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Research Paper

Effects of Soursop (*Annona muricata*) Leaf Extract on the Prefrontal Cortices of Maternal and Fetal Wistar Rats

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ABSTRACT

Background:

Annona muricata (soursop) leaf extract contains acetogenins, which inhibit mitochondrial complex I, and alkaloids, known neurotoxins. This study investigates the neuroanatomical and biochemical effects of crude aqueous A. muricata leaf extract on the prefrontal cortices of maternal and foetal Wistar rats.

Materials and Methods:

Twenty-four adult female Wistar rats were randomly assigned to three groups (n=8 each). Treated groups received 0.5 ml of crude aqueous soursop leaf extract during the second and third trimesters, while controls received distilled water ad libitum. Rats were sacrificed a day before delivery via cervical dislocation. Prefrontal cortex tissues were processed for histological examination using Hematoxylin & Eosin staining and biochemical analysis of oxidative stress markers: Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glucose-6-Phosphate Dehydrogenase (G6PDH), and Lactate Dehydrogenase (LDH). Serum progesterone and oestrogen levels were also measured.

Results:

Treated groups exhibited a significant increase in SOD and GPx activities ($p<0.05$) compared to controls. Conversely, G6PDH, LDH, progesterone, and estrogen levels decreased significantly ($p<0.05$) in treated groups. Histological analysis revealed a reduction in neuronal density in the prefrontal cortex of treated rats.

Conclusion:

Maternal consumption of Annona muricata leaf extract during pregnancy may lead to oxidative stress, hormonal imbalance, and neuroanatomical alterations in developing foetuses. Given these potential neurotoxic effects, pregnant women should exercise caution, and further research is needed to assess its safety for foetal development.

KEYWORDS: *Annona muricata,* Oxidative stress, Neurotoxicity, Pregnancy, Wistar rats.

BACKGROUND

Teratogens are substances or environmental factors that can cause birth defects in a foetus[1]. These agents disrupt normal foetal growth and can lead to physical malformations, neurological disorders, or even pregnancy loss[2]. Dosokyand Setzer demonstrated that the effects of teratogens depend on the timing of exposure, with the first trimester being the most critical period for organ formation[3]. Broussard and colleagues in their study in 2011 further indicated that the severity of the impact also varies based on the dosage and duration of exposure. Preventing teratogenic effects involves avoiding harmful substances during pregnancy and proper prenatal care against exogenous agents[4].

Reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, singlet oxygen, and hydroxyl are active forms of reactive molecular oxygen[5]. ROS are bye product of normal metabolism of oxygen within the mitochondrial matrix, which acts as their precursor serving as physiological regulator of normal cell multiplication and differentiation[6]. If the balance of ROS increases more than the scavenging potentials of the intracellular antioxidant system, the cell undergoes a state of oxidative stress which impairs significantly the cellular structures and functions. However, excessive levels of ROS, often result in severe damage to DNA and proteins[7].

Complementary medicine includes a diverse range of traditional and alternative healing practices that are used alone or alongside conventional Western medicine[8]. These approaches are deeply rooted in cultural beliefs, indigenous knowledge, and the rich biodiversity of most continents of which Arica is not left out. Natural medicinal plants often exert their effects through several mechanisms including oxidative stress depending on their bioactive components[9].

Soursop (*Annona muricata*) is a medicinally valuable traditional medicinal plant across tropical regions because of the well-known antioxidant and anti-inflammatory effects of its leaves, bark, root, and other components[10]. Biochemical studies on leaves of *Annona muricata* have detected anxiolytic, anti-inflammatory, and anticonvulsant properties as well as neuroprotective properties[11]. The plant is a rich source of alkaloids, and flavonoids together with acetogenins[12]. However, the safety limit and potential neurocytotoxic effects of crude aqueous extracts of soursop leaves on the developing brain, particularly the pre-frontal cortex(PFC), remain largely unexplored.

The Pre-frontal cortex (PFC) is known to play a vital role in decisions making, social and emotional regulation[13]. Developmental changes in this region before birth strongly impact neuronal functions which conversely impairs cognitive functions and behavioral manifestations[14]. The periods of organogenesis are usually a pivotal point in teratogenic interference of the developmental process[15]. The gestational development of the PFC becomes vulnerable because of multiple factors that include environmental toxins as well as pharmaceuticals and herbal remedies[16].

Brain development of foetuses faces potential risks when pregnant women are exposed to bioactive compounds from plants[17]. Studies have shown both protective and toxic neurological outcomes in connection with CBD usage which depends on how much CBD is given and how long people use it[18].

This research aims to examine the gestational brain structural alterations of maternal administration of crude aqueous soursop leaf extract on the prefrontal cortices of both mother and pups of Wistar rats. Focusing on neurodevelopmental safety and effects through evaluation of both histological and neuronal densities together with investigations into potential neuroprotective or neurotoxic impacts.

MATERIALS AND METHODS

Animal Procurement and Breeding

Procurement:

A total of 24 female and 10 male healthy adult Wistar rats, weights (150-200g) were procured from the Department of Veterinary Medicine, University of Ilorin. The rats were transported in plastic transparent cages to the animal facility of the Department of Human Anatomy, Faculty of Basic Medical Sciences College Health Sciences, University of Ilorin where they were transferred into their home cages.

Breeding and Acclimatization:

The rats were acclimatized for two weeks in the animal facility of the Human Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin.

They were kept under a 12-hour light-dark cycle at room temperature. The male and female rats were housed separately in wooden wire-gauzed cages measuring 12" x 12" x 16". The cage floors were designed with wire mesh to facilitate waste removal. The cages were cleaned daily, and the rats were fed with pelletized feeds (Topfeeds Nigeria Limited, Sango, Ilorin) and provided distilled water *ad libitum*.

Experimental Plant

Fresh *Annona muricata* leaves were collected from the Department of Plant Biology, University of Ilorin. The leaves were air-dried, ground into a fine powder, and sieved. A crude aqueous extract was prepared by dissolving 10g of the powdered leaves in 100ml of distilled water, which was subsequently evaporated to dryness. A final extract concentration of 10g dissolved in 100ml of normal saline was prepared for oral administration.

OTHER MATERIALS

Wire-gauzed cages, rat pellets, feeding trough, dissecting sets, calibrated syringe, oro-gastric tube, measuring cylinder, feeds, staining trough, oven, microtome, sensitive weighing scale, slide cover slips, slides, specimen bottles, hand gloves, dissecting board, cotton wool, xylene, 10% formalin, beakers, glass rods, hot plate, plastic embedding mould, Bunsen burner, paraffin wax, absolute alcohol, distilled water, haematoxylin stain, eosin stain, hydrochloric acid, egg albumin, Distrene Plasticizer Xylene (DPX), forceps, scalpels, surgical blades, paper tape, and permanent markers.

Determination of Mating

Mating was determined using the vaginal smear test to monitor the estrous cycle, which includes proestrus, estrus, metestrus, and diestrus phases. A micropipette containing 0.5ml of normal saline was introduced into the vagina of the female rats between 7:00-9:00 am to collect vaginal secretions. The collected fluids were placed on glass slides and examined under a light microscope (x10 magnification). In rats, ovulation occur in oestrous phase[19]. Ovulation was confirmed during the estrous phase[19], and female rats were paired with males at a 2:1 ratio from 4:00 pm to 8:00 am. The presence of spermatozoa in the vaginal smear confirmed mating and was considered day 0 of pregnancy.

Animal Grouping and Treatment

After pregnancy confirmation, the rats were randomly grouped as follows:

Group	Duration	Treatment (0.5ml)	Administration Duration	No. of Animals	Expected No. of Fetuses
A	2nd Week	0.5ml aqueous extract	Days 9-11	8	8
B	3rd Week	0.5ml aqueous extract	Days 15-17	8	8
C	Control	0.5ml distilled water	Entire gestation	8	8

Tissue Processing for Microscopic Analysis

Fixation and Dehydration:

Fixation and dehydration were carried out using the method of Drury and Wallington (1980). The extracted tissues were fixed in 10% formalin to preserve their structural integrity. Following fixation, the tissues underwent a series of graded dehydration steps to remove water content in preparation for embedding. Initially, the tissues were immersed in 50% alcohol for one hour, followed by subsequent immersion in 70% and 90% alcohol, each for one hour. Finally, the tissues were placed in absolute alcohol in two changes, with each change lasting one hour. This stepwise dehydration process ensured the optimal removal of water from the tissues, facilitating proper infiltration during the embedding phase[20].

Clearing, Embedding, and Sectioning:

This was done using the protocol indicated by Feeback[21]. The clearing was performed using xylene in two changes of 1 hour each. Impregnation with molten paraffin wax (60°C) was done in two changes at 1-hour intervals. Embedded tissues were trimmed, mounted on wooden blocks, and sectioned using a microtome.

Staining Techniques:

The procedure of staining was carried out as described by Bakerand colleagues[22]. Haematoxylin and eosin (H&E) staining techniques were employed to visualize tissue morphology. In this method, haematoxylin stains cell nuclei a blue-black color, while eosin imparts varying shades of red, orange, and pink to the cytoplasm and connective tissues. The haematoxylin solution was prepared using 5 g of haematoxylin crystals, 100 g of calcium alum, 5 ml of 95% alcohol, 100 ml of distilled water, and 2.5 g of mercuric oxide. For eosin preparation, 1 g of eosin Y was dissolved in 640 ml of 95% alcohol and 160 ml of distilled water, with one drop of acetic acid added per 100 ml of the solution[22].

The staining procedure began with dewaxing tissue sections in xylene for 3 to 5 minutes, followed by rehydration through descending grades of alcohol—absolute, 90%, 70%, and 50%. The sections were then rinsed in distilled water and stained in haematoxylin for 12 to 15 minutes. Differentiation was carried out using 1% acid alcohol for approximately 2 seconds, after which the sections were blued under running tap water for 15 minutes. Counterstaining with eosin was performed for 2 to 3 minutes. The sections were then dehydrated through ascending alcohol grades, cleared in xylene, and finally mounted using DPX mountant and covered with a coverslip to preserve the stained tissues for microscopic examination[22].

Photomicrography

Slides were photographed using a Canon Digital Camera (10 megapixels).

Biochemical Assays

Prefrontal cortex samples were preserved in 0.25M sucrose solution, homogenized, and centrifuged at 5000 rpm for 10 minutes using a centrifuge (Model 90-1). The supernatants were stored in the deep freezer (GC-B207WVQ) at – 20°C - 20°C and analyzed for:

- Superoxide Dismutase (SOD)
- Glutathione Peroxidase (GPx)
- Glucose-6-Phosphate Dehydrogenase (G6PDH)
- Lactate Dehydrogenase (LDH)

Superoxide Dismutase (SOD) Assay:

Beauchamp's method was used to measure superoxide dismutase (SOD). To assess SOD activity, both standard and sample solutions (10 µl each) were prepared. To each solution, 200 µl of a radical detector was added. The reaction was initiated by adding 20 µl of xanthine oxidase, and the mixture was then incubated at room temperature for 30 minutes to allow the enzymatic reaction to proceed. Following incubation, the absorbance of each reaction mixture was measured at 450 nm using a spectrophotometer to determine SOD activity[23].

Glutathione Peroxidase (GPx) Assay:

As demonstrated by Weydert, the activity of glutathione peroxidase (GPx) was determined using a spectrophotometric assay. The reaction mixture was prepared containing phosphate buffer, reduced glutathione (GSH), nicotinamide adenine dinucleotide phosphate (NADPH), and glutathione reductase (GR). The enzymatic reaction was initiated by the addition of the sample serving as the enzyme source. Subsequently, hydrogen peroxide or an organic hydroperoxide was added as the substrate to start the reaction. The decrease in absorbance at 340 nm, resulting from the oxidation of NADPH to $NADP^+$, was monitored continuously. GPx activity was then calculated based on the rate of NADPH consumption, using the molar extinction coefficient of NADPH ($6.22 \times 10^3\ M^{-1}\ cm^{-1}$)[24].

Glucose-6-Phosphate Dehydrogenase (G6PDH) Assay:

To check how active the G6PD enzyme is, Minunci demonstration was employed for this study. A buffer was first made using Tris-HCl and magnesium chloride. Then, two important ingredients — $NADP^+$ and glucose-6-phosphate — were added. After that, the sample containing the enzyme was mixed in, and everything was warmed up to 37°C. As the enzyme worked, it caused a change that was measured by watching how the mixture absorbed light at 340 nm. The faster the light absorbance increased, the more active the enzyme was[25].

Lactate Dehydrogenase (LDH) Assay:

Hochella & Weinhouse's description was adopted to measure lactate dehydrogenase (LDH) activity, an assay buffer was first prepared using phosphate buffer. For the forward reaction, NADH and pyruvate were added to the mixture, while for the reverse reaction, NAD^+ and lactate were used instead. The enzyme sample was then added, and the mixture was incubated at 37°C. As the reaction took place, changes in absorbance at 340 nm were monitored over time — either a decrease in NADH or an increase in NAD^+. The activity of LDH was calculated based on how fast the absorbance changed, which reflects the speed of the reaction[26].

STATISTICAL ANALYSIS

The data were analysed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, California, USA) and displayed as mean ± standard error of mean (SEM). Two-way ANOVA were used to test for statistical significance, with a significance level of $p < 0.05$.

RESULTS

BODY WEIGHT CHANGES

The body weights of the pups were taken immediately as they were removed from the mother before they were sacrificed. There was no statistically significant mean difference in the mean body weight of the pups in 2nd week of gestation when compared with the control. Meanwhile, there was a statistically significant reduction in the mean body weight of the pups in the 3rd week of gestation when compared with the control ($p<0.05$). However, the 2nd week and 3rd week of gestation showed no statistical significance in their mean body weight when compared with the control as shown in Figures 8 & 9.

BIOCHEMICAL

At the dosage of 0.5ml administered per oral, Superoxide dismutase (SOD) activities were observed to be elevated to 145.025±4.22 and 130.595±18.66 U/L for mother and foetus respectively, in the treated groups of 3rd week, compare to the control group with values 40.000±2.00 and 65.30±9.33 (mother and pups) as shown in Figure 1. However, there was an increase in the level of GPx in both the mother and pups of the 2nd week and 3rd week of the treated group from 84.500±17.50 and 90.000± 8.00 (U/L) against the control group as shown in Figure 2 while there was observed decrease in the levels of the activity of Lactate dehydrogenase (LDH) in the treated groups slightly when compared with the control group as shown in Figure 3. Meanwhile, there was no statistically significant difference in the activity of G6PDH of the treated groups when compared with the control groups FIG 4. In addition, the results of the hormonal assay involving progesterone and estrogen in the treated groups slightly reduced when compared with the control. However, this decrease was not statistically significant Figure 5.

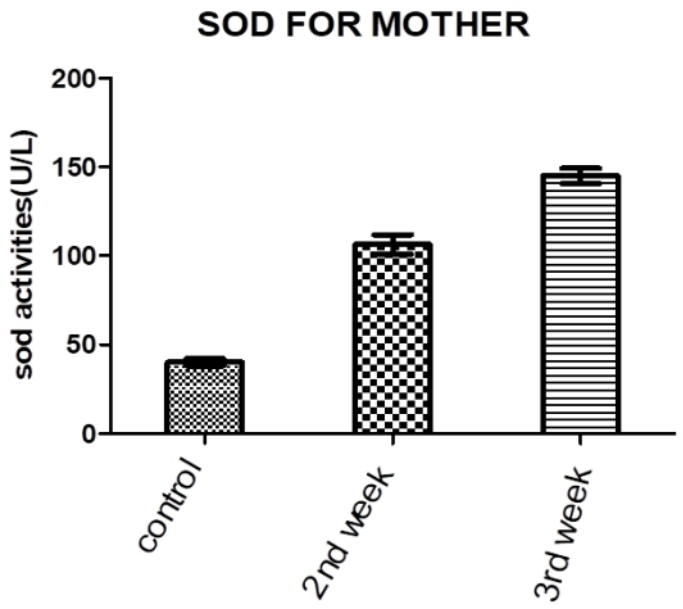

Figure 1: Bar Chart showing the Result of Superoxide dismutase (SOD) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group (Adult Female Rats)

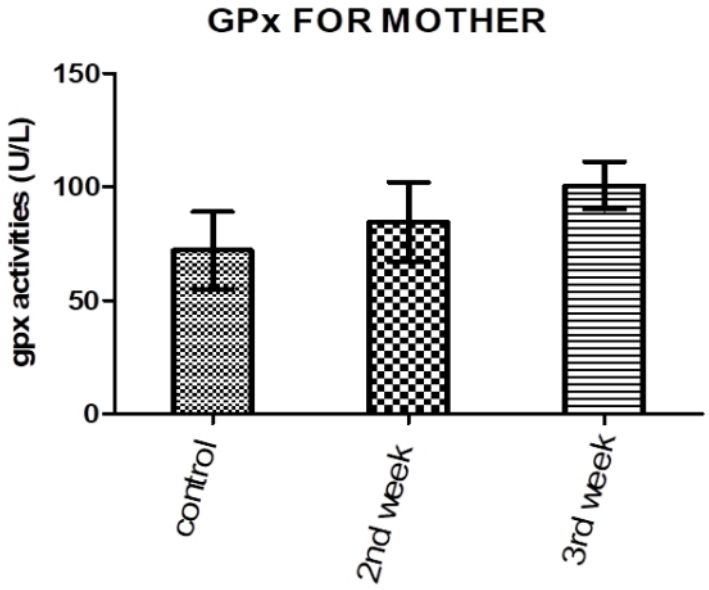

Figure 2: Bar Chart showing the Result of Glutathione peroxidase (GPx) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group (Adult Female Rats)

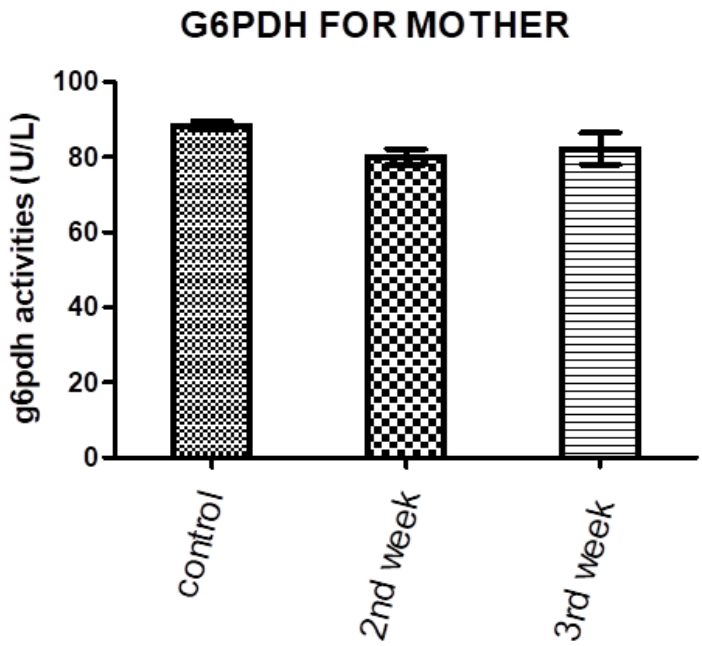

Figure 3: Bar Chart showing the Result of Glucose 6 Phosphate dehydrogenase (G6PDH) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group (Adult Female Rats)

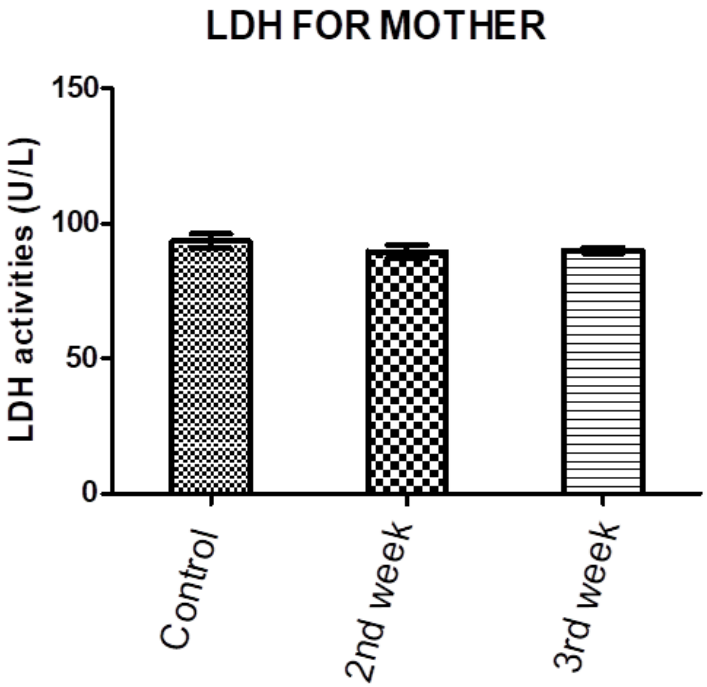

Figure 4: Bar Chart showing the Result of Lactate Dehydrogenase (LDH) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group (Adult Female Rats)

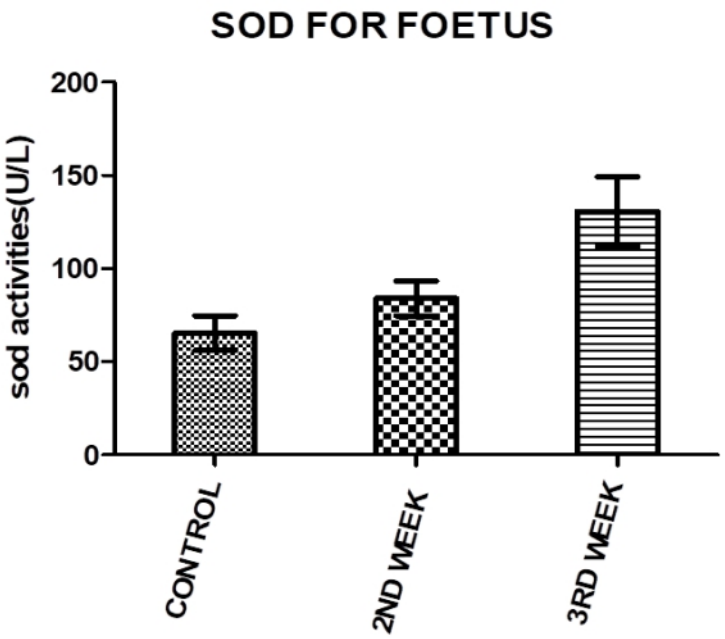

Figure 5: Bar Chart showing the Result of Superoxide dismutase (SOD) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group

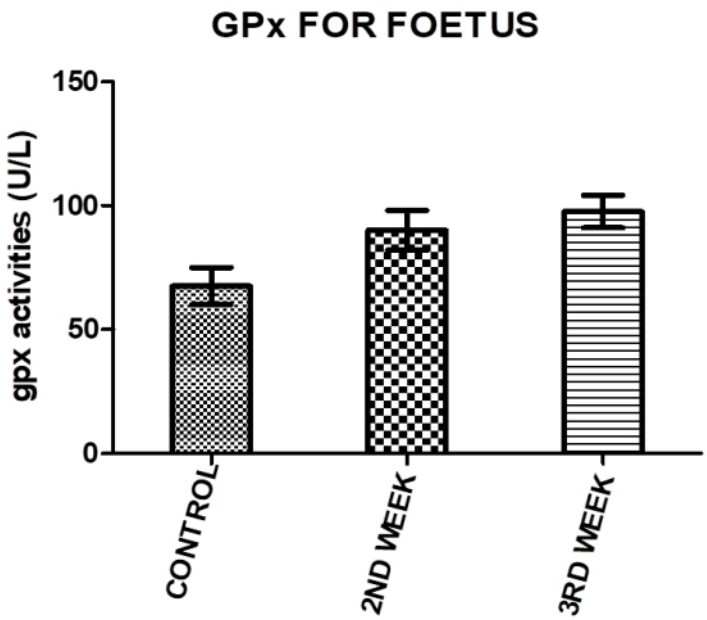

Figure 6: Bar Chart showing the Result of Glutathione peroxidase (GPx) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group

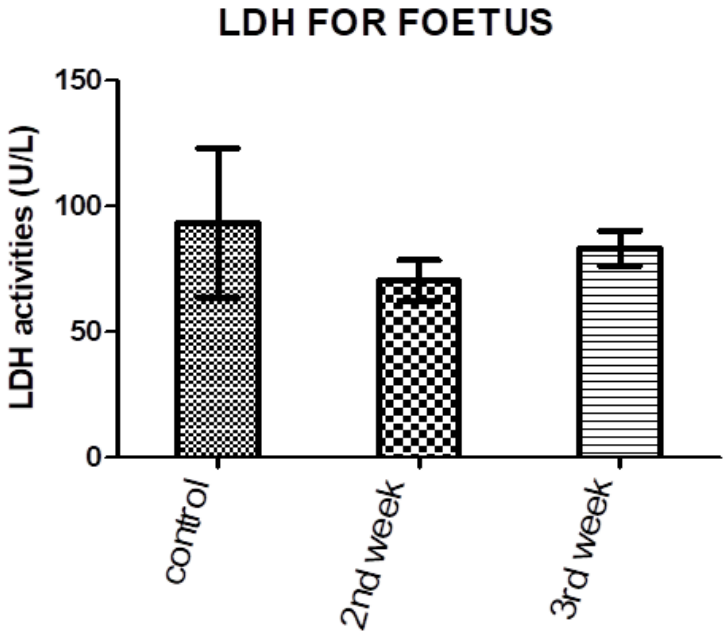

Figure 7: Bar Chart showing the Result of Lactate Dehydrogenase (LDH) Enzyme Analysis exhibiting Control, 2nd Group and 3rd Group (Foetuses)

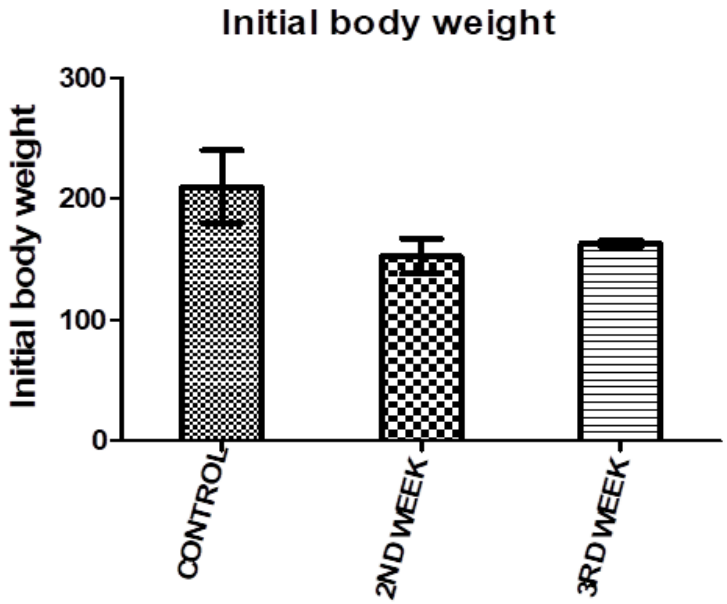

Figure 8: Bar Chart showing the Result of Initial Body Weight Analysis exhibiting Control, 2nd Group and 3rd Group

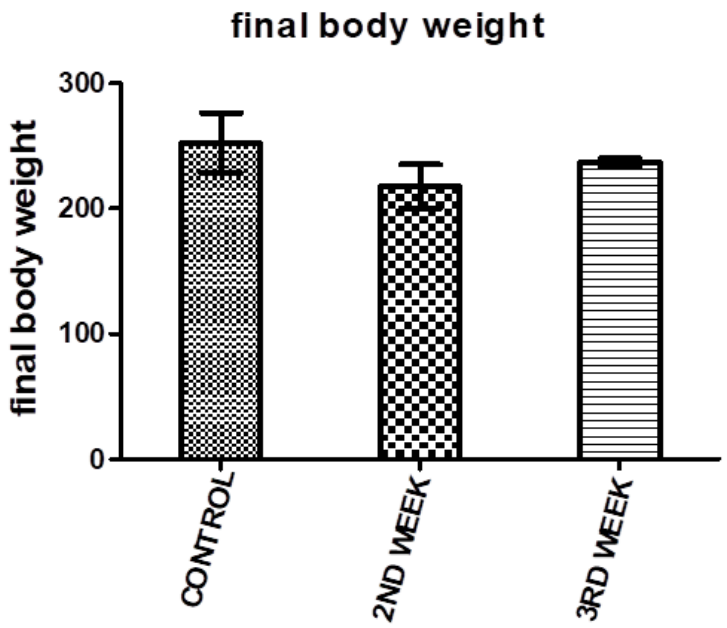

Figure 9: Bar Chart showing the Result of Final Body Weight Analysis exhibiting Control, 2nd Group and 3rd Group

HISTOLOGY

The histological observation revealed normal cytoarchitecture of the pre-frontal cortices of both the mother and foetus of the rats in the control with normal Pyramidal cells as shown in Plates A & D. However, the rats treated with the soursop leave extract at the second trimester revealed several levels of neurodegenerations such as membrane disbandment, cellular aggregation, and vacuolation as evidenced in plates B & E. Meanwhile, the rats that were administered with the extract in their third trimester were observed to have shown mild degeneration of cells when compared with the control as shown in plates C & F.

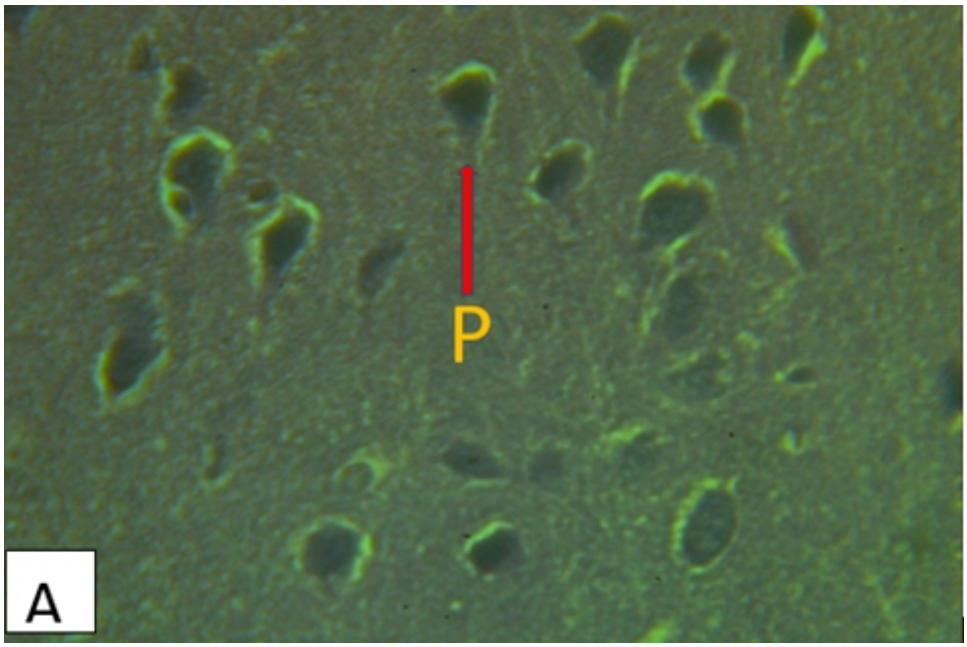

Plate A: Photomicrograph showing Normal Pyramidal Cell (PC) of the Section of the Prefrontal Cortex of Wistar Rat (Mother) in the Control Group using H&E Stain (x400)

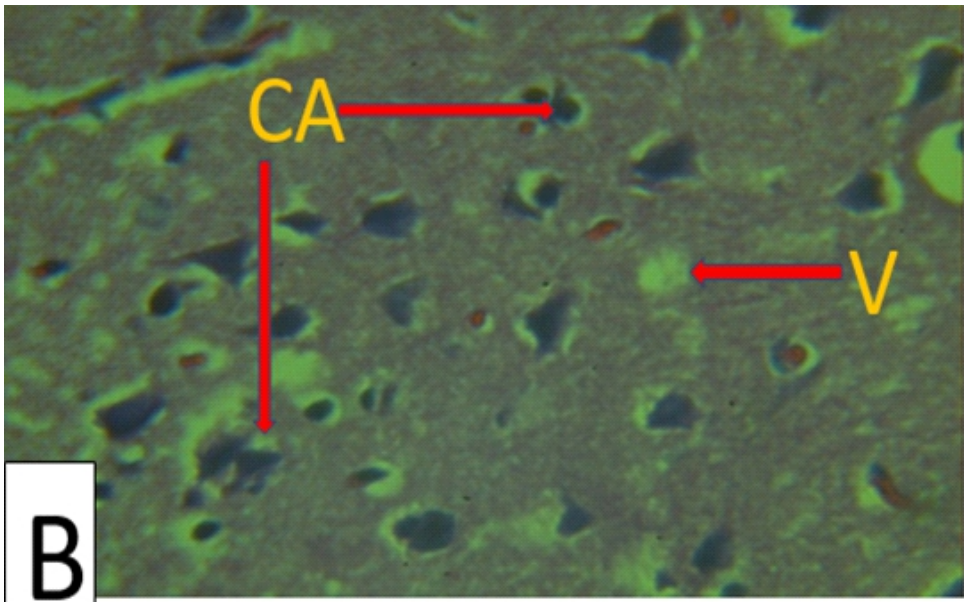

Plate B: Photomicrograph showing Cellular Aggregations (CA), Vacuolations (V) of the Section of the Prefrontal Cortex of Wistar Rat (Mother) in the 2rd Trimester of 2nd Group using H&E Stain (x400)

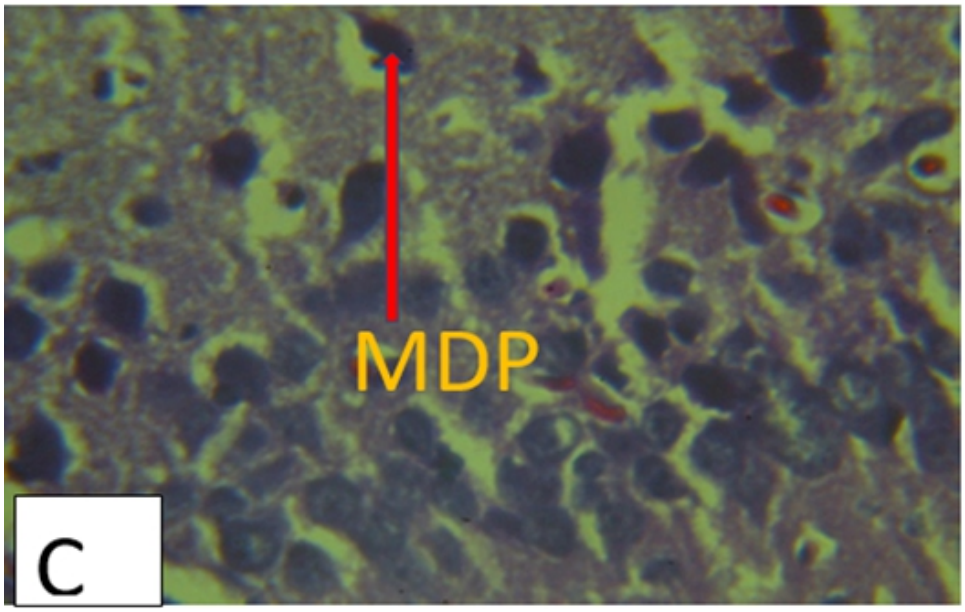

Plate C: Photomicrograph showing Mild Degeneration of Pyramidal Cells (MDP) of the Section of the Prefrontal Cortex of Wistar Rat (Mother) in the 3rd Trimester of the 3rd Group using H&E Stain (x400)

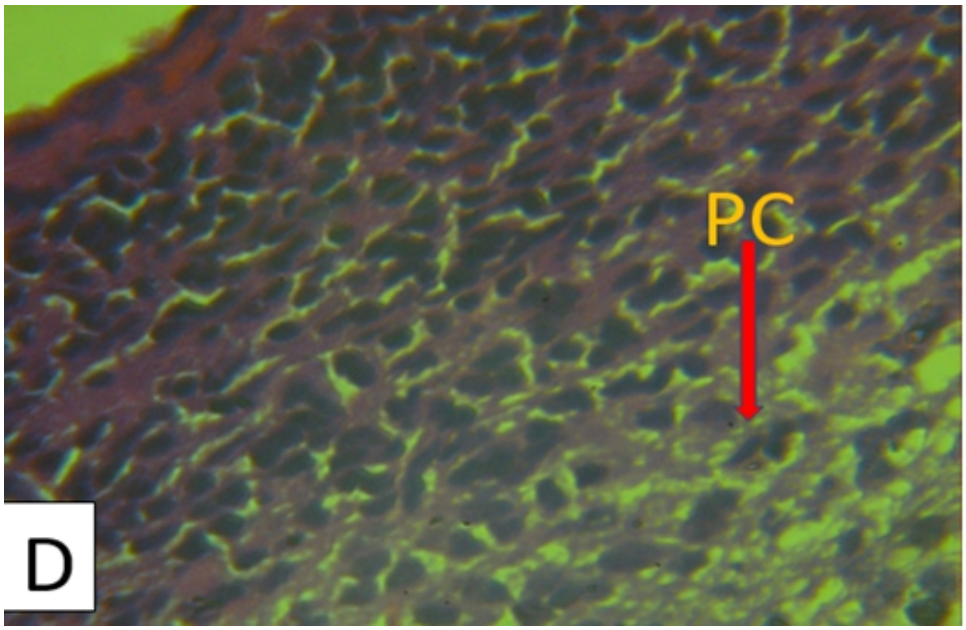

Plate D: Photomicrograph showing Normal Pyramidal Cells (PC) of a section of the Prefrontal Cortex of Young Wistar Rat (Foetus) in the Control Group using H&E Stain (x400)

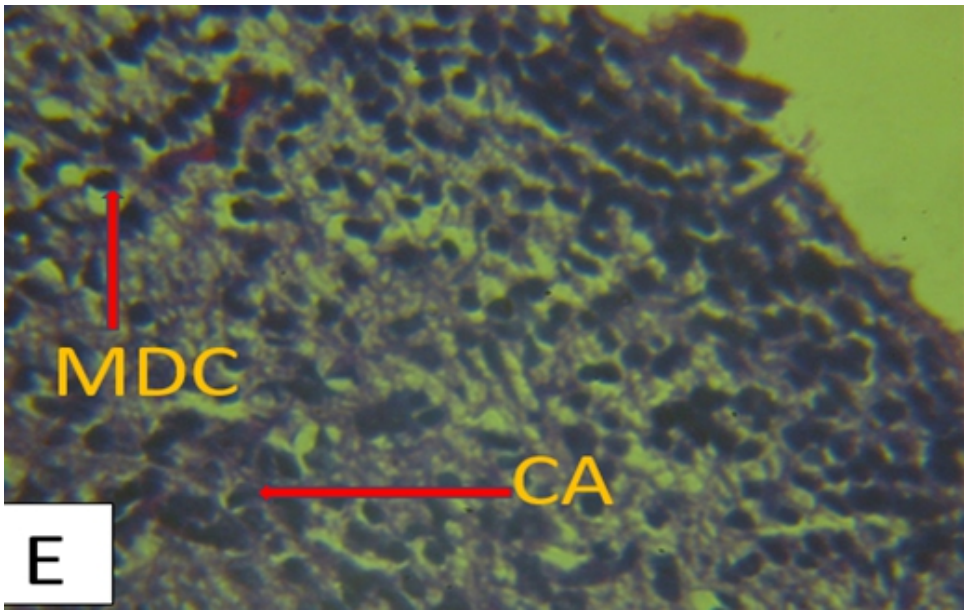

Plate E: Photomicrograph showing Mild Degeneration of Cell (MDC), Cellular Aggregation (CA) of a Section of the Prefrontal Cortex of Young Wistar Rat (Foetus) in the 2nd Trimester of Rats in 2nd Group using H&E stain (x400)

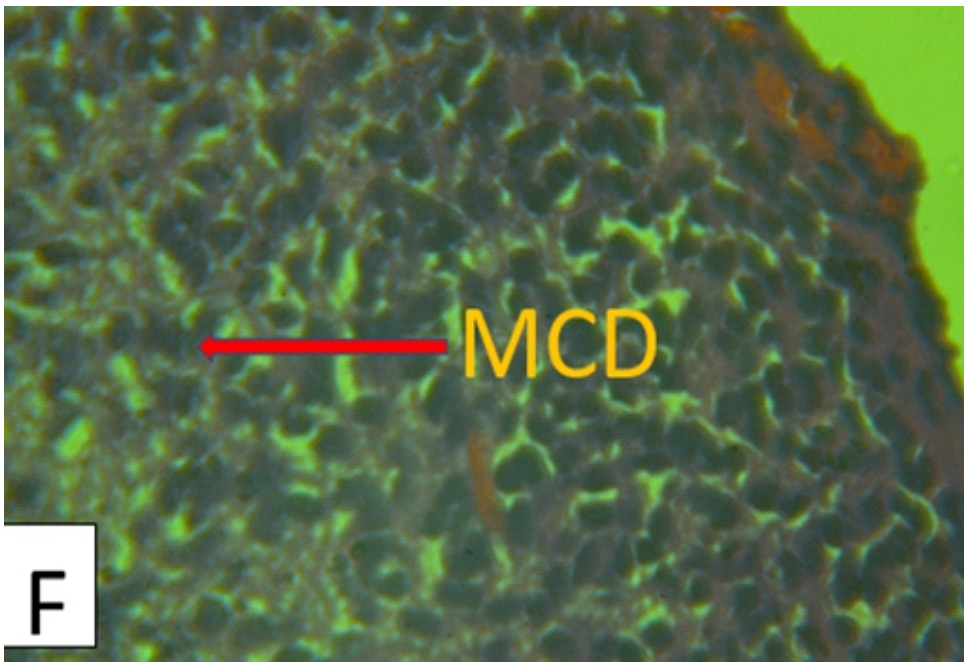

Plate F: Photomicrograph showing Mild Cellular Degeneration (MCD) of a Section of the Prefrontal Cortex of Young Wistar Rat (Foetus) in the 3rd Trimester of Rats in the 3rd Group using H&E Stain (x400)

DISCUSSION

Findings from this study revealed no statistically significant difference in the mean body weight of the pups in the second week of gestation compared to the control group. However, in the third week of gestation, a statistically significant reduction in mean body weight was observed ($p<0.05$). This suggests that while foetal growth appeared unaffected in the early stage, the later stage of gestation may be more vulnerable to the effects of Annona muricata leaf extract.

Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of toxic superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), which is relatively stable. It plays a crucial role in cellular defense against oxidative stress[27]. Following oral administration of 0.5 mL of the extract, SOD activity significantly increased in pups compared to the control group ($p<0.05$). This suggests an induced oxidative response in the developing brain, potentially leading to redox imbalance. Interestingly, the stability of SOD levels in mothers implies that the plant extract's antioxidant properties may have provided some level of protection in maternal tissues. This aligns with Carole et al. (2022), who also reported a significant increase in SOD activity following Annona muricata extract administration[28].

Glutathione peroxidase (GPx) plays a critical role in detoxifying hydrogen peroxide and mitigating oxidative stress[29]. In this study, GPx levels increased in both mothers and pups during the second and third weeks of gestation in the treated group. Although not statistically significant, this trend suggests an adaptive antioxidant response. This aligns with the findings of Basker et al. (2007), who observed a similar pattern in their in vitro antioxidant study of Annona species leaves[30].

Lactate dehydrogenase (LDH) is a key enzyme involved in cellular energy metabolism and is commonly used as a marker of cell damage[31]. In this study, LDH activity in the treated groups showed a slight decrease compared to the control group. While a decrease in LDH may suggest metabolic adaptation rather than direct neuronal damage, the presence of annonacin, a potent neurotoxin in *Annona muricata* leaf extract, raises concerns about its potential to induce neurodegeneration through the mitochondria-mediated apoptosis pathway[32].

Glucose-6-Phosphate Dehydrogenase (G6PDH) plays a pivotal role in the pentose phosphate pathway, generating NADPH essential for antioxidant defense and biosynthetic reactions[33]. The observed decrease in G6PDH activity in the treated groups compared to the control suggests that the extract may induce metabolic stress, potentially affecting neuronal proliferation and maturation. This finding is consistent with the work of Lannuzel et al. (2002), who identified *Annona muricata* alkaloids and acetogenins as neurotoxic compounds capable of interfering with ATP synthesis and leading to neuronal dysfunction[34].

Teratogens disrupt normal intrauterine development, with the extent of interference depending on gestational age and organ sensitivity[35,36]. The brain, being highly susceptible to exogenous agents, is particularly vulnerable to alterations in neuronal cytoarchitecture[35]. Histological analysis in this study revealed a reduction in neuronal density in the prefrontal cortex of pups exposed to *Annona muricata* leaf extract during the second trimester. Accompanying changes such as vacuolation, membrane disintegration, and cellular aggregation further highlight potential neurotoxic effects. These findings align with Kim et al. (2020), who reported similar neuroanatomical alterations following *Annona muricata* exposure in female Wistar rats[37].

Moreover, Handayani & Nugraha (2015) demonstrated that *Annona muricata* extract induces neuronal degeneration, increases neuroglial proliferation, and contributes to neuroinflammation, particularly in the substantia nigra and cerebral cortex[38]. The photomicrographic analysis in this study corroborates these findings, suggesting that annonacin, a potent neurotoxin present in *Annona muricata* leaves, disrupts mitochondrial function by reducing ATP supply and inhibiting mitochondrial transport, ultimately leading to tau protein dysfunction, neuronal degeneration, and cell damage[39].

These findings suggest that while *Annona muricata* leaf extract exhibits antioxidant activity, its potential to induce oxidative stress, metabolic alterations, and neuroanatomical changes in developing fetuses warrants further investigation. Given the vulnerability of the developing brain to exogenous compounds, caution is advised regarding its use during pregnancy.

CONCLUSION

In conclusion, *Annona muricata* is a potent antioxidant and anti-inflammatory medicinal plant. However, its administration during pregnancy should be carefully considered, particularly concerning the timing and duration of exposure. This study suggests that exposure to *Annona muricata* leaf extract during the second and third trimesters may result in severe teratogenic effects on embryonic nervous tissues and contribute to maternal neurodegeneration.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1. Tantibanchachai C: Teratogens. Arizona State University. School of Life Sciences. Center for Biology and Society. *Embryo Project Encyclopedia*. Arizona Board of Regents; 2014.
2. Alwan S, Chambers CD: Identifying human teratogens: an update. *J Pediatr Genet*. 2015;4(2):39-41.
3. Dosoky NS, Setzer WN: Maternal reproductive toxicity of some essential oils and their constituents. *Int J Mol Sci*. 2021;22(5):2380.
4. Broussard CS, Rasmussen SA, Reefhuis J, Friedman JM, Jann MW, Riehle-Colarusso T. Maternal treatment with opioid analgesics and risk for birth defects. *Am J Obstet Gynecol*. 2011;204(4): 314.e1.
5. Nosaka Y, Nosaka AY: Generation and detection of reactive oxygen species in photocatalysis. *Chem Rev*. 2017;117(17):11302-36.
6. Madkour LH: Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. *Pharm Sci Anal Res J*. 2019; 2:180023.
7. Zhang Y, Dai M, Yuan Z. Methods for the detection of reactive oxygen species. *Anal Methods*. 2018;10(38):4625-38.
8. Tangkiatkumjai M, Boardman H, Walker DM. Potential factors that influence usage of complementary and alternative medicine worldwide: a systematic review. *BMC Complement Med Ther*. 2020; 20:1-15.
9. Enioutina EY, Salis ER, Job KM, Gubarev MI, Krepkova LV, Sherwin CM. Herbal medicines: challenges in the modern world. Part 5. Status and current directions of complementary and alternative herbal medicine worldwide. *Expert Rev Clin Pharmacol*. 2017;10(3):327-38.
10. Sarkar T, Salauddin M, Roy A, Sharma N, Sharma A, Yadav S. Minor tropical fruits as a potential source of bioactive and functional foods. *Crit Rev Food Sci Nutr*. 2023;63(23):6491-535.
11. Keskin E, Elmas Ö, Şahin HHK, Guven B. Efficacy of *Annona muricata* (graviola) in experimental spinal cord injury: biochemical and histopathological analysis. *Turk J Trauma Emerg Surg*. 2022;28(3):233.
12. Mutakin M, Fauziati R, Fadhilah FN, Zuhrotun A, Amalia R, Hadisaputri YE. Pharmacological activities of soursop (*Annona muricata* Lin.). *Molecules*. 2022;27(4):1201. doi:10.3390/molecules27041201.
13. Dixon ML, Thiruchselvam R, Todd R, Christoff K. Emotion and the prefrontal cortex: an integrative review. *Psychol Bull*. 2017;143(10):1033.
14. Schubert D, Martens GJM, Kolk SM. Molecular underpinnings of prefrontal cortex development in rodents provide insights into the etiology of neurodevelopmental disorders. *Mol Psychiatry*. 2015;20(7):795-809.
15. Demirtaş MS: The pathogenesis of congenital anomalies: roles of teratogens and infections. InCongenital Anomalies in Newborn Infants-Clinical and Etiopathological Perspectives 2020 Jun 17. IntechOpen.
16. Chini M, Hanganu-Opatz IL: Prefrontal cortex development in health and disease: lessons from rodents and humans. *Trends Neurosci*. 2020. doi: 10.1016/j.tins.2020.10.017.
17. Mohsenpour H, Pesce M, Patruno A, Bahrami A, Pour PM, Farzaei MH. A review of plant extracts and plant-derived natural compounds in the prevention/treatment of neonatal hypoxic-ischemic brain injury. *Int J Mol Sci*. 2021;22(2):833.
18. Alam A, Suen KC, Hana Z, Sanders RD, Maze M, Ma D. Neuroprotection and neurotoxicity in the developing brain: an update on the effects of dexmedetomidine and xenon. *NeurotoxicolTeratol*. 2017; 60:102-16.
19. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the oestrus cycle phases of rats: some helpful considerations. *Braz J Biol*. 2002;62(4a).
20. Drury RA, Wallington EA: *Chalton's Histochemical Technique*. 5th ed. New York: Oxford University Press; 1980. p.195.
21. Feeback DL, Ketring-Hanna JL, Leech RW, Benningfield LK, Brumback RA. Methyl methacrylate embedding of large nervous tissue blocks for neurohistologic, immunocytochemical, and ultrastructural studies. *J Histotechnol*. 1991;14(2):89-95.

22. Baker F., Silverlon R., Luck C.E. An introduction to medical laboratory technology. 5th edition, London Butterwoths. (1976) Pg 34-37.

23. Beauchamp C, Fridovich I: Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44:276–287.

24. Weydert CJ, Cullen JJ: Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue. Nature protocols. 2010 Jan;5(1):51-66.

25. Minucci A, Giardina B, Zuppi C, Capoluongo E. Glucose-6-phosphate dehydrogenase laboratory assay: how, when, and why? *IUBMB Life*. 2009;61(1):27-34.

26. Hochella NJ, Weinhouse S: Automated assay of lactate dehydrogenase in urine. *Anal Biochem*. 1965;13(2):322-35.

27. Hayyan M, Hashim MA, AlNashef IM. Superoxide ion: generation and chemical implications. *Chemical reviews*. 2016 Mar 9;116(5):3029-85.

28. Carole NC, Ekpe IP, Ifeancho NW, Andrea OE, Damilola EF, Juachi AE. Evaluation of phytochemical profile and comparative free radical scavenging activities of ethanolic extract of *Annona muricata* leaf and fruit. *Int Res J Mod Eng Technol Sci*. 2022.

29. Pei J, Pan X, Wei G, Hua Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. *Front Pharmacol*. 2023; 14:1147414.

30. Baskar R, Rajeswari V, Kumar TS. In vitro antioxidant studies in leaves of Annona species.

31. Khan AA, Allemailem KS, Alhumaydhi FA, Gowder SJ, Rahmani AH. The biochemical and clinical perspectives of lactate dehydrogenase: an enzyme of active metabolism. *EndocrMetab Immune Disord Drug Targets*. 2020;20(6):855-68.

32. Smith RE, Shejwalkar P: Potential neurotoxicity of graviola (*Annona muricata*) juice. In: Safety issues in beverage production. Academic Press; 2020. p. 429-49.

33. Nwizugbo KC, Ogwu MC, Eriyamremu GE, Ahana CM. Alterations in energy metabolism, total protein, uric and nucleic acids in African sharptooth catfish (*Clarias gariepinus* Burchell) exposed to crude oil and fractions. *Chemosphere*. 2023; 316:137778.

34. Lannuzel A, et al. Toxicity of Annonaceae for dopaminergic neurons: potential role in atypical parkinsonism in Guadeloupe. *Mov Disord*. 2002; 17:84-90.

35. Genetic Alliance; DC Department of Health. *Understanding Genetics: A DC Guide*. Washington (DC): Genetic Alliance; 2010.

36. Belanger BG, Lui F. Embryology, teratology TORCH. *StatPearls* [Internet]. 2025.

37. Kim WS, Kim YE, Cho EJ, Byun EB, Park WY, Song HY, et al. Neuroprotective effect of *Annona muricata*-derived polysaccharides in neuronal HT22 cell damage induced by hydrogen peroxide. *BiosciBiotechnolBiochem*. 2020;84(5):1001-12.

38. Handayani ES, Nugraha ZS: Soursop leaf extract increases neuroglia and hepatic degeneration in female rats. *Universa Med*. 2015;34(1):17-24.

39. Escobar-Khondiker M, et al. Annonacin, a natural mitochondrial complex I inhibitor, causes tau pathology in cultured neurons. *J Neurosci*. 2007; 27:7827-37.