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RESEACH ARTICLE

Ameliorative Potentials of Black Seed Oil (*Nigella Sativa*) on Short-Term Memory Impairments and Histological Changes on Ethanol Treated Rats

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Abstract

Background and Objective: Alcohol is the sixth leading risk factor contributing to most deaths and other related diseases. This study aims at investigating the ameliorative potentials of black seed oil on short-term memory impairments and histological changes on ethanol treated rats. **Methods:** Twenty-five Wistar rats with an average weight of 157g were randomly grouped into five groups of five rats each, after two weeks of acclimatization. Animals in group I served as the control and were administered with distilled water, group II, III, IV and V were administered with 2.8ml/kg of black seed oil, 1.4ml/kg of ethanol, 1.4ml/kg of ethanol and 1.4ml/kg of black seed oil, 1.4ml/kg of ethanol and 2.8ml/kg of black cumin oil respectively orally, for three weeks. A short term memory was assessed using novel object recognition test. The rats were humanly sacrificed and the brains were removed and fixed in formal saline before being processed using routine histological techniques. Tissues were stained with hematoxylin and eosin stain for histological studies. One-way analysis of variance was used to compare the mean value using the Statistical Package for Social Sciences (SPSS version, 21), and the data were presented as mean ± standard deviation. **Result:** The result indicates that there is a slight improvement of memory in the group administered with 1.4 mg/kwt of ethanol and black seed oil. There is no significant alternation of memory in the control group and the group administered with 1.4 mg/kg bwt of ethanol. The group administered with 1.4 mg/kg bwt of ethanol and 2.8 mg/kg bwt of black cumin oil shows a significant difference in impairment in short term memory. The ameliorative effects of black seed oil was observed on the histomorphology of the prefrontal cortex due to a higher number of pyramidal cells, granular cells, reduction in pyknotic cells and vaculations. **Conclusion:** It was concluded from the present study that black seed oil reduces the toxic effects of ethanol on the prefrontal cortex of adult Wistar rats.

Key words: *Nigella sativa,* Prefrontal cortex, alcohol, short term memory.

INTRODUCTION

Nigella sativa (N.sativa) is an annual flowering plant, which belongs to the Ranunculaceae family (Hamza and Al-Harbi, 2015). It grows in three different regions: Eastern Europe, the Middle East, and Western Asia (Fararh *et al*., 2004). The plant produces small black seeds that are flat, trigonous, angular in appearance, about 2 to 3.5mm long, and 1 to 2 mm wide (Harzallah *et al*., 2011). In addition, these dark gray- or black-colored seeds are similar in appearance to sesame seeds and are thought to be the most impressive part of the plant in terms of their valuable health impacts (Fararh *et al*., 2004). Moreover, the plant reaches a height of about 20–90cm and has linear-lanceolate leaves and flowers that are usually colored white, yellow, pink, pale blue or pale purple. The N. sativa fruit is large, balloon-like in shape, and is composed of 3–7 united follicles containing several seeds (Ahmad *et al*., 2013). N. sativa is named differently in different parts of the world; for example, it is known as black cumin (English), kalonji (South Asia), Al-Habba Al-Sawdaa or Al-Kammoon Al Aswad (Arabic) (Harzallah *et al*., 2011), Habbatussauda (Hausa), Asofeyeje (Yoruba), and Irugbin Dudu (Igbo). For thousands of years, N. sativa seeds (black seeds or black cumin) have been used for nutritional and medicinal purposes in many countries (Jansen, 1981).

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Some pharmacological effects have been attributed to the black cumin seed extracts and/or its oil, including anti-hypertensive (El-Tahir*et al*., 1993), analgesic and anti-inflammatory (Al-Ghamdi, 2001), anti-bacterial and anti-fungal (Hanafy and Hatem, 1991), anticonvulsant and anti-ischemia (Hosseinzadeh *et al*., 2006), anti-tumor (Salomi and Nair, 1992) and anti-oxidant activities (Burits and Bucar, 2000).

Animal studies have shown that the extracts of the Nigella sativa seed have significant therapeutic effects against variety of ailments such as headache, fever, cough, bronchitis, asthma, skin diseases, eczema, warts, jaundice, liver damage, anorexia, gastrointestinal problems, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension and intrinsic hemorrhage, amenorrhea, dysmenorrhea, scorpion poisoning and snake bites (Sobhi *et al*., 2016; Forouzanfar *et al*., 2014). They have beneficial antitussive, antinociceptive effects, antiosteoporotic property, hypotensive, antibacterial effect, antifungal, anticestodal, hepatoprotective effects, potent analgesic, anti-asthmatic effect, anti-inflammatory, antioxidant, spasmolytic, lactagogue, vermifuge, galactagogue, diaphoretic, antineoplastic, antihistaminic, bronchodilating effect, carminative, a blood pressure regulating effect as well as a bile flow stimulating effect (Tembhurne*et al*., 2014; Boskabady*et al*., 2010; Halawani, 2009).

Alcohol is the sixth leading risk factor contributing to most deaths and disabilities (Institute of Health Metrics and Evaluation, IHME, 2017). Many people drink alcohol, whether out of curiosity, ignorance, peer pressure or other reasons (Agberotimi, 2021). Alcohol interacts with three powerful neurotransmitters–chemical messengers that are responsible for communication.

Between alcohol's interaction with GABA and Glutamate, the net effect is a depression of brain activity and all the nerves in your spinal cord (also known as the Central Nervous System). This effect does not just result in general drowsiness, but it also slows breathing, thinking, and even suppresses the gag reflex. The frontal lobes of the brain are responsible for cognition, thought, memory, and judgment. By inhibiting its effects, alcohol impairs nearly every one of these functions.

The main component of black seed oil extracts is thymoquinone which is abundant in black seed oil and has a strong antioxidant potential due to its free radicals scavenging activity. It was shown that ethanol provokes toxic effects through generation of reactive oxygen species (ROS) and lipid peroxidation in different tissues and cell types. In addition to oxidative stress, ethanol can provoke apoptosis in several cells through the induction of the mitochondrial pathway or death receptor signaling (Yang, 2012). It was reported that oxidative stress and decreased antioxidant capacity of cells and tissues may be an important mediator of apoptotic cell death and this process can be suppressed by various antioxidants (Hossein*et al.*, 2007).

It is a serious public health concern, which leads to high spending and costly medical expenses associated with alcoholic accidents. For that, it is important to study a natural occurring plant that has antioxidant potentials. The present study is designed to access the ameliorative effects of black cumin oil on ethanol induced histopathological and neurobehavioral changes on the prefrontal cortex of Wistar rats.

MATERIALS AND METHODS

REAGENTS

Haematoxylin and Eosin stain (H and E stain),10% Normal Saline (NS), Graded alcohol, Xylene and Paraffin wax were used.

INSTRUMENTS AND APPARATUS

The materials used during this study include aluminum cages, plastic bottles for drinking water by the rats, syringes (1ml), digital weighing balance, distilled water, normal saline, plain bottles, disposable hand gloves, chloroform, surgical blade and small size plastic bucket, glass slides and cover slips, dissection kit, metallic mold, metallic canula, white plastic cassettes, transparent tissue containers, cotton wool, water bath, oven refrigerator, camera, microtome, towel, objects (bottles), disinfectant (spirit), square arena and microscope.

SAMPLE (OIL) COLLECTION

A sealed bottle of black cumin oil manufactured by El-Hawag Company for extraction and packaging of natural oils was collected at BSI Islamic Chemist. El-

Hawag extracted the oil via the Pharaonic method by cold pressing, preserving all the useful materials and qualities under the supervision of the Ministry of Health Egypt, License 150/2/190 of 2009.

EXPERIMENTAL ANIMALS

Twenty-five apparently healthy adult Wistar rats with the average weight of 157 mg were purchased from the Animal House of Department of Biological Sciences, Bayero University, Kano. The animals were housed in the Animal House of Anatomy Department enclosed in steel cages and fed with nutritious grower chicken food purchased from Agro Feed Mills and a feeder containing water. The animals were acclimatized for one week at a normal room temperature under a 12-hour light-dark cycle before administration of the oil and throughout the experimental period. All experimental protocols were in compliance with ethical standards of Bayero University, Kano animal committee as well as internationally accepted principles for laboratory animal use and care.

EXPERIMENTAL DESIGN

After a period of one week of adaption, the experimental animals were weighed and randomly classified into five groups, with five rats per group. The experimental groups were subjected to the following treatments for three consecutive weeks (21 days) orally; animals in group I serves as the control group and were administered with distilled water, group II, III, IV and V were administered with 2.8ml/kg of black cumin oil, 1.4ml/kg of ethanol, 1.4ml/kg of ethanol and 1.4ml/kg of black cumin oil, 1.4ml/kg of ethanol and 2.8ml/kg of black cumin oil respectively.

NOVEL OBJECT RECOGNITION TEST FOR SHORT TERM MEMORY

All experimental procedures were done in the Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria.

TRAINING AND SETTING UP OF NOVEL OBJECT TEST

The selected objects are different enough to be easily discriminated by rats, but have a similar degree of complexity (texture, shape, color patterning and brightness, etc.). Diffuse and low lighting were used to minimize the stress of bright lighting. Temperature and humidity were similar to regular housing conditions. The rats were handled properly prior to testing, because the test relies on the natural tendency of the rodents to explore novelty. For the main arena, a square chamber (around 40 cm x 40 cm x 40 cm) made from wood was used, and it is grey in colour. The camera was placed directly overhead the squared arena for optimal view of exploration.

Experimental Procedures

Habituation

Each rat was removed from its home cage and placed in the middle of the open, empty arena to explore the arena for 5 mins. At the end of 5 minutes, the rats were removed and placed in a holding cage. The apparatuses were thoroughly cleaned between each rat using a disinfectant.

Testing (T1)

Twenty-four (24) hours after the habituation, two identical objects were placed in opposite quadrants of the arena (NE corner and SW corner). Each rat was removed from its home cage and placed in the center of the arena, equidistant from the 2 identical objects. The rats were allowed to explore the arena for 5 mins. The apparatus and objects were thoroughly cleaned between each rat using a disinfectant.

Testing (T2)

Twenty-four hours (24hrs) after T1, one object used during T1 (familiar objects) and one novel object were placed in opposite quadrants of the arena. Each rat was removed from its home cage and placed in the center of the arena, equidistant from the familiar object and novel object. The rats were allowed to explore the arena for 5 minutes. For both T1 and T2, the first 5 minutes were scored. If the mouse does not meet the minimum exploration time of 20s for both objects, scoring continues past 5 minutes.

Animal Sacrifice and Tissue Collection

The rats were humanely sacrificed after the last day of the experiment by putting them in the sacrifice chamber containing cotton wool soaked in chloroform (anesthesia) for about 2-3 minutes. Then, the rats were removed and then placed in a prone position with limbs pinned outwards. Incisions were made through the skin and skull in order to expose the brain tissues.

TISSUE PROCESSING

The brains were collected immediately and fixed in a white rubber container containing 10% formal saline (fixative) for the minimum of 24 hours, before carrying out routine histological processing of the frontal lobe and stained using hematoxylins and eosin (H & E) stain.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compared the means between the groups. The data were expressed as mean ± standard deviation. All statistical analyses were done using the SPSS version 21.

RESULTS

The results of the short term memory using novel object recognition test show that the exploration time ((ET1) for both identical objects was higher in the control group as compared to all the treated groups with a value of 26.4 ± 11.72, 7.40 ± 3.29, 9.50 ± 5.07, 11.75±14.24 and 15.50±11.85 for control, 2.8 mg/kg bwt of BSO group, 1.4 ml/kg bwt of ethanol, 1.4 mg/kg bwt of ethanol and 1.4 mg/kg bwt of BSO group and 1.4 mg/kg bwt of ethanol and 2.8 mg/kg bwt of BSO, respectively, though not statistically significant. Also, the exploration time 2 (ET2) is higher in group 1 (control) 22 ± 15.36 compared to group 2 (2.8 ml/kg of black cumin oil) 12 ± 8.63 seconds, group 3 (1.4 ml/kg of ethanol) 17.50 ± 13.48 (seconds), group 4 (1.4 ml/kg of ethanol + 1.4 ml/kg of black cumin oil) 20.25 ± 9.64 and group 5 (1.4 ml/kg of ethanol + 2.8 ml/kg of black cumin oil) 11 ±8.29. Also, the discrimination time for both exploration time 1 and exploration time 2 is slightly higher in group 4 compared to group 1, group 2, group 3 and group 5 with a value of 5.75 ± 6.08,2.80 ± 4.27, 1.20 ± 4.44, 2.50 ± 1.29 and 1.50 ± 9.75. Also, the exploration time for the novel object was higher when compared to the familiar objects with a value of 12.40 ± 8.76, 6.60 ± 5.59, 10 ± 6.78, 13 ± 7.35 and 4.75 ± 2.99 for group control, 2.8 mg/kg bwt of BSO group, 1.4 ml/kg bwt of ethanol, 1.4 mg/kg bwt of ethanol and 1.4 mg/kg bwt of BSO group and 1.4 mg/kg bwt of ethanol and 2.8 mg/kg bwt of BSO, respectively as can be seen in figure 4.1 below:

Table 2: Memory Performance (sec) in Novel Object Recognition Test in Male and Female Wistar Rats Expressed as Mean ± SEM

Variables	Control	2.8 mg/kg bwt BSO	1.4 mg/kg bwt of eth	1.4 mg/kg bwt of BSO & 1.4 mg/kg Eth	1.4 mg eth & 2.8 mg of BSO	p-value
Object 1	15.20±5.85*	4.20±2.95*	5 ± 2.16	5.75 ± 7.14	8.50 ± 5.97	0.02
Object 2	11.20 ± 6.06	3.20 ± 2.17	4.50 ± 3.51	4.50 ± 7.35	4.50 ± 6.48	0.22
ET1	26.4 ± 11.72	7.40 ± 3.29	9.50 ± 5.07	11.75±14.24	15.50±11.85	0.06
FOBJ	9.60 ± 7.09	5.40 ± 3.97	7.50 ± 6.76	7.25 ± 3.30	6.25 ± 8.54	0.86
Novel	12.40 ± 8.76	6.60 ± 5.59	10 ± 6.78	13 ± 7.35	4.75 ± 2.99	0.33
ET2	22 ± 15.36	12 ± 8.63	17.50±13.48	20.25 ± 9.64	11 ± 8.29	0.54
DT	2.80 ± 4.27	1.20 ± 4.44	2.50 ± 1.29	5.75 ± 6.08	1.50 ± 9.75	0.51

ET1 = Exploration Time 1, ET2 = Exploration Time 2, DT = Discrimination Time, Familiar Object = FOBJ, BSO

The results from the histological study reveals no histo-architectural distortion in the group 1 (distilled water) prefrontal cortex of Wistar rats, while there is distortion in the histo-architecture in the group administered with 2.8ml/kg of black cumin oil) with slight vaculations, but normal pyramidal cells. Group 3 (1.4ml/kg of ethanol) shows more vaculations, pyknosis and decreased number of pyramidal cells. Group 4 (1.4ml/kg of ethanol + 1.4ml/kg of black cumin oil) shows normal pyramidal cells, granular cells less vaculations, while group 5 (1.4ml/kg of ethanol + 2.8ml/kg of black cumin oil) shows normal pyramidal cells and normal granular cells which is comparable to the control group.

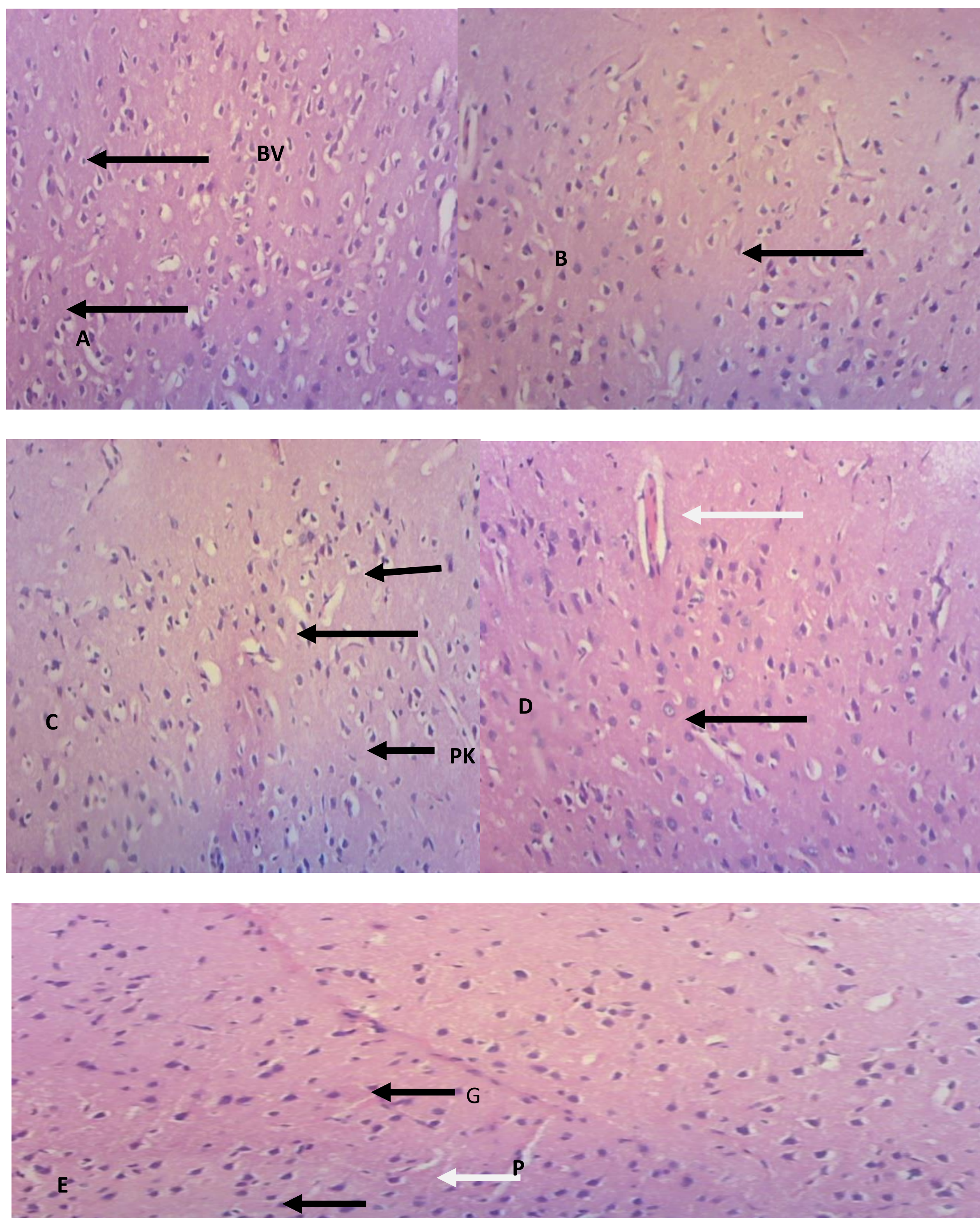

Plate A: shows a photomicrograph of the prefrontal cortex (pyramidal cells) in Wistar rats administered with distilled water as negative control, normal pyramidal cells and blood vessels. Plate B: 2.8ml/kg of N.sativa oil used as positive control. Mild vacuolation of pyramidal cells., Plate C: shows a photomicrograph of the prefrontal cortex (pyramidal cells) in wistar rat administered with 1.4ml/kg of ethanol used as positive control. Showing, Degenerated, shrunken, vacuolated and necrosis. Plate D: shows a photomicrograph of the prefrontal cortex (pyramidal cells) in Wistar rats administered with 1.4ml/kg of ethanol + 1.4ml/kg of black cumin oil as treatment showing Mild vacuolation, degenerated and shrunken and patches of normal cells, Plate E: shows a photomicrograph of the prefrontal cortex (pyramidal cells) in Wistar rats administered with 1.4ml/kg of ethanol + 2.4ml/kg of black cumin oil as treatment 2, Presents a normal cells with a visible outline of dendrites, pyramidal cells (P), granular cell (G) Stained with routine Heamatoxtyin and Eosin (H & E X100)

DISCUSSION

The novel object recognition task is very useful to studying short-term memory, intermediate-term memory, and long-term memory, through manipulation of the retention interval, i.e., the amount of time animals must retain memory of the sample objects presented during the familiarization phase before moving to the test phase, when one of the familiar objects is replaced by a novel one (Taglialatela *et al*., 2009). In 1988, Ennaceur and Delacour conclude that these tests are simple behavioral assays of memory that rely primarily on a rodent's innate exploratory behavior in the absence of externally applied rules or reinforcement.

The result obtained in this study indicates that all the experimental animals approach frequently and spend more time exploring the novel object than the familiar object. This result is in total agreement with (Ennaceur, 2010) who states that "the preference for a novel object means that presentation of the familiar object exists in animals' memory". The recognition of novelty requires more cognitive skills from the subjects, relative to tasks measuring exploration of novel environments or a single novel object (Silvers *et al.,*. 2007).

Ajao *et al.,* 2016 report that oral administration of saline and black seed oil to adult rats at 1 ml/kg for 14 days to investigate the potential efficacy of black seed oil (BSO) in anxiety-like behavior, motor activity, cerebellar architecture and Purkinje morphometry in Wistar rats. He concludes that black seed oil could improve anxiety-like behavior, locomotors activity and Purkinje or cerebellar architectonic in the Wistar rats. Observations from this study show that group 4 in the experimental animals explore the novel object the most and show more cognitive skills which is in agreement with the work of (Ajao *et al.,* 2016). However, this disagrees with the findings of Fry et al., 2020, who report non-observable difference in the novel object recognition abilities in developmental prosopagnosia, as compared to the control individuals. This is due to the fact that developmental prosopagnosia is characterized by absence of brain injury or co-occurring social, intellectual or visual impairments

In this study, light microscopic examination of routine Haematoxylin and Eosin (H&E) stained cerebral (prefrontal cortex) sections of Wistar rats reveals that there is no histo-architecture distortion in group 1 administered with distilled water, while in group 2 administered with 2.8 ml/kg of black cumin oil, the pyramidal cells were slightly vacuolated which is in disagreement with Ajao *et al.,* 2016, who orally administered saline and black seed oil to adult rats at 1 ml/kg for 14 days to investigate the potential efficacy of black seed oil (BSO) in motor activity, cerebellar architecture and Purkinje morphometry in Wistar rats. He concludes that black seed oil could improve locomotors activity and Purkinje or cerebellar architectonic in the Wistar rats. The mild vacuolation of pyramidal cells in group 2 may be due to individual drug sensitivity, amount administered (dose), period of administration, specific disease processes and metabolic disorders, such as lysosomal storage disorders which can lead to vacuolation in various brain regions.

Considering the histomorphology in group 3 of the experimental animals administered with 1.4ml/kg of ethanol, there were necrosis, vacoulation, degeneration and shrunken of the pyramidal cells as a result of direct toxic injury of ethanol to the prefrontal cortex interfering with the functions of the frontal lobe. This result concords with the work of Yang, 2012 who says "that ethanol provokes toxic effects through the generation of reactive oxygen species (ROS) and lipid peroxidation in different tissues and cell types". In addition to oxidative stress, ethanol can provoke apoptosis in several cells through the induction of the mitochondrial pathway or death receptor signaling. It is well established that even uncomplicated alcoholics who have no specific neurological disease show signs of regional brain damage and cognitive dysfunction (Harper and Matsumoto, 2004).

In group 4 and 5 which are treated with varying dose of 1.4 ml/kg and 2.8 ml/kg of Nigella Sativa oil respectively, the result concords with the work of Hossein *et al.*, 2007, who report that oxidative stress and decreased antioxidant capacity of cells and tissues may be an important mediator of apoptotic cell death and this process can be suppressed by various antioxidants. N. sativa seed contains more than 30% fixed oil and 0.4 to 0.45% of volatile oil (Nergiz and Otles, 1993). Thymoquinone (TQ) is an abundant component of black seed oil extract (Ghosheh *et al.,* 1999).). TQ has a strong antioxidant potential due to its

free radical scavenging activity (Hossein *et al.*, 2007). Group 4 section shows patches of normal pyramidal cells amidst slightly degenerated and shrunken cells, while group 5 section presents a virtually normal pyramidal cells with visible outline of dendrites.

CONCLUSION

It can be concluded from this study that oral administration of ethanol at 1.4 ml/kg has no significant effect on short term memory, likewise black cumin oil at 2.8ml/kg. Similarly, histomorphological changes induced by alcohol were ameliorated by black cumin oil in dose-dependent manner.

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Authors' Contribution

AS design the work and wrote the manuscript, MMM carried out the work, RIF proof read the manuscript, IAT interpreted the histological slides and contributed in designing the manuscript, ANJ conducted the statistical analysis and TA contributed in conducting the research and writing the manuscript

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