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RESEARCH ARTICLE

Study of Induced Inflammation Effect on Motor Activities and Coordination in Wistar Rats

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ABSTRACT

This study was designed to investigate the effect of egg albumin extract on motor activities and coordination using 20 adult wistar rats; they were divided into four groups, each consisting of five animals in each compartment. The rats were all induced with inflammation using egg albumin. Group 1 which was the control not subjected to any inflammation. Group 2 were given 0.3ml of albumin on their forelimb paws intradermally, a known extract that causes inflammation. Group 3 were given 0.3ml of albumin on their hind limbs paws intradermally. Group 4 were given 0.3ml of albumin on their forelimbs paws intradermally and after four hours, 1ml of indomethacin was given intraperitoneally (A non-steroidal anti-inflammatory drug). The research work as an acute experiment lasted for 14 days. After induction of egg albumin administration of same dose, there was gradual inhibition of motor activities and coordination. The data derived shows that induced inflammation using egg albumin has influence on the motor activities and coordination when compared to the control. In membrane stability study with aspirin (indomethacin) shows about 91% effectiveness in stability.

Keywords: inflammation, indomethacin, motor activities, intradermal, intraperitoneal.

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1. Introduction

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators (Fagundes, 2015). Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Inflammation is therefore normally closely regulated by the body (Hagerstown, 2007).

Egg albumin emboldens many chemical compounds, which have a potential value in the laboratory but some of the compounds can also be poisonous. All the proteins of the albumin family are water-soluble, moderately soluble in concentrated salt solutions, and experience heat denaturation. Albumins are commonly found in blood plasma and differ from other blood proteins in that they are not glycosylated. Josse et al. (2010) concluded that milk (albumin) supplementation during RT effectively promoted changes in body composition. Nevertheless, there is a lack of reports in the field that explore such effects in wistar rats and athletes.

2. Materials and Methods

Collection of Experimental Animals:

Twenty (20) healthy wistar albino rats of mixed sex were used for this study, and were bred and housed in the pre-clinical house, college of Medicine, University of Port Harcourt, Nigeria. The animals were kept and maintained under laboratory conditions of temperature, humidity and light; and were allowed free access to food (standard pellet) and drinking water *Ad libitum*. The experimental protocols and procedures used in this study were approved by Ethical Committee, University of Port Harcourt, and Rivers State, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH Publication No. 85-93, revised 1985)

Acclimatization of the Experimental Animals:

After identification, the animals were housed in a wire mesh cage under standard condition (Temperature 25-29°C, Hours light/ darkness cycle), for four (4) weeks as to acclimatize with the environmental condition of the University of Port Harcourt. Following 14 days of acclimatization, the twenty wistar rats were divided into four groups. Each group consisted of five rats and was housed in separate compartments of the wooden cage.

A Control group 1(non-inflamed)

They were fed with standard rat feed and distilled water *ad libitum*. This group serves as the control.

- Group 2 (inflammation on the forelimbs)
- Members of this group they were induced with the 0.3ml of egg albumin just before tests for motor activities and coordination were carried out.
- Group 3 (inflammation on the hind limbs)

Members of this group, they were induced with the 0.3ml of egg albumin just before tests for motor activities and coordination were carried out.

C Group 4 (inflammation + treatment)

Members of the group, they were inflamed with the egg albumin on their paws and after four hours they were treated intraperitoneally then after 30 minutes motor activities and coordination test was carried out.

Administration of Extraction and Standard Drugs:

The extracts were given to the rats intraperitoneal using 1ml syringe and the standard drugs (indomethacin) was given intraperitoneally. 0.3ml of each dosage of egg albumin were given to members of group two, group three for 14 days.

20ml of water (saline) was poured into the beaker and was mixed with one capsule of indometacin. Which the animals in group 4 received 1ml of the dilute (normal saline) with indomethacin via intraperitoneal injection.

Experimental Test For Inflammation

Five experimental tests were done in this study to determine the motor activities and coordination of the experimental animals. Some of the test were time based (i.e. measured in the amount of time spent in carrying out in a task) whereas others were qualitative assessment of the level of response or performance of the rats showed. These tests are as follows;

Light-Dark Box Test:

This test was performed using the light dark box which has two compartments. The light compartment is 2/3 of the box and is brightly lit and open. The dark compartment is 1/3 of the total box and it's covered and dark. A door of 7cm connects the two compartment (ennaceur et al, 2009).The rats were thrown into the light phase and was allowed to enter the dark box phase and back to the light phase for 5 minutes. Light or dark box entries were recorded using a stop watch as timer. Light entries simply refers to the number of times the rats enters the light box, light box time spent refers to the time spent in the light phase. Dark box entries refer to the number of times the rats enters the dark box phase. Dark box time spent refers to the time spent in the dark box phase. Behavioural scoring was performed according to. And during each five minutes test session, two behaviours were observed according to their motor activities and coordination in different phases of the box.

Elevated Plus Maze:

This test was performed using the elevated plus shaped maze. The model was designed in such a way that it shows some behavioural tendencies of the experimental animals. It has two sections; the enclosed arms and open arms.

A. The enclosed arms has an enwrapped covering on it, which an open end on the extremes.

B. The open arms don't have any covering on it and it has an free open end at both extremes.

Opaque Maze Test (Navigation Maze Test):

The design is in a form of maze which has many compartments and outlets and it was used to perform the test. The animal was thrown into the maze box from one end and was allowed to navigate its way through many outlets to the other end. Then the total time spent was recorded in seconds.

Hand Grip Test:

This test was performed with a tiny iron rod which the rat was made to grip the iron rod with its forelimb paws while its hind limbs and tail were held downward and after some seconds the forelimb paws got weakened and fell. The total time spent during the hand grip was measured in seconds.

Climbing Test:

The test was performed with a tiny stick and was slantly placed, with its extreme side slightly deep into the water to allow the rat to climb through the slanted stick and the other extreme side was above the water. The total time the rat climbed through the tiny stick was measured in seconds.

Statistical Analysis:

The data obtained from this study were statistically analysed using one way analysis of variance (ANOVA). Statistical comparison of groups 1,2,3,4 were made by one way post hoc analysis. Means were considered at $p < 0.05$.

3. Results and Discussion

The purpose of this study was to evaluate the effect of induced inflammation on motor functions. The experimental procedures were carried out on wistar albino rat using the following tasks; elevated plus maze test, light-dark box test, opaque maze test (navigation maze), hand grip test, climbing test. Observation from the navigational maze study as shown in Table 4.8 revealed that there was a significant ($P < 0.05$) change in the time it took to navigate through the maze from 32.20 ± 8.47 of the 1st session to 128.80 ± 56.72 of the 3rd session of the control group compared to the test groups. Comparatively, the induced-inflammation- group performed the slowest, followed by group 4 (induced + treatment). Quantitatively, the percentage performance analysis revealed that group 1 (control) performance was enhanced significantly ($p < 0.05$) than other groups followed by group 3 then Group 2 (inflamed group), group 4 (inflammation + treatment) followed in that order.

The elevated plus maze is an animal model of anxiety that usually uses rodents as a screening test for putative anxiolytic or anxiogenic compounds and as a general tool in neurobiological anxiety research. The model is based on the test animal's aversion to open spaces and tendency to be thigmotactic (a preference to remain near to, or touching, vertical surfaces). In the elevated maze plus, this is expressed as the animal spending more time in the enclosed arms (Pellow, 2005). Results from the elevated maze test as shown in Table 4.0 revealed that, the group 4 (inflamed + treatment) rats spent more significant time in the closed arm compared to the open arm with the control in all the groups, while group 2 spent more significant time in the open arm compared to the control. In these inflammatory models, hypersensitivity and drug effects are usually quantified by observational rating of paw pressure against the floor during standing or walking but even by evoked responses to thermal and mechanical stimuli such as paw withdrawal, vocalization and struggling (Coderre and Wall, 1987; Yu et al., 2003). Results from the climbing test using the Morris Water Maze as shown in Table 5.0 revealed that group 4 (inflamed + treatment) was significantly ($P < 0.05$)

the fastest compared to group 3 then group 1. Group 2 performed the slowest. Equally, the percentage performance analysis revealed that the performance of the inflamed + treated group was enhanced significantly ($P < 0.05$) All groups were positively enhanced.

The Morris water navigation task, also known as the Morris water maze (swimming test/climbing), is a behavioural procedure mostly used with rodents. It is widely used in behavioural neuroscience to study spatial learning and memory (D'Hooghe De-deyn, 2001). It can be a very accurate study of learning, memory, and spatial working and can also assess damage to cortical regions of the brain (O'Keefe and Burgess, 1996). It is used largely by neuroscientists to measure the effect of neurocognitive disorders on spatial learning and possible neural treatments, to test the effect of lesions to the brain in areas focused on memory, motor activities and to study how age influences cognitive function and spatial learning (Sharma and Savil, 2010).

Results from the light – dark box test as shown in Table 4.4 revealed that group 2 (inflamed) significantly ($P < 0.05$) preferred light phase compared to group 1 (control) then group 4. Group 3 performed the slowest in the light compartment phase. While in the dark compartment phase group 1 (control) significantly ($P < 0.05$) preferred the dark phase compared to group 2 and group 4. group 3 performed the slowest. Equally, the percentage performance analysis revealed that the performance of the group 2 (inflamed) was enhanced significantly ($P < 0.05$).

Rodents prefer darker areas over lighter areas. However when presented in a novel environment, rodents have a tendency to explore. These two conflicting emotions lead to observable anxiety like symptoms. Rodents typically spend more time in the dark compartment than in the light compartment. If rodents are injected with anxiolytic drugs, percentage of time spent in the light compartment will increase. Locomotion and rearing, which is when the rodent stands up on its hind legs and is a sign of exploration, in the dark compartment also increase. When injected with anxiogenic drugs, more time is spent in the dark compartment (Bourin and Hascoet, 2003).

Increased locomotion effects can affect the percentage of time spent in the compartments and rearing. The hand grip test results shown in Table 4.9 revealed that group 3 (inflamed) was significantly ($P < 0.05$) has more muscle strength compared to group 1 (control) then group 2. Group 4 (inflamed + treatment) performed the slowest.

Equally, the percentage performance analysis revealed that the performance of the group 3 (inflamed) was enhanced significantly ($P < 0.05$). Models with neuromuscular disease has more muscle strength. The Hand Grip Test has been documented in numerous literatures, and is included in the Functional Observational Battery (FOB) to screen for neurobehavioral toxicity. This can have a clinical significance.

4. Conclusion

From the experimental data gathered from this research work, it can be concluded that egg albumin has an inflammatory impact on motor activities and coordination in albino wistar rats. The study clearly showed that some groups inflamed with egg albumin exhibited some degrees of partial inhibition of motor activities and coordination and while also showing some significant behavioural activity.

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