

A STUDY ON THE EFFECTS OF NANO ALGINATE/CHITOSAN/LOVASTATIN ON LOCOMOTION, EXPLORATION AND RECOGNITION ACTIVITY IN OBESITY RAT MODEL

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ABSTRACT

Objective: To investigate the effects of nano-Alginate/Chitosan/Lovastatin on locomotor activity, exploratory behavior, and cognitive performance in a high-fat diet-induced obese rat model.

Subjects and methods: An experimental, controlled, descriptive cross-sectional study on white male rats, including 30 rats (Group 1) fed a normal diet and 30 rats (Group 2) induced with obesity using a high-fat diet for 7 weeks. After 7 weeks, group 1 was randomly divided into 3 subgroups (1, 2, 3) and continued with a normal diet (group 1 received saline; group 2 received Lovastatin at a dose of 4 mg/kg; group 3 received nano Alginate/Chitosan/Lovastatin at a dose of 4 mg/kg) for 12 weeks; Group 2 was divided into 3 subgroups (4, 5, 6) and continued with a high-fat diet (group 4 received saline; group 5 received Lovastatin at a dose of 4 mg/kg; group 6 received nano Alginate/Chitosan/Lovastatin at a dose of 4 mg/kg) for 12 weeks. Rat behaviors were assessed after 12 weeks of intervention using the open-field and novel object recognition tests.

Results: The locomotion of rats in the open-field: No significant differences were found among the experimental groups in total traveled distance, moved time within the open-field arena, or traveled distance in the central zone of the open field test. The exploration and recognition of rats in the object Recognition: There was a significant difference in the discrimination ratio to explore object between old and new objects of rats groups in groups 1, 2, 3 and 6. While no significant differences ($p > 0.05$) were observed in Groups 4 and 5.

Conclusions: Nano Alginate/chitosan/Lovastatin improved locomotor activity, exploration and increased cognitive exploration and activity memory in obese rats fed a high-fat diet than in obese rats using Lovastatin alone and obese rats receiving NaCl.

Keywords: High-fat diet, locomotion, recognition, rat, Alginate, Chitosan, Lovastatin.

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1. INTRODUCTION

Overweight and obesity are worrying public health issues, not only in Vietnam but also globally [1]. Overweight and obesity are defined as having excess body fat, affecting health and potentially leading to many dangerous diseases [1], [2]. Many studies in the world have shown that obesity is related to central nervous system disorders (such as impaired locomotion, cognition and memory...) in both humans and experimental animals [3].

Among statin drugs, Lovastatin has the main effect of reducing blood lipids and preventing cardiovascular complications. Many studies have shown that drugs of statins also have ameliorative effects on functions of the central nervous system in obese patients and animals, such as improving learning ability, memory, and exploring ability...

[4], [5]. However, these drugs often have a short half-life and poor gastrointestinal tract absorption, so they are not highly effective. Therefore, many scientists are interested in the design of nanocarrier complex which is capable of delivering drugs to the right place, at the right time and the correct dose, helping to improve the drug stability, increase the duration, therapeutic effect [6].

Recently, research into the development of slow-release drugs has emerged as one of the solutions to improve treatment effectiveness. Applying this technology, the Institute of Tropical Technology (in the Vietnam Academy of Science) has successfully developed a slow-release nanoparticle-containing drug, Lovastatin (named the Alginate/Chitosan/Lovastatin nano composite), created from readily available natural materials in Vietnam [6].

In this study, we used a slow-release Lovastatin complex in experimental animals to evaluate the effects of the nano Alginate/Chitosan/Lovastatin complex on locomotion, exploration, and cognition function in experimental animals with an induced obesity model.

2. SUBJECTS AND METHODS

2.1. Subjects, chemicals

- Subjects: 60 white male rats, aged 8 -9 weeks old (100-150 g). White rats were provided by Animal Center of Vietnam Military Medical University (VMMU) ; Rats were raised in good conditions and 12/12 hour light-dark cycle; enough food, drinking water and Experimental procedures and care for experimental animals were performed according to the guidelines of the Laboratory Animal Supply Division, VMMU.

- Chemicals: Nano Alginate/Chitosan/Lovastatin with ratio 8:2:10, power,(Institute of Tropical Technology, Vietnam Academy of Sciences) [6]), natri clorid 0,9% (NaCl 0,9%), lovastatin powder (Sigma Aldrich).

2.2. Methods

- Research design: This is an experimental, controlled, cross-sectional study.

- Equipment, instruments, and chemicals used in the research:

+ Quiet laboratories, the stable temperature at $25 \pm 1^{\circ}\text{C}$.

+ Open-field - evaluating behavior: a cylindrical open-field made of composite with diameter 80cm and height 25 cm, black-painted inside, divided according to the behavioral program design into the central zone (centre, 25 cm in diameter) and the peripheral zone (Figure 1A).

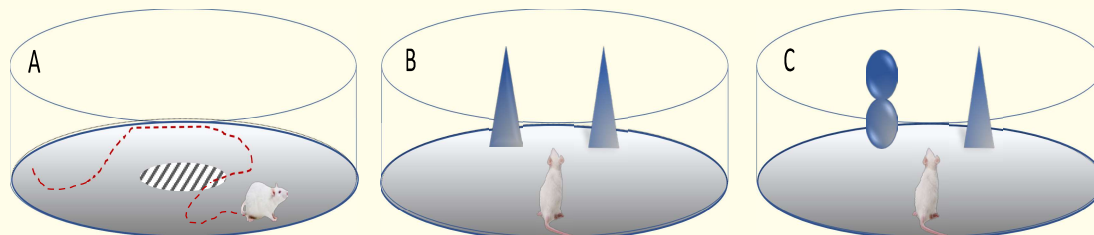


Figure 1. Open-field: A - behavioral assessment (including the central area as the striped region and the motor pathway as the dashed line); B - assessment of the recognition of two identical cone-shaped objects; C - assessment of the recognition of two objects with a new object (snowman)..

+ Object recognition maze: using open-field. The designed object consists of two identical white ABS plastic objects, cones and a snowman-like object consisting of two overlapping spheres, of the same height (15.5 cm high, diameter base 7.5 cm) (Figures 1B and 1C).

+ Behavioral recording and analyzing system: using C525 HD camera (Logitech, USA) connected to a computer with ANY-MAZE (Stoelting, USA) for recording and analyzing rat activity during the open-field and object recognition tests.

- Randomly divide 60 rats into 2 groups, each group with 30 rats. Group 1 rats were fed a normal diet and Group 2 rats were given an obesity model diet with high-fat food according to Nguyen Thi Hoa's research [7], for 7 weeks. Then, the rat in each group were divided into 3 subgroups, each subgroup with 10 rat (Group 1 into subgroups 1, 2, 3; Group 2 into subgroups 4, 5, 6). The rat in each subgroup were fed (for 12 weeks) with the following diet:

- + Group 1: normal diet-saline group (NaCl 0,9%).
- + Group 2: normal diet-lovastatin receiving Lovastatin at 4 mg/kg.

+ Group 3: normal diet - Alginate/Chitosan/ Lovastatin nano combination of 4 mg/kg.

+ Group 4: high-fat diet-saline.

+ Group 5: high-fat diet-lovastatin dosed 4 mg/kg.

+ Group 6: high-fat diet - Alginate/Chitosan/ Lovastatin nano combination dosed 4 mg/kg.

- Assessing the motor, cognitive, and exploratory behavior of rats in an open field after 12 weeks of treatment:

+Open-field test (assessing locomotion, freedom of exploration, related to anxiety and fear when contacting new or unfamiliar environments) [4], [5]): Rats were allowed to explore the maze freely for 5 minutes and its behaviors were recorded and analyzed by Any-maze program in the zone of the open-field (bottom zone - the entire open-field; central zone - 25 cm in diameter and peripheral zone). These include the following indicators: distance traveled (the length of distance the mouse moves in 5 minutes, measured in meters); and locomotion duration in the zones (the total time the mouse spends in the respective zones, measured in seconds).

+ The Novel Object recognition test (NOR): evaluates new object exploratory and memory behavior in the open-field): The NOR test was conducted sequentially in three phases, each phase lasted 5 minutes, including Habituation phase (The animals were allowed to freely explore the environment - figure 1A); The sample phase (two identical objects in shape, material, and color were added bilaterally inside the open-field and the animals could explore and remember the environment - Figure 1B); The test phase (different shape but same material and color, which had been replaced for one of the two objects in the sample phase); The rats were free to explore the environment (old and new objects) - figure 1C.

The ability to remember and recognize familiar and novel objects was demonstrated by the number of times exploration and the ratio of time exploration, defined as the percentage (%) of time exploring the novel object to a total time of exploring objects.

- Ethics: The research was conducted in accordance with the procedures of the Military Medical University. The research data was authorized for use and publication by the Department of Physiology, Military Medical University. The authors commit to no conflict of interest in the research.

- Data analysis: Statistical algorithms run on SPSS Statistics 20.0 software (IBM, USA). Differences in research indicators between groups were analyzed using a two-factor analysis of variance (diet and drug). Statistically significant differences were determined with $p < 0.05$. The data in this study were expressed as mean values ($X \pm SD$).

3. RESULT

3.1. Spontaneous locomotion, exploration activity in the open-field

The total distance traveled and mobile time in the entire open-field arena and central zone of the open-field are shown in Figures 2 and 3.

Figure 2 shows that the distance traveled by the analysis of variance showed no interaction between the two factors (diet and drug), with $p > 0.05$, but there was a difference in diet ($F_{1, 56} = 6.194$; $p = 0.016$). Analysis of each diet showed that, there were no significant difference between the three groups of a normal diet (groups 1, 2, and 3) and the three groups of a high-fat diet (groups 4, 5, and 6) ($p > 0.05$). Two-way ANOVA showed that there were no differences in mobile time between diet and drug, there was no interaction between these two factors ($F_{2, 56} = 0.112$, $p = 0.895$).

With the drug factor of each diet, there were no difference between the three groups in normal diets (groups 1, 2, 3) and between the three groups in high-fat diets (groups 4, 5, 6). ($F_{\text{normal diet (2, 28)}} = 0.308$, $p = 0.737$; $F_{\text{high-fat diet (2, 28)}} = 0.673$, $p = 0.518$).

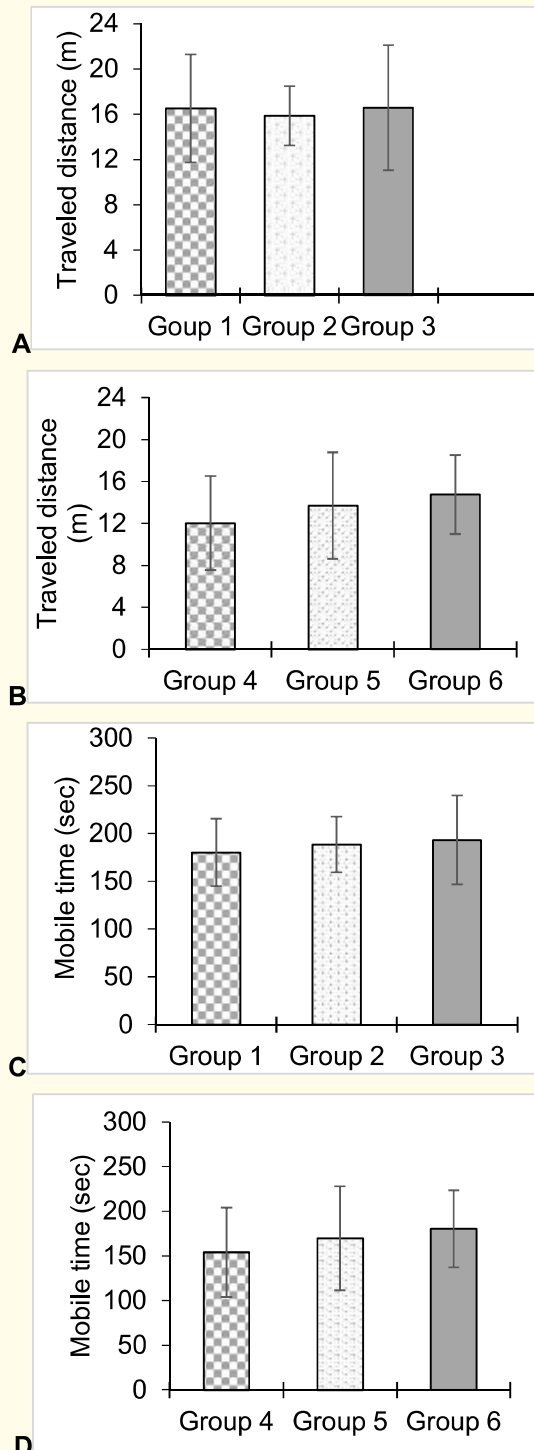


Figure 2. Traveled distance (A: groups 1, 2, 3; B: groups 4, 5, 6) and mobile time (C: groups 1, 2, 3; D: groups 4, 5, 6) in the open field of rats

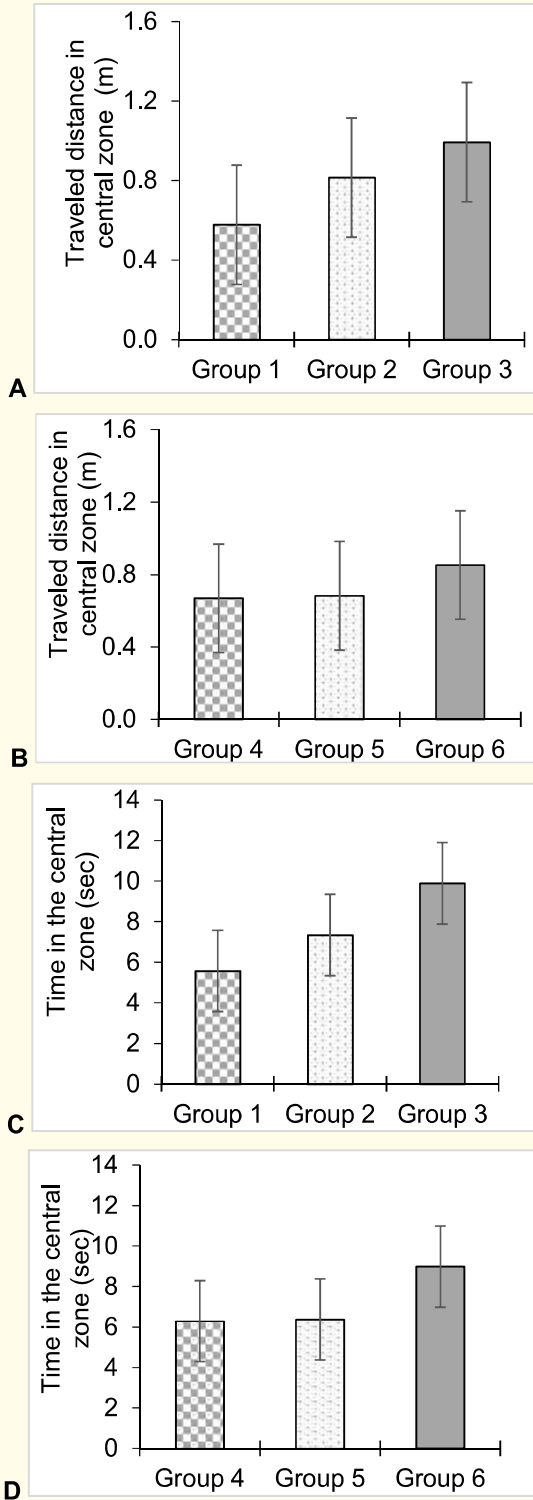


Figure 3. Travelled distance (A: groups 1, 2, 3; B: groups 4, 5, 6) and mobile time (C: groups 1, 2, 3; D: groups 4, 5, 6) in the central zone of rats in the open field.

Figure 3 shows that the traveled distance in the central zone did not differ significantly with respect

to either diet and treatment factors ($p > 0.05$), and no interaction was observed between these two factors ($F_{2, 56} = 0,330, p = 0,721$). Evaluation of treatment effects within each dietary condition revealed no significant differences among Groups 1, 2, and 3 (normal diet) ($F_{\text{normal diet}}(2, 28) = 1,377, p = 0,269$); Similarly, no significant differences were found among Groups 4, 5, and 6 (high-fat diet) ($F_{\text{high-fat diet}}(2, 28) = 0,497, p = 0,614$). The mobile time in the central zone also showed no significant differences with respect to diet and treatment ($p > 0.05$), and no interaction was detected between the two factors ($F_{2, 56} = 0,144, p = 0,866$). Analysis of treatment effects within each dietary condition demonstrated no significant differences among the three treatment groups under the normal diet condition (Groups 1, 2, and 3) or under the high-fat diet condition ($F_{\text{normal diet}}(2, 28) = 1,153, p = 0,330$; $F_{\text{high-fat diet}}(2, 28) = 0,988, p = 0,385$).

3.2. Cognitive exploratory behavior in the novel object recognition test

The exploration time ratio of object in the experimental groups under both dietary conditions during the sample and the testing phases post-intervention are presented in Figures 4 and 5.

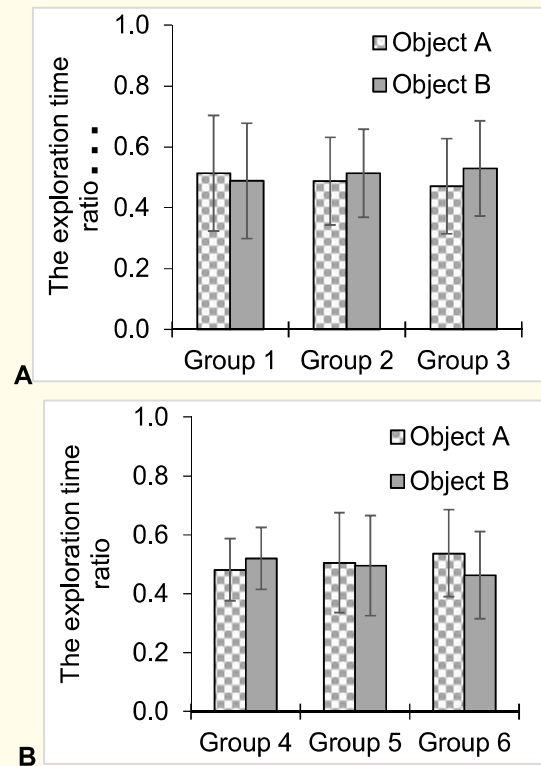


Figure 4. The exploration time ratio in the sample phase in the open-field of the investigated groups of rats fed with normal diet (A) and high-fat diet (B) after drug intervention.

The results presented in Figure 4 for The exploration time ratio showed no interaction among the three factors (diet, object, and group) ($p > 0.05$).

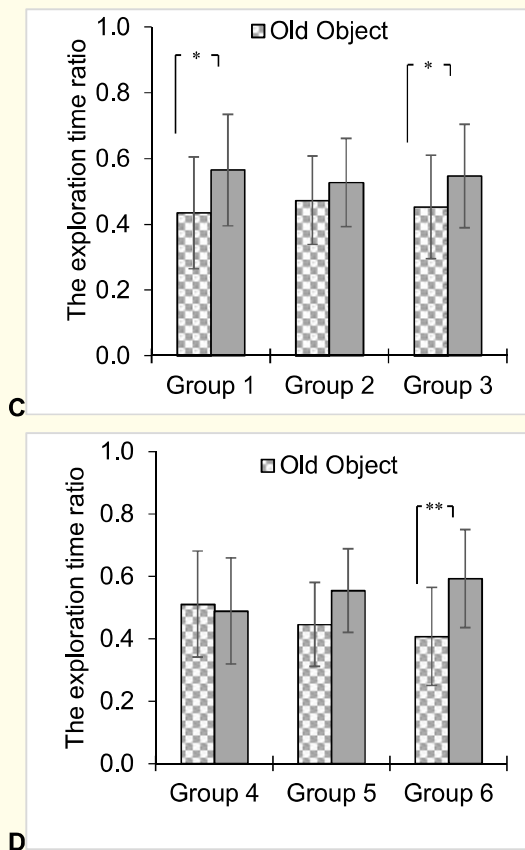


Figure 5. The exploration time ratio in the test phase in the open-field of the investigated groups of rats fed with normal diet (C) and high-fat diet (D) after drug intervention.

*: $p < 0.05$; **: $p < 0.01$.

During the testing phase, Figure 5 shows a significant difference in the exploration time ratio between the familiar and novel objects ($F_{1,110} = 12.000, p = 0.001$). However, no significant differences were observed in the exploration time ratio between the two dietary conditions ($F_{1,110} = 0, p = 1.0$) or among the treatment groups ($F_{2,110} = 0, p = 1.0$). Two-way ANOVA followed by Bonferroni analysis revealed a significant difference in the exploration time ratio between the two objects in Groups 3 and 6 treated with the nano-Alginate/Chitosan/Lovastatin complex under both dietary conditions ($p = 0.002$). In contrast, no significant differences were observed between the two lovastatin-treated groups (Groups 2 and 5) ($p = 0.082$) or between the two NaCl-treated groups (Groups 1 and 4) under the two dietary conditions ($p = 0.244$). A significant difference in the exploration time ratio between

the two objects was observed in the NaCl-treated group under the normal diet condition (Group 1) ($p = 0.049$), whereas no significant difference was found under the high-fat diet condition (Group 4) ($p = 0.737$). In the groups treated with nano-Alginate/Chitosan/Lovastatin, no significant difference in the exploration time ratio between the two objects was observed under the normal diet condition (Group 3) ($p = 0.153$), whereas a significant difference was detected under the high-fat diet condition (Group 6) ($p = 0.004$).

4. DISCUSSION

The open-field locomotor task is widely used to assess locomotor activity, exploratory behavior, and certain “emotional” responses in rodents [4, 8]. In a freely moving environment, locomotion is typically accompanied by exploratory behavior. The commonly used parameters to quantify these behaviors include the travelled distance, time, and average speed within the entire open-field arena as well as in specific zones of the arena (depending on the experimental design). In the present study, statistically significant differences were observed among the experimental groups across all three measured parameters, including mean locomotor speed, total distance traveled, and movement time both in the entire arena and in the peripheral zone of the open field. Obese model rats exhibited reduced traveled distance, lower average speed, and shorter movement time compared with rats fed a standard diet. The results also indicated that obese model rats showed impaired exploratory locomotor activity, characterized by reduced movement across all areas of the open field, compared with the normal diet group. [3], [4], [5].

The object recognition task is designed to assess exploratory behavior, learning ability, memory, and discrimination in animals, based on their natural tendency to explore novelty. The task consists of two phases: a sample phase (memory acquisition phase) and a testing phase (memory retrieval phase). During the task, animals are allowed to freely explore objects in the environment (in the sample phase, two identical objects are presented; in the testing phase, one of the objects is replaced with a novel object). To evaluate these abilities, commonly used parameters include exploration time or exploration time ratio and the number of interactions with each object.

In the present study, during the sample phase (learning phase), when two identical objects A and

B were presented, animals in both groups showed similar exploration time ratios and similar numbers of interactions with both objects. During the testing phase, when object B was replaced by a novel object, rats in Groups 1, 2, and 3 exhibited higher exploration time ratios and a greater number of interactions with the novel object compared with the familiar object. In contrast, no significant differences in these exploratory indices were observed between the two objects in Groups 4, 5, and 6.

These findings suggest that a high-fat diet may impair exploratory cognitive function in animals, which is an important step in memory formation. Chen (2017) [3] used the object recognition task to evaluate the effects of an obesity-inducing diet on exploratory and cognitive performance by comparing total exploration time between two groups across both phases, as well as the percentage of time spent exploring each object. The results demonstrated that rats fed an obesity-inducing diet exhibited impaired object recognition performance compared with normal diet.

After the 12-week intervention period involving continuous administration of the pharmacological agent in combination with diet, the open-field test results indicated that the total distance traveled, movement time, and exploratory activity in both the central zone and the entire arena in Group 6 tended to increase compared with Group 5 and were higher than those observed in Group 4. Previous pharmacological studies have shown that oral administration of *Morus alba* (white mulberry) extract increases locomotor activity in obese rats compared with untreated obese controls [8]. Go (2017) [9] also reported that Ephedra extract improved locomotor function in diet-induced obese rats. The present study similarly demonstrated that Group 4 (obese rats receiving only 0.9% NaCl) exhibited lower distance traveled and reduced time spent in the central zone compared with Group 5 (lovastatin-treated group) and Group 6 (nano-Alginate/Chitosan/Lovastatin-treated group). Reduced exploratory activity in untreated obese rats may contribute to depression- and anxiety-like behaviors [10]. The higher level of central zone activity observed in Group 6 compared with Groups 5 and 4 suggests an anxiolytic-like effect of the nano-Alginate/Chitosan/Lovastatin formulation. In general, reduced anxiety is associated with increased locomotor and exploratory activity; accordingly, animals exhibit greater movement and explore all areas of the open-field arena more extensively. The mechanisms underlying the effects

of lovastatin on locomotor activity, exploration, and anxiety may involve neuroprotective actions, inhibition of inflammatory cell activation, and reduction of dopaminergic neuronal oxidative stress, as reported by several authors [4, 5]. Overall, these findings suggest that obese rats treated with nano-Alginate/Chitosan/Lovastatin (Group 6) showed partial improvement in locomotor activity, enhanced exploratory behavior, and reduced anxiety-like responses, leading to increased movement and more uniform exploration across all regions of the open field compared with obese rats treated with lovastatin alone (Group 5) and untreated obese controls (Group 4). Several studies have demonstrated that lovastatin influences cognitive function and memory retrieval processes [4, 5]. To evaluate the effects of nano-Alginate/Chitosan/Lovastatin on cognitive performance in obese rats, we employed the object recognition task, which consists of two phases: a sample phase (memory acquisition) and a testing phase (memory retrieval). The results of the sample phase showed that the three treatment groups receiving NaCl, lovastatin, and nano-Alginate/Chitosan/Lovastatin under both normal diet (Groups 1, 2, 3) and high-fat diet conditions (Groups 4, 5) exhibited similar exploration time ratios for the two identical objects, with no significant differences observed. Notably, during the testing phase, when one familiar object was replaced with a novel object, the results indicated that Groups 1, 2, and 3 showed a higher exploration time ratio for the novel object compared with the familiar object. Group 6 also exhibited a higher exploration time ratio for the novel object than for the familiar one. Group 5 showed a tendency toward differences in exploration time ratios between the two objects, whereas Group 4 showed no significant difference in exploration between the novel and familiar objects. Untreated obese rats (Group 4) spent equal time exploring both objects, suggesting impaired cognitive function and short-term memory deficits [4]. In contrast, Group 6 showed a significant increase in the exploration time ratio between the two objects, with a preference for the novel object. Our findings indicate that nano-Alginate/Chitosan/Lovastatin improves cognitive exploratory function and memory in obese rats more effectively than lovastatin monotherapy and NaCl treatment. These results are consistent with previous studies, such as Metwally (2019) [8], which reported that *Morus alba* extract improved exploratory cognitive performance and working memory in diet-induced obese rats after 8 weeks of continuous treatment compared with untreated obese controls.

5. CONCLUSION

Based on the obtained results, nano-Alginate/Chitosan/Lovastatin improved locomotor activity, exploratory behavior, and anxiety-like states in high-fat diet-induced obese rats more effectively than in obese rats treated with lovastatin alone or NaCl.

Nano-Alginate/Chitosan/Lovastatin also enhanced exploratory cognitive function and working memory in high-fat diet-induced obese rats to a greater extent compared with obese rats receiving lovastatin monotherapy or NaCl treatment.

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