



***Nigella sativa* (BLACK SEED) EXTRACT IMPROVES SPATIAL LEARNING ABILITY IN ALBINO MICE**

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ABSTRACT

This study was carried out to assess the memory enhancing effect of Nigella sativa Extract on mice using Morris Water Maze. The study was conducted on 30 Albino mice of both sexes randomly divided into 5 groups with 6 animals each. Group 1 served as control and was treated with oral distilled water, Groups 2, 3 and 4 were treated orally with 1ml/kg, 2ml/kg and 4ml/kg body weight of the Nigella sativa Extract (NSE) respectively for 3 days, and Group 5 was treated with 100mg/kg body weight memory enhancing drug-Piracetam. Latency to locate the platform and the frequency of crossing the platform was measured. The result showed a decrease though not significant in latency to locate platform between training days 1 and 2 in 2ml& 4ml Nigella sativa groups (49.92±10.75 NSE 1 ml/kg; 29.63±7.12 NSE 2ml/kg; and 34.04±6.51 NSE 4ml/kg), also when compared to control and Piracetam groups, with the Piracetam group having a similar latency to control. The increase in there frequencies of platform crossing compared to control was significant at p=0.045 and was dose dependent (1.67±0.72 1ml/kg NSE; 1.83±1.60 2ml/kg NSE; 3.50±0.76 4ml/kg NSE; and 4.17±0.87 100 mg/kg Piracetam). These findings conclude that acute administration of Nigella sativa has a beneficial effect on learning and memory and has a better effect on learning but not memory than piracetam.

Key words: *Nigella sativa, learning, memory, Morris water maze*

INTRODUCTION

Nigella sativa (*N. sativa*) seed, popularly known as Black Seed or Black Cumin in English and Habbat-As-saudah in Arabic is well known for its culinary uses and as a natural remedy for array of ailments including, bronchitis, asthma, diarrhoea, and skin disorders around the world especially in the Muslim world, the Middle East and the Indian subcontinent. Scientific studies have shown it to possess amongst others, anti-inflammatory, antioxidant, antispasmodic and neuroprotective properties (Ahmad et al., 2013). NS has a long history of folklore usage in different civilizations and has been recognized as a 'miracle cure' for its ability to treat various diseases and assist the body in its own natural healing process (Goreja, 2003). In ancient texts and historical documents, NS has been mentioned as a notable healer for a range of ailments (Randhawa, 2008 & Sahak, 2016). The traditional practice of its usage in the muslim world is primarily due to the authentic prophetic statement that NS is a cure for all, except death; that was quoted by a renowned Muslim scholar, Al-Bukhari (1976).

The efficacy of the NS oil is mostly attributed to its quinone constituents in the NS fixed and essential oil, which is especially endowed with thymoquinone (TQ), a significant bioactive constituent making up 30–48% of the total compounds. Other functional components of the NS oil include *p*-cymene, carvacrol, thymohydroquinone (THQ),

dihydrothymoquinone(DHTQ), α -thujene, thymol, *t*-anethole, β -pinene, α -pinene, and γ -terpinene (Ahmad et al., 2013; Sahak, 2016; Khan, 1999).

The decline in or loss of the ability for learning and memory is a prominent feature of dementia, which affects millions of individuals all over the world. Alzheimer's disease (AD) is one of the most common subtypes of dementia (Fratiglioni, 1999). It is a neurodegenerative disease characterized by deposition of amyloid plaques, neurofibrillary tangles, cerebral oxidative stress, inflammation, neuronal loss and atrophy, and impaired neuronal function (Bamberger & Landreth, 2002; Di Patreet al., 1999, & Nicolakiset al., 2006). Although a lot of information is known about the pathology involved, treatment remains elusive at best.

The Black Seed of *Nigella sativa* has been historically and religiously used for thousands of years for preventing and treating many different kinds of diseases. Its antioxidant, anti-inflammatory, and neuroprotective properties could serve a therapeutic role in the management of neurodegenerative diseases in which memory impairment is associated.

The aim of this study was to assess the memory enhancing effect of *Nigella sativa* Extract on mice using Morris Water Maze. Its effect was also compared to piracetam, a nootropic derivative of GABA used in the treatment of cognitive disorders.

MATERIALS AND METHODS

Preparation of *Nigella sativa* Extract

Nigella sativa seeds were purchased from Samaru market in Zaria Nigeria in its ground form. 68.57g of the powder was extracted with 500mg of petroleum ether in a Soxhlet extractor for 12 hours. The solvent extracted was concentrated under reduced pressure by rotary evaporator.

Animals and Groupings

Thirty (30) albino mice of both sexes weighing 10-25g were obtained from the animal house of Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria. They were housed in metal cages and fed standard rodent pellet and tap water ad libitum. The animals were randomly divided into five groups of six animals each. Group 1 served as control and was treated with oral distilled water, Groups 2, 3 and 4 were treated orally with 1ml/kg, 2ml/kg and 4ml/kg body weight of the *Nigella sativa* Extract (NSE) respectively for 3 days, and Group 5 was treated with 100mg/kg body weight memory enhancing drug-Piracetam. All animal procedures were carried out in accordance with the guidelines of the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

Morris Water Maze Test

A Morris Water Maze modified for mice was used. The water maze was constructed out of a circular plastic pool that measured 110 cm in diameter and 20 cm in depth. The pool was filled to a depth of 14 cm with room-temperature tap water.

The pool was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. Boundaries of these quadrants were marked on the edges of the pool with masking tape and labeled: North, South, East and West. A Plastic rubber was used as the escape platform in the maze. The platform was filled with water to weigh it down in the pool. The level of the water in the pool was adjusted to within 0.5 cm below the surface of the striped top, thus creating an invisible escape platform.

Testing in the Morris Water Maze lasted for three days. The first two days were acquisition training days

(learning phase) with a visible platform and the third day was for probe trial (or memory retrieval phase) without a platform. There were four training trial sessions during the acquisition phase (2 minutes for each trial) per day with inter-trial interval of six minutes, during which latency to locate platform was recorded in seconds and averaged for the day. During the probe trial, frequency of platform crossing in sixty seconds was noted. This served as a measure of memory (Morris, 1984).

During the test period, each mouse was placed in a clean empty cage (no bedding). Tissue paper was torn and placed in the bottom to allow the mice to dry more quickly; this paper was replaced when it became completely wet.

Statistical Analysis

Results were presented as mean ± SEM. Data were analyzed using one way analyses of variance (ANOVA) followed by Tukey HSD multiple comparison test and a p value ≤ 0.05 was considered significant. The analyses were performed using SPSS statistical software for Windows Version 20.0 (SPSS Inc., Chicago, IL, 2011)

RESULTS

Table 1 shows result obtained for latency to locate platform and frequency of platform crossing in the MWM. There was a decrease though not significant in latency to locate platform between training days 1 and 2 in 2ml & 4ml *Nigella sativa* groups (49.92±10.75 NSE 1 ml/kg; 29.63±7.12 NSE 2ml/kg; and 34.04±6.51 NSE 4ml/kg). There was also a decrease in latencies to locate platform in all NSE treated groups when compared to control and Piracetam groups between training days 1 and 2. These increase in latencies were not significant. The Piracetam group had similar latencies to control. The increase in there frequencies of platform crossing compared to control was dose dependent (1.67±0.72 1ml/kg NSE; 1.83±1.60 2ml/kg NSE; 3.50±0.76 4ml/kg NSE; and 4.17±0.87 100 mg/kg Piracetam) and was significant for 4ml/kg NSE and Piracetam group.

Table 1: Effect of *Nigella sativa* on learning and memory in Morris water maze

GROUPS	LATENCY		FREQUENCY OF PLATFORM CROSSING
	DAY 1	DAY 2	
Control	60.58±10.35	43.04±13.25	1.67±0.42
NSE (1ml/kg)	39.92±3.39	49.71±10.75	1.67±0.72
NSE (2ml/kg)	39.67±6.81	29.63±7.12	1.83±1.60
NSE (4ml/kg)	57.21±6.93	34.04±6.51	3.50±0.76*
Piracetam (100mg/kg)	60.33±9.7	42.92±6.75	4.17±0.87*

NSE (*Nigella sativa* extract). Values are mean ±SEM (n=6). * significant at p≤0.05 compared to control

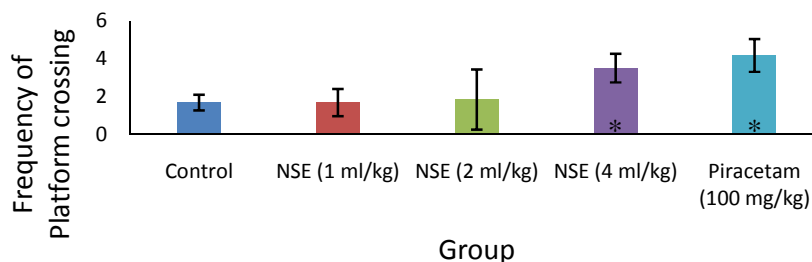


Figure 1: Frequency of platform crossing in Morris water maze. Results presented as Mean ± SEM; n=6 *significant at p≤0.05 compared to control

DISCUSSION

There was a decrease even though not significant in latency to locate platform between training days 1 and 2 in all groups and compared to control except for the Piracetam group which was similar to control. The increase in frequency of platform crossing which is a measure of memory was significant and dose dependent and highest in Piracetam group.

Among the earliest signs of Alzheimer's disease is the progressive deterioration in spatial memories (Baddeley et al., 1991). The MWM tests for visuospatial learning and memory which is hippocampal dependent (Brown & Aggleton, 2001). Acquisition trial is used to assess learning capacity composed of escape latency and swimming distance. Probe trial measures memory ability evaluated by time spent in different areas of the maze and number of target area (platform) crossing.

Tamadonfard et al. (2014) reported a significant increase in learning and memory following chronic administration of higher doses of *N. sativa* extract in Barnes maze in rats, El-marasy et al., (2012) in T maze model of scopolamine induced memory impairment, and Sahaket al. (2013) in Radial arm maze. Azzubaidi et al. (2012) demonstrated noticeable spatial cognitive preservation in rats challenged with chronic cerebral hypoperfusion in MWM indicative of a neuroprotective effect.

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