

Acute Oral Neurotoxicity Study of Petroleum-Derived Spray Oil In Female And Male Wistar Rats: A Comprehensive Review

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Abstract

In the present study, 20 male and 20 female adult healthy Wistar rats were used to examine neurotoxicity potential as per OECD 424 and 423 guidelines. Animals were acclimatised for 5 days prior to test item administration. After randomisation and grouping, G2 animals were kept fasting overnight, and the test item, petroleum-derived spray oil, was administered through oral gavage as neat. The G1 group was not given a vehicle, as no vehicle was used in this study. The animals were observed at 30 min, 1, 2, and 4 hours post-administration for any visual clinical toxicity. The grip strength and motor activity in 10 animals were observed on day 0, days 1, 7 and 14 using the UGO BASILE grip strength meter and Opto-M3 Activity Monitor (Columbus), respectively. The locomotor activity was measured for 30 minutes; however, results are given only for the first 10 minutes due to time constraints. On terminal sacrifice, 5 animals were perfused with 50 ml PBS and 50 mL 4% paraformaldehyde, and the brain, sciatic nerve, eye with optic nerve, spinal cord, and tissues were collected; however, three representative brain images are shown in this report. All other collected organs are still being processed. The gross pathology was recorded for all experimental animals. Peak time and peak force values of Group 1 (control) and Group 2 (treated) animals in each sex were compared using Student's t-test separately. Results showed normal body weight gain amongst all groups on days 7 and 14. There was no mortality in any of the groups. There were no adverse clinical findings in any of the experimental animals (G1 and G2). Grip strength analysis showed no significant differences, indicating that the test chemical did not produce consistent or permanent alterations in neuromuscular coordination over the study period. Over the course of the investigation, the plotted graph revealed similar patterns of locomotor activity in both groups (G1 and G2). The graphical findings showed that, under the study's experimental settings, petroleum-derived spray oil had no discernible negative impact on locomotor activity. Gross pathology on terminal sacrifice showed no abnormality in any of the organs due to test item administration at 2000mg/kg b. wt. dose. Histopathology findings showed normal morphology in H&E-stained slides. In the present study, when male and female Wistar rats were administered with petroleum-derived spray oil through oral gavage @ 2000mg/kg b.wt. did not result in any significant abnormal changes in body weight gain (on Day 7 and 14), clinical observations, Dyspnea, Larymation, Salivation, Diarrhoea, Gait, Posture, Tremors, Circling, Lethargy, Bizarre behaviour; grip strength (peak time and peak force) and locomotor activity (vertical and horizontal movements) under the conditions of the study. Therefore, this chemical may be categorised in GHS class V through the oral route. These findings may be useful to select a dosage regimen for sub-acute and chronic studies.

Keywords: Neurotoxicity , petroleum derived spray oil (PDSO), pesticides, Neurotoxicity assesement ,OECD Guidelines 423,424 , Histopathology.

Introduction:

Pesticides are widely employed in contemporary agriculture because they are cost-effective, increase crop yields and quality, protect crops from pests, and help feed a growing global population [1]. Pesticide use has been on the rise globally, which has serious consequences for both human and environmental health. Overuse of pesticides depletes natural resources and reduces biodiversity [2]. Crude oil, often known as refined petroleum, is a combination of saturated hydrocarbons (paraffinic) and aromatic hydrocarbons (C and H atoms). Metal ions (such as arsenic), sulphur, nitrogen, and oxygen compounds in the asphaltene and resin components, and a trace quantity of sulphur are also present [3]. The use of horticultural oils and petroleum-derived spray oils (PDSOs) for pest management dates back to the late 1800s [4]. Chemical pesticides are widely used in agriculture and other areas, and their persistence and bioaccumulation pose great risks to the environment and human health. While designed to control pests, these chemicals can accidentally affect non-target organisms, contaminate ecosystems, and degrade air, water, and soil quality, thereby impacting wildlife, human health, and the safety of the food supply [5]. Human exposure is via food, the environment and occupational use through skin contact, ingestion, inhalation and eye contact. Exposure can cause both short- and long-term health effects. Pesticide poisoning is a serious public health problem worldwide, affecting millions of people every year [6]. The research reveals that pesticide formulations intentionally include petroleum-derived substances, such as polycyclic aromatic hydrocarbons (PAHs), rather than being incidental pollutants. These substances have the potential to make the formulation more harmful or carcinogenic than the named active components alone, and their effects might last for generations (epigenetic effects) or longer (long-term consequences) [7]. Environmental Protection Agency (EPA) Federal Pesticide Regulation (FPR); EPA Works to Ensure that Pesticides Will Not Harm Human Health or the Environment. The U.S. Environmental Protection Agency (EPA) primarily regulates pesticides under federal laws like the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to protect human health and the environment. It registers products, reviews safety data, sets residue tolerances in food, and collaborates with states on compliance and worker safety [8]. Long-term exposure to pesticides, both directly and indirectly, is linked to a number of health issues, including gastrointestinal disturbances, neurological conditions, reproductive disorders, respiratory diseases and cardiovascular problems [9].

Literature review;

Shah, M. A., et al. (2025) apple trees could be protected from the green apple aphid (*Aphis pomi* De Geer) by spraying them with petroleum oil prior to blooming. At the dormant or early bud stage, when the aphids are still attached to the twigs and buds but have not yet opened their flowers, they sprayed the oil. Early season shoots and leaves were far less damaged by aphids after the treatment. As an effective component of integrated pest control systems, the oil effectively killed aphids by covering them on bark and buds, smothering them. By reducing the need for further insecticide treatments later in the year, pre-bloom oil sprays may further minimise expenses and chemical residues. The oil has few negative effects on the environment and is helpful to insects when applied correctly and under favourable weather conditions. According to the research, a simple and low-chemical way to manage green apple aphid in apple orchards is to spray petroleum oil before bloom. This is especially helpful in regions where aphid outbreaks are frequent, and producers want to use fewer conventional pesticides [10].

Sood *et al.* (2025) examine pesticide use patterns in India's individual states and union territories from 2013 to 2024 in depth in this research. It delves into the variations at the state and territorial levels, as well as the worldwide and Indian trends in pesticide usage throughout time. While the general trend is toward a decline, the annual growth rate of household pesticide use has fluctuated greatly. An upward trend in pesticide use is discernible when comparing usage across various states and Union territories. In terms of overall pesticide use, the states of Uttar Pradesh, Maharashtra, Punjab, and Haryana were at the top. Trends in production, import, and export, as well as the worldwide and national consumption of various pesticide classes, are also included in the study. It also delves into the pesticide-treated area for important crops. Due to the significant differences in pesticide usage between crops and regions, specific interventions are required to promote more consistent and sustainable agricultural practices. It discusses how pesticide use has evolved over time and across countries, including India. Domestic pesticide usage has grown at a very varied pace each year, even if overall pesticide use is down. According to the data, pesticide use has increased in some EU states and

territories. In terms of overall pesticide use, the states of Uttar Pradesh, Maharashtra, Punjab, and Haryana were at the top. Pesticide-treated area for important crops, production, import/export patterns, and worldwide and national consumption of several pesticide classes are also examined in the study. Targeted initiatives are needed to promote more sustainable and consistent agricultural practices, since there are significant disparities in pesticide usage between crops and regions [11].

Jungers, G., et al. (2022). Toxicology studies of pesticides ignore the whole unknown compositions in favour of studying the active components, which make up just 20%-50% of the formulation. Out of 24 pesticides, 16 PAHs were detected using GC-MS analysis. The toxicity thresholds for each PAH ranged from 2 to 8288 times, and there were dozens of formulations for each active ingredient. This suggests that 2- and 3-ring PAHs derived from crude oil predominated in the persistent petroleum leftovers, which were most likely considered garbage. Toxicological evaluations are the only basis for ADI. Adjust the ADI by dividing it by 1000 since petroleum enhances toxicity by about 1000 times. Long-term exposure to PAHs may be more carcinogenic than that of designated actives. There are thousands of formulations for each active ingredient, and GC-MS analysis of 24 pesticides revealed 16 key PAHs with toxicity levels ranging from 2-8288 times. All of these point to the 2-3 ring PAHs seen in crude oil being the most abundant in the long-lasting petroleum leftovers, which are probably trash. Toxicological assessments are the only basis for ADI. Petroleum is approximately one thousand times more poisonous. Therefore, divide ADI by 1000. Compared to stated actives, PAHs may have a greater long-term carcinogenic risk [12].

Baliota, G. V., et al. (2022). The possibility of phytotoxicity has prevented the widespread use of insecticidal paraffin (petroleum) oils in agriculture, despite their long history of usage. Their potential use in IPM (integrated pest management) initiatives has garnered attention as of late. Processing improvements have led to commercial formulations that are superior to their early versions. Nevertheless, there is a paucity of evaluations of the new oils' compatibility with IPM and their agricultural uses. Examining petroleum oils' insecticidal effectiveness in the context of decades of knowledge on pesticidal and phytotoxic components, this paper examines their use in arboriculture and other applications. Commercial formulation, non-target organism toxicity, and regulatory frameworks in the US and EU are also covered [13].

Elmorsy et al., 2022. The objective of the research is to find out how the pesticides paraquat, chlorpyrifos, and malathion affect various regions of the brain. Examined regions of the brain

such as the hippocampus, cerebral cortex, cerebellum, and corpus striatum that are responsible for learning, memory, movement, and coordination. Determining if these chemicals may be a factor in neurological issues was the primary goal. The outcome shows that all three pesticides impede essential brain enzymes involved in proper nerve transmission and function, namely acetylcholinesterase and neuropathy target esterase. By harming brain cells and decreasing the brain's natural antioxidant defence, pesticides exacerbated oxidative stress. Paraquat and chlorpyrifos were the most lethal of the insecticides that were evaluated. According to the research, neurodegenerative illnesses like Parkinson's and Alzheimer's may be accelerated by chronic exposure to these pesticides, which may impact brain function via oxidative stress, enzyme disruption, and mitochondrial damage.[14].

Rani *et al.*'s (2021) Examining the effects of chemical pesticides on both humans and the environment: A review was published in the Journal of Cleaner Production. Rani's point claims that pesticides increase crop yields at the expense of serious environmental and health risks. Some of the health problems that have been associated with long-term exposure include cancer, neurological illnesses including Parkinson's, diabetes, problems with reproduction, and paediatric developmental abnormalities. Skin rashes, nausea, difficulty breathing, and irritation of the eyes may all result from an acute encounter. These side effects are caused by DNA damage, endocrine disruption, neurotoxicity, and mutagenicity. Pesticides harm beneficial insects, pollinators, soil microbes, and animals while poisoning the air, water, and soil. That has a negative impact on ecosystems and biodiversity. According to studies, a lot of pesticides don't work as intended and can make pests more resistant. In an effort to reduce the use of synthetic chemicals and increase the use of biopesticides and organic farming practices, the authors generally call for stricter regulations, more integrated pest management, and the promotion of cleaner, more sustainable agriculture [15].

M.A. van den Dries, et al. (2019). Children whose mothers were exposed to organophosphate (op) insecticides when they were pregnant may have a higher chance of developing attention deficit hyperactivity disorder (ADHD) or genetic autism. Organophosphate metabolite levels in pregnant women's urine were examined at three different phases of pregnancy to determine pesticide exposure. Next, they assessed autistic characteristics and ADHD-related behaviours in children at 3, 6, and 10 years of age. Researchers assessed pesticide exposure by measuring levels of organophosphate metabolites in pregnant women's urine at three different phases of the pregnancy. After that, at 3, 6, and 10 years of age, they assessed autistic characteristics and ADHD-related behaviours in children. Although the women in the study had a history of

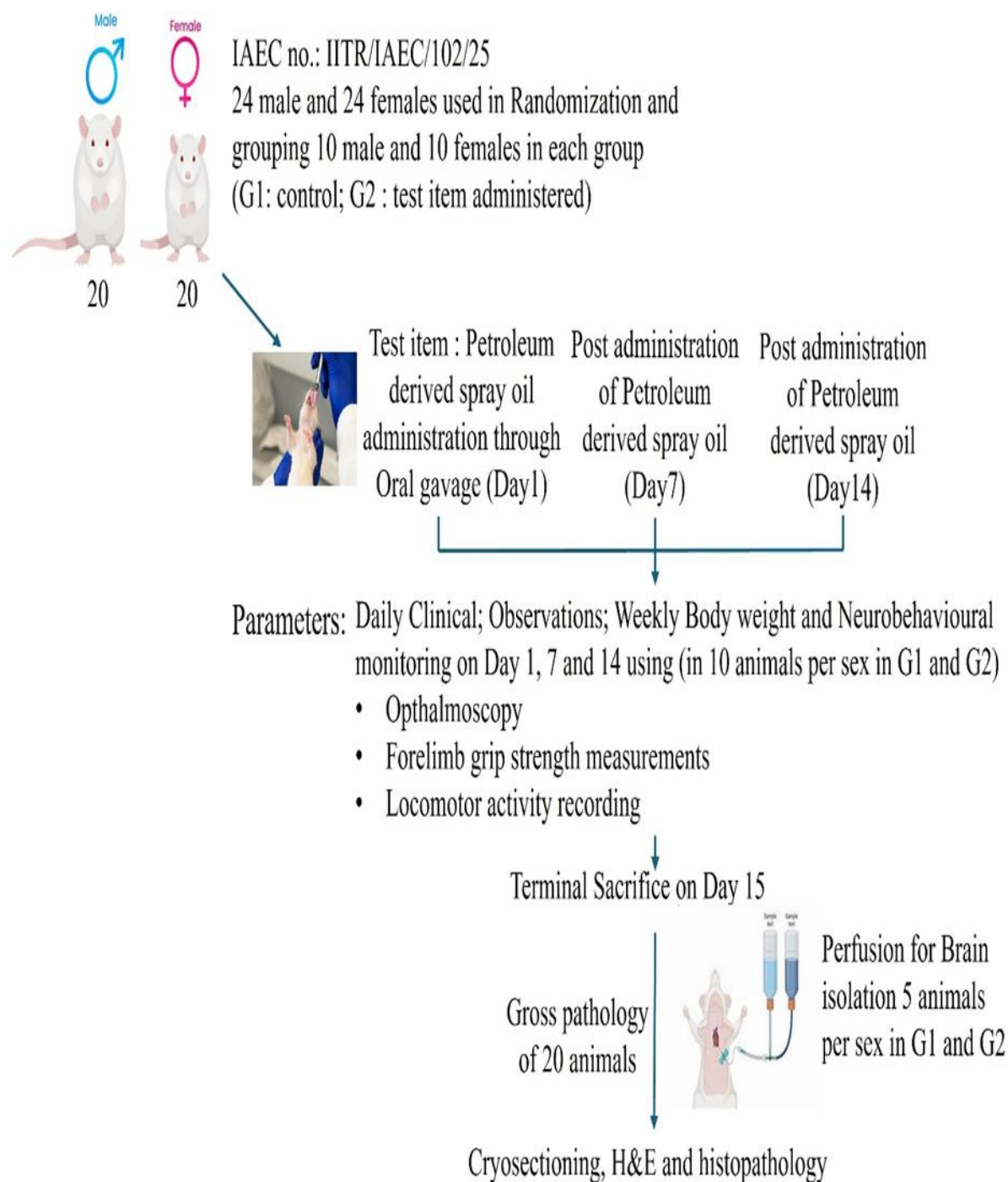
exposure to OP pesticides, the researchers were unable to find any link between prenatal pesticide exposure and an elevated risk of ADHD or autism in the children. This conclusion was reached after taking into consideration other variables that potentially impact these features. This Dutch population did not show any elevated risk of attention deficit hyperactivity disorder (ADHD) or autism-related characteristics in children whose mothers were exposed to organophosphate pesticides during pregnancy, according to researchers M.A. van den Dries. Nevertheless, it was noted that one urine sample would not be enough to draw conclusions about exposure during pregnancy; future research should include several measures to be more precise [16].

Nile, A., et al. (2019). When compared to conventional chemical pesticides, horticultural oil is much safer for the environment and just as effective. Unlike synthetic pesticides, which pollute soil, water, and air and cause major health concerns in people, horticultural oils operate via a physical action, covering and smothering bugs and their eggs. They are often used to control soft-bodied insects, including aphids, whiteflies, scales, and mites on fruits, vegetables, ornamentals, and field crops. These pesticides are produced from either petroleum or plants. These have a number of benefits, the most important of which are their low toxicity to people and animals, their safety for beneficial insects, their quick biodegradability, their low risk of pesticide resistance, and their appropriateness for organic farming and integrated pest management (IPM). These days, refined oils are safer, more versatile, and simpler to work with than ever before. They also produce much less harmful byproducts. It is crucial to avoid damaging plants by applying at the correct time, using the correct emulsification, and avoiding application at high temperatures. The Environmental Protection Agency (EPA), the Food and Agriculture Organisation (FAO), and the World Health Organisation (WHO) all endorse horticultural oils as a long-term solution to the problem of pesticide abuse, particularly in nations like India [17].

McKee, R. H., et al. (2014). A wide variety of compounds come together in petroleum-based goods. Compounds with aromatic rings, straight chains, and other shapes fall within this category. In order to study the possible health impacts of these compounds, the EPA in the US examined 400 of them and classified them into 13 categories. The skin, lungs, and brain are all vulnerable to the irritant and potentially toxic effects of lighter petroleum molecules, which are also more volatile. Heavier petroleum oils may contain polycyclic aromatic compounds (PACs), some of which are linked to cancer, developmental problems and damage to organs. Research on male rats has shown that exposure to certain chain-like hydrocarbons may lead to

renal and liver problems, along with subtle alterations in blood parameters. Crude petroleum oils can also contain impurities such as sulphur and nitrogen-containing compounds, although many of these are removed during refining. In addition, harmful chemicals such as benzene or hexane may sometimes be present and require careful monitoring due to their toxic effects on health. An increased risk of cancer, developmental delays, and organ damage has been linked to polycyclic aromatic chemicals (PACs), which may be present in heavier petroleum oils. Some of the chain-like hydrocarbons have been shown to cause effects on the liver and kidneys in male rats, as well as mild changes in blood parameters. Crude petroleum oils may also contain impurities such as sulphur- and nitrogen-containing compounds, but many of these are removed during refining. Besides, sometimes toxic chemicals like benzene or hexane are present and should be monitored carefully because of their toxic effects on health [18].

Stadler, T. et al. (2009) Petroleum-distilled spray oils (PDSOs) may enter insects mainly through the cuticle by disrupting the protective waxy layer, thereby increasing its permeability and reducing its water retention capacity. These oils may also enter the tracheal system through the spiracles, depending on the insect species and exposure conditions. Although cuticular penetration is considered an important mechanism of action, blockage of spiracles and subsequent suffocation may also contribute to insect mortality under certain conditions. These mechanisms support the potential use of PDSPs as pest control agents in integrated pest management (IPM) programs[19].

Plan of work :

Methodology:

The OECD guidelines 423 & 424 were used as guidance documents to conduct this acute single-dose neurotoxicity study of petroleum-derived spray oil.

Selection of animal species: In accordance with OECD guidelines 423 & 424, rats are the species of choice for conducting preclinical acute toxicity studies. Fulfilling regulatory requirements and OECD guidelines, the present acute neurotoxicity test was performed in Wistar rats.

Ethics statement;

40 healthy Wistar rats, 6 to 8 weeks old, were used for the experiment, IAEC no. IITR/IAEC/102\25

Test system: Wistar rats were selected as the experimental animal model for the neurotoxic effects of petroleum-derived spray oil.

Table 1. Test system used for the acute neurotoxicity in Wistar rats

Sr. no	Species	Rats
1	Strain	Wistar: conventionally bred (in-house randomly bred)
2	Source	Animal Facility CSIR-Indian Institute of Toxicology Research, CRK Campus, Gheru, Sarojini Nagar Industrial Area, Kanpur Road, Lucknow-226008, UP, India
3	Justification for the selection of species	Rats are one of the standard test systems used to assess toxicity and are acceptable to the regulatory authorities.
4	Justification for route of administration	The route of test item administration was through oral gavage. This route has been chosen since it was the intended route of exposure in humans.
5	No. & the sex of animals	20 male, 20 female
6	Age at the start of treatment	6–8 weeks
7	Body weight	At the commencement of the treatment, the weight variation of animals used did not exceed $\pm 20\%$ of the mean body weight in each group.

Justification for the Selection of the Vehicle

The product being tested was a spray oil, which was a liquid emulsion, and its intended application is to protect seasonal crops, vegetables, and fruit orchards from pests and insects. To ensure the stability of the test item, it was given neatly.

Dose levels and Justification for their Selection

The OECD 423 standard sets a maximum dosage of 2000 mg/kg for a single acute neurotoxicity test.

Homogeneity Analysis

Due to the liquid nature of the test item, no examination of homogeneity or concentration of active ingredients was performed.

Grouping. All rats, both male and female, were randomly assigned to control and treatment groups according to their weight, which was kept within a narrow range.

Test item administration

The test item was administered to the G2 animals based on a computation using animal body weight. In order to conduct the test, 20 female rats and 20 male rats from the G2 group were administered the test formulation orally at a limit dose of 2000 mg/kg body weight after fasting overnight. The test item will be administered to the lower-dose animals if neurotoxicity occurs at the upper-limit dose of 2000 mg/kg b.wt.

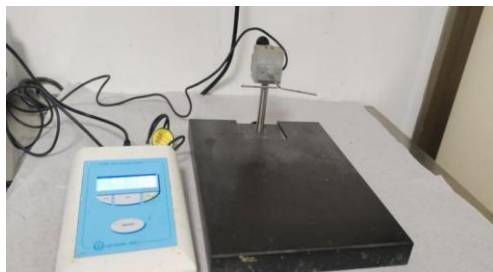
TABLE 2: The table shows dose volume and no. of males and females.

GROUP NO	DOSE VOLUME	NO. OF RATS MALE	NO. OF RATS FEMALE
G1	0 Mg/Kg	20	20
G2	2000 Mg/Kg	20	20

Observation: Twenty male and twenty female rats were monitored for clinical signs and mortality on the dosing day and daily for 14 days. Observations included behavioural , neurological , respiratory , and autonomic changes . Gross necropsy and pathological examinations were performed according to standard operating procedures.

Functional tests :

Ugo Basile Grip Strength Meter (GSM): Grip strength was measured using a rodent grip strength meter to assess forelimb muscle strength and sensorimotor function. Reduced grip strength indicated potential muscle weakness and neurobehavioral impairment .



(a)



(b)

Figure 1 : (a) Experimental setup of the grip strength meter
(b) Grip strength performance test conducted in Wistar rats

Motor activity:**OPTO -M3 Activity monitor (Columbus)**

The locomotor activity of Wistar rats was assessed using an infrared photocell-based animal activity monitoring system. The apparatus detects interruptions of infrared beams caused by animal movement and records locomotor activity. The resulting measurements are used to evaluate potential treatment-related alterations in spontaneous motor activity and exploratory behaviour.

The **X-axis** runs along the length of the cage (front to back).

The **Y-axis** runs along the width of the cage (side to side).

The **Z-axis** is vertical (up and down).

Ophthalmoscopy: Male and female rats underwent ophthalmoscopy before the test items were administered, as well as on days 1, 7, and 14, and the results were documented. To help identify any negative effects of a test chemical on the eyes and nervous system, ophthalmoscopy, or an inspection of the eyes using an ophthalmoscope, is a crucial component of acute neurotoxicity testing according to OECD Test Guideline 424 (Neurotoxicity Study in Rodents).

Body weight:

During the observation period, body weights were recorded on four separate days: Day 0, which is one day before the test item is administered; Day 1, which is the fasting body weight just before the test item is administered; Day 7; and Day 14.

Pathology:**Necropsy:**

An overdose of anaesthetic was administered to surviving rats on day 14. Neurohistopathology was performed on a minimum of ten animals (five males and five females). We included the animals in the perfusion group that showed symptoms of neurotoxicity if there were just a few animals in a specific dosage group. The method of choice for studying acute neurotoxicity in OECD TG 424 is perfusion fixation. This allows for the quick preservation of sensitive neural structures by bringing the fixative solution directly via the blood circulation. Reduced tissue disintegration and artefacts from delayed fixation are two benefits of this method.

Organ collection :

The organs were collected and preserved for histopathological examinations. Organs that were collected are: brain, Eye with optic nerve, Calf muscle, Spinal cord, Sciatic nerve

Cryosectioning:

After the neurotoxicity investigation of petroleum-derived spray oil, brain tissue from Wistar rats was fixed in paraformaldehyde (PFA) and then cryoprotected using sucrose solution. Using a standard procedure for rodent brain histology, the cryoprotected tissue was ready for cryostat sectioning. A cryostat microtome was used to cut thin slices at a thickness suitable for histological analysis, which were then gathered onto labelled microscope slides. Hematoxylin and eosin (H & E) staining were then applied to the slices in order to see the overall tissue architecture. Cell nuclei are stained blue to purple by hematoxylin, whereas the cytoplasm and extracellular proteins are stained pink by eosin. In order to evaluate the appearance of brain tissue and detect potential treatment-related histopathological changes, such as modification in cellular structure and tissue integrity, the stained slices were inspected under a light microscope.



Figure 2: Cryosectioning of brain tissue for histological analysis

Result :

Effect of Petroleum-based oil spray on Body Weight: The average animal body weight in males and females in all experimental animals of each group was summarised in Figure 3. The body weight gain table is given in Table 5.

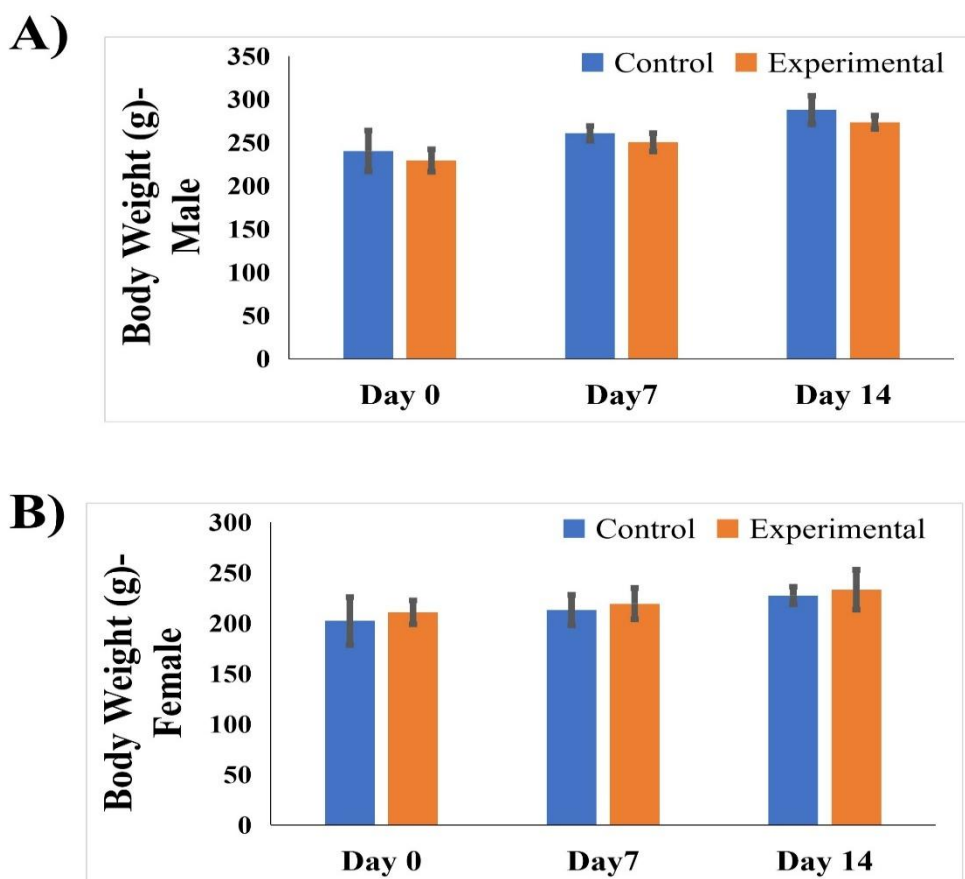


Figure 3: **A)** Graph showing Weekly body weight (male) comparison of G1 (Control) and G2 (Experimental) groups; **B)** Graph showing weekly body weight (female) comparison of G1 (Control) and G2 (Experimental) groups.

No statistically significant differences in body weight were observed in the G2 group, with a mean of 40 animals on any of the days 1,7 and 14, indicating that the administration of the test item did not negatively impact the animals' normal development or overall health state. All experimental animals showed a gradual increase in body weight that was comparable to the control group, also indicating the lack of severe neurotoxic effects that might affect food intake, nutrient utilisation, or general physiological functioning.

Table 3: Body weight gain of male and female Wistar rats during the 14-day acute neurotoxicity study following exposure to petroleum-derived spray oil.

	Body weight gain (g)			
	Day 7		Day 14	
	G1	G2	G1	G2
Male	20.3 ± 18.2	21.105±8.16	47.34±33.79	44.27±9.79
Female	10.84±9.89	8.66±3.79	25.28±12.23	22.49±10.58

Effect of petroleum-based oil spray on clinical observation:

Male and female rats were observed twice a day to track treatment-related neurotoxicity and death. Throughout the trial, neither the control group nor the groups treated with petroleum-based oil spray showed any unusual clinical symptoms or death.

FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

The most critical analysis includes the functional observational battery (FOB) and motor activity. There are several regulatory guidelines available for neurotoxicity tests such as the U.S. EPA Neurotoxicity Testing Guideline (1998); OECD 424 (Neurotoxicity study in Rodents), OECD SERIES ON TESTING AND ASSESSMENT Number 20 (Guidence document for neurotoxicity testing) European Agency for the Evaluation of Medicinal Products Q published in June 2001 a guideline, (ICH S7A: Safety Pharmacology studies for human pharmaceuticals, 2001) and safety pharmacological studies for human pharmaceuticals, these includes detail procedures to investigate the potential effects on central nervous system.

Effect of petroleum-based oil spray on forelimb grip strength :

Grip strength assessment was performed in both male and female Wistar rats at days 0, 1, 7, and 14 to evaluate neuromuscular function. Peak time and peak force values of Group 1 (control) and Group 2 (experimental) animals were compared using Student's t-test. In the present study, when we analysed the grip strength of male rats on day 1 of test item oral administration to experimental animals, on the remaining observation days, no significant differences were observed, indicating that the test chemical did not produce consistent or permanent alterations in neuromuscular coordination over the study period. For peak force, no statistically significant differences were observed between the control and treated groups on any observation day ($p > 0.05$). Grip strength values remained within the normal physiological range throughout the study, suggesting no treatment-related impairment in muscular strength.

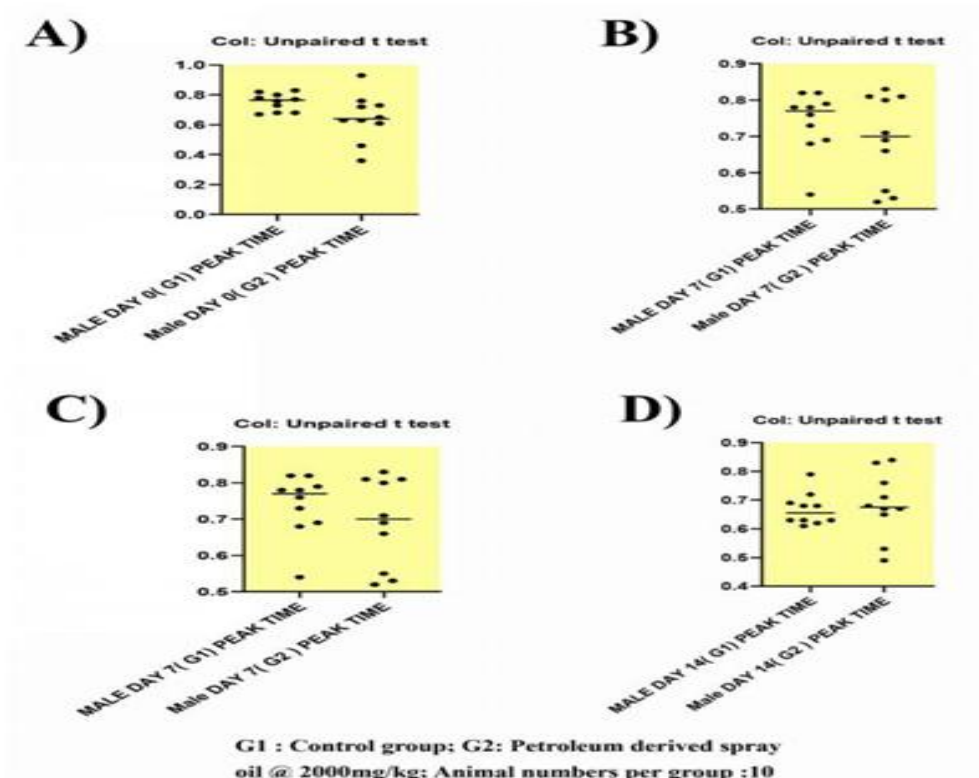


Figure 4 : Figure A–D: Graphical representation comparing the male peak time between Group 1 (G1) and Group 2 (G2) recorded on (A) Day 0, (B) Day 1, (C) Day 7, and (D) Day 14 during the study

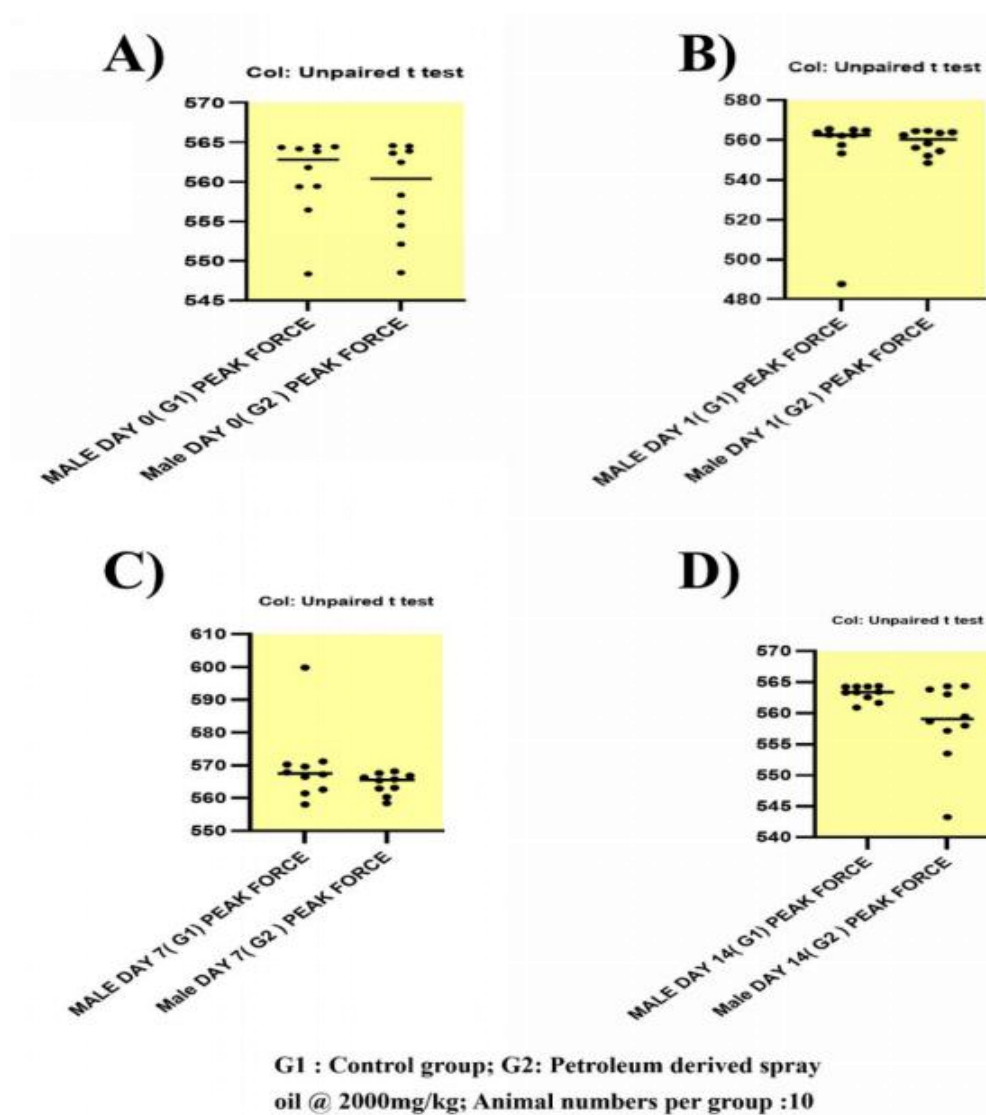


Figure 5: A to D: Graphical representation comparing the male peak force between Group 1 (G1) and Group 2 (G2) recorded on (A) Day 0, (B) Day 1, (C) Day 7, and (D) Day 14 during the study.

In female rats, peak time values did not show any statistically significant differences between Group 1 and Group 2 on any observation day ($p > 0.05$). Day 7 showed a statistically significant difference in peak force ($p < 0.05$), but all other observation days were determined to be non-significant. The change was isolated to a single time point, and no dose-related trend was observed. The finding may be considered incidental and not indicative of treatment-related neurotoxicity

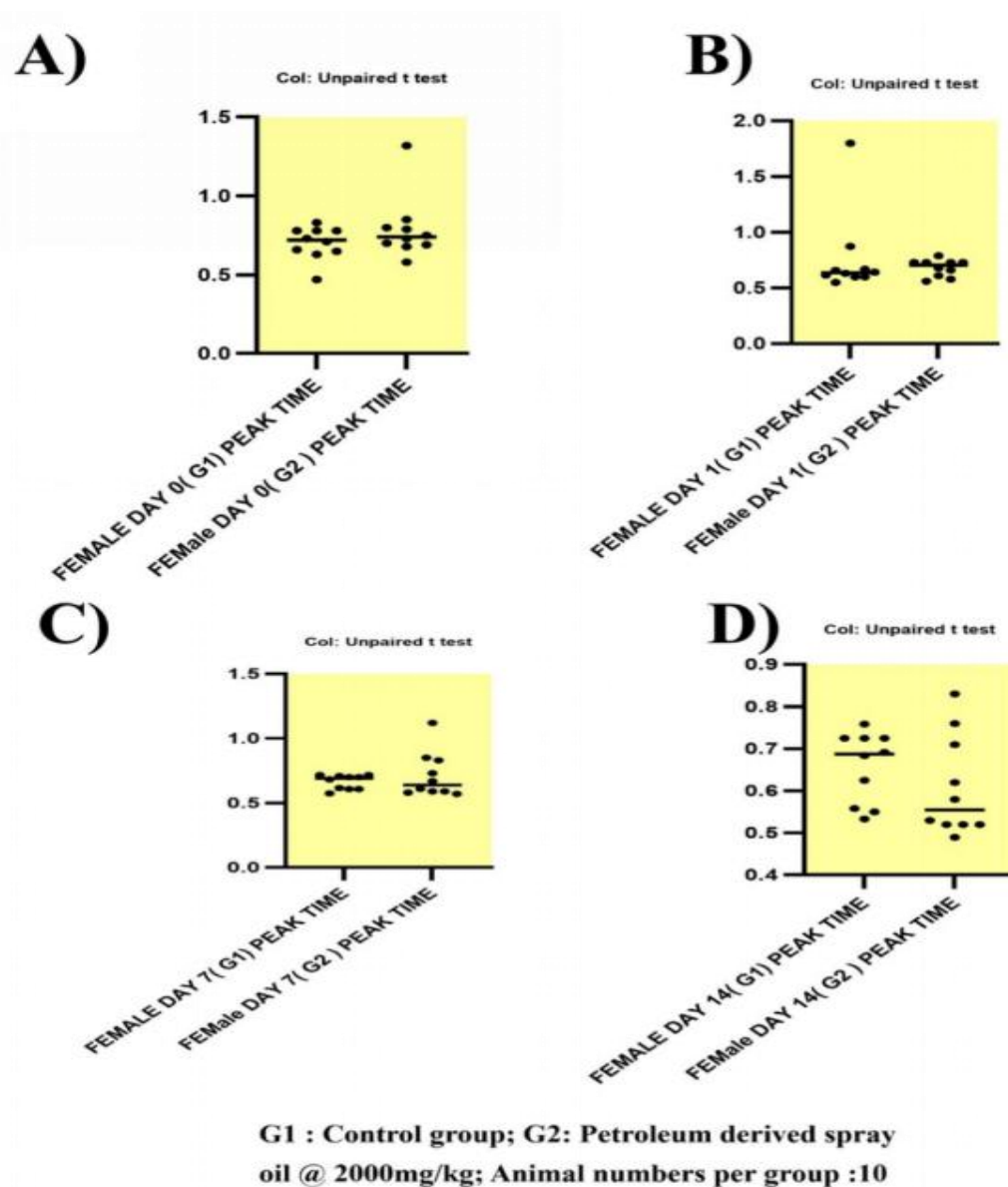


Figure 6: A–D: Graphical representation comparing the female peak time between Group 1 (G1) and Group 2 (G2) recorded on (A) Day 0, (B) Day 1, (C) Day 7, and (D) Day 14 during the study.

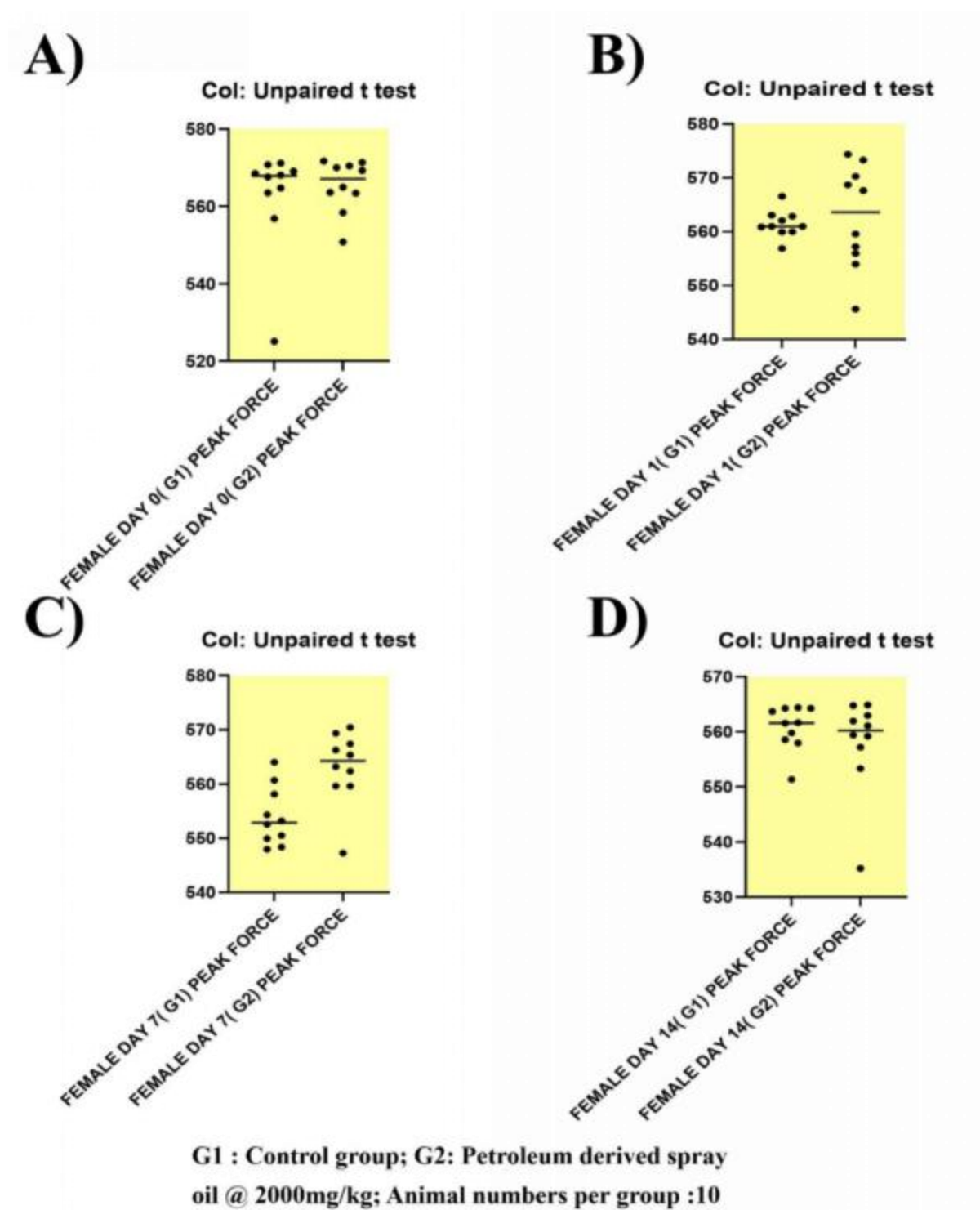


Figure 7: A–D: Graphical representation comparing the female peak force between Group 1 (G1) and Group 2 (G2) recorded on (A) Day 0, (B) Day 1, (C) Day 7, and (D) Day 14 during the study.

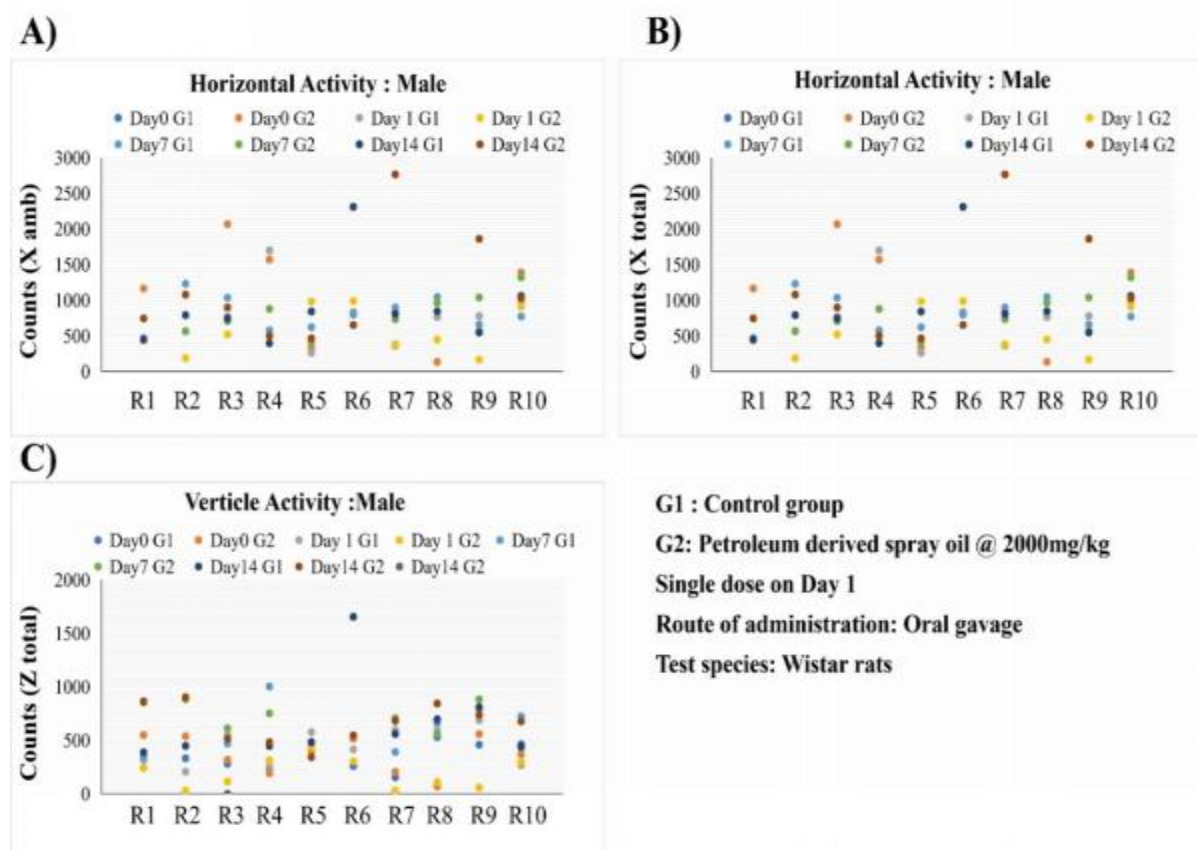
Effect of petroleum-based oil spray on motor activity:

Figure 8: Graphical representation of motor activity observation in experimental and control Wistar rats (A – Horizontal activity x amb: male, B – Horizontal activity x total: male, C – Vertical activity z total: male).

Both the control (G1) and experimental (G2) groups' locomotor activity in male Wistar rats was measured, and the results were graphically shown in Figure 8. Over the course of the investigation, the plotted graph revealed similar patterns of locomotor activity in both groups. When compared to the control groups, the experimental animals showed no noticeable alterations in their spontaneous movements, gaits, posture or behavioural activity. The graphical findings showed that, under the study's experimental settings, petroleum-derived spray oil had no discernible negative impact on locomotor activity.

Both the control (G1) and experimental (G2) groups' locomotor activity in female Wistar rats was measured, and the results are graphically shown in Figure 9. The results, when evaluated using statistical methods, showed minor significant changes in vertical movements in males and females on day 1; however, based on our historical data, these values were within normal

biological values. The locomotor activity, when measured in the morning and post-lunch sessions, may show variations due to food habits.

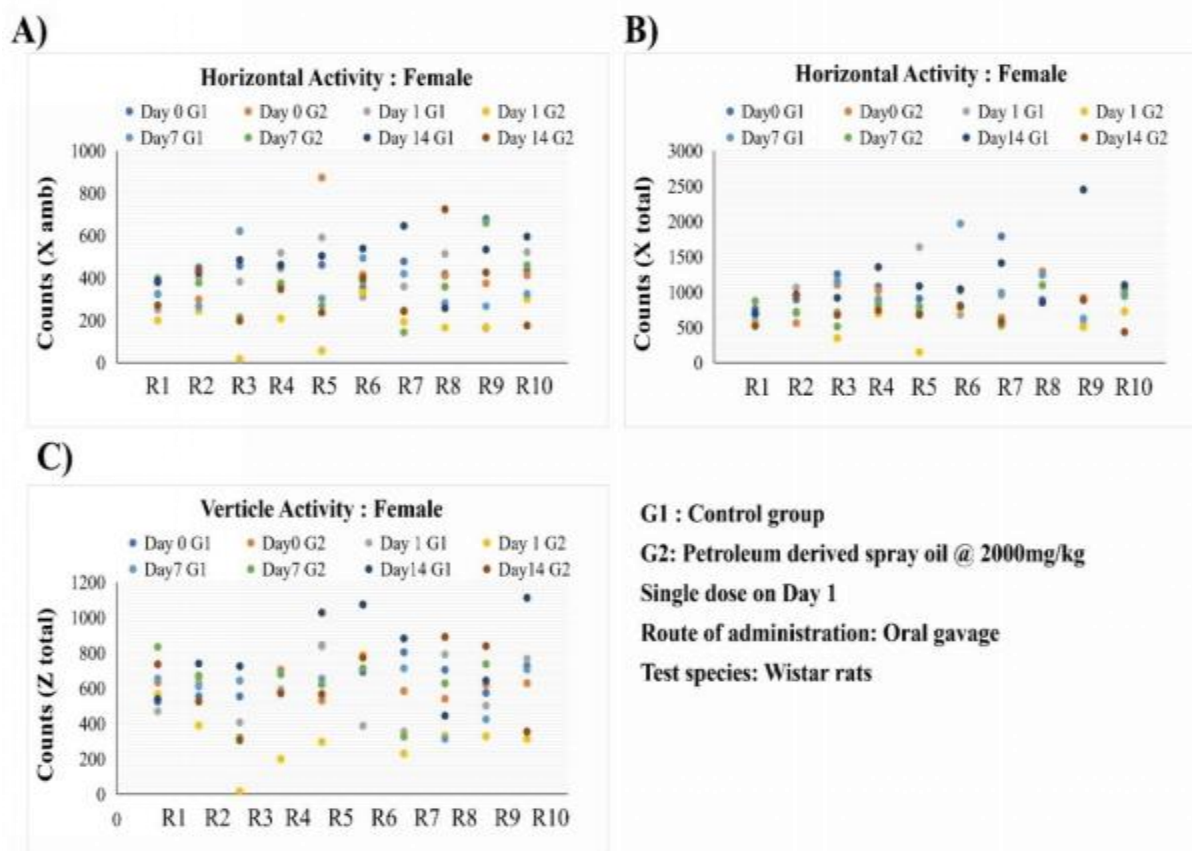


Figure 9: Graphical representation of motor activity observation in experimental and control Wistar rats (A – Horizontal activity x amb: female, B – Horizontal activity x total: female, C – Vertical activity z total: female).

Histopathology

Throughout the course of the investigation, neither male nor female Wistar rats showed any unusual changes in spontaneous motor activity, gait, posture, or exploratory behaviour. The dot plot data point distribution showed that the Experimental animals remained in the normal range. No treatment-related clinical symptoms or signs were seen, including tremors, circling, convulsions, hypoactivity, or hyperactivity. Overall, the locomotor activity findings indicated normal neurobehavioral performance and the absence of observable neurotoxic effects in treated animals.

Neither the treatment group (G2: 2000 mg/kg body weight) nor the control group (G1: 0 mg/kg body weight) had any discernible pathological abnormalities in the organs investigated by gross necropsy. Organs were taken from five male and five Wistar rats in each group at the time of final sacrifice. Owing to the time restrictions for the dissertation, histopathology

results from three typical brain tissue samples are reported: the study pathologist determined that all these samples had normal histological architecture.

Microscopic evaluation of brain histology:

Histopathological examination of the brain tissue (H&E, 10x), Figure 18 (A, B, C), revealed normal cytoarchitecture, well-preserved neurons and neuropil. No significant histopathological alterations, including neuronal degeneration, inflammatory cell infiltration, haemorrhage, necrosis, or vascular changes, were observed.

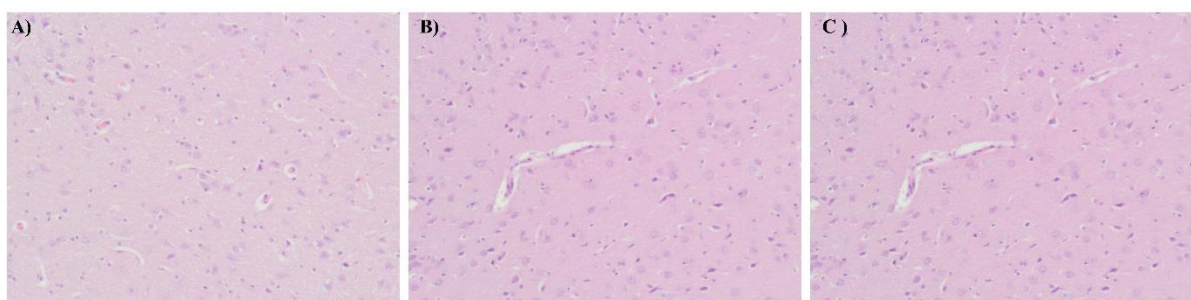


Figure 10: photomicrograph of the treatment rats' brain tissue stained with hematoxylin and eosin (H&E) showing normal histological architecture with intact neuronal cells with no observable treatment-related pathological changes.

Conclusion

In the present study, when male and female Wistar rats were administered petroleum-derived spray oil through oral gavage @ 2000mg/kg b.wt. did not result in any significant abnormal changes in body weight gain (on Day 7 and 14), clinical observations (Fur, Dyspnea, Larymation, Salivation, Diarrhoea, Gait, Posture, Tremors, Circling, Lethargy, Bizarre behaviour), grip strength (peak time and peak force) and locomotor activity (vertical and horizontal movements) under the conditions of the study. Therefore, this chemical may be categorised in GHS class V through the oral route. These findings may be useful to select a dosage regimen for sub-acute and chronic studies.

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