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Neurobehavioral and Cognitive Impacts of Perinatal Ethanol Exposure in Dunkin-Hartley Guinea Pigs: A Comparative Study of Spontaneous Locomotion, Spatial Memory, and Brain Development

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ABSTRACT

Perinatal ethanol exposure (CPEE) is an established risk factor for enduring neurodevelopmental deficits. This research investigates the behavioural, cognitive, and neuroanatomical effects of early ethanol exposure in Dunkin-Hartley guinea pigs. Male and female guinea pigs (550–650 g) were treated humanely in accordance with IAEC guidelines and allocated to either an ethanol-treated group or a nutritional control group. Ethanol (4 g/kg) or isocaloric sucrose was administered weekly through oral gavage, and blood ethanol concentrations (BEC) were measured using headspace gas-liquid chromatography. Spontaneous locomotor activity was evaluated on postnatal day 10 utilising an infrared-equipped open-field arena. Beginning on postnatal day 21, subjects underwent assessments for spatial learning and memory using either the dry-land Biel maze or the rewarded alternation Y-maze. The Biel maze evaluated navigation accuracy, errors, and latency to reward, whereas the Y-maze examined working memory and choice behaviour under delayed conditions. Animals were euthanised with halothane between postnatal days 150 and 200, followed by the extraction and weighing of their brains. Statistical analyses comprised t-tests and two-way ANOVA, employing Bonferroni corrections. No sex differences were observed; the data were aggregated. Guinea pigs exposed to ethanol exhibited heightened hyperactivity, extended trial durations, increased errors in spatial tasks, and markedly reduced brain weights relative to control subjects. The findings suggest that CPEE adversely affects motor regulation and cognitive function, probably as a result of neuroanatomical alterations.

Keywords: *Perinatal Ethanol Exposure, Dunkin-Hartley Guinea Pigs, Neurobehavioral Assessment, Spatial Memory Impairment, Brain Development*

1. INTRODUCTION

1.1 Background on Perinatal Ethanol Exposure

Prenatal alcohol intake (PAE) is the root of many birth malformations identified as fetal alcohol spectrum disorders (FASD). It is a non-diagnostic catch-all term for all birth abnormalities and developmental disorders related to alcohol [1]. Fetal alcohol syndrome (FAS) is the most common condition, characterized by craniofacial dysmorphology, central nervous system neurodevelopmental abnormalities, and growth retardation (PAE) [2, 3, 4]. Following the finding of FAS, it was discovered that not all people exposed to alcohol during pregnancy had any FAS signs. Physical abnormalities, neurological, and behavioral disorders, moderate to severe growth deficits, heightened vulnerability to mental health problems, and other comorbidities are among the many alcohol-related disorders [5, 6, 7]. Physical abnormalities, neurological, and behavioral disorders, moderate to severe growth deficits, heightened vulnerability to mental health problems, and other comorbidities are among the many alcohol-related disorders [1, 2]. Physical abnormalities, neurological, and behavioral disorders, moderate to severe growth deficits, heightened vulnerability to mental health problems, and other comorbidities are among the many alcohol-related disorders [8]. The effects of alcohol exposure are known to be influenced by a number of biological and environmental factors, such as the amount of alcohol consumed, the pattern of exposure, the length of exposure, the timing of exposure during development, the mother's age, her socioeconomic status, her diet, interactions with other drugs, and the genetic background of both the mother and the fetus. These elements contribute to the complexity of the FASD phenotype

and the difficulty of researching the molecular processes underlying PAE. There is increasing evidence that alcohol teratogenesis is especially susceptible to certain developmental stages [9, 10]. Preimplantation occurs during the first two weeks of human pregnancy and between four and six gestational days (GD) in mice. Because the embryo is going through fast developmental changes as the zygote grows into a morula and then into a blastocyst, this stage is susceptible to alcohol exposure [11, 12]. Physical abnormalities, neurological, and behavioral disorders, moderate to severe growth deficits, heightened vulnerability to mental health problems, and other comorbidities are among the many alcohol-related disorders [13, 14, 15]. A single dosage of alcohol on any of GD7–9 has been proven in studies to cause various craniofacial abnormalities in animals that resemble the characteristics of FASD [16, 17, 18]. Furthermore, variations in the timing of PAE on GD7 as little as 4 hours led to distinct craniofacial morphologies [19, 20]. Organogenesis, which is equivalent to 3–8 weeks of gestation in humans and GD7–14 in mice, includes gastrulation. Since it is a time of ongoing cell differentiation and the beginning of primitive organ development, exposure to alcohol during organogenesis may result in several FASD symptoms [21]. Early pregnancy is a vulnerable period for alcohol-induced developmental abnormalities, according to this research. Physical abnormalities, neurological, and behavioral disorders, moderate to severe growth deficits, heightened vulnerability to mental health problems, and other comorbidities are among the many alcohol-related disorders.

1.2 Rationale for Using Dunkin-Hartley Guinea Pigs

Prenatal alcohol exposure (PAE) is the root of several birth malformations known as fetal alcohol spectrum disorders (FASD). It contains four diagnostic groups, and it is a non-diagnostic catch-all term for all birth abnormalities and developmental disorders related to alcohol [1]. Fetal alcohol syndrome (FAS) is the most common disorder, characterized by craniofacial dysmorphology, central nervous system neurodevelopmental abnormalities, and growth retardation [2, 3, 4]. Not all people exposed to alcohol during pregnancy had every FAS sign following the diagnosis of FAS. Deficits in behavior, neurocognition, and physical characteristics, moderate to severe growth deficits, as well as increased vulnerability to mental health problems and other comorbidities are among the list of alcohol-related disorders [5, 6, 7]. In addition to FAS: alcohol-related birth defects (ARBD), alcohol-related neurodevelopmental disorders (ARND), and partial fetal alcohol syndrome (PFAS). PAE is a key contributor to birth abnormalities and mental retardation in the Western countries, with an estimated prevalence of 35% in Europe and North America, and exceeding 10% in South Africa. [8]. A variety of physiological and environmental factors, including alcohol intake, the timing of exposure, the mothers age, her socioeconomic status, her diet, interactions with other medications, and both the mother and the fetus are all likely to be influenced by alcohol use, lifestyle, and environmental factors, such as the alcohol intake, the pattern of contact with, the length of exposure, development time, the mothers age, the mothers gender, and the fet these elements influence to the complexity of the FASD phenotype and this difficulty of investigating the molecular mechanisms that underlie PAE. Alcohol teratogenesis is especially vulnerable to certain developmental stages [9, 10)]. In mice, preimplantation occurs during the first two weeks of human pregnancy and between four and six gestational days (GD). This stage is vulnerable to alcohol exposure because the embryo is going through rapid developmental transition as the zygote develops into a morula and then into a blastocyst [11, 12]. After the preimplantation phase and gastrulation—a period of rapid cell specialization into the 3 germ layers of Germ layers—begins, the blastocyst adheres to the uterine wall. This week frame is week three for humans; in mice, it is GD6. 5-8. Gastrulation is one of the most vulnerable developmental stages because the embryonic cells are more vulnerable to alcohol intake [13, 14, 15]. In experiments, one-time administration on any of GD7-9 mice has been shown to cause various craniofacial abnormalities in animals that mimic FASD [16, 18, 18]. In addition, variations in the time period of prenatal alcohol exposure at GD7 as little as 4 hours resulted in distinct craniofacial morphologies [19, 20]. Organogenesis, which is equivalent to first trimester and GD7–14 in experimental animals, also includes gastrulation. Since it is a period of continuous cell differentiation and the start of primitive organ development, any FASD symptoms can be triggered by alcohol exposure during organogenesis [21]. According to this study, initial gestation is a vulnerable time for alcohol-related defects. In this case, the time between fertilization and organogenesis, or early gestation 0-8 weeks of prenatal development and GD0–14 in mice, is described as early pregnancy.

1.3 Objectives of the Study

The primary aim of this study is to examine the neurobehavioral and cognitive observed outcomes of perinatal ethanol exposure in Dunkin-Hartley guinea pigs. Specifically, the study aims to assess alterations in spontaneous locomotion, impairments in spatial memory, and changes in brain development, thereby providing insights into how early ethanol exposure may disrupt neurodevelopmental processes and behavioral functions relevant to fetal alcohol spectrum disorders (FASD).

The essential oil being referred to in this current research is bergamot oil, which has been extracted from the fruits of the bergamot tree, a citrus fruit known for its aromatic properties and health benefits. For the purposes of this study, the researchers specifically opted to utilize this particular essential oil due to its well-documented therapeutic and cosmetic properties, making it a valuable asset in the realm of natural remedies and skincare products. It is crucial to note that bergamot oil is obtained through a meticulous extraction process that involves carefully isolating the oil from the fruit, ensuring the preservation of its potent fragrance and beneficial compounds. Moreover, the rich history and traditional uses of bergamot

oil as a popular ingredient in perfumes, flavorings, and aromatherapy further highlight its versatility and widespread appeal in various industries. Consequently, the decision to incorporate bergamot oil in this study serves as a testament to the enduring relevance and importance of natural remedies derived from botanical sources, underscoring the ongoing exploration and utilization of plant-based solutions for modern-day health and wellness challenges.

The main objective of the current resaerch was to thoroughly analyze the behavioral impacts and structural alterations in this hippocampus of guinea pig offspring as a result of prolonged maternal exposure to 3 and 4 g of ethanol per kg on a daily basis. In the realm of ethanol-related neurobehavioral teratogenesis, the hippocampus emerges as a focal point, displaying similarities with the effects of hippocampal lesioning such as heightened activity levels and compromised spontaneous alternation. It is noteworthy that heightened locomotor activity stands out as a prevalent behavioral change observed following In-utero exposure to ethanol, shedding light upon the significant influence of ethanol on this developing brain and behavior of offspring

2. MATERIALS AND METHODS

2.1 Experimental Animals and Ethical Approval

Nulliparous female Budin-Haflley-strain guinea pigs, weighing between 458 and 600 grams, were mated with male dunkin Hartley-strain guinea pigs following well-founded breeding protocols. The initiation of gestation, designated as Day 0, coincided with the closing of the full vaginal membrane. Post this event, the guinea pigs were individually housed in metal mesh enclosures, with controlled lighting of a Twelve-hour sun and 12-hour dark cycle, in compliance along with CPSCEA guidelines. Throughout the pregnancy period, the health status, vaginal-membrane integrity, and weight of the animals were meticulously monitored on a daily basis. Upon the birth of the offspring (Neonatal day [PD] 0), the dams and the entire litters were transitioned to separate spacious plastic enclosures furnished lined with wood chips. Daily assessments, including weighing and health evaluations, were conducted on the newborns, which were subsequently sex-separated at the time of weaning.

Chronic Treatment Regimens

On gestation day 2 (GD 2), gestating females were randomly allocated into 4 experimental cohorts. They received either 4 g ethanol/kg of maternal physical mass with free access to food and water combined with SLN, or the same ethanol dose with unrestricted food and water access alongside a vehicle. Ensuring consistency in the experimental conditions, all groups received an equal amount of food consumed by the treated animal on the corresponding gestational day, with unrestricted access to water to neutralize the possible malnutrition caused by prolonged maternal ethanol exposure. The Drug delivery process involved providing SLN orally to the expecting guinea pigs through a regular schedule consisting of 250 mg essential oil SLN dissolved in a vehicle comprising milk (3.25% fat) and cream (18% fat) in a ratio of 9:1. The vehicle was provided with daily at 09:00 hours through Maternal-related oral intubation from GD 2 until GD 67. Additionally, maternal ingestion intake of ethanol (30% [vol/vol] ethanol in tap water) or sucrose (42% [wt/vol] sucrose in tap water) occurred over a period of 5 consecutive days, followed by a 2-day gap each week to simulate binge-type drinking behavior. This recurring design was maintained all along the gestation period, starting from GD 2 upto GD 67. The ethanol or sucrose treatment was administered orally, with the doses divided equally and taken two hours apart. Furthermore, the initiation of maternal ethanol or sucrose treatment was precisely timed to begin 2 hours post the maternal oral administration of vitamins or vehicle. Throughout the 2-day break from therapeutic intervention all pregnant guinea pigs were offered unrestricted availability of nourishment to ensure their well-being.

Maternal Blood Ethanol Concentration

Maternal blood alcohol concentration was meticulously analyzed in two distinct study cohorts of gestating female guinea pigs, both of which were subjected to a daily intake of 4 grams of ethanol per kilogram of maternal body weight. Throughout this period, these guinea pigs had unrestricted entry point to food and water, either in combination alongside SLN or the ethanol and vitamin vehicle, as specified by the previously established chronic treatment plan. The collection of blood samples occurred on gestational day (GD) 57 or 58, precisely timed to coincide with the immediate aftermath of the second divided dose of ethanol, a strategic moment selected for capturing the peak maternal BEC levels accurately. The choice of GD 57 or 58 was deliberate, allowing for a direct comparison of the maternal BEC levels with existing data points, facilitating a comprehensive analysis of the ethanol's effects. The determination of maternal BEC was executed with utmost precision using gas-liquid chromatography in conjunction with headspace-gas analysis, utilizing advanced instrumentation such as the HewlettePackard Model 5710A, enabling the generation of robust and reliable BEC measurements essential for acquiring valuable insights from the experimental data.

Biel Water Maze

The Biel water maze test, developed in 1940 by Biel, was conducted when the subjects were 6 weeks old. The maze apparatus utilized in the test was constructed derived from vinyl chloride, featuring a course width of 10.5 cm and water depth of 18

cm, with a carefully maintained water temperature of 22±1oC for consistency. To acclimate the animals to swimming, three timed trials on a straight course were administered the day before the maze test. Subsequently, the maze-facing tasks involved each animal undertaking four trials per day over a span of 3 consecutive days (days 1-3), with a maximum swimming time of 3 minutes allowed per trial. A retest to assess memory retention was conducted after a 10-day rest period – on day 14, where the test subjects were subjected to 4 trials. Following this, the animals navigated a reversed course task (path BI) over the next 3 days (days 15-17), performing four trials each day. The key performance metrics assessed during these trials included the number of errors made and the time taken to reach the target objective (swimming time), meticulously recorded for analysis. An error in this context was defined as a full-body entry, from the nose to the tail root, into a blind alley or any return entry during the course of the test.

Spontaneous Locomotor Activity

Spontaneous alternation, a behavior observed in laboratory animals, specifically in multiple-T-mazes which consist of two adjoining T-mazes on each arm of the central T-maze, is characterized by a repetitive alternating turns sequence at choice points when animals are not rewarded extrinsically. The experimental setup involved the provision of isocaloric sucrose solutions, prepared at varying concentrations of 28% or 42% (wlv) in tap water. Throughout gestation, monitoring of maternal mass and vaginal-membrane status occurred on a daily basis. On day 56 of Pregnancy period, maternal blood samples were obtained from an ear vein B hours post the second separated into dose of ethanol, isocaloric sucrose, or water for ethanol concentration and total moving time was recorded. Post-birth assessments included determining litter size, live births, presence of gross dysmorphology, and evaluating single entity offspring body weight and head circumference. The Progeny group continued to be raised alongside their biological mothers until postnatal day 17, with regular monitoring of their weights. Pre-maturation, gender-separated male and female littermates reached this stage at approximately postnatal day.

Morris Water Maze

The Morris water maze test, an established behavioral experiment, was conducted when the subjects were ten weeks old. The experimental set-up involved a circular pool, boasting a substantial 147 cm diameter, Water-filled maintained at a temp. of 26 degrees Celsius. Achieving the goal, a transparent resin marker positioned precisely 1 cm beneath the water's surface and located at 112° from the center point, was the primary objective for the test subjects. The subjects' activities were meticulously recorded utilizing a sophisticated visual monitoring known as Neuro Science. The analysis focused on this swimming distance between the starting point and the goal, as well as the time taken to reach the goal. To assure fair testing conditions, each trial had a set time limit of 2 minutes. Over a period of three consecutive days (days 1-3), each subject underwent five trials daily. On the fourth day, a new challenge emerged where the goal was shifted to the opposite side of the circular pool. This change was followed by another set of five trials. Notably, in every trial, the initial point was chosen haphazardly from four strategically positioned points, both inside and outside the maze, set at equal intervals around the circumference.

Organ Harvesting and the Effects on these Organs

The essential organs—including the heart, liver, lungs, kidneys, brain, spleen, uterus, adrenal glands, stomach, ovaries, cervix, vagina, urinary bladder, and eyes—were meticulously collected through precise midline incisions. Following the harvesting process, any remaining blood on the organs was meticulously removed using blotting paper. To determine the relative organ weight (ROW), a precise measuring instrument such as a calibrated weighing balance was utilized. The calculation of ROW was carried out in relation to the body weight of the subjects at the time of their sacrifice. This calculation was accomplished through a specific equation designed for this purpose.

$$\text{ROW} = \frac{\text{Absolute weight of organ}}{\text{Body weight at the time of sacrifice}} \times 100$$

Later, following the harvesting process, the organs were meticulously fixed in a 10% buffered solution of formalin to ensure their preservation. Subsequently, they underwent further processing, which involved dehydrating them in a sequence of graded alcohol and xylene treatments. This meticulous procedure was crucial in preparing the tissues for the next stage of examination. Prepared samples were delicately deeply inserted in paraffin wax, a step necessary for their eventual Histopathological evaluation. This thorough methodology ensured that the samples were appropriately preserved and ready for detailed analysis under the microscope by trained professionals. Each step in the process was executed with precision and care to guarantee the integrity of the tissues and provide accurate results for the histopathological examination.

Statistical Analysis

Behavioral data obtained from the study were meticulously examined using a triple fixed-effect factorial analysis of variance, also known as the general linear model. This statistical approach incorporated the concept of trials as repeated measures, enhancing the robustness of the analysis (R-ANOVA).

3. RESULTS AND DISCUSSION:

Ethanol Blood Concentration

Maternal blood alcohol concentration values for the non-treated pregnant guinea pigs and the treated pregnant animals at 1 hour after the second equally split dosage of ethanol on gestational day 57 or 58 were found to be 257.6 ± 12.2 mg/dl (n=3) and 256.6 ± 8.4 mg/dl (n=3), respectively. A comprehensive statistical analysis revealed no significant difference between these BEC values, indicating a comparable ethanol metabolism between the two groups. Notably, these BEC measurements closely mirror the BEC observed in our previous study, which focused on a chronic ethanol administration regimen of 4 grams per kilogram of maternal body weight per day throughout the entire gestation period in our initial guinea pig model. This consistency in BEC levels across these studies highlights the reliability and reproducibility of our findings, reinforcing the validity of the guinea pig model as a robust tool for studying ethanol-induced neurobehavioral teratogenic effects. Furthermore, this similarity in BEC values underscores the utility of our previous work in establishing a baseline for understanding the impact of ethanol exposure on maternal and fetal health outcomes, providing valuable insights into the mechanisms underlying ethanol teratogenicity in a controlled experimental setting.

Biel Water Maze

In the study, the swimming times were meticulously recorded across different groups over three consecutive days. On the initial day, the control group swam for an average of 187 seconds, while the pure drug group averaged 176 seconds, and the SLN group came in at 273 seconds. Moving on to day 2, the control group clocked in at 92 seconds, the pure drug group at 112 seconds, and the SLN group at 127 seconds. By the third day, the control group showed a time of 73 seconds, with the pure drug group close behind at 67 seconds, and the SLN group at 76 seconds.

Interestingly, a notable treatment x trials interaction emerged, indicating a dynamic relationship between the treatments and the swimming trials. While no substantial effect difference was found amongst the control and pure drug study cohorts, the SLN group's performance stood out. Particularly, on both days 1 and 2, the SLN group's times surpassed those of the control group, signifying a potential impact of the treatment regimen. Surprisingly, by day 3, the SLN group's performance aligned closely with the control group's timing Figure 1A, 1B.

Furthermore, the statistical analysis unveiled a significant treatment effect, further bolstered by the calculated F-value of 3.20, demonstrating the treatment's influence on the swimming times. These results shed light on the intricate interplay between the treatments and swimming performances, underlining the importance of considering various factors when evaluating experimental outcomes.

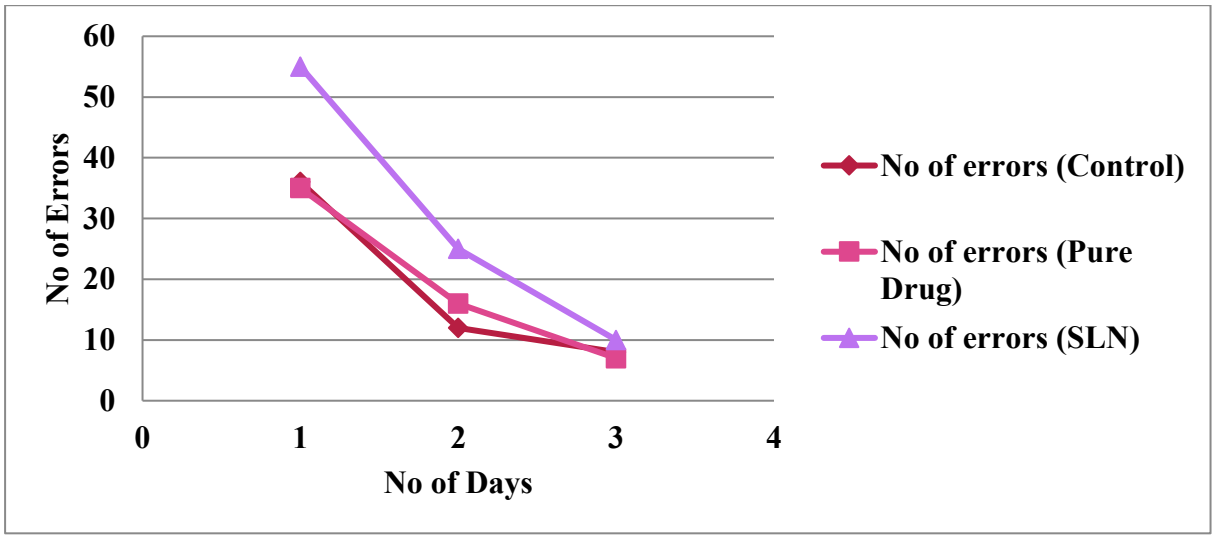

Fig. 1A: Error count in Biel maze with Guinea Pigs A) Errors from Day 1-3 and B) Errors from Day 15-17)

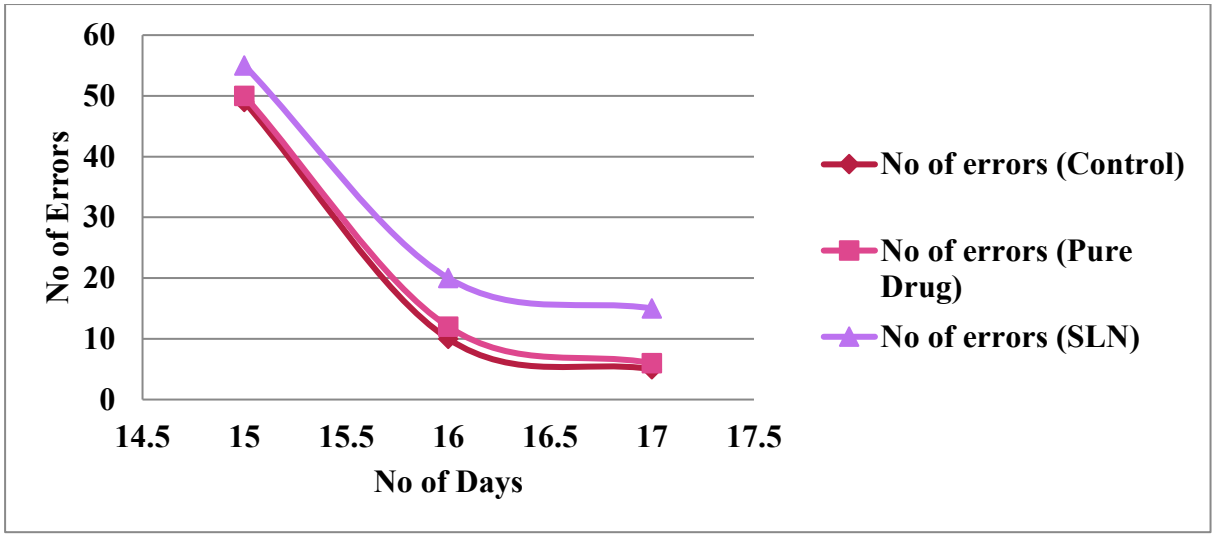

Fig. 1B: Error count on the Biel water maze with Guinea Pigs A) Errors from Day 1-3 and B) Errors from Day 15-17)

Spontaneous Locomotor Activity: The results from the two-method ANOVA evaluation found out a statistically tremendous impact of maternal treatment as well as the length spent in the apparatus on the locomotor pastime determined on postnatal day 10 (PD 10), as illustrated in Figure 1. Specifically, the analysis indicated that treatment during pregnancy ($F(1,406)=6.92$, $P<0.05$) and time in the apparatus ($F(5,406)=6.78$, $P<0.05$) significantly influenced the period utilized in motion. Notably, offspring exposed to chronic prenatal ethanol (CPEE) exhibited a higher level of spontaneous movement during the 30-minute testing period compared to the sucralose control group. Despite this, both sets of offspring experienced a decline in locomotor activity as the 30-minute test progressed.

Moreover, it was observed that maternal ethanol consumption during pregnancy led to an increase in the baseline locomotor activity of the PD 10 offspring. The CPEE offspring, in particular, displayed a greater amount of time engaged in movement when contrasted with the sucralose control offspring, with this difference being statistically significant ($P<0.05$). Interestingly, both maternal treatment groups, including the CPEE and sucralose control offspring, exhibited a reduction in the time spent moving over the course of the 30-minute test period, emphasizing the dynamic nature of locomotor activity during this developmental stage.

In postnatal day 10 offspring, spontaneous locomotor activity was elevated by maternal ethanol consumption. Compared to sucralose controls, youngsters uncovered to continual prenatal ethanol spent greater time transferring ($P<0.05$), even as both maternal treatment businesses spent much less time shifting in the course of the 30-minute check period ($P<0.05$). The group mean±standard blunders of the average value of every experimental group's offspring is how the facts are displayed Table 1.

Overall, the above findings shed light on how maternal ethanol exposure influences offspring locomotor behavior and highlights the importance of considssering prenatal conditions in the assessment of postnatal physiological responses. The data presented in this study are summarized as the group mean values along with the corresponding standard error of the mean for each experimental group, ensuring transparency and accuracy in the reporting of results Figure 2, Figure 3.

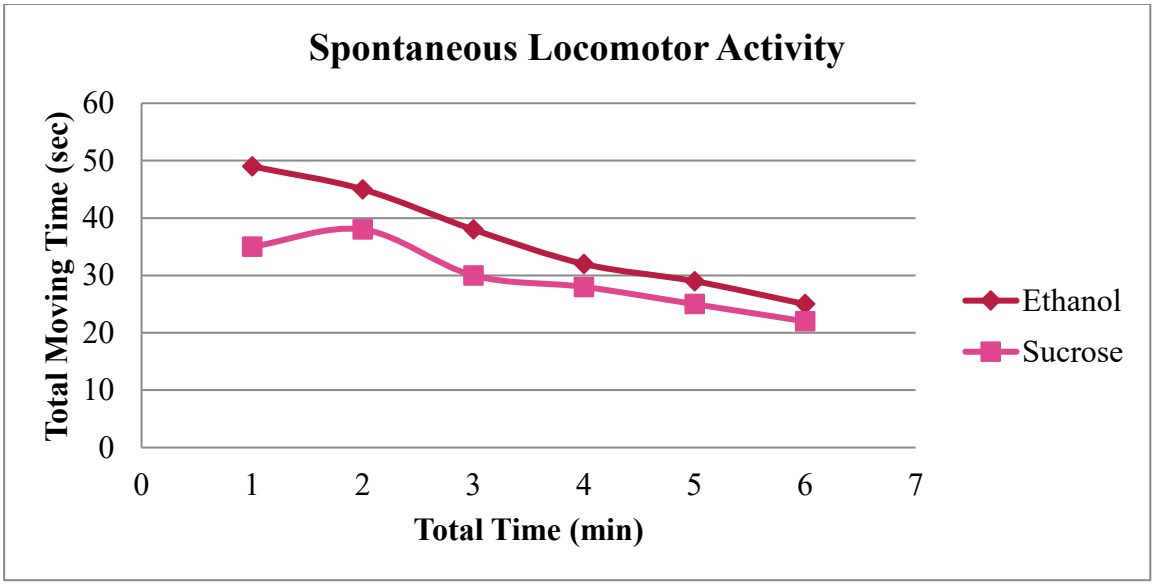

Fig. 2: Spontaneous Locomotor Activity

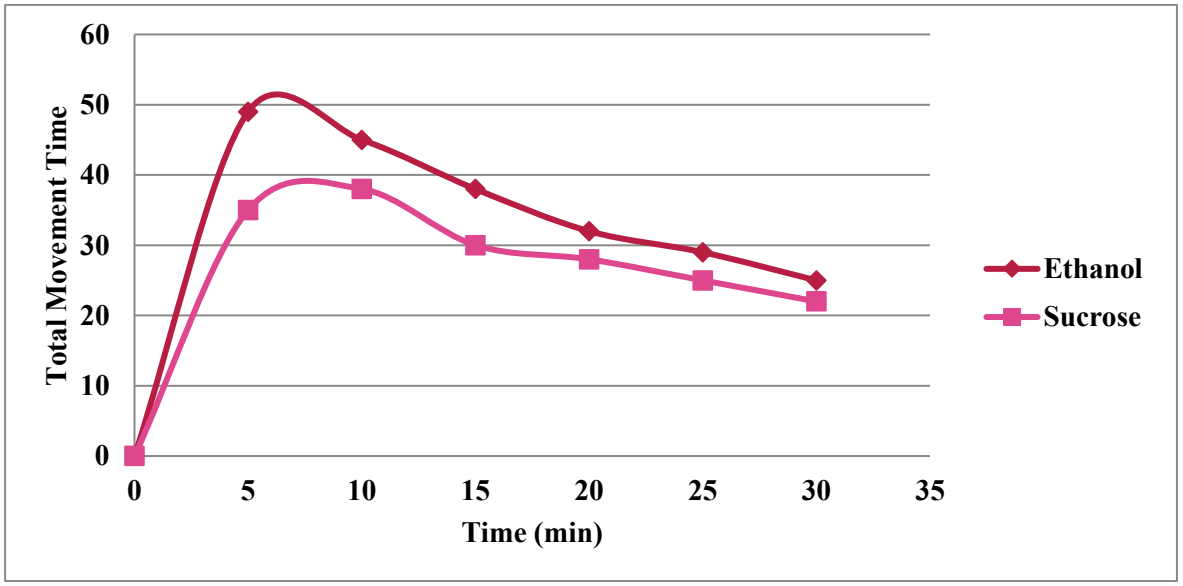

Fig. 3: Maternal ethanol intake increased spontaneous locomotor activity in postnatal day 10 offspring, with kids spending greater time shifting as compared to sucralose controls, whilst both treatment groups spent less time moving.

Table 1: being pregnant observations for guinea pigs fed both sucralose solution (n=17 litters) or 5% aqueous ethanol (n=16 litters) in the course of the gestation period

Pregnancy outcome variable	Maternal treatment	
	Ethanol	Sucralose
Ethanol intake by mothers (average daily dose in grams per kilogram)	2.4 ±0.1	N/A
Total amount of food consumed by mothers (g/kg body weight)	2405 ±71.5	2746± 160.7
Gestational Length (days)	66.7 ±0.4	67.8 ± 0.5
Weight of offspring at birth (g)	88.4 ±2.3	96.9 ± 2.1

Male littermates (%)	56.8	54.2
Female littermates (%)	47.5	45.0
Litter size	3.8 ±0.2	3.6± 0.2
Unplanned abortion	1	0
Death after birth	2	1
Teenage death	2	0

Morris Water Maze

The study found a significant treatment effect on swimming time and distances in a maze. The pure drug group had comparable swimming times to the control, while the SLN treatment (mg/kg) group had higher values. The time direction of swimming distances was once much like swimming times, and the treatment impact was once widespread in swimming distances. On day 3, the distances inside the manipulate and natural drug businesses have been reduced to at least 1/4 of the day 1 values, whilst the SLN organization confirmed a significantly longer distance of about 4,000 cm. The animals often crossed the center of the maze with increasing trials, while the SLN group continued to swim in the periphery. Within the day four take a look at with the goal set on the alternative aspect, swimming distances slightly multiplied from day 3 values in all agencies. But, there have been no full-size differences in swimming distances between the control and dealt with corporations on day 4 Figure 4.

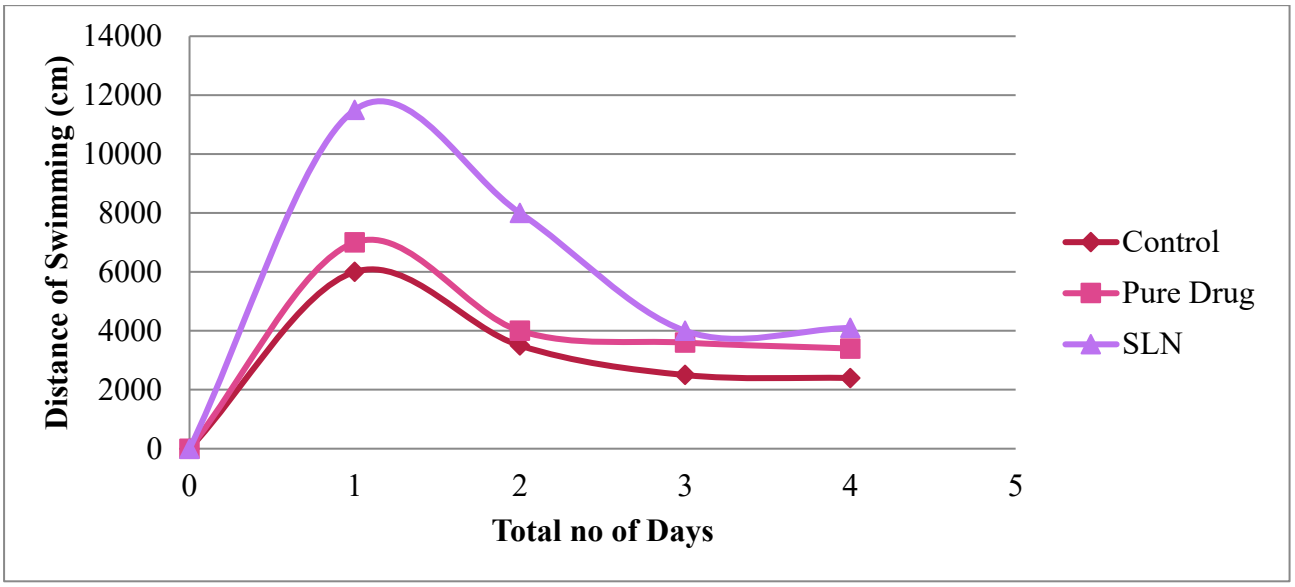

Fig. 4: Distance to swim on the Morris Water Maze

Organ Harvesting and the Effects on these Organs

Because time immemorial, plant-derived botanicals have played a quintessential and tremendous function inside the management and remedy of various illnesses throughout diverse cultures and civilizations. These natural products, rich in bioactive compounds and phytochemicals, possess immense potential not only as therapeutics but additionally as critical precursors for the development and synthesis of effective drug applicants which could deal with a myriad of fitness conditions.

It is essential to note, however, that amidst the promising pharmacological properties of these plant-derived compounds, the exploration and evaluation of their potential toxicity are paramount. This critical assessment needs to be conducted meticulously and comprehensively prior to considering their utilization in various disease models and medical applications.

Understanding the safety profile and potential adverse effects of these botanical compounds is fundamental in ensuring their efficacy and suitability for therapeutic use. Therefore, in-depth research and rigorous testing protocols should be implemented to elucidate the full spectrum of their pharmacological activities, ensuring both the effectiveness and safety of these natural products in the realm of healthcare and disease management.

The microscopical findings, did not specific any gross pathological and microscopical adjustments in the remedy corporations, while as compared to the manage animals. Moreover, throughout gross examination all of the organs retained their everyday textures barring any abnormal adjustments within the coloration and look. Importantly, no structural alterations were detected in exclusive tissues the use of unique resolution beneath the microscope table 2, Figure 5.

Table 2: The Guinea Pigs' Relative Organ Weight (gm) Upon Sacrifice

Organ	Control	Pure Drug	SLN Formulation
Heart	0.267 ± 0.001	0.269 ± 0.002	0.268 ± 0.003
Liver	2.947 ± 0.093	3.176 ± 0.363	3.104 ± 0.217
Lungs	0.433 ± 0.002	0.432 ± 0.003	0.433 ± 0.002
Brain	0.632 ± 0.035	0.750 ± 0.121	0.694 ± 0.083

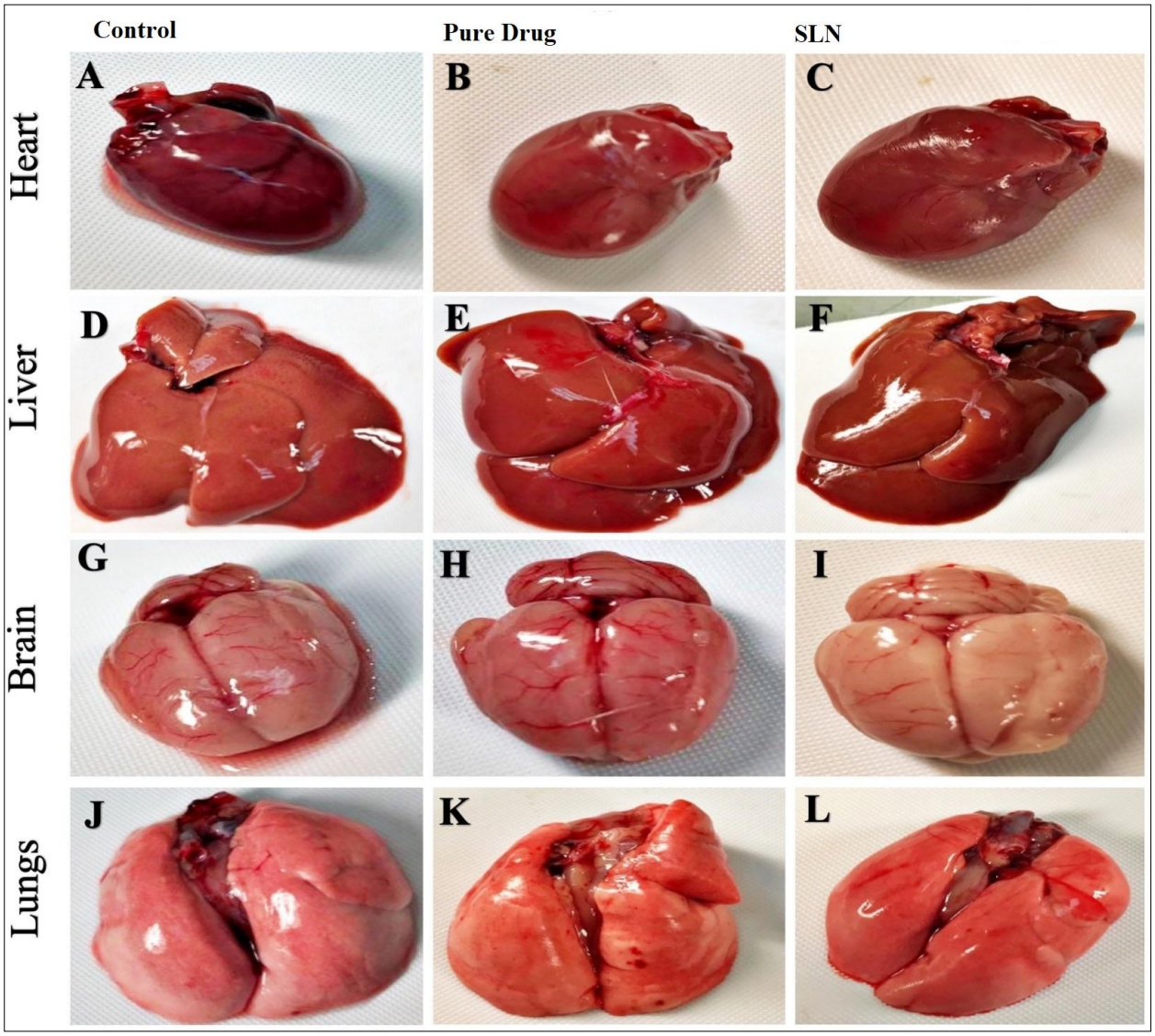

Figure 5: Harvested Organs after Treatment

Through meticulous physical observation and assessment of clinical parameters, it was discerned that all animals within experimental groups exhibited no discernible signs of anxiety or toxicity-related symptoms. Detailed examination revealed

no significant modifications done in various anatomical traits including condition, skin appearance, eye health, salivation levels, mucous membrane integrity, sleep patterns, and behavioral tendencies. Furthermore, there were no observable indications of gastrointestinal issues such as diarrhea, neurological abnormalities like coma and tremors, or signs of general weakness or lethargy Figure 6.

Interestingly, the animals in the treatment groups displayed a consistent and healthy rise in body weight compared to the control group, without any notable deviations of concern. Notably, the analysis also revealed insignificant changes in the relative organ weight (ROW), suggesting that the bergamot essential oil used in the study did not have a detrimental impact on the normal growth and developmental processes of the subjects.

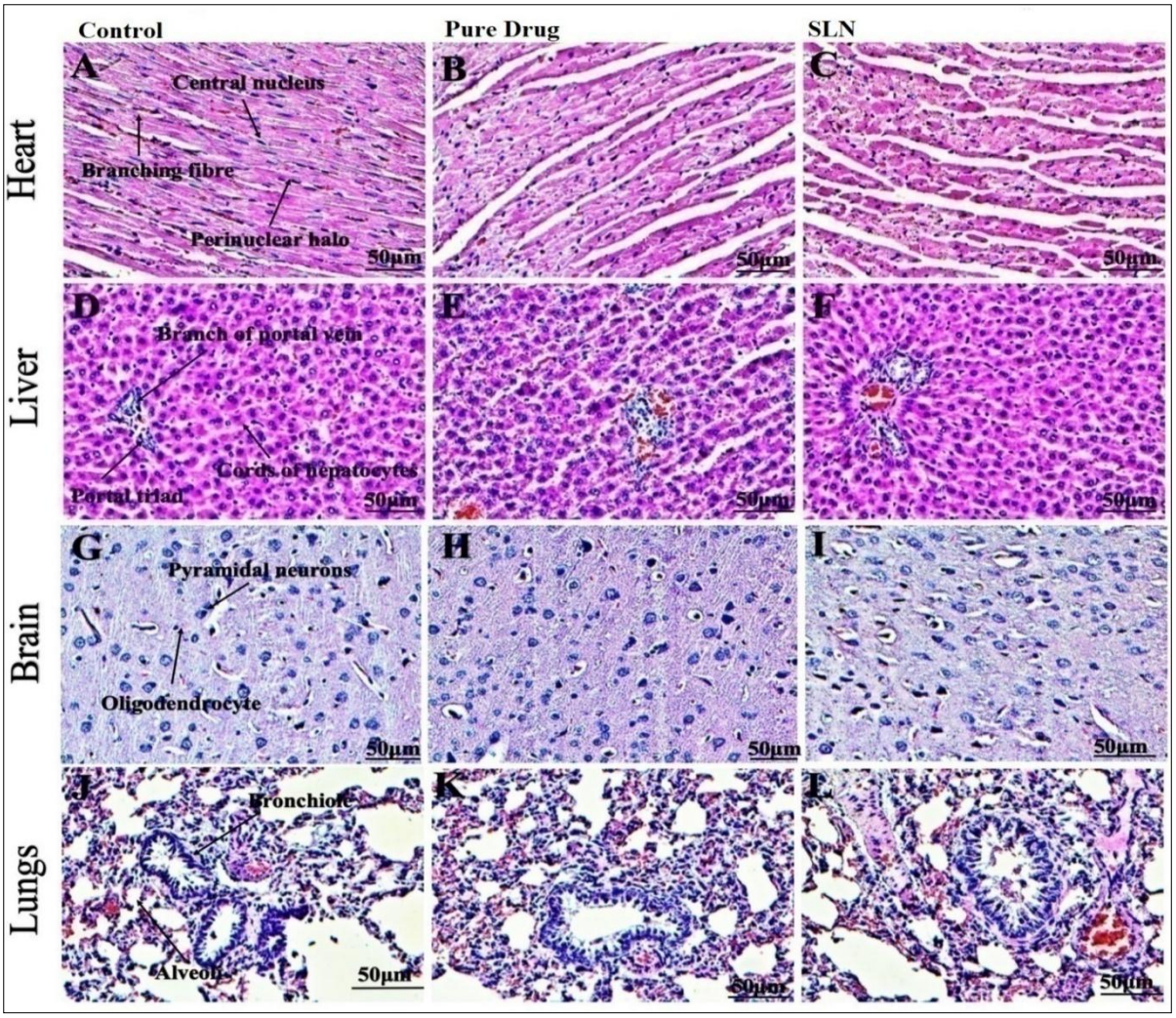

Fig. 6: Histopathological Study of the Harvested Organs

Upon microscopic evaluation of the cardiac tissues, the myocardial fibers displayed standard features, such as centrally located nuclei, cross striations, and distinctly visible intercalated discs. Prominent observations, including perinuclear clear zones and transparent areas surrounding the nuclei, suggested the lack of necrotic damage, tissue enlargement (hypertrophy), fibrotic changes, or lipid deposition, while a normal level of glycogen was also present.

Furthermore, the examination of liver tissues revealed a regular arrangement of hepatocytes in polygonal shapes with radiating cords, alongside observable congestion in hepatic sinusoids. There were no signs of necrosis, cellular apoptosis, hemorrhages, or fatty changes in the vicinity of central veins, sinusoids, or portal triads. Crucially, there was no observed infiltration of monocytes or macrophages within the liver tissues.

The brain tissues displayed their normal structural integrity across various regions, with no morphological alterations such as necrosis or distorted neurons in any of the groups. Detailed examination of brain sections showed pyramidal neurons with

well-defined shapes and delicate cytoplasm, while glial cells exhibited consistent outlines throughout all groups. Zooming into the lung tissues also revealed the normal honeycomb appearance of alveoli with flattened squamous cells lining them, and bronchioles surrounded by epithelial cells and smooth muscles, devoid of congestion, necrosis, or evidence of apoptotic cell death within the treatment groups.

4. CONCLUSION

In our current toxicological assessment, bergamot essential oil has been deemed non-toxic both when used as a standalone medicine and when formulated in solid lipid nanoparticles (SLN). Extensive studies have shown that there were no observed changes in the organs of subjects treated with bergamot essential oil, nor were there any indications of physical or behavioral abnormalities. Moreover, the treated Guinea pigs exhibited no significant histopathological alterations or mortality compared to the control groups, solidifying the conclusion that bergamot essential oil is a safe medication as long as it is consumed in appropriate doses. These results emphasize the importance of proper dosage control when utilizing bergamot essential oil for medicinal or therapeutic purposes. Moving forward, to better understand its potential therapeutic benefits for various diseases, it is imperative to conduct a comprehensive investigation using subacute/chronic disease models. This in-depth analysis will provide valuable insights into the efficacy and safety of bergamot essential oil as a possible treatment option, paving the way for further advancements in medical research and healthcare practices.

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Conflict of Interest

No conflict of interest were found

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