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FOETAL BRAIN DIMENSIONS AND NEUROGLIAL CELLS FOLLOWING ADMINISTRATION OF COSTUS AFER IN PREGNANT WISTAR RATS

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Abstract

Costus afer commonly used as medicinal plant in tropical Africa during pregnancy may have teratogenic effects on the developing nervous system. Twenty four adult female Wistar rats weighing between 150g and 200g was shared into 4 groups of 6 rats each. The females were caged overnight with sexually mature male rats of the same strain. The presence of sperm (tailed structures) in vaginal smears the following morning was designated day zero of pregnancy. Group A (Control) received 0.5ml of distilled water, Group B, C and D received 250, 500, and 1000mg/kg bodyweight of ethanolic leaf extract of *Costus afer* respectively via oral route from day 6 to 12 of gestation. Pregnancy was terminated on 20th day of gestation and fetuses harvested by uterectomy. Brain dimensions was measured using vernier slide calipers. The brain was processed and cerebral cortex stained for neuroglial cells using Phosphotungtic Acid Haematoxylin staining technique. Results showed no significant alteration in brain dimensions evaluated and no evidence of histological distortion of neuroglial cells of the cerebral cortex in the litters of albino rats. This study suggests *Costus afer* may not have teratogenic potentials on brain morphology and neuroglial cells in foetal cerebral cortex of albino rats.

Key words: Costus afer, foetal brain, neuroglial cells

Introduction

The use of plants for medicinal purposes dates back to thousands of years ago as the earliest humans used various plants to treat illness[1,2]. The use of medicinal plants has significantly increased over recent years as it is easily accessible, cheap and the strong belief that herbal remedies are natural and therefore non-toxic[3]. According to world Health Organization, approximately 80% of World population currently use herbal medicines in healing different ailments. Study by Melchias[4], also showed that about 70%-80% of the World population depends on traditional healthcare based on traditional medicinal plants. The use of medicinal plants and different herbs in the treatment of different ailments by the populace including pregnant women may have some teratogenic effects on the fetuses. *Costus afer* is commonly used as a medicinal plant throughout tropical Africa. Aweke[5] reported its use to treat tachycardia, urethral discharges, venereal diseases, jaundice and to prevent miscarriage. Leaf extracts of *Costus afer* appears to be safe for use during pregnancy and probably no toxic effects on the morphology of litters and histology of the cerebral cortex of fetuses of albino Wistar rat[6]. Phytochemical analysis of the leaf of *Costus afer* reveal the presence of alkaloids, saponins, flavonoids, phenols, glycosides and terpenoids[3,7,8,9]. *Costus afer* hexane leaf extract treated arthritic rats were not exposed to free radicals and also showed its effectiveness in endogenous antioxidant defense system[10]. Throughout the world, many pregnant women consume a great variety of herbal products during pregnancy for a variety of reasons, including pregnancy related conditions (nausea, vomiting, constipation), to prepare for labour[11]. Exposure to teratogens affects the fetus or embryo in variety of ways, such as the duration of exposure, the amount of teratogenic substance and the stage of development the embryo or fetus is in during the exposure[12]. Teratogens may affect the embryo or fetus in a number of ways, causing physical malformations, problems in behavioural or emotional development of the child, and decreased intellectual quotient (IQ) of the child. Additionally, teratogens may also affect pregnancies and cause complications such as preterm labours, spontaneous abortions, or miscarriages. The result of the research on Ethanolic extract of *momordica feoetida* leaf at high dose (500mg/kg body weight) prevents implantation, induces abortion, and significantly reduces fetal parameters in Sprague Dawley rats[13]. *Trigonella foenum*-graecum leaves aqueous extract has also been studied to have teratogenic effect on the organogenesis stage of Sprague-Dawley rat fetus[14]. Study has shown that some components of *ginkobuloba* extract has teratogenic effects when administered in high dosages, leading to intrauterine growth retardation in fetuses of Wistar rats[15]. Maternal administration of Bonny light crude oil has effect on the fetal brain by causing significant reduction in, brain weight and dimensions, neuroglia cells of developing cerebral cortex and induce malformations of the cerebrum[16,17]. Mesembe *et al.*[18] reported a dose dependent reduction in cerebral lateral anteroposterior diameter (LAPD) and medial anteroposterior diameter (MAPD) of the groups of rats treated with graded doses of artesunate which was significantly ($P<0.05$) lower than the control. The study further reported a reduction in the cerebral transverse diameter in the group that received high dose of artesunate. However, there was no report of morphological malformation, but suggest that high doses of artesunate may lead to high level of intrauterine growth retardation and could also be toxic to the fetal nervous system. Exposure to some of these herbs/medicinal plants taken orally as decoction or order wise may have teratogenic effects on the developing nervous system. This study on the foetal brain dimensions and neuroglial cells following administration of Costus afer in pregnant Wistar rats will provide knowledge and verify the safety of this herb on foetal brain during pregnancy .

Methods

This research was carried out in the Department of Anatomical Sciences, University of Calabar, Calabar, Nigeria with ethical approval number 032AN20717. The plant was identified and authenticated in the Department of Botany, University of Calabar, and with Voucher number: Herb/Bot/002. *Costus afer* leaves was air-dried at room temperature, grinded into powdered form and ethanol extraction process

was carried out in the Endocrine laboratory of Biochemistry Department, University of Calabar. Twenty four adult female Wistar rats weighing between 150g and 200g was used for this study. The animals were grouped into 4 groups of 6 rats each and kept in separate wooden cage. The rats were fed with normal rat chow, and water was provided *ad libitum* throughout the duration of the experiment. The females were caged overnight with sexually mature male rats of the same strain. The presence of sperm (tailed structures) in the vagina smears obtained the following morning confirmed coitus and the sperm positive day were designated as day zero of pregnancy. Group A served as control and received 0.5ml of distilled water orally for same period with the experimental groups. Group B, C and D served as experimental groups and were given 250mg/kg, 500mg/kg, and 1000mg/kg bodyweight of ethanolic leaf extract of *Costus afer* respectively via oral route of administration from day 6 to 12 of gestation. Pregnancy was terminated on the 20th day of gestation and fetuses were harvested by uterectomy. The fetuses were blotted dry and examined for gross malformations. The heads were severed and fixed in Bouins fluid. The brain dimensions was measured using vernier slide calipers. The brain was processed histologically and cerebral cortex stained for neuroglial cells using Phosphotungtic Acid Haematoxylin (PTAH) staining technique. Data obtained was statistically analyzed using SPSS and ANOVA followed by Scheffes post hoc test.

Results

The mean medial anteroposterior diameter (MAPD) of the groups treated with 250 mg/kg and 500 mg/kg of *Costus afer* extract (0.29±0.00 and 0.28±0.00) showed no significant reduction and a significant decrease was observed in the group treated with 1000 mg/kg (0.20 ± 0.00) at ($P<0.05$) compared to the control (0.31±0.00). The mean transverse diameter (TD) of the 250 mg/kg and 500 mg/kg treated groups (0.50±0.00 and 0.49±0.00) was similar to the control group, however, the group treated with 1000 mg/kg *Costus afer* extract showed a significant decrease (0.40±0.00) when compared to the control group (0.50±0.00). The mean Lateral anteroposterior diameter (LAPD) of the Groups B and C showed no difference (0.28±0.00 and 0.28±0.00) while Group D was significantly decreased (0.20±0.00) when compared to the control group (0.28±0.00) (Table 1).

Sections of the developing cerebral cortex stained with PTAH showed the arrangement of astrocytes, neurons and layers of the developing cortex. The control group (Group A) showed distribution of the astrocytes and other supporting neuroglial cells in the marginal zone, cortical plate, intermediate zone, subventricular and ventricular zone. (Fig. 1a&b). The staining intensity of the astrocytes and other supporting neuroglial cells in the intermediate zone is higher than that on the marginal zone and cortical plate zones. No significant variation was observed in the staining intensity of the astrocytes and other supporting neuroglial cells in the layers of the developing cerebral cortex in the treated groups B, C and D (Fig 2a&b, 3a&b,& 4a&b) when compared with the control.

Discussion

Some studies carried out on teratology has shown that there is relationship between the various brain dimensions; (medial anteroposterior diameter (MAPD), lateral anteroposterior diameter (LAPD) and transverse diameter (TD)) and malformation of the nervous system[19,20]. In this study, ethanolic leaf extract of *Costus afer* did not show significant alteration of the brain dimensions (MAPD, LAPD and TD) evaluated. This is in-line with report of no observable malformations on the external structures of fetuses following maternal administration of *Costus afer* in rat model[6]. One of the most important mechanisms that have been postulated to explain the causes of teratogen-induced fetal malformations is excessive formation of reactive oxygen species or impaired antioxidant defense[21]. However, *Costus afer* has been reported to possess antioxidant activity[3,22,23] and with this, we may suggest ethanol leaf extract of *Costus afer* as non teratogenic. The result of this study using Phospho-tungstic Acid Haematoxylin (PTAH) stain also showed no histological changes in astrocytes on the sections of cerebral cortex cells of the tissue when compared with the control. Fischer *et al.*[6], reported no evidence of alteration of the

developing cerebral cortex in rats after administration of *Costus afer* using Haematoxylin and eosin staining technique. There was no observable significant difference between the control and groups treated with 250, 500 and 1000mg/kg of ethanol leaf extract of *Costus afer.* No distortion, atrophy, dysplasia or hyperplasia of the cells. The astrocytes were distinct in all the layers of the sections as seen in Fig 1-4. However, the astrocytes were not prominently differentiated from other cells of the cerebral cortex. This may be in line with the report by that immature neurons and glials do not show cytological recognizable differentiation[12]. Bandeira *et al.*[24] reported that, at birth, non-neuronal cells (most of which are glia) in rat comprise of about 6% of brain cells, in adult rats, however, they account for nearly 50% of brain cells. Miller and Gauthier[25] also reported that, just before birth, the radial glials accelerate the expansion of the neuronal population and switch to gliogenesis to produce astrocytes. This is in-line with the non-differentiation of the astrocytes from other glias observed in this study. Studies has also shown flavonoids to be one of the active compounds found in *Costus afer* leaf extract and its consumption is associated with reduced risk of a number of chronic diseases, including cancer, cardiovascular disease, and neurodegenerative disorders[26]. Maternal consumption of high levels of flavonoids on reproductive and developmental outcomes in a mouse model revealed similar number of implantations, litter size, body weight, skeletal development and post-natal survival amongst both the control and treated groups, indicating that high consumption of flavonoids during gestation and fetal development has no negative effect[27]. This can possibly be suggested as a reason the administration of various doses of ethanol leaf extract of *Costus afer* had probably no adverse effect on the brain dimensions and neuroglia cells

References

1. Agbanyim, A.N., Ukpabi, F.I. and. Chinwendu, S. (2013). Phytochemical composition of *Piper guineresnce* (Uziza leaves) and *Telfania occidentalis* (Fluted pumpkin).Proceeding of 8th annual conference. *Association of Analytical chemists,* 3 (2): 54-57.
2. Ajiwe, V.I., Dimonyejiaku, N.N., Chinwuba, A.J. and. Chendo, N.M. (2008). Preliminary studyon the pharmaceutical constituents of *Emilia sonchifolia* leaf. *Association of Analytical chemists Journal,* 2 (2): 302 – 309.
3. Ezejiofor, A.N., Odowelle, N.A., and. Orisakwe, O.E. (2016). Protective and antioxidant effect of aqueous leaf extract of *costus afer* Ker Gawl on cyclosporine-a(csa) induced nephrotoxicity. *International Journal of Phytomedicine and Phytotherapy* 2:11.
4. Melchias, G. (2001). *Biodiversity and Conservation.* Science Publishers, Inc. Pp121-139.
5. Aweke, G. (2007). *Costus afer* Ker Gawl. In: Schmelzer, G.H. and. Gurib-Fakim, A. (Eds).*PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale). http://database.prota.org/search.html*. Retrieved 3rd April 2017.
6. Fischer, V.A., Onwukwe, E. F., Fischer, C. E., Ebong, P. E. (2020). Evaluation of Leaf extracts of *Costus Afer* on foetal morphology and cerebral cortex using rat model. *Asian J. Anim. Sci.*, 14: 88-92
7. Momoh, S., Yusuf, O.W., Adamu, M.M., Agwu, C.O.C. and. Atanu, F.O. (2011). Evaluation of the phytochemical composition and hypoglycaemic activity of methanolic leaves extract of *Costus afer* in Albino Rats. *British Journal of Pharmaceutical Research,* 1(1):1-8.
8. Ukpabi, C.F., Agbafor, K.N., Ndukwe, O.K., Agwu, A and Nwachukwu, S.N. (2012). Phytochemical composition of *costus afer* extract and its alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. *International Journal of Modern Botany,* 2(5):120-126.
9. Anaga, A.O., Njoku, C.J., Ekejiuba, E.S., Esiaka, M.N. and. Asuzu, I.U. (2004). Investigations of the methanolic leaf extract of *Costus afer.* Ker for pharmacological activities *in vitro* and *invivo*. *Phytomedicine,* 11:242-248.
10. Anyasor, G.N., Onajobi, F., Odutola, O., Adebawo, O, and. Oboutor, E.M. (2014).

Antiinflammatory and antioxidant activities of *costus afer* ker Gawl hexane leaf fraction in arthritic rat models. *Journal of Ethnopharmacology* 115:543-551.
11. Gideon, K. (2007). *Medication Safety in Pregnancy and Breastfeeding.* U.S.A. McGraw-Hill Inc, Jaypee Brothers Medical Publisher, Pp 77.
12. Waney, S. and. Anna, J. (2010). Abnormal development of the human cerebral cortex. *Journal of Anatomy,* 217(4):312-323.
13. Odulande, A.K., Nwaoha, O.C., Ashade,O.O., Ojukuku, S.A., Taiwo, I.A., Abebambo, A.O. and. Adeoye. A.A. (2014). Teratogenic effect of the ethanolic leaf extract of *momordicafoetida* scum (cucurbitaceae) on the morphology of foetal sprague dawley rats. *Carribbean Journal of science and technology,* 2:471-481.
14. Taloubi, L.M., Rhouda, H., Belahcen, A., Thmou, A., and. Mdaghri, A.A. (2013). An overview of plants causing teratogenicity: Fenugreek (Trigonella Foenum Graecum). *International Journal of Pharmaceutical Sciences and Research,* 4(2):516-519.
15. Fernandes, E.S., Pinto, R.M., DePaulareis, J.E., Guerra, M.O. and Peters, V.M. (2010). Effects of Ginko bilola extract on the embyofetal development in Wistar rats. *Birth defect research,* 89:133-138
16. Fischer, V.A., Anibeze, C.I.P., Igiri, A.O., Eyong, E.O., Mesembe, O.E. and. Fischer, C.E. (2006a). Effects of bonny light crude oil on the morphology of litters of wistar rats. *Global journal of medical sciences,* 5(1):51-53.
17. Fischer,V.A., Anibeze, C.I.P., Igiri, A.O., Ekanem, T.B., Mesembe, O.E, and. Fischer, C.E,. (2006b). Effects of maternal administration of bonny light crude oil on brain dimensions of wistar rat fetuses. *Global Journal of Pure and Applied Sciences,* 13:95-97.
18. Mesembe, O. E., Ivang, A.E., Udoaffah, G., Igiri, A.O., Fischer. V.A., Akpaso, M., Eluwa, M.A. and. Akpa, A.O. (2004). A morphometric study of the teratogenic effect of artesunat on the central nervous system of the wistar rat fetuses. *Nigeria Journal of Obstetrics and Gynecology,* 19:92-97.
19. Singh,S. and. Padmanabhan, R. (1978). Effect of chloropromazine (CPZ) in developing rat brain: morphological and histological study. *Congenital Anomalies,* 18: 251-256
20. Igiri, A.O., Aniakot, I.C.,Osayande. O., Akpa, O.A. and. Anita, U.E. (1999). Effect of halofantrine hydrochaloride on the morphology and histology of the cerebral cortex of wistar rat fetuses. *Mary Slessor Journal of Medicine,* 2 (1): 58-63.
21. Wells, P.G., Bhuller, Y., Chen, C.S., Jeng, W., and. Kasapinovic, S. (2005). Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicology* and *Applied Pharmacology,* 207:354–366.
22. Armelle, D.T., Lauve, R.Y.T, and. Protus, A.A. (2015). *Costus afer* possesses carbohydrate hydrolyzing enzymes inhibitory activity and antioxidant capacity in vitro. *Evidence-based Complementory and Alternative Medicine,* 13(51):1-10.
23. Anyasor, G.N., Funmilayo, O., Odutola, O., Olugbenga, A. and. Oboutor, E.M. (2015). Evaluation of *Costus afer* Ker Gawl.in vitro anti-inflammatory activity and its chemical constituents identified using gas chromatography-mass spectrometry analysis. *Journal of Coastal Life Medicine,* 3 (2):132-138.
24. Bandei ra, F., Lent, R, and. Herculano-Houzel, S. (2009). Changing numbers of neuronal and nonneuronal cells underlie postnatal brain growth in the rat. *Proceedings of the National Academy of Sciences of the United States of America,* 106:244-249.
25. Miller, F.G and. Grauthier, A.S. (2007).Timing is everything: Making neurons versus glial in the developing cortex. *Neuron,* 15:805-819.
26. Kozlowska, A. and Szostak-Wegierek, D. (2014). Flavonoid-Food Sources and Health Benefits. *Rocz Panstw Hig,* 65(2)79-85
27. Mary, N.R.L., Carl, L.K. and Louise, L. (2015). Reproductive and developmental outcomes, and influence on maternal and offspring tissue mineral content of (-)-epicatechin, (+)-

catechin, rutin ingestion prior to, and during pregnancy and lactation in C57BL/6J mice. Toxicology, (2) 443-449

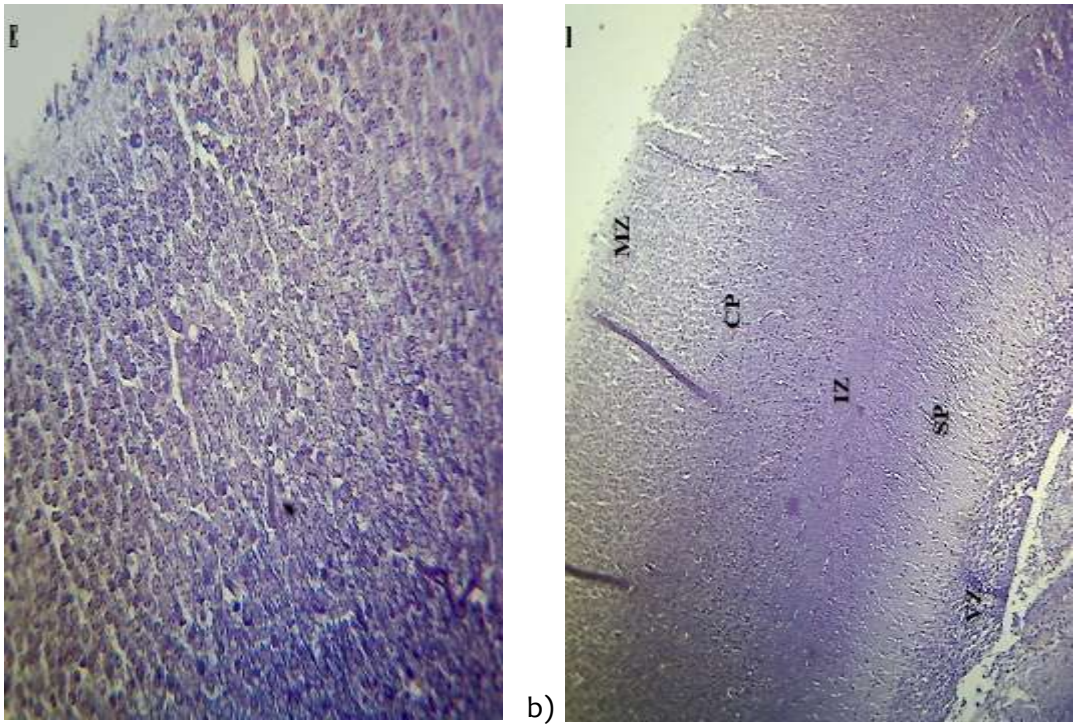
Table 1.

Maternal administration of ethanolic leaf extract of *Costus afer* on brain dimensions of the fetuses of albino Wistar rats.

Parameters	Group A (Control)	Group B (250mg/kg)	Group C (500mg/kg)	Group D (1000mg/kg)
MAPD (cm)	0.30 ± 0.00	0.29 ± 0.00	0.28 ± 0.00	0.20 ± 0.00
TD (cm)	0.50 ± 0.00	0.50 ± 0.00	0.49 ± 0.00	0.40 ± 0.00
LAPD (cm)	0.28 ± .00	0.28 ± 0.00	0.28 ± 0.00	0.20 ± 0.00

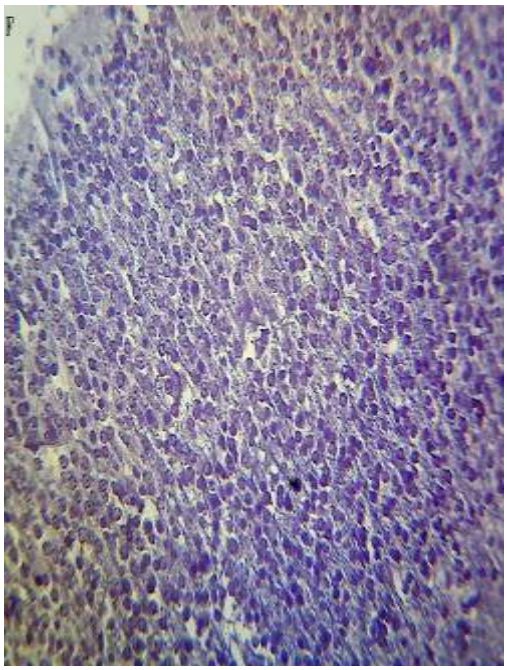
Results are presented as mean ± SEM (P<0.05) when compared with control.

a) b)

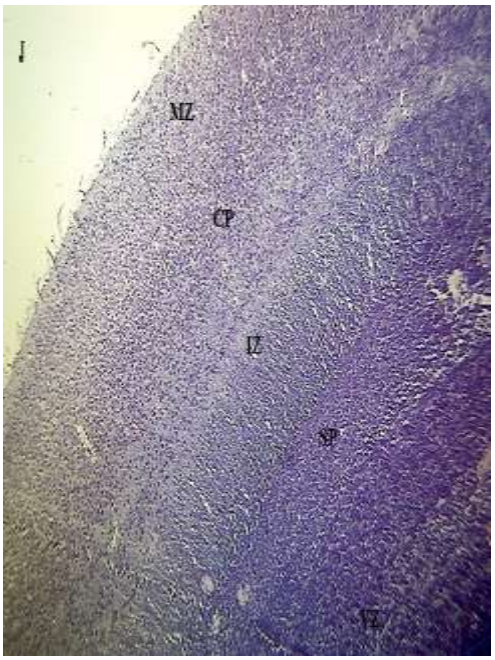


(mag.×400) (mag.×100) PTAH

Figure 1a&b. Photomicrograph of the developing cerebral cortex (Group A) showing five basic histological zones. MZ- marginal zone, CP- cortical plate, IZ- intermediate zone, SP- subventricular zone, VZ- ventricular zone

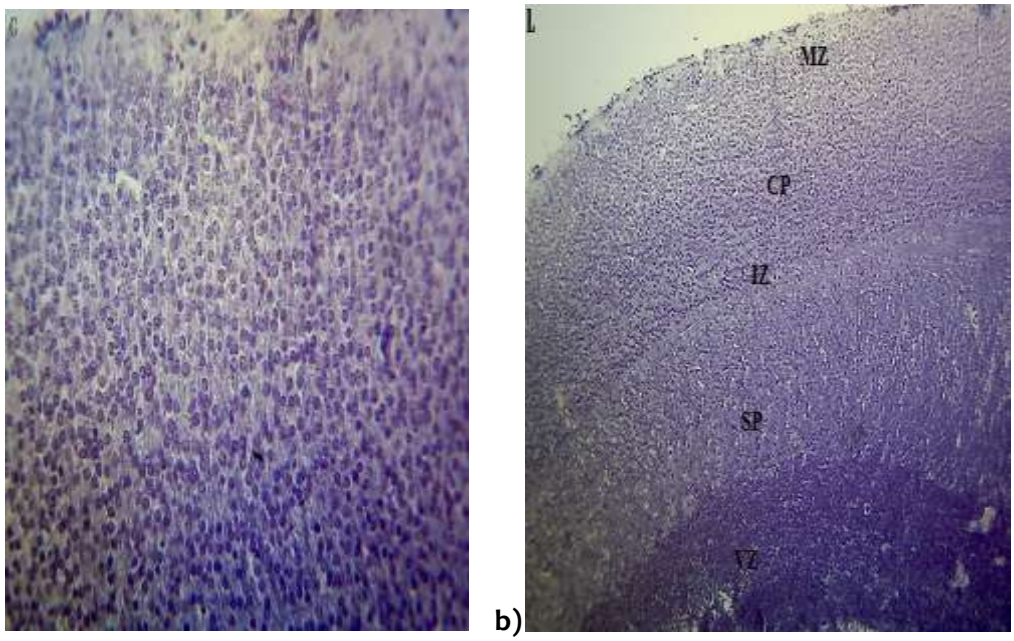


a) (mag. ×400)



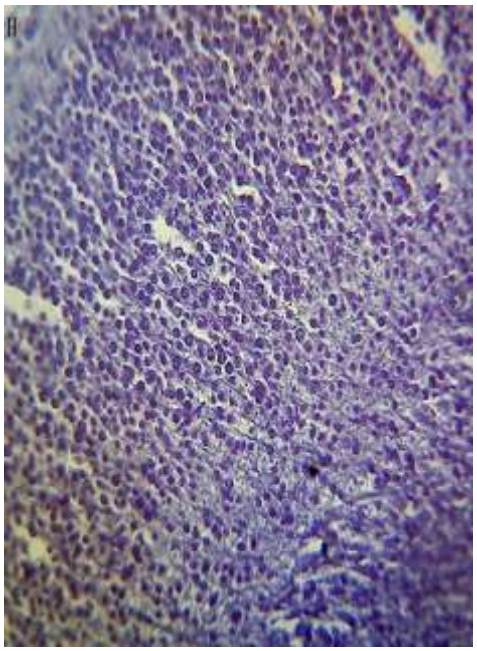
b) (mag. ×100) PTAH

Figure 2a&b. Photomicrograph of cerebral cortex of fetuse (Group B), showing staining intensity in layers of cerebral cortex . MZ- marginal zone, CP- cortical plate, IZ- intermediate zone, SP- subventricular zone, VZ- ventricular zone

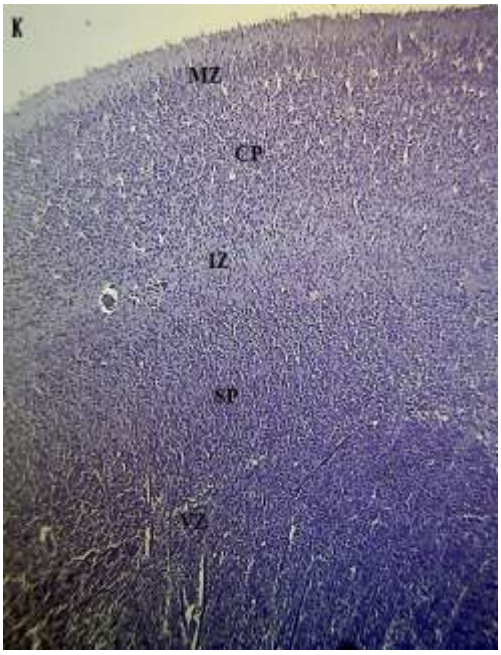


a) (mag. ×400) **b)** (mag. ×100) PTAH

Figure 3a&b. Photomicrograph of the cerebral cortex of fetuse (Group C), showing staining intensity in layers of cerebral cortex . MZ- marginal zone, CP- cortical plate, IZ- intermediate zone, SP- subventricular zone, VZ- ventricular zone



a) (mag. ×400)



b) (mag.×100) PTAH

Figure 4a&b. Photomicrograph of the cerebral cortex of fetuse (Group D) showing staining intensity in layers of cerebral cortex . MZ- marginal zone, CP- cortical plate, IZ- intermediate zone, SP- subventricular zone, VZ- ventricular zone