

## A HISTOPATHOLOGY STUDY ON THE EFFECTS OF ACUTE EXPOSURE OF TOPICAL ANALGESIC SPRAY ON BRAIN TISSUES OF SPRAGUE DAWLEY RATS

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### ABSTRACT

Topical analgesic sprays are a group of drugs that inhibits the action of pain receptors (nociceptors) resulting in a pain relieving effect. In Malaysia, the abuse of topical analgesic sprays via inhalation is now growing in popularity amongst teenagers as it causes a “high” effect and it is easily available in pharmacies. This study aimed to determine the morphological changes in the brain tissue of Sprague Dawley rats exposed acutely for 14 days to two different brands of topical analgesic sprays. 15 rats were divided into 3 groups; control group, Group A for Medgeneral Ethyl Chloride spray and group B for Perskindol Cool Spray. Each group consisted of 5 rats. The rats were exposed to their specific sprays twice a day for 14 days. On the final day, all rats were sacrificed and the brain tissues underwent gross examination and histological preparations for microscopic viewing. The tissues were examined via comparison microscope and comparisons were made between the control group and the exposed group and also between the two exposed groups. For the brain morphology, both Group A and B showed an increase in pyknotic nuclei, elongated microglia and macrophages compared to control group. Group A showed a higher number of the three morphological findings compared to group B. These results were evident that solvents present in the sprays causes cellular damage and cell death. The results showed that acute exposure of topical analgesic sprays via inhalation causes morphological changes in the brain tissues due to cellular damage and death. However, biochemical tests should be done to determine the mechanism of toxicity caused by the topical analgesic sprays.

**Key words:** Histopathology, topical analgesic sprays, acute toxicity, inhalants, brains

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### INTRODUCTION

Inhalant misuse is the deliberate attempt of inhaling volatile substances in order to obtain euphoric, disinhibiting, and exciting effects. Examples of inhalants are solvents, glues, adhesives, paints, paint removers, nail polish removers, and aerosol propellants (Ramón *et al.*, 2003). Inhalant abuse is a problem that is becoming more prevalent all around the world. The increase in prevalence of inhalant abuse escalates morbidity and mortality rates (Aldemir *et al.*, 2015).

The statistics of inhalant abuse are staggering as well. According to the Health Research Foundation in the USA, 1.1 million teenagers between the ages of 12 to 17 in the United States have used inhalants at least once in the past 12 months. Also, there are an estimated 14,000

teenagers living on the streets of Karachi, Pakistan. Up to 90% of them are using inhalants regularly and 20% of youths in Europe, between the ages of 12 to 16 have tried inhalants. In Malaysia, the abuse of organic volatile solvents has been observed since the early 1980s (Kin & Navaratnam, 1995).

On May 15th 2015, Berita Harian released an article titled “*Remaja Malaysia dapat khayal dengan hidu ubat kebas sukan*” (Malaysian teenagers getting high by sniffing pain relief spray). According to the article, teenagers would spray the pain relief medicine on their clothes and sniff their clothes multiple times to get high. Sniffing of pain relief spray is gaining popularity in Malaysia as the sprays are easily available in Malaysia. Two of the most common pain relief sprays (topical analgesic sprays) in Malaysia are the Perskindol spray (as was shown in the news) and the Medgeneral Ethyl Chloride spray. This should be a worrying matter as teenagers are mostly unaware of the consequences

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of abusing those sprays. Sniffing chemicals from topical analgesic sprays falls under the category of inhalant abuse. This research aimed to determine the harmful effects of acute exposure to topical analgesic spray, namely Perskindol Cool Spray and Medgeneral Ethyl Chloride Spray on the brains and lungs tissues of Sprague Dawley rats. Rodents such as rats are used in research as their biological and behavior characteristic resemble those of humans and symptoms of human conditions can be simulated in rodents.

## MATERIALS & METHODS

### Materials

All chemicals and reagents used were of analytical grade. Fifteen males, 2 months old Sprague Dawley rats were used in this research because these rats grow rapidly during their childhood and become sexually mature at about the sixth week, but attain social maturity 5-6 months later (Sengupta, 2013). Rats were divided into 3 Groups (Control, A and B) and each Group contained 5 rats. Group A was exposed to Medgeneral Ethyl Chloride Spray while Group B was exposed to Perskindol Cool Spray.

### Administering Inhalants to Rats

The control group was exposed to ambient air. The rats from Group A and Group B were placed in separated containers. The rats were then exposed to the solvent from the spray can in the container. This was done by spraying the solvents interally through the hole created on the lid. The experimental rats were exposed to the solvents until at least 50% of the rat's population from each group showed signs of narcosis or any behavioural changes. The volume of the solvents needed to induce narcosis or behavioural changes was measured by weighing the cans before and after spraying (1g equals to 1cm<sup>3</sup>). Exposure to the solvent was carried out in 8-hour interval. A multi-perforated petri dish containing 50g of NaOH pellets were placed inside the container to trap exhaled CO<sub>2</sub> (Carabez-T *et al.*, 1998). The time taken for the rats to show signs of narcosis or behavioural changes was taken and recorded (onset of action). This entire procedure was repeated for 14 days.

### Sampling for Morphological Examination (Histology)

On the 14<sup>th</sup> day, the rats were sacrificed using cervical dislocation method and necropsy procedure was done for the collection of brains tissue samples. Gross examination was done on the tissue samples. The cerebrum was trimmed into three sections

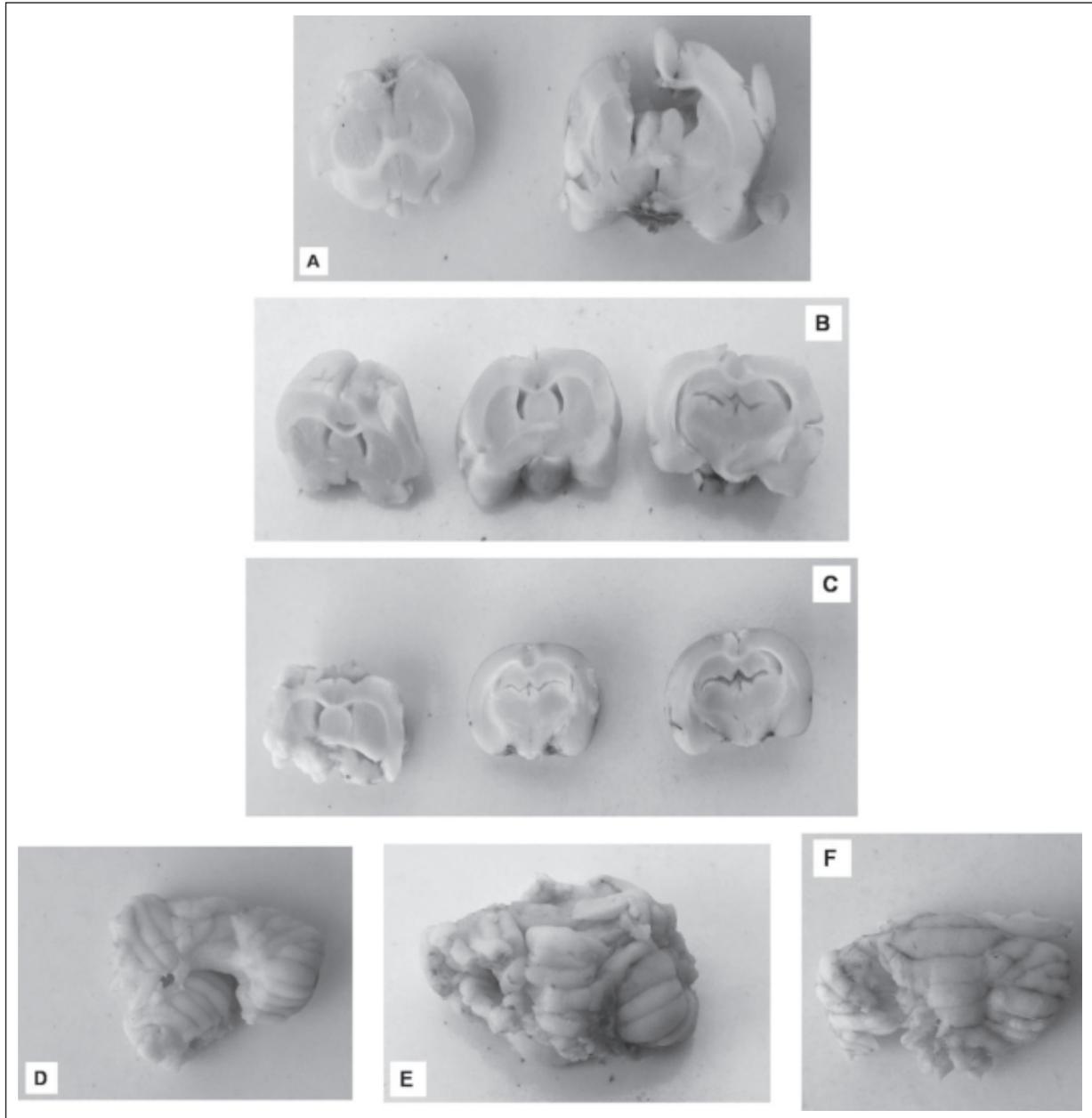
(frontal, medial and dorsal part of the cerebrum was obtained), while cerebellum was taken as a whole. All the samples were then immersed in 10% formalin solution separately according to their group for 24 hours for fixation. The tissues were then processed using increasing concentration of ethanol and xylene. Lastly, the tissues were stained using Hematoxylin & Eosin dyes. The sample slides were viewed under comparison chloride spray. The comparisons are done by comparing the control group with Group A and Group B. Comparison were also done between Group A and Group B.

## RESULTS & DISCUSSION

When Group A was exposed to Ethyl chloride spray, the rats showed signs of narcosis by becoming unconscious and when Group B was exposed to Perskindol Cool Spray, the rats showed signs of behavioural changes from panicking to a relaxed state causing a decrease in their mobility.

For the first administration, the average volume needed for the rats to show signs of narcosis and behavioural changes were 7.77 cm<sup>3</sup> for Group A while Group B was 8.29 cm<sup>3</sup>. Also, the average onset of action was 1.93 minutes for Group A while Group B was 2.64 minutes. As for the second administration, the average volume needed for the rats to show signs of narcosis and behavioural changes were 7.03 cm<sup>3</sup> for Group A while Group B was 7.29 cm<sup>3</sup>. Also, the average onset of action was 1.64 minutes for Group A while Group B was 2.23 minutes. Based on gross examination, no abnormalities were observed in all parts of the brains from the 3 groups (Figure 1). However, changes can be seen during histological examination. When compared with the control group, both Group A and B showed an increase in pyknotic nuclei (Figure 2G and Figure 2J), macrophages (Figure 2H and Figure 2L) and elongated microglia (Figure 2I and Figure 2K). This can be seen in the cerebrum and cerebellum of both the exposed groups. When both the exposed groups were compared, Group A had higher numbers of pyknotic nuclei, macrophages and elongated microglia (Figure 3).

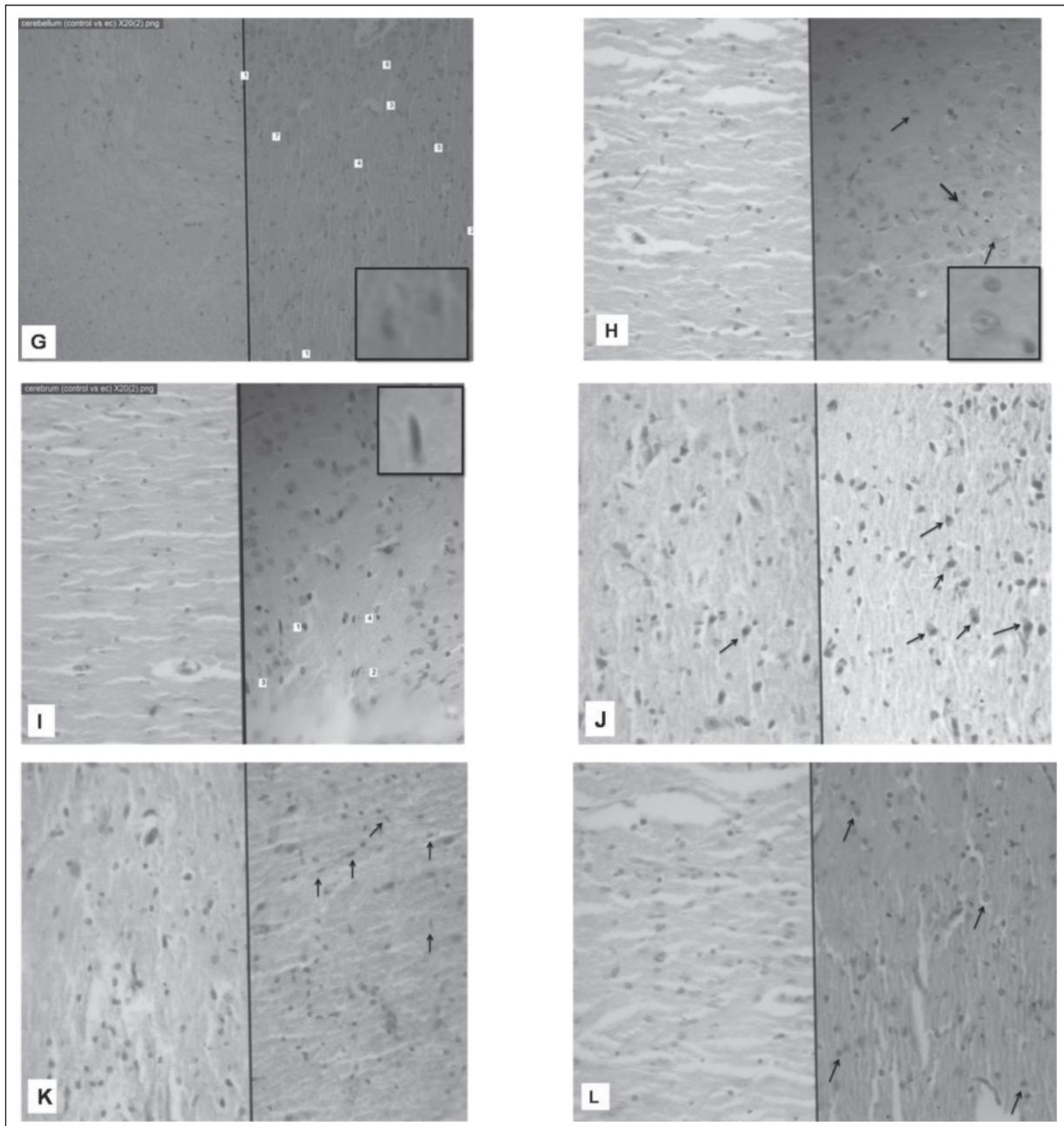
Pyknotic nucleus is one of the morphological changes of a cell undergoing cell death (Fuller & Burger, 2012). The mechanisms are dependent on the type of cell death. The increased presence of pyknotic nuclei in Group A and Group B compared to the control are indications that these chemical agents were causing cell injury or death. The increase of cell death causes the increase in elongated microglia and macrophages. Microglia are the resident macrophage-like cell in the brain involved in eliminating damaged neurons or cell



**Fig. 1.** (A) The cerebrum of the control group; (B) The cerebrum of Group A; (C) The cerebrum of group B. No abnormalities were observed at the cerebrum of all the groups. The cerebral cortex, the white matter, the putamen and the lateral ventricle appear to be of normal colour and shape; (D) The cerebellum of control group; (E) The cerebellum of Group A; (F) The cerebellum of Group B. Similar to the cerebrum, no abnormalities could be observed on the cerebellum of all the groups. The anterior and posterior lobes appear to be of normal colour and shape.

residues. The increased presence of elongated microglia shows the occurrence of microgliosis which is the accumulation of microglia cells as a reaction to cell injury. Macrophages on the other hand, rapidly undergo mitosis when there is an increase in cell injury or cell death. These cells remove damaged cells or cellular residue from dead cells by engulfing them. Thus, this explains why there were higher numbers of macrophages.

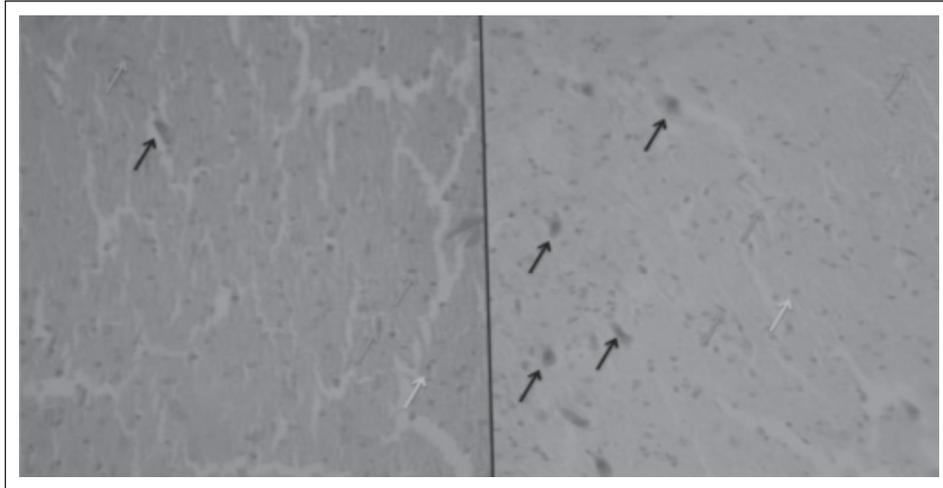
At certain concentrations, ethyl chloride causes the relaxation of muscles such as the cardiac muscle and the diaphragm (Senussi & Chalise, 2015). This causes a reduction in cardiac output and induces shallow breathing which eventually decreases the blood supply to brains. Due to the low blood volume, tissues will be deprived of oxygen. This condition is called ischemia. Deprivation of oxygen causes the neurons to undergo cell death.



**Fig. 2.** (G & H) Comparison between the control group (left) and Group A (right) at the cerebellum at 200X magnification. In Fig. 2. (G) number of pyknotic nuclei (number markings) increased in Group A compared to the control group. A pyknotic nucleus is characterized by condensation of the nucleus where it appears to be darker than normal and intense eosinophilic cytoplasm, where the cytoplasm appears to be darker in red colour compared to the normal cytoplasm. Fig. 2. (H) showed an increase in macrophages (arrow) in Group A. Macrophages are characterized by their small nucleus and large cytoplasm; (I) Comparison between control group (left) and Group A (right) at the cerebrum at 200X magnification. This figure showed an increase in elongated microglia (arrow). These neurons were characterized by the rod-shaped appearance. Fig. 2. (J & K) Comparison between control group (left) and Group B (right) at the cerebellum at 200X magnification; (L) Comparison between control group (left) and Group B (right) at the cerebrum at 200X magnification. In Fig. 2. (J) There was a higher number of appearances in pyknotic nuclei (arrow) in Group B compared to the control group while in Fig. 2. (K) Showed a higher number of elongated microglia (arrow) in Group B in comparison to the control group. Lastly, in Fig. 4. (L) Higher numbers of macrophages (arrow) were observed in Group B.

Organic chemicals such as iso-butane and propane present in the Perskindol spray can cause hypoxia which deprives the neurons of oxygen.

Another chemical present in the Perskindol spray that causes neuronal damage or loss is denatured alcohol which is ethanol with additives. Study have



**Fig. 3.** The Comparison between Group B (left) and Group A (right) at the cerebrum at 200X magnification. There was an increased in pyknotic nuclei (black arrow), elongated microglia (blue arrow) and macrophages (green arrow) in Group A compared to Group B.

shown that denatured alcohol does cause neurologic damages; however, the mechanism of toxicity is still poorly understood (Harper, 2007).

One possible reason why Group A has a higher number of pyknotic nuclei, elongated microglia and macrophages is the differences in concentration of solvent present in both the spray cans. Ethyl chloride spray has no additional ingredient; this spray can use the solvent's own vapour pressure to eject the solvent out of the can (Proctor *et al.*, 2004), thus causing the rats in Group A to obtain a higher concentration of ethyl chloride as the rats only inhaled this solvent. While for the Perskindol spray, there are 6 substances present in the can with different concentrations. When inhaled, the rats in Group B obtained lesser concentration of substances that caused the morphological changes.

This research recommends that biochemical tests such as Caspase and RIP1 activity done to determine the mechanism and type of cell death these chemical agents causes.

## CONCLUSION

It was evident that from the findings that the acute exposure of topical analgesic sprays namely Medgeneral Ethyl Chloride and Perskindol Cool Spray do have histopathological effects on the brains tissue as morphological changes could be observed in both the exposed group.

## ACKNOWLEDGEMENT

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