Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 10 (2)

Neuroanatomical studies of crude aqueous *annona muricata* leaf extract on hippocampus of the maternal and foetal Wistar rats.

[1]Enaibe BU, [1]Ajibade AE, [1]Lewu FS,[1]Olajide OJ, [2]Arogundade TT, [2]Yawson EO, [2]Tokunbo OS,[1]Bamisi O, [1]Atoyebi A, [1]Keji ST, [1]Ojo OD and [1]Alabi AS

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin. Nigeria

[2]Department of Anatomy, Faculty of Basic Medical Sciences, Adeleke University, Ede, Osun State. Nigeria.

Correspondence Author: Dr Bernard Ufuoma Enaibe,
Email - benaibe@unilorin.edu.ng; +2348035672393

ABTRACT

*The period of conception to child birth is a very sensitive period and occurs naturally without any interference but the food or drinks consumed during this period has a major role to play on the developmental stages of the fetus either positively or negatively. Hence, the critical study on some herbs/fruit that are commonly consume by pregnant women.*The research was carried out basically to examine and evaluate the morphological and biochemical effects of crude aqueous soursop leaf extract *(Annona muricata) on the hippocampus of both mother and foetus of wistar rats following prenatal administration.*Thirty four adult wistar rats (24 females and 10 males) with an average weight of 180g $\pm$ 22g were used for the research. They were fed with rat pellets and had access to clean water *ad libitum* and were acclimatize for 14 days. The males were put in a separate cage to prevent mating during acclimatization before the commencement of the experiment. Oestrous cycle was carried out. Fresh soursop leaves were obtained and was certified original and prepared for use. 0.5mls of aqueous soursop leaf extract was administered orally using a calibrated syringe fitted with oral cannula. The animals were sacrificed on the 22nd day of gestation before delivery by cervical dislocation and the foetuses were removed from the mothers. The brain of both the mothers and the foetuses were excised and weighed. The hippocampus were removed and homogenized for subsequent analyses for biochemical studies. There was no statistically significant increase in the weight gained by the animals across the groups, $p<0.05$, when compared with control. There was slight increase in the weight gained by the group in the 2nd week of gestation and 3rd week of gestation while comparing group two with three, there was no statistically significant increase in weight gained. Progesterone level shows no statistically significant difference comparing 2nd week gestational group with control. There was statistically significant increase in 2nd week gestational group compared with 3rd week gestational group while there was significant reduction in 3rd week gestational group when compared with control and 2nd week gestational groups.These results confirmed the deleterious effects of PEE generally on growth and further confirmed the adverse effects of PEE in both the SC and LGB of the rats investigated.

Key words: Superior Colliculus, Prenatal, Annona Muricata,

INTRODUCTION

The period of conception to child birth is a very sensitive period and occurs naturally without any interference but the food or drinks consumed during this period has a major role to play on the developmental stages of the fetus either positively or negatively. Hence, the critical study on some herbs/fruit that are commonly consume by pregnant women[1]. Soursop is a fruit of *Annona muricata*; a broadleaf, flowering, evergreentree native to Mexico, Cuba, Central America and northern South America [2]. Soursop is also found among tropical parts of Africa, especially in Eastern Nigeria and the Mambilla Plateau [3]. The compound annonacin, which is contained in the seeds of soursop, is a neurotoxin associated with neurodegenerative disease and research has suggested a connection between consumption of soursop and atypical forms of Parkinson's disease due to high concentrations of annonacin [4]. In 2010 the French food safety agency, Agence française de sécurité sanitaire des produits de santé, concluded that based on the available research findings, it is not possible to confirm that the observed cases of atypical Parkinson syndrome are linked to the consumption of *Annona muricata*, calling for further study on potential risks to human health [5]. The hippocampus is an essential part of human brain which is located in the temporal lobe. It helps in information processing and the reproductive cycle. [6]. It was noted that any damage to the hippocampus can lead to epilepsy and Alzheimer's disease [7]. The hippocampus proper is composed of two different parts namely the dentate gyrus and the cornu ammonis (literally "Amun's horns", abbreviated CA). The dentate gyrus contains the fascia dentata and the hilus, while the CA (cornu ammonis) is differentiated into fields CA1, CA2, CA3, and CA4 [8]. The overall developmental processes reflect

more specific change in neural patterning within all regions of the embryonic nervous system. These changes in neural patterning begin in the embryonic period and extend for many years. The changes are gradual and follow an ongoing course of continuous specification and refinement [9]. Embryonic patterning affects all brain regions from the forebrain through the spinal column, such that by the end of the embryonic period in GW3 primitive patterning of sensorimotor regions within the neocortex is established [10] major compartments within diencephalic and midbrain regions have differentiated [11], and the segmental organization of the hindbrain and spinal column have been specified [6,12].

The dry leave extract of *Annona muricata* contained saponins, tannins/polyphenols, flavonoids and steroids [13]. Flavonoid compounds contained in extracts of leaves of *Annona muricata act as* an antibacterial for being able to inhibit the growth of bacteria by destroying the cell wall permeability, microsomes, lysosomes and bacterial cells as a result of interaction between flavonoids with DNA [14]. The tannins/polyphenolic also act as an antibacterial by reacting with cell membranes to inactivate essential enzymes function and metabolism of the cell's genetic material so impeding and disrupted cell wall synthesis [15]. Saponins have a beneficial biological effect that is hypocholesterolemic and anti-carcinogen and can boost the immune system. Saponins also interacts with cell membrane steroids to inhibit the growth of microbe by releasing bacterial proteins and enzymes of the cells [16].

The research was carried out basically to examine and evaluate the morphological and biochemical effects of crude aqueous soursop leaf extract *(Annona muricata)* on the hippocampus of both mother and foetus of wistar rats following prenatal administration.

MATERIALS AND METHODS

Laboratory Animals: Thirty four adult wistar rats (24 females and 10 males) with an average weight of 180g ± 22g were used for the research. They were kept in the animal house of the Department of Anatomy, University of Ilorin. The rats were fed with rat pellets and had access to clean water *ad libitum.* The animals were allowed to acclimatize for fourteen days. The males were put in a separate cage to prevent mating during acclimatization before the commencement of the experiment. Oestrous cycle was carried out using [17]. method. Adult male rats were introduced to the females in their proestrous phase between 5.00pm to 7.00 am the next morning. The presence of spermatozoa in the vaginal smear the following morning when examined under microscope established a successful mating and confirmation of pregnancy [17].

Grouping

Table 1: Pregnant rats groupings and treatment

GROUPS	DURATION	TREATMENT (0.5mls)	ADMINISTRATION DURATION	NO. OF ANIMALS
A	2^{ND} WEEK	0.5mls of aqueous crude leaf extract of Soursop	DAY 9 -11	8
B	3^{RD} WEEK	0.5mls of aqueous crude leaf extract of Soursop	DAY 15 -17	8
C	CONTROL	0.5mls of Distilled water *ad-libitum*	ALL THROUGH GESTATION	8

Purchase and Preparation of Extract: Fresh soursop leaves were obtained from a local merchant in Ilorin. It was certified original by the Department of Plant Biology at the University of Ilorin. The extract was prepared according to the method describe by [18].

Administration: 0.5mls of aqueous soursop leaf extract was administered orally using a calibrated syringe fitted with oral cannula.

Sacrifice and Sample Collection: The animals were sacrificed on the 22nd day of gestation before delivery using cervical dislocation and the foetuses were removed from the mothers. The brain of both the mothers and the foetuses were excised and weighed. The hippocampus were removed and homogenized for subsequent analyses of lactate dehydrogenase (LDH), Glutathione peroxidase (GPx) and Glucose -6 Phosphate Dehydrogenase (G6PDH) levels.

RESULTS

The results from this study include rat's relative body weight and relative brain weight in both mothers and foetuses, biochemical assays and hormonal results.

Morphological Observations

Relative Body Weights Observation of Adult (Mothers) Rats: Relative body weight changes across all the experimental groups were examined by

subtracting the initial weight from final weight and divided by initial weight multiply by 100 (final-initial weight/initial weight x 100). After the analysis, there was no statistically significant increase in the weight gained by the animals across the groups, $p<0.05$ (Fig 4), when compared with control. There was slight increase in the weight gained by the group in the 2nd week of gestation and 3rd week of gestation while comparing group two with three, however, there was no statistically significant increase.

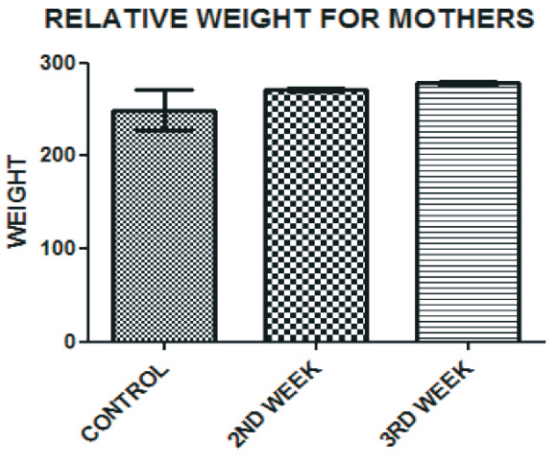

Figure 1: Bar Graph Showing Relative Body Weight Changes of Mothers over the Period of Administration. There was no statistically significant changes in the relative body weight between 2nd week , 3rd week of gestation and the control group after administration.

Relative Brain Weight Changes of Adult (Mothers) Rats

The relative weight of the whole brain was obtained by using brain weight/body weight x 100 across groups. The was no statistically significant mean difference (Figure 4.2) when the relative brain weight of 2nd week and 3rd week gestational groups were compared with that of control group at $p<0.05$.

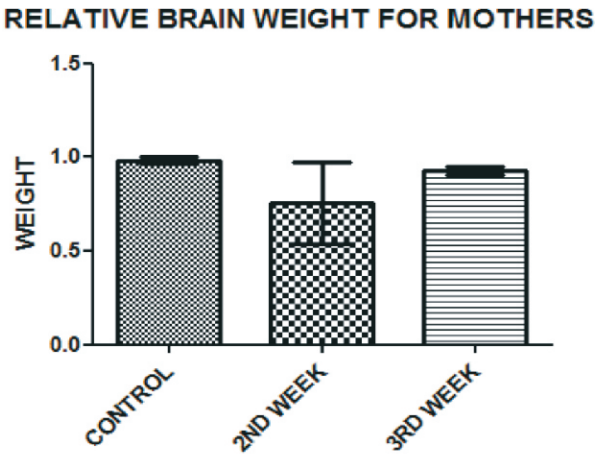

Figure. 2: Bar Graph Showing the Relative Brain Weight of Adult(Mothers) Rats. There was no statistically significant difference in the relative brain weights across groups ($p<0.05$).

Relative brain weight for pups: The animals were sacrificed on the 23rd day of gestation before delivery using cervical dislocation, the foetuses were removed from the mothers and weighed thus no relative body weight for pups could be taken because the pups were also sacrificed immediately. The pups brains both the control group and treated groups were weighed and compared after sacrifice to determine their relative weight.

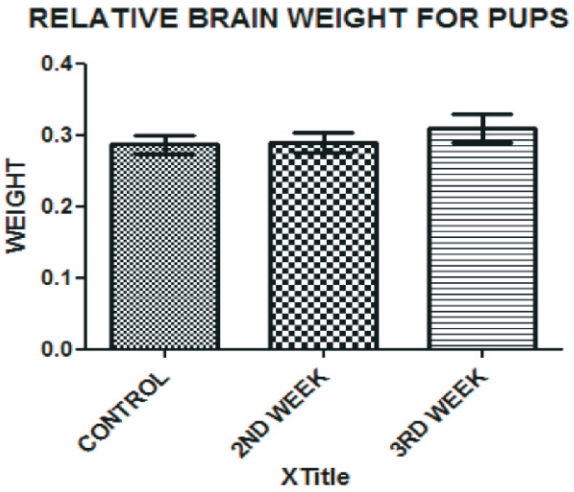

Figure 3: Bar Graph Showing the Relative Brain Weight for Pups: There was no statistically significant difference in the relative brain weights of the pups across groups ($p<0.05$).

Biochemical Analysis

Lactate Dehydrogenanes (LDH): is expressed extensively in body tissues. It is released during tissue damage. LDH levels showed a non significant reduction changes in 2nd week of gestational group when compared with the control. when compared with control, group 3rd week of gestation showed a statistically significant reduction of LDH while the comparison between 2nd week and 3rd week gestational groups showed no statistically significant reduction at $p<0.05$

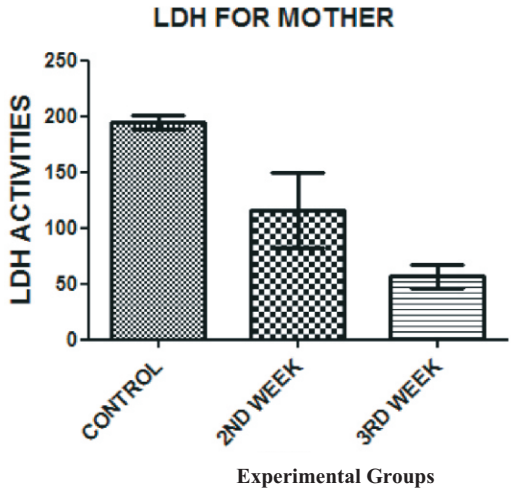

Figure 4: Result of Lactate Dehydrogenase Enzyme analysis. The 2nd and 3rd week gestational groups showed non statistically significant reduction difference while 3rd week of gestation showed a statistically significant reduction of LDH when compared with control at $p< 0.05$.

Glucose 6 Phospate Dehydrogenase (G6PDH): is an enzyme that is involved inpentose phosphate pathway wherebyco-enzymenicotinamide adenine dinucleotide phosphate (NADPH) level is maintained. The NADPH helps protect cells against oxidative damage from compounds like hydrogen peroxide by maintaining the level of glutathione. The level of G6PDH shows a significant decrease of 2nd week and 3rd week gestational groups when compared with the control group while there was no statistically significant decrease between 2nd and 3rd weeks of gestation.

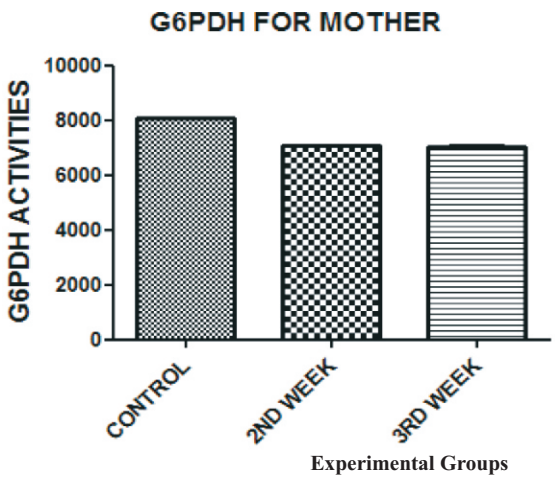

Figure 5: Result of Glucose 6 Phospate Dehydrogenase (G6PDH). The 2nd week and 3rd week gestational groups shows significant reduction compared to control while there was no statistically significant difference between 2nd and 3rd gestational group (p<005).

Superoxide Dismutase (SOD): Superoxide as one of the main reactive oxygen species in the cell. it also serves as a key antioxidant role. SOD decrease reactive oxygen species generation and oxidative stress and inhibits endothelial activation and indicate that modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. Superoxide dismutase levels in 2nd week and 3rd week of gestation increased compared to the control group, but no statistically significant increase comparing 2nd week and 3rd week gestational groups.

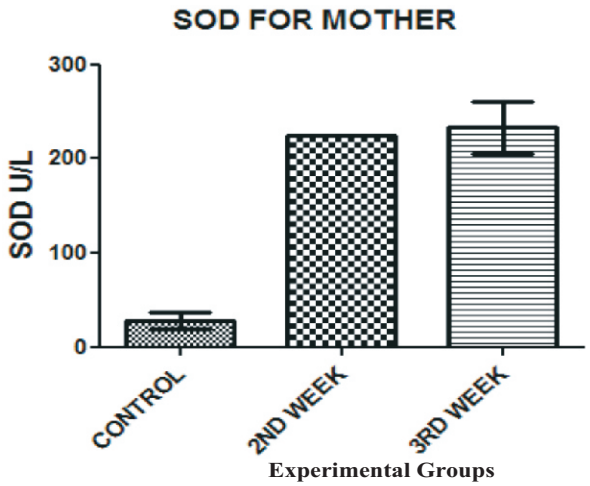

Figure 6: Chart Showing the Result of Superoxide Dismutase Enzyme Analysis. Data showing 2nd week and 3rd week of gestational groups with * represent (p< 0.05)- statistically significant difference compared to control group.

Glutathione Peroxidase (GPx): This helps in the detoxification of peroxides in livingcells. This reaction plays a crucial role in protectingcells from damage by free radicals, which are formedby peroxide decomposition. The administration of Annona Muricata in the 2nd week, and 3rd week gestational groups shows no statistically significant difference compared to control group while there was also no significant different between 2nd week and 3rd week gestational groups when compared.

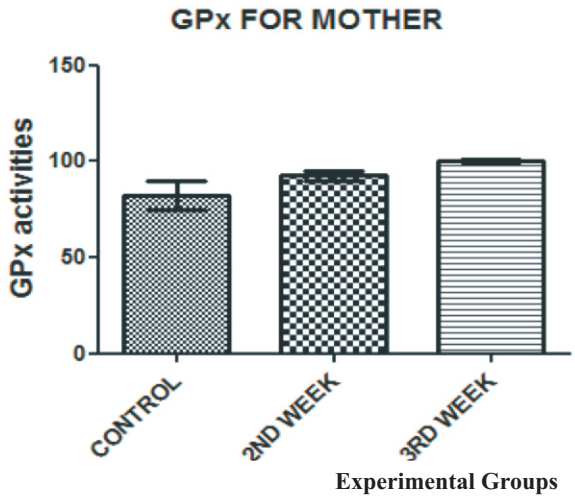

Figure 7. Graph Shows Glutathione Peroxidase Enzyme Analysis; whereby all groups shows no statistically significant difference compared to control.

SUPEROXIDE DISMUTASE (SOD): Superoxide is one of the main reactive oxygen species in the cell. As a consequence, SOD serves a key antioxidant role. Superoxide dismutase level in foetuses from 2nd week and 3rd week gestational groups shows no statistically significant increase compared to control group.

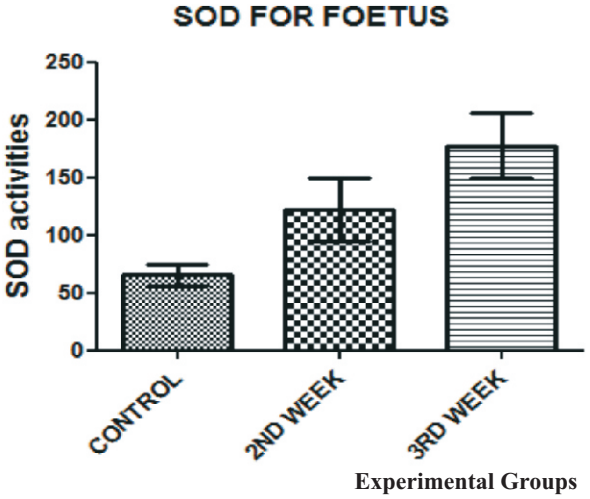

Figure 8: Chart Showing the Result of Superoxide Dismutase Enzyme Analysis for the Foetus. Data showing 2nd week and 3rd week of gestational groups with no statistically significant difference compared to control group * represent (p< 0.05)

Glutathione Peroxidase (GPx) for Foetuses: Glutathione peroxidase (GPx) provides amechanism for detoxification of peroxides in livingcells. GPx level shows statistically significant increase comparing 2nd week and 3rd week gestational group with the control group while comparing 2nd week and 3rd week gestational groups shows no statistically significant increase.

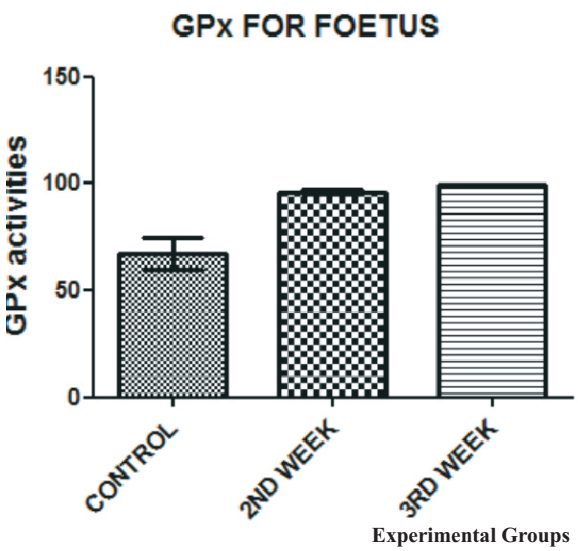

Figure 9: Chart Showing the Result of Glutathion Peroxidase Enzyme Analysis for Foetus. Data showing 2nd week and 3rd week gestational groups with statistically significant difference compared to control group * represent ($p < 0.05$).

Lactate Dehydrogenase (LDH) for Foetuses: LDH is highly express during tissue damage. The LDH level shows no statistically significant difference comparing 2nd week and 3rd week gestational groups to the control group.

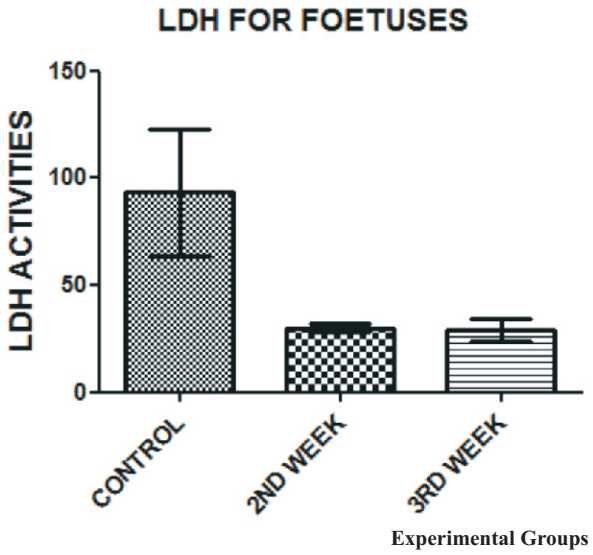

Figure 10: Chart Showing the Result of Lactate Dehydrogenase Enzyme Analysis for Foetus. Data showing 2nd week and 3rd week gestational groups with no statistically significant difference compared to control group * represent ($p < 0.05$).

Glucose 6 phosphate Dehydrogenase (G6PDH): G6PDH is an enzyme that is involved inpentose phosphate pathway, wherebyco-enzymenicotinamide adenine dinuclotide phosphate (NADPH) level is maintained. The NADPH helps protect cells against oxidative damage from compounds like hydrogen peroxide by maintaining the level of glutathione. The level of G6PDH across the groups (2nd week and 3rd week) compared to control group shows no statistically significant difference.

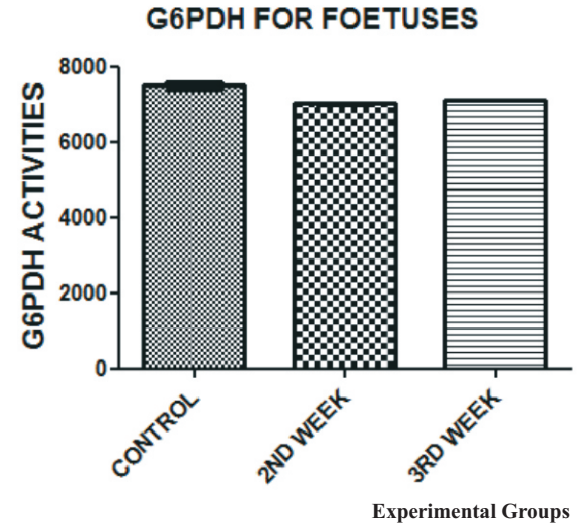

Figure 11: Chart Showing the Result of Glucose 6 Phosphate Dehydrogenase Enzyme Analysis for Foetus. Data showing 2nd week and 3rd week gestational groups with no statistically significant difference compared to control group * represent ($p < 0.05$).

Hormonal Analysis

Progesterone:is a steroid hormone secreted by corpus luteum after ovulation. Before ovulation, progesterone level is always low but increased after ovulation usually 5-7 days. If no incidence of pregnancy, progesterone levels usually decrease. Progesterone level shows no statistically significant difference comparing 2nd week gestational group with control. There was statistically significant increase in 2nd week gestational group compared with 3rd week gestational group while there was significant reduction in 3rd week gestational group when compared with control and 2nd week gestational groups.

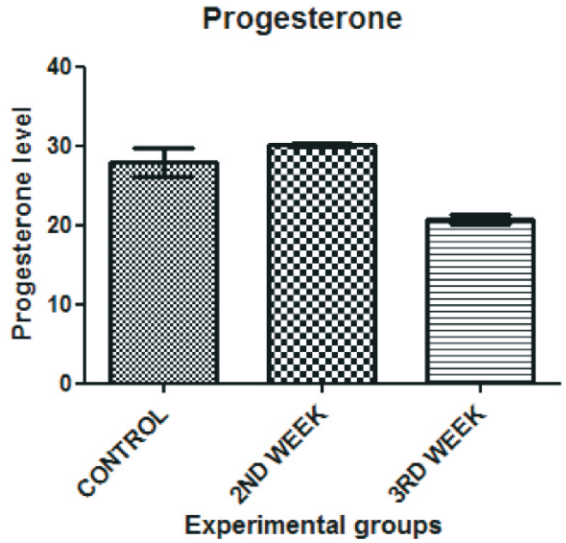

Figure 12: Chart Showing the Result of Progesterone Analysis. Data shows significant increase in 2nd week gestational group compared with 3rd week gestational groups while 3rd week gestational group shows statistically significant reduction compared to 2nd week and control group * represent ($p < 0.05$).

Medicine during pregnancy, especially herbs/ natural products has been a great concern to all and sundry because the constituent of the herbs and the extent of damage can not be quantied. The importance of the active ingredients can not be over emphasized but the adverse effect depending on the stage of organogenesis and duration of exposure can not be quantied.

Relative body weight of the maternal wistar rats were analyzed using Graph-pad@ and the outcomes were plotted in ANOVA followed with Tukey's multiple comparisons test and the maternal relative body weight of 2nd week and 3rd week of gestation shows no statistically significant difference when compared with the control, also 2nd week and 3rd week of gestation were of no difference on comparison which suggest that the administration of 0.5mls aqueous *annona muricata* has no significant effect on maternal body weight. However, feeding and appetite of the rats were not affected except little withdrawal few seconds after administration, $p<0.05$ (Fig. 4.1). Relative maternal brain weights were taken and analysed after sacrifice. The analysis shows no statistically significant difference when 2nd week and 3rd week gestational groups were compared with control while there was no statistically significant difference comparing 2nd and 3rd week gestational groups, which means the administration 0.5mls of soursop did not have a significant effect on the brain weight of the pregnant rats ($p< 0.05$).

Evaluation of the relative brain weight of the pups after sacrifice were analysed using Graph-pad@ and the outcomes were plotted in ANOVA followed with Tukey's multiple comparisons test, shows no statistically significant difference when 2nd week and 3rd week of gestation were compared with the control group, also comparing 2nd week and 3rd week gestational groups shows no statistically significant difference however, administration of 0.5mls of annona muricata did not have significant prenatal effect on the pups ($p<0.05$).

Biochemical evaluation serves as maker for assessing tissue integrity. The measurement of activities of various enzymes in the tissue and body fluids plays important role in disease investigation and diagnosis. Biochemical evaluation was done on maternal and foetal wistar rats and the results were analysed using Graph-pad@ and the outcomes were plotted in ANOVA followed with Tukey's multiple comparisons test. Lactate dehydrogenase is an enzyme that helps in the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD^+ [19]. Lactate Dehydrogenase (LDH) is expressed extensively in body tissues. It is released during tissue damage [20]. The LDH levels in hippocampus of maternal brain shows statistical decrease comparing 2nd week and 3rd week of gestational group with control group while there was no statistically significant difference when 2nd week and 3rd week gestational group were compared ($p<0.05$). The decrease of LDH shown in the 2nd and 3rd week compare to control indicates that there was no rapid cell proliferation which means no cancerous cell growth. Also LDH evaluation in foetal hippocampus shows slight reduction but not statistically significant when 2nd week and 3rd week gestational groups were compared with the control. The 2nd week and 3rd week gestational groups show no statistically significant difference when compared with each other, this indicates that there are slight cell proliferations which are not significant to become cancerous since foetal stage requires cell growth and proliferation for organ formation and maturity($p<0.05$).

Glucose 6 Phoshate Dehyrogenase (G6PDH) is the enzyme that helps to maintain the co- enzyme nicotinamide adenin dinucleotide phosphate (NADPH) level.The NADPH in turn maintains the level of glutathione in the cells which helps to protectcells against oxidative damage from compounds like hydrogen peroxide .Levels of G6PDH in the maternal and foetal wistar rats were evaluated, it showed a statistically significant reduction when 2nd week and 3rd week gestational groups were compared with control and while comparing 2nd week with 3rd week there was no statistically significant difference, this means that oxidation were prevented within the tissue and glutathione level was preserved to inhibit cell proliferation which is in agreement with Xu *et al.,* 2016.The foetal G6PDH was evaluated, and it shows no statistically significant difference comparing 2nd week and 3rd week gestational groups with the control and while comparing 2nd week with 3rd week of gestational groups it also shows no statistically significant difference. This is a pointer that the administration of 0.5ml of *annona muricata* does not have significant effect on the G6PDH level vivo ($p<0.05$).

Superoxide Dismutase (SOD) serves a key antioxidant role. SOD decrease reactive oxygen species generation and oxidative stress and inhibits endothelial activation which indicates modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. SOD level when evaluated shows a statistically significant increase comparing 2nd week and 3rd week gestational groups with the control while 2nd week and 3rd week gestational groups shows no statistically significant difference when compared thus the increase in the SOD level in the treated groups is an indication that administration of 0.5mls of *annona muricata* have a protective effect on tissue against oxidative damage which agrees with [1]. stating that elevated activity of SOD protect tissue against oxidative damage to accelerate wound healing process. The evaluation of SOD level in the foetus shows no statistically significant difference when 2nd week and 3rd week gestational group are compared with control also no significant difference comparing 2nd week with 3rd week of gestational groups. This is suggestive that effect of SOD is not much to be statistically significant in prenatal administration ($p<0.05$).

Glutathione peroxidase (GPx) provides amechanism for detoxification of peroxides in livingcells[21]. This reaction plays a crucial role in protectingcells from damage by free radicals, which are formedby peroxide decomposition. Lipid components of thecell are

especially susceptible to reactions with freeradicals, resulting in lipid peroxidation. GPx enzymesreduce peroxides to alcohols using glutathione, thuspreventing the formation of free radicals. The results of GPx level evaluation in mother shows no statistically significant difference comparing the 2nd week and 3rd week gestational groups with control and comparing 2nd week with 3rd week of gestation show no statistically significance. The evaluation of Glutathione peroxidase (GPX) in foetus shows statistically significant increase when 2nd week and 3rd week of gestational groups were compared with the control while no statistical increase between the 2nd week and 3rd week groups when compared, this is suggestive that the administration of soursop did not show significant difference as there are less free radicals while in the foetus there is increase in other to protect hippocampal cell from damage by detoxifying the peroxides in the cells which is in agreement with [22] ($p<0.05$).

Hormonal profile evaluation of progesterone and estrogen was done. Progesterone is a steroid hormone secreted by corpus luteum after ovulation. Before ovulation, progesterone level is always low but increased after ovulation usually 5-7 days. If no incidence of pregnancy, progesterone levels usually decrease. Progesterone level shows significant increase comparing 2nd week and 3rd week of gestational groups with the control group but no statistically significance comparing 2nd week of gestation with control group which is suggestive that administration of 0.5mls of annona muricata did not altered the pregnancy hormone.

REFERENCE

1. Moghadamtousi, S.Z.; Goh, B.H.; Chan, C.K.; Shabab, T.; Kadir, H.A.. Biological activities and phytochemicals of Swietenia macrophylla king. Molecules, 2013; 18: 10465–10483.
2. *Morton, Julia F. "Soursop (Annona muricata)". Fruits of warm climates. Purdue University. 1987; 75–80.*
3. *Le Ven, J.; Schmitz-Afonso, I.; Touboul, D.; Buisson, D.; Akagah, B.; Cresteil, T.; Lewin, G.; Champy, P. "Annonaceae fruits and parkinsonism risk: Metabolisation study of annonacin, a model neurotoxin; evaluation of human exposure". Toxicology Letters 2011; 205: S50.*
4. Lannuzel, A., Hoglinger, G.U., Champy, P., Michel, P.P., Hirsch, E.C. and Ruberg, M. Is a typical parkinsonism in the Caribbean caused by the consumption of Annonacae?. *J. Neural Transmission Supplementa.,* 2006; 70(70):153 –157.
5. *Caparros-Lefebvre, Dominique; Elbaz, Alexis. "Possible relation of atypical parkinsonism in the French West Indies with consumption of tropical plants: A case-control study". The Lancet 1999; 354 (9175): 281–6.*
6. Gavalas, A., Ruhrberg, C., Livet, J., Henderson, C. E., & Krumlauf, R. Neuronal defects in the hindbrain of Hoxa1, Hoxb2 and Hoxb2 mutants reflect regulatory interactions among these Hox genes. *Development,* 2003; *130*(23): 5663–5679.
7. *Amaral, D; Lavenex P. "Ch 3. Hippocampal Neuroanatomy". In Andersen P; Morris R; Amaral D; Bliss T; O'Keefe J. The Hippocampus Book. Oxford University Press. 2006; ISBN 978-0-19-510027-3*
8. Andersen, P., Morris, R., Amaral, D., Bliss, T. and O'Keefe, J. Hipocampal Formation: The Hippocampus Book. Oxford University Press; New York: 2007; 872.
9. Sur, M., & Rubenstein, J. L. Patterning and plasticity of the cerebral cortex. *Science,* 2005; *310*(5749): 805–810.
10. Bishop, K. M., Rubenstein, J. L., et al. Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. The Journal of Neuroscience, 2002; 22(17): 7627–7638.
11. Kiecker, C., & Lumsden, A. Hedgehog signaling from ZLI regulates diencephalic regional identity. Nature Neuroscience, 2004; 7(11): 1242–1249
12. Lumsden, A., & Keynes, R. Segmental patterns of neuronal development in the chick hindbrain. Nature, 1989; 337(6206): 424–428.
13. Wisdom S, Ugoh GO and Mohammed B. Phytochemical Screening and Antimicrobial Activities of*Annona muricata* (L) leaf extract. American Journal of Biological, Chemical and Pharmaceutical Sciences, 2014; 2:44-47.
14. Sabir, A., Konservasi, B., Kedokteran, G F., Makassar-indonesia, H. Aktivitas antibakteri flavonoid propolis Trigona sp terhadap bakteri Streptococcus mutans (in vitro). 2005
15. Roslizawaty Nita Y. Fakhrurrazi dan Herrialfian R. Aktivitas Antibakterial Ekstrak Etanol dan Rebusan Sarang Semut (Myrmecodia sp) Terhadap Bakteri Escherichia coli. Jurnal Medika Veterinaria. 2013; 7(2).
16. Zablotowitcz RM, Hoagland RE, Wagner SC. Effect of Saponin on The Growth and `activity of Rizophere Bacteria. CRC Press, USA. 1996.
17. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: Some helpful considerations. Braz. J.Biol., 2002; 62:609-614.
18. Bitar R, Fakhoury R, Fahim R, Borjac J. Histopathological effect of the Annona Muricata Aqueous leaves extract on the liver and kidneys of Albino Mice. Transl Med., 2017; 7: 194. Dol: 10.4172/2161-1025.1000194.
19. Butt AA, Micheals S, Greer D, Clark R, kissinger P, Martin DH. Serum LDH level as a clue to diagnosis of histoplasmosis, AIDS. Read, 2002; 12(7):317-21.
20. Selwood T., and Jaffe E.K. Dynamic dissociating homo-oligomers and the control of protein function. Arch. Biochem. Biophys. 2012:519 (2):131-43.
21. *Mannervik, B., Methods in Enzymol., 113, 490-495(1985).*
22. Gupta, B.L., and Baquer, N.Z., *Biochem. Mol. Biol.Int*., 46, 1145-1152 (1998).