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Ultrastructural changes of Basolateral Amygdaloid nuclei in the Sprague Dawley rats brain following exposure to naphthalene balls

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ABSTRACT

Naphthalene is a bicyclic aromatic constituent commonly used in different domestic and marketable applications comprising soil fumigants, lavatory scent disks and mothballs. Accidentally, workers, children and animals are exposed to naphthalene mothballs, so there is a need to study the pathology behind this chemical toxicity. The current study was carried out to assess the ultra structural changes of basolateral amygdaloid nuclei in the Sprague Dawley rats brain in association to naphthalene toxicity. The toxicity model group was administered with naphthalene (200 and 400mg) using corn oil as a vehicle for 28 days. The post delayed toxicity of naphthalene high dose ingestion was also assessed in rats. After the experimental period, the brain tissue was processed to observe the ultra structural changes using a transmission electron microscope. The alterations in cell organelles, nuclei damage, mitochondrial swelling, chromatin condensation suggested naphthalene induced damage in the neurons of the basolateral amygdala of the brain in the toxicity model group. These experimental trials provide information about the alert of mothball usage in the home and identify risks linked with accidental exposure and misuse.

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INTRODUCTION

Naphthalene is a bicyclic aromatic hydrocarbon that has been used as an insect repellent, anthelminthic, insecticide, and vermicide. Different aspects of naphthalene's interaction with biological organisms, including metabolism and bio accumulation, have come under intensive research. Environmental health professionals should be aware of mothball use at every home. The occurrence of naphthalene exposure and its potential to become a widespread environmental contaminant emphasizes the requirement for inclusive toxicity testing in mammals (Pannu and Singla, 2020).

Coal tar and petroleum are the major sources of naphthalene. Naphthalene is used directly as an insecticide, moth repellent, anthelmintic, and intestinal antiseptic. Human exposure to naphthalene can take place in the home, in the workplace, and through water consumption contaminated by oil spills and industrial waste. The main toxicological indexes of exposure of naphthalene in humans

are haemolytic anemia with related jaundice and cataract formation (Volney *et al.*, 2018). People have developed nausea, dizziness, headaches, and/or vomiting after being contacted with naphthalene vapors. If someone inhales in enough of the vapour or eats a mothball having naphthalene, they may progress to hemolytic anemia. Children have also established fever, abdominal pain, diarrhea, and discolored urine with painful urination after accidental intake of naphthalene balls (Angurana *et al.*, 2019).

There are a few available studies on naphthalene teratogenicity Shafer *et al.* (2020) reported that naphthalene crosses the placenta in humans and can result in neonatal toxicity. When naphthalene gas is breathed in, the body breaks it down into other chemicals that counter cells in the body and results in brain tissue damage. Kidney, brain, eyes, lungs and liver injury may also occur due to naphthalene toxicity (Sotnichenko *et al.*, 2019). The neurologic symptoms of ingestion of naphthalene reported in human case studies comprise altered sensorium, confusion, lethargy, listlessness, and vertigo. Few data is available concerning the naphthalene toxicity on the human brain. Till now, detailed research was not conducted on naphthalene balls oral toxicity induced brain damage. Therefore, the current study was carried out to evaluate the ultra structural changes of basolateral amygdaloid nuclei in the Sprague Dawley rats brain upon oral exposure to naphthalene balls using a Transmission electron microscope.

METHODOLOGY

Animals

Twenty-five Sprague Dawley male rats, aged between 8 and 9 weeks, were taken for the current study. The experiments were approved by IAEC, Sri Ramachandra Institute of Higher Education and Research and performed out in accordance with standard operating procedures in "Guidelines on the regulation of scientific experiments on animals" (CPCSEA guidelines) by the Ministry of Environment and Forest, Government of India. The animals were kept in polycarbonate cages in the standard day-night cycle and the temperature kept at 22 ± 2^0C. Animals were fed with laboratory rodent pelleted feed obtained from M/s. VRK Nutritional Solutions, Pune, *ad libitum* (IAEC/60/SRIHER/674/2019).

Experimental Grouping

The rats (n=25) were grouped into five, composed of 5 animals in each group. The vehicle control group administered with 5ml corn oil/kg/day for 28 days (Group I); Experimental groups orally induced with 200mg/kg naphthalene per day for 28 days (Group II) and 400mg/kg naphthalene per day for 28 days (Group III). The satellite control vehicle group was given orally 5ml of corn oil/kg/day for 28 days and 14 days post treatment observation period (Group IV). The satellite high dose naphthalene group was treated with 400/mg/kg naphthalene for 28 days and 14 days post treatment observation (Group V). The satellite groups were observed for 14 days post treatment to check for any withdrawal symptoms associated with a recovery period. After completion of 28 days, the rats in Group I to III were euthanized by deep anesthesia with intraperitoneal injection ketamine (50mg/kg bwt) + xylazine (5mg/kg b.wt), death occur as the fixative is perfused and the heart decease to beat and in Group IV and V (satellite groups), the same procedure was followed for euthanasia after completion of 42 days.

Transmission electron microscopy

The brain tissue specimens were collected in the fixative containing gluteraldehyde (2.5%) in cacodylate buffer (pH-7.4). They were cut into small bits and shifted on to the fixative and were fixed about 6-8 hrs in the fridge. Bits were rinsed in cacodylate buffer three times for ten minutes each. It was fixed with 1% Osmium tetroxide in the same buffer (-8 degree Celcius). After 2 hours, it was rinsed thrice with the same buffer. The bits were treated with series (30%, 50%, 70%, 80%, 90%) of acetone and treated twice with 100% acetone for 10minutes each. It was infiltered with the resin mixture (EPON 812 resin- 2ml, DDSA- 1.5ml, and MNA-0.75 ml) and kept at room temperature. The same resin mixture is made, but 3 drops of catalyst DMP 30 were added for embedding. One bit of infiltered specimen is kept in one well, which contains resin mixture with catalyst. For every specimen, various bits are taken. A small paper piece has been inserted onto the well (mould: 60 degree Celcius, 48 hrs). The specimen bocks were easily removed from the mould after bringing it back to normal room temperature and stored for further sectioning. Semithin sections were cut with ultramicrotome using glass knives and ultrathin sections were cut with diamond knives. Ultrathin sections were applied on the grids and stained using lead citrate and uranyl acetate for ten minutes. It was air dried and then monitored in electron microscopy.

RESULTS AND DISCUSSION

Naphthalene exposure is related to various toxic manifestations in laboratory animals and humans, with the lens of the eye, lungs and the brain being the most sensitive (Lee *et al.*, 2018). Naphthalene

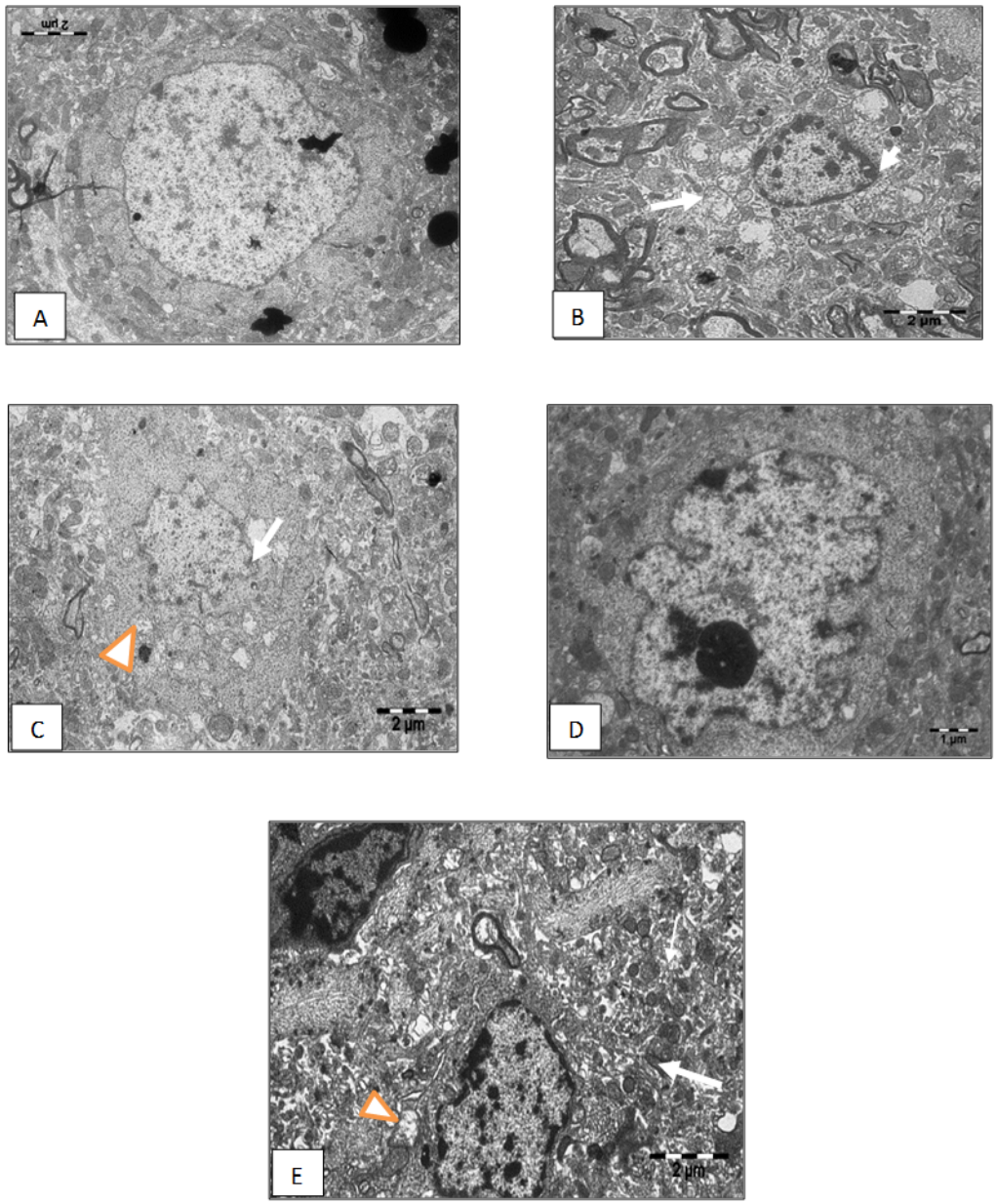

Figure 1: TEM images of basolateral amygdala nucleus of rat's exposure with naphthalene balls

endures wide microsomal metabolism. The initial step in the metabolism of naphthalene is oxidative in nature and is catalysed by cytochrome P-450 oxygenases in the microsome, producing 1,2-naphthalene oxide, an electrophilic arene epoxide intermediate (Shafy and El-Sherif, 2019). Due to the ingestion of mothballs, very few cases of naphthalene poisoning have been reported in the literature (Kundra *et al.*, 2015). Previous research has exposed that unilateral amygdala damage is enough for impairing autonomic responses to conditioned stimuli (Fadok *et al.*, 2018). The accidental ingestion of mothball, which consists either of naphthalene or paradichlorobenzene, is very common in children, which leads to acute and chronic toxicity in the human body.

The amygdala has a significant function in the emotional memory like fear, anxiety, decision making and other behvioural disturbances. The basolateral nucleus is the biggest among all the three nuclei and has been powerfully concerned as key sites for stress and anxiety/fear roles (Fadok *et al.*, 2018).

The ultra structural changes in the neurons of the basolateral amygdala were examined by transmission electron microscopy in control and experimental groups (Figure 1).

The Basolateral nuclei of the amygdala in the rats of the control group (Group I) exhibited normal structural morphology. The neurons in the normal control Basolateral amygdala, displaying abundant

organelles such as the endoplasmic reticulum, mitochondria, and ribosomes. The nucleus is round and large, a uniform density of chromatin is found, and a very clear nucleolus in the control vehicle group also found (Figure 1A).

After treatment with a low dose (200mg) of naphthalene (Group II) showed chromatin condensation (long arrow) and nucleolus disappearance (small arrow) is shown in the neurons of basolateral amygdaloid nuclei (Figure 1B).

The high dose of naphthalene 400 mg induced group (Group III) showed destructive processes and changes in cells increased, nucleus showed clear signs of degeneration (long arrow) and chromatin in the nucleus was condensed, cristae and membrane were broken (shown with orange arrowhead) in the neurons of basolateral amygdale (Figure 1C).

The neurons in the satellite control vehicle group (Group IV) of the basolateral amygdala had clear margins and a huge central nucleolus, the plasma membrane was incessant and clear, and various organelles were visible (Figure 1D).

It should be noted that 28 days after the start of naphthalene administration and 14 days observed period of delayed toxicity group (Group V) indicates structural changes in the mitochondria swelling (long arrow) and vacuolation (shown with arrowhead) was noted (Figure 1E).

The obtained results indicate that ultrastructural alterations in the basolateral nuclei of the amygdala in rats brain take place when the dosage of treatment with naphthalene increases. The satellite vehicle control group showed normal cellular organelles and no damage in basolateral amygdaloid nuclei was observed upon administration of corn oil and the delayed toxicity group proved less pathological nuclei and other cellular injury compared to the toxicity model group.

CONCLUSIONS

The ultrastructural variations in neurons morphology confirmed that naphthalene oral ingestion induced damage on basolateral amygdaloid nuclei of the rat brain. The results of this study highlight that many organelles are damaged in the basolateral amygdala, which may lead to the development of anxiety or emotion related disorders. Further studies are necessary to confirm the presence of structural abnormality in other areas of the brain following oral exposure of naphthalene in relevance to the development of neuropsychiatric disorders.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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