Original Research Article

HISTOLOGICAL EFFECTS ~~OF~~ AND PRENATAL EXPOSURE ~~OF~~ TO CRUDE AQUEOUS EXTRACT OF *MORINDA LUCIDA* LEAVES ON THE FRONTAL CORTEX OF GROWING WISTAR RATS.

ABSTRACT

Background: The use of medicinal plants has always been part of human culture and is common in Africa. Amongst the medicinal plants commonly used in Nigeria for management~~/~~ or treatment of various types of ailments is *Morinda lucida* Benth.

Aims: This research work was designed to investigate ~~d some of~~ the effects~~, if any, the~~ of prenatal exposure of morinda lucida on the frontal cortex in wistar rats.

Methods and Materials: 25 pregnant wistar rats with an average weight of 150g were randomly divided into five groups (A-E) of five (~~n=~~5) rats each. ~~The treated g~~ Groupd A served as control group and were given normal saline. Groups B, C, D and E were orally administered with M~~m~~orinda lucida (64000mg/kg/bw) on the first, second, third and all weeks of pregnancy respectively ~~while the control were given normal saline~~. The litters in each group were ~~then~~ weighed and sacrificed ~~by cervical dislocation~~ on days 1, 7, 14, 21, 28 and 35 after birth. ~~The brains were also weighed after sacrifice and the frontal cortex excised, fixed in formocalcium for routine histological processing.~~ The photomicrographs of the brain and frontal cortex in the control~~,~~ and ~~the~~ treated groups were observed and compared for changes and differences.

Results: The findings showed that ~~for~~ the brain ~~stained with heamatoxylin and eosin shows that~~in the treated groups were not different from the control groups in terms ~~he~~ of ~~development of their~~ neuronal development. ~~s as t~~There were also no alterations in ~~their~~ neuronal microarchitecture. ~~that is, n~~No vacuolations were visible ~~suggesting cell death were seen and the~~ neuronal cells appear well defined.

Conclusion: Morinda lucida have no toxic ~~or deleterious~~ effect on the brain and frontal cortex in rats ~~as it does not alter carbohydrate metabolism~~, does not cause any loss of Nissl substance and did not affect the microarchitecture of the neurons if administered during pregnancy.

Comment [I1]: Over 20 words in one sentence. Too long

Comment [I2]: This was not mentioned in the methodology in this abstract

Comment [I3]: Find a way of including this in the materials and method above

Comment [I4]: Long sentence. Over 20 words in one sentence

Keywords: *Morinda lucida*, Frontal cortex, teratogen, neurons, weight, growing wistar rat, blood brain-barrier.

1. INTRODUCTION

Many researchers in the field of embryology have in several years carried out researches in order to ascertain the teratogenicity of various chemical substances and herbs taken by women during pregnancy and this has led to many of these substances/ herbs having potential of being teratogenic. Morinda lucida is among the widely used antimalarial plant around the world most especially in Africa and therefore its use among pregnant women cannot be undermined. Teratology is the branch of science that studies the abnormal development of embryo and the causes of congenital malformation [1]. It was believed until 1940s that the mammalian embryo developed in the impervious uterus of the mother but Gregg and Lenz made it apparent and acceptable that the developing embryo could be highly vulnerable to certain environmental agents that have negligible or non-toxic effects in adult~~s~~ individuals. Although the human embryo is well protected in the uterus by the ~~extra-embryonic/foetal membranes (~~amnion and chorion~~),~~ and their mothers' abdominal and uterine walls' environmental agents may cause developmental disruptions following maternal exposure to them. These environmental agents are therefore referred to as teratogen. A teratogen can therefore be defined as any agent that can produce a congenital anomaly or raise the incidence of an anomaly in the population. Animal research has shown that there is a dose-response relationship for teratogens; so, for a drug to be considered as a teratogen, a dose-response relationship has to be observed; i.e., the greater the exposure during pregnancy, the more severe the phenotypic effect. Awareness that certain agents can disrupt human prenatal development offers the opportunity to prevent some congenital anomalies; for example, if some are aware of the harmful effects of drugs (e.g. alcohol and some herbs), environmental chemicals, and some viruses, they will not expose their embryos to these teratogenic agents. The general objective of teratogenicity testing of drugs, chemicals, food additives and pesticides is to identify agents that may be teratogenic during human development and to alert physicians and pregnant women of their possible danger to the embryo/ fetus.

The use of medicinal plants has always been part of human culture and is common in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of

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Comment [I6]: 46 words. Break this sentence into two or three. Add references also

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Comment [I10]: The last 13to 14 lines is referring to teratogens. Is that the main focus of this work?

Comment [I11]: Why the interest in Ghana?

medicine rather than expensive imported drugs. Also in Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach [2, 3].

Amongst the medicinal plants commonly use in Nigeria for management/treatment of various types of ailments is Morinda lucida Benth. Morinda lucida (L.) (Rubiaceae) is a tropical West Africa rainforest commonly known as Brimstone tree [4]. Morinda lucida is a medium size tree that is about 15m tall with scaly grey bark, short crooked branches and shining foliage [5]. The leaves are used as ~~oral teas~~beverage, which are usually taken orally for the traditional treatment of malaria~~,~~ and as a general febrifuge, analgesic, laxative and antibiotic [6]. Two known triterpenic acids (Ursolic and oleanolic acids) were isolated from the leaves which are known to have protective effects on the brain and also which exhibit anti-microbial features against numerous strains of bacteria, HIV and HCV viruses and *plasmodium* protozoa causing malaria [7]. This research work aimed at investigating ~~if any~~ the effects of ~~the~~ aqueous leaf extract of morinda lucida on the frontal cortex of growing wistar rats exposed to it prenatally.

Comment [I12]: Not necessary Information

Comment [I13]: 43 words in one sentence. Too long.

Comment [I14]: There is no real flow in describing the subject matter and purpose of this research. Authors should please improve this introduction.

2. MATERIALS AND METHOD

2.1 Extract

Fresh leaves of Morinda lucida was gotten and authenticated at the botanical garden of the department of plant biology, LAUTECH, Ogbomoso, Nigeria. The leaves were weighed (306g) then air~~, weighed, ~~dried.~~,~~ The air dried leaves where then pulverised and ~~blended,~~ reweighed~~, pounded, reweighed and~~. The pulverised leaves were subsequently sieved~~,~~ and then weighed ~~finally~~again. The aqueous extract was prepared ~~using~~ dissolving 10 g of the powdered leaves~~, it was dissolved~~ in 100 mL~~s~~ of distilled water~~,~~ and evaporated to dryness~~,~~. ~~t~~The residue was weighed and ~~10g~~ was further dissolved in 100 mL~~s~~ of distilled water for oral administration to the rats at a dose of 6400mg/kg body weight.

Comment [I15]: Consider "obtained "??

2.2 Experimental Design

25 female and 10 male adult wistar rats weighing between 120g-180g were u~~sed for this research work~~tilized. The male rats were caged separately from the female rats. The females

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rats were randomly selected into five groups as follows A, B, C, D, and E, each containing five rats. They were kept in the animal house of University of Ilorin, Nigeria and given water and feed twice daily. The treatments for the various groups were administered accordingly, following strictly, the ethical approval and guides of the ethical committee of College of Health Sciences, University of Ilorin, Nigeria.

Comment [I17]: Insert IACUC number

2.3 Determination of Mating

Comment [I18]: Please find literatures that explains methods of determination of mating in rats : simple method is the vaginal smear method as described here in the corrections

Mating was done by natural copulation method. Vaginal smear test was performed between 7.00am and 9.00am on daily basis prior to matingto determine successful mating. This was done in order to observe the oestrous cycle of the female rats which is on four phases prooestrus, oestrus, dioestrus, and metoestrus. In rats, ovulation occurs in the oestrus phase. The Briefly, vaginal smear was done performed by introducing a micro-pipette containing 0.5 mLl normal saline into the vaginal of the female rats. This was performed in the morning between 7.00am and 9.00am in order to get absolute result. The vVaginal fluid was withdrawn with the pipette and placed on theand examined under a light microscope (Insert brand, Company, Country) slide. This was viewed under the light microscope without the condenser to identify determine the presence of spermatozoathe cells, thereby determining the phase of the oestrus cycle of each female rat. A normal oestrus cycle takes a period of about 4-5 days [8].

2.4 Animal Grouping

Group	Number of Animals	Days of Administration	Dosage
A	5	Day 0-7 days after of pregnancy was confirmed	6 400mg/kg/bw
B	5	Day 8-14 days after pregnancy	6 400mg/kg/bw
C	5	Day 15-21 days of after pregnancy	6 400mg/kg/bw
D	5	Receive extract throughout the pregnancy period i.e. 0-35 days	6 400mg/kg/bw

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E			Control group receive ~~only~~ normal saline throughout	Normal saline

After confirming pregnancy, pregnant rats were randomly divided into five groups of five animals each. Administration of the extract follows the animal grouping as shown ~~below~~above.

2.5 Procedure of Animal Sacrifice

The rats were sacrificed through cervical dislocation. The skulls were dissected and the brains were harvested. The brain tissues were fixed in 40% formal calcium. The tissues were Processed and stained with Heamatoxylin and Eosin to demonstrate the microarchitecture of the cells~~,~~ Cresyl fast violet was also used to demonstrate Nissl substances and biochemical analysis was done to assess tissue damage in the brain.

PHOTOMICROGRAPHS DEMONSTRATION FOR H&E.

3.1 Group A

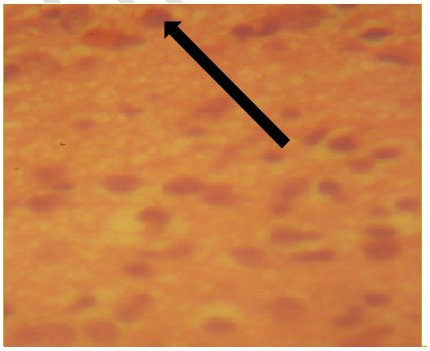

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Fig.1.Histological demonstration of the frontal cortex using H&E staining techniques (×200) showing normal neurons (N, black arrow) at postnatal days 0-7.

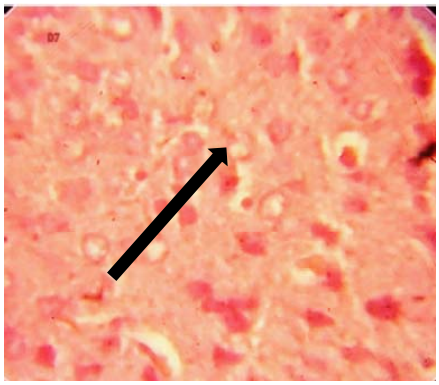


Fig.2. Histological demonstration of the frontal cortex using H&E staining techniques (×200) showing normal neurons (N, black arrow) at postnatal days 8-14.

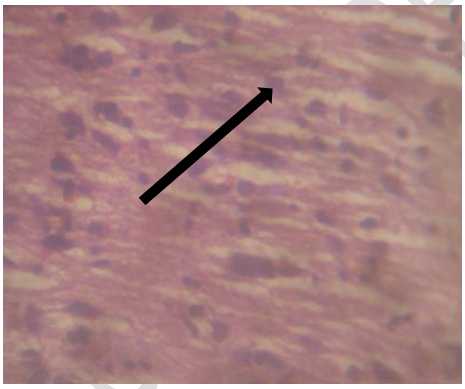


Fig.3.Histological demonstration of the frontal cortex using H&E staining techniques (×200) showing normal neurons (N, black arrow) at postnatal days 15-21.

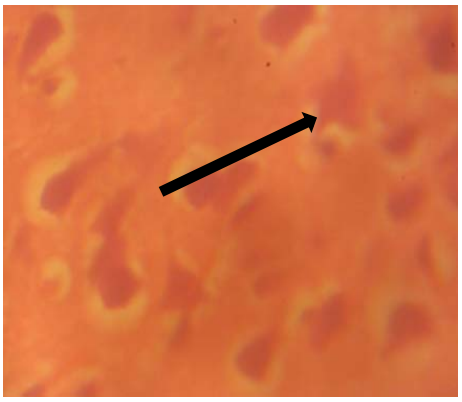


Fig.4.Histological demonstration of the frontal cortex using H&E staining techniques (×200) showing normal neurons (N, black arrow) at postnatal days 0-35.

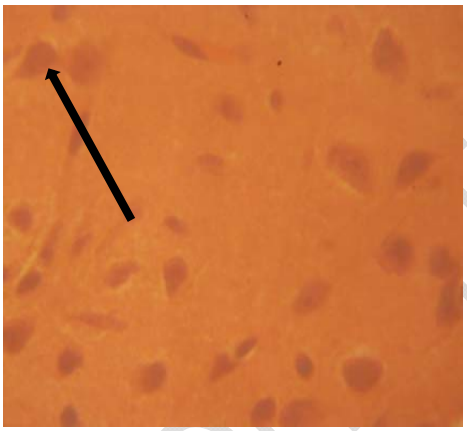
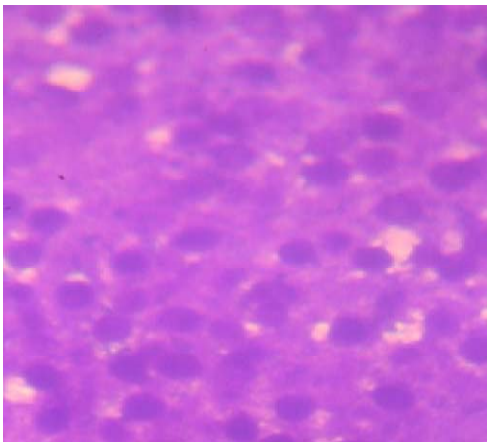


Fig.5.Histological demonstration of the frontal cortex using H&E staining techniques (×200) showing normal neurons (N, black arrow) showing control group.



PHOTOMICROGRAPHS FOR CREYSL VIOLET STAIN.

Fig. 6. The extensive dark purple coloration indicating an abundance of Nissl bodies characteristic of a normal cell. Cresyl violet x200 at postnatal days 0-7.

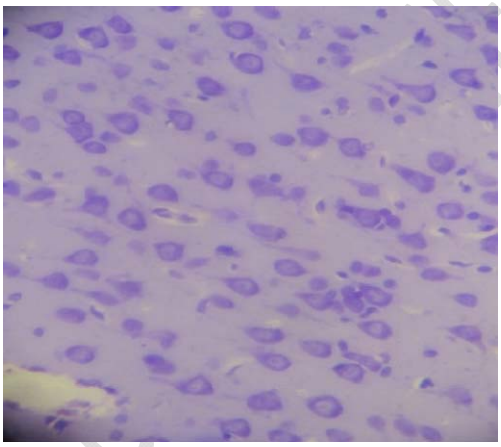


Fig. 7. The extensive dark purple coloration indicating an abundance of Nissl bodies characteristic of a normal cell. Cresyl violet x200 at postnatal days 8-14.

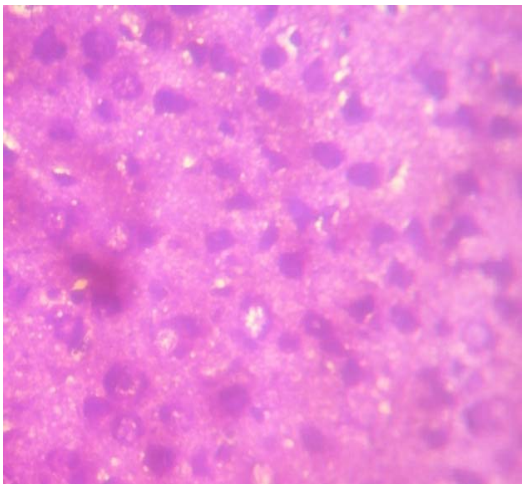


Fig. 8. The extensive dark purple coloration indicating an abundance of Nissl bodies characteristic of a normal cell. Cresyl violet x200 at postnatal days 15-21.

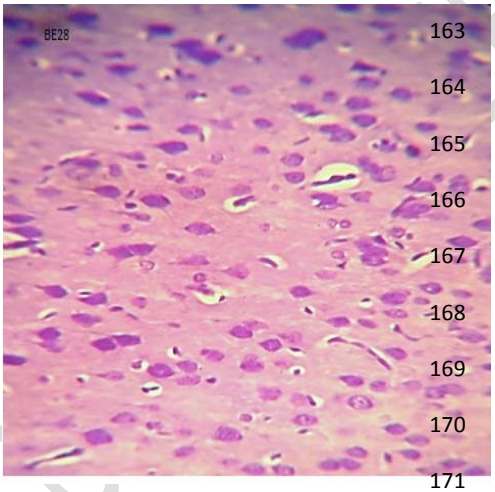


Fig. 9. The extensive dark purple coloration indicating an abundance of Nissl bodies characteristic of a normal cell. Cresyl violet x200 at postnatal days 0-35.

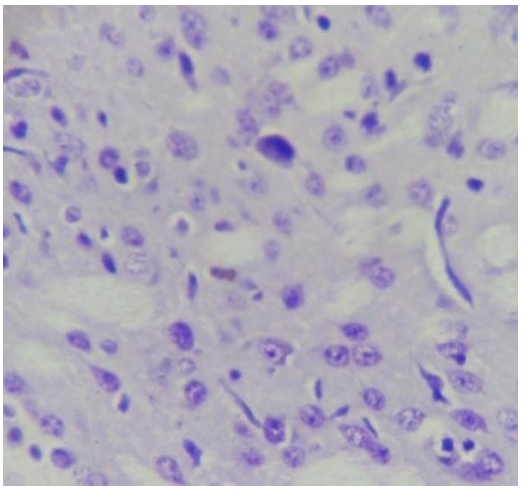


Fig. 10. The extensive dark purple coloration indicating an abundance of Nissl bodies characteristic of a normal cell. Cresyl violet x200 at normal control group.

QUANTITATIVE HISTOCHEMICAL OBSERVATION

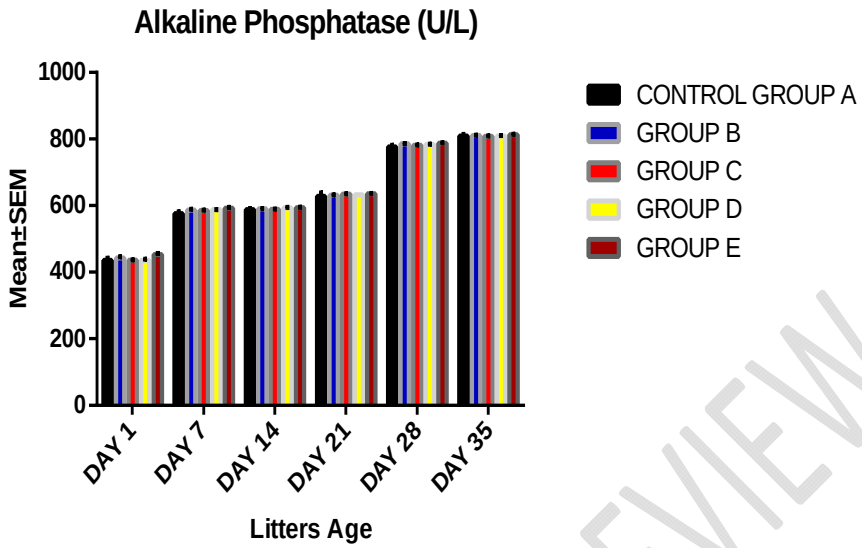
Comment [I20]: Which statistical test did you performed?

	Control E	Group A	Group B	Group C	Group D
Day 1	435.0±5.0	442.5±2.5	436.5±0.5	435.5±2.5	452.5±2.5
Day 7	576.5±3.5	586.5±1.5	585.5±0.5	587.5±0.5	592.0±2.0
Day 14	587.5±2.5	590.5±0.5	588.5±0.5	593.0±1.0	594.0±1.0
Day 21	627.5±7.5	631.0±1.0	634.5±0.5	633.0±0.0	635.5±0.5
Day 28	776.0±4.0	785.5±0.5	781.0±1.0	783.5±0.5	787.0±1.0
Day 35	808.5±3.5	810.5±0.5	808.0±1.0	809.0±1.0	813.0±1.0

TABLE1: SHOWING THE LEVEL OF ALKALINE PHOSPATASE (U/L)

Mean±SEM, P <0.05- Values For Alkaline Phospatase (u/l)

CHART 1: SHOWING THE LEVEL OF ALKALINE PHOSPATASE (U/L)



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199

200 **TABLE 2: SHOWING THE LEVEL OF LACTATE**
201 **DEHYDROGENASE (IN U/L)**

202 **Mean±SEM, P <0.05- Values For Lactate Dehydrogenase**

Comment [I21]: State statistical test used ?

Litters Age	Control Group	Group A	Group B	Group C	Group D
Day 1	677.5±18.5	687.5±0.5	690.5±0.5	695.5±0.5	701.0±1.0
Day 7	832.5±7.5	834.5±0.5	837.0±1.0	839.5±0.5	842.0±0.0
Day 14	1002.5±22.5	991.5±1.0	1008.5±0.5	1015.5±0.5	1024.5±0.5
Day 21	1197.5±4.5	1189.5±0.5	1195.0±0.0	1200.5±0.5	1205.5±0.5
Day 28	1287.5±7.5	1282.5±0.5	1288.5±0.5	1291.0±1.0	1295.5±0.5
Day 35	1380.0±10.0	1377.0±0.5	1381.5±1.5	1386.0±1.0	1391.0±1.0

203

CHART 2:SHOWING THE LEVEL OF LACTATE DEHYDROGENASE(U/L)

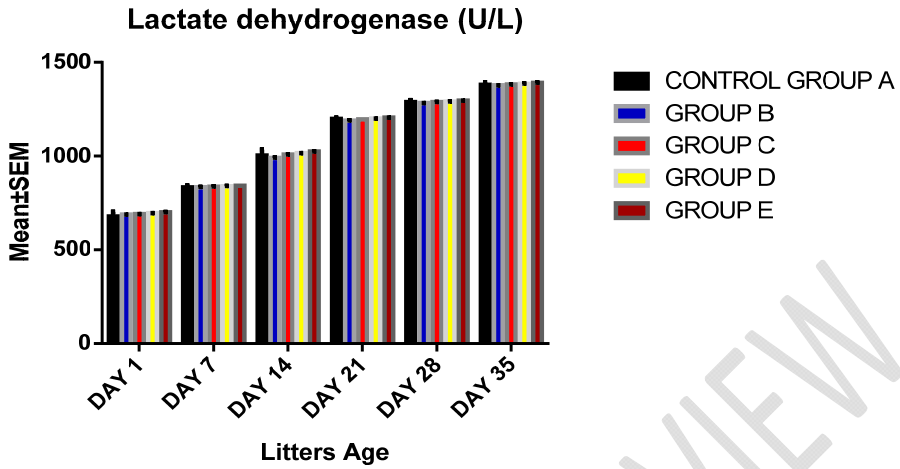
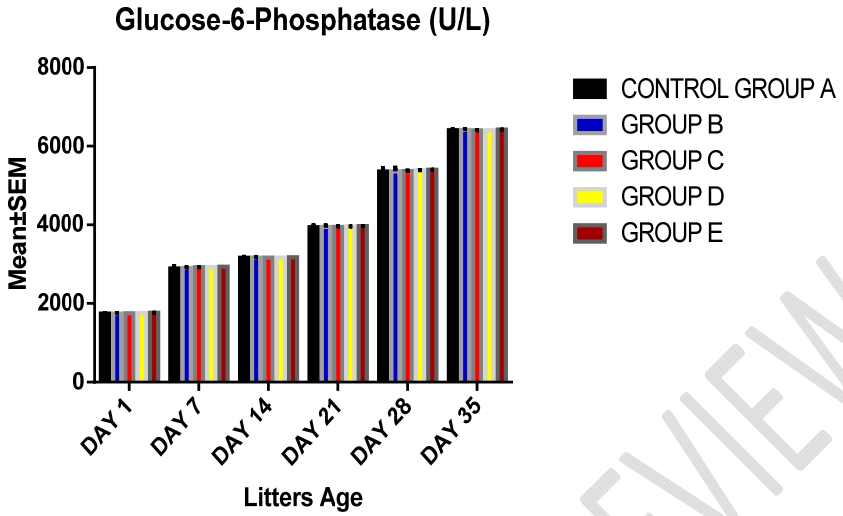


TABLE 3: SHOWING THE LEVEL OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (U/L)

Mean±SEM, P <0.05- Values For Glucose-6-Phosphate Dehydrogenase

Litters Age	Control Group	Group A	Group B	Group C	Group D
Day 1	1745.0±5.0	1746.5±1.5	1751.0±1.0	1755.0±0.5	1761.0±1.0
Day 7	2892.5±42.5	2900.0±10.0	2922.5±2.5	2931.5±1.0	2936.5±1.0
Day 14	3164.0±12.0	3167.5±7.5	3168.5±0.5	3171.5±1.0	3176.5±1.0
Day 21	3945.0±25.0	3952.5±22.5	3952.5±2.5	3962.5±2.5	3967.5±1.5
Day 28	5358.0±62.0	5362.5±62.5	5371.5±1.5	5391.5±1.5	5402.5±2.0
Day 35	6408.5±16.5	6413.5±14.5	6406.5±1.5	6411.5±1.0	6420.5±0.5

CHART 3: SHOWING THE LEVEL OF GLUCOSE-6-PHOSPHATE DEHY



DROGENASE (U/L)

E

Dehydrogenase

A

B

C

D

RESULTS AND DISCUSSION

Evidences are also seen in the photomicrographs obtained from the histological part of this study because histology is good in early assessment of any brain damage caused by teratogenic agents. In the histological study, Heamatoxylin /Eosin were used to study the microarchitecture of the developing neurons while Cresyl fast violet was used to study Nissl substances.

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Study with Heamatoxylin and eosin shows that the litters in the groups given Morinda lucida leaf extract were not different from the control group in the development of their neurons as there were no alterations in their microarchitecture that is, no vacuolations suggesting cell death were seen and the cells appear well defined. This is supported by the low level of G-6-

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PDH in the litters whose mothers were given ~~the~~ Morinda lucida during pregnancy when compared with the control.

Nissl bodies ~~are known to function just like endoplasmic reticulum and golgi apparatus that is; to~~ manufacture and release ~~certain chemicals, namely proteins~~ (8). The ultrastructure of Nissl bodies suggests they are primarily concerned with the synthesis of proteins for intercellular use (9). The staining intensity of the Nissl substance in this study both in the control and in the treated group are similar which may suggest Morinda lucida did not affect the synthesis of protein for neuronal functions because there was no loss or degeneration of the Nissl substance– an indication that the extract administered to the mother rats during pregnancy has no neurotoxic effect on the frontal cortex of the litters.

The biochemical analysis done in this study are useful 'markers' for assessing ~~tissue~~ brain damage ~~in the brain~~ as enzymes measurement are used to study and diagnose the presence of different diseases and abnormalities in the body (10).

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids and they are most effective in an alkaline environment (11). ALP is resistant to inactivation, denaturation and degradation. ~~, and~~ It also has a higher rate of activity and ~~it~~ is believed to be a means for bacteria to generate free phosphate group for uptake and use is supported by the fact that ALP is usually produced by ~~the~~ bacteria during starvation and not when phosphate is ~~plentiful~~ (11).

There was no significant difference in the level of ALP in treated groups as compared to the which shows that there are no increase in the function of alkaline phosphatase and therefore no degradation which is a major function of lysosomal enzymes and therefore no deleterious effect on the treated group which could have led to the death of the cells hence there is no need for the degradation of cell debris, or rather self- death (apoptosis) of cells and this is supported by the fact that alkaline phosphatase is usually produced by the bacteria only during phosphate starvation and not when phosphate is ~~plentiful~~ (12).

The levels of LDH in the treated group when compared with the control also shows that there are no irregularly rising and falling rates in metabolism, an indication of a normal

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development that is, no tissue breakdown or cell destruction as supported by Butt et al., 2002 that tissue breakdown elevates levels of LDH and

G6PDH is an enzyme in the pentose phosphate pathway. It converts glucose -6 – phosphate into 6- phosphoglucono-δ- lactone. It supplies reducing energy to cells by maintaining the level of co- enzyme nicotinamide adenine dinucleotide phosphate (NADPH). It is also known to function in glucose metabolism which is the primary source of energy needed to support life and this has been reported to increase in growing cells (12) and decrease in cell undergoing cell death (13).

The levels of G6PDH in the treated groups when compared with the control group shows no significant difference statically which suggest that there are no degradative enzymes but there are proliferation and cell maturation.

Tian et al., 1998 reported that inhibition of G6PDH may inhibit cell proliferation, by inhibiting tyrosine phosphorylation.

Quantitative histochemical analysis results correlated with the histological observations. The levels of alkaline phosphatase, lactate dehydrogenase and Glucose-6-Phosphate dehydrogenase were not higher in the treated group when statistically compared with the control and also in the histological result it does not alter the microarchitecture of the neurons.

Since the above result show that Morinda lucida does not act as a teratogen to pups during pregnancy, it therefore suggests that the extract does not cross the placenta or the blood brain barrier to affect the developing embryo.

It can therefore be concluded that aqueous extract of Morinda lucida have no toxic or deleterious effect as it does not alters carbohydrate metabolism, does not cause any loss of Nissl substance and did not affect the microarchitecture of the neurons if administered during pregnancy.

Comment [130]: And what ?

Comment [131]: Too long sentence

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Comment [133]: Which statistical analysis did the authors use

Comment [134]: Too long sentence and vague

Comment [135]: Was this investigated in this study??

CONCLUSION

It ~~can~~ is therefore ~~be~~ concluded from the present study that morinda lucida does not cause any ~~teratogenic~~ effect on the brain – and frontal cortex of ~~the~~ growing wistar rats following administration prenatally.

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Comment [136]: 11 out of 14 were more than 10 years old and too few references for this work

Comment [137]: Year?

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