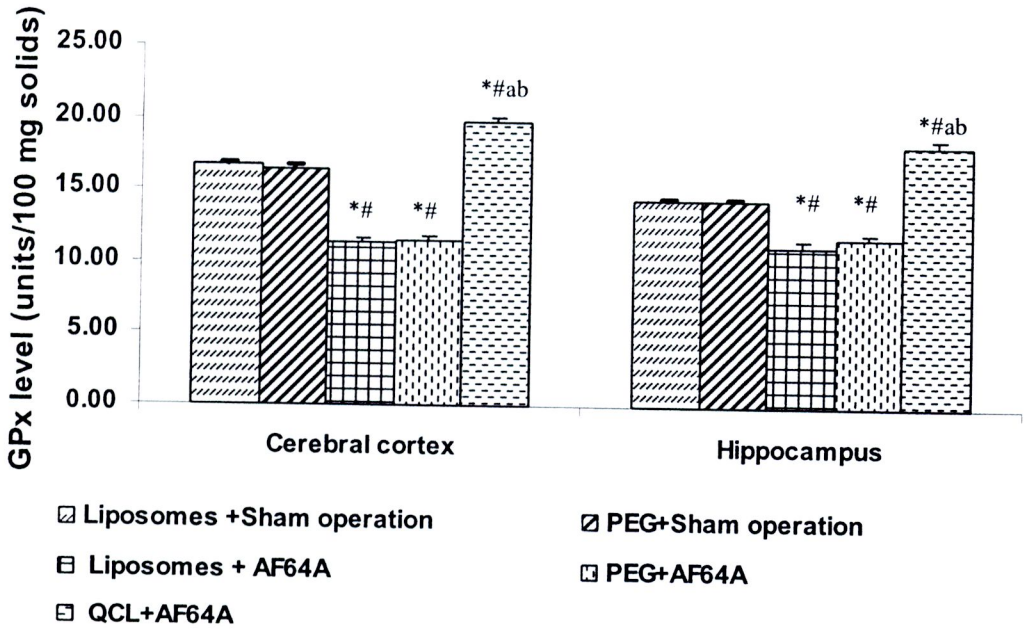

Figure 53 Effect of quercetin via nasal administration on the activity of glutathione peroxidase (GPx) enzyme in both cerebral cortex and hippocampus of cognitive deficit rats induced by AF64A. Each rat had been treated with free liposomes, vehicle (PEG) or quercetin liposomes administered via nasal route 2 weeks before and 1 week after AF64A administration, then the animals were sacrificed and the brains were removed in order to determine the level of glutathione peroxidase (GPx) activity in both cerebral cortex and hippocampus. Data were presented as mean ± S.E.M. (n=8/group).

$^{*}p < 0.05$ vs. free liposomes + Sham operation, $^{\#}p < 0.05$ vs. vehicle (PEG) + Sham operation, ^{a}p vs. free liposomes + AF64A and $^{b}p < 0.05$ vs. vehicle (PEG) + AF64A.