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Research article

Protective Effect of *Carica papaya* Fruit Extract Against Gamma Radiation-Induced Oxidative Damage in Postnatal Developing Rat Cerebellum

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ABSTRACT

Radiation side effects have been reported to induce oxidative stress by free radical generation. The protective effect of Carica papaya (CP) fruit extract, vitamins C and E against gamma radiation-induced oxidative damage on postnatal developing rat cerebellum was studied. Forty-two female Wistar rats were mated and divided into six groups (n=7). Group A served as control and received water, Group B received a dose of 2.5 Gy irradiation only, Group C received 200 mg/kg aqueous extract of CP only, Group D received 200 mg/kg aqueous extract of CP + 2.5 Gy irradiation, Group E received 500mg/kg Vitamin E + 2.5 Gy irradiation and Group F received 200 mg/kg Vitamin C + 2.5 Gy irradiation. The pregnant rats were exposed to irradiation with radiation from Cobalt 60 delivered by an AECL machine in prone position on 7th day of pregnancy, while aqueous extract of CP, Vitamin E and Vitamin C were administered orally from day 7 of gestation to postnatal day 28. Behavioural and haematological assessments of pups were done on day 21, and killed. Some cerebella of the pups of days 1, 7 14 21 and 28 were fixed in 10% formol-saline for histological and histomorphometric studies, while other cerebella of day 21 were preserved in phosphate buffered saline at 4oC and pH 7.4 for biochemical assays. Data were presented as Mean ± Standard Deviation and analysed using ANOVA at $p < 0.05$. Results showed in the irradiated rats, a significant reduction in body weight, increased brain weight, decreased time spent on the forelimb grip, decreased haemoglobin (Hb) concentration and increased lipid peroxidation (LPO) and H2O2 levels in the cerebellum compared with the control, CP, vitamins C and E rats at $p<0.05$. Histologically, there was persistent external granular layer (EGL) as well as reduction in molecular layer (ML) thickness in the irradiated group compared with the control and other treated groups on postnatal day 21. In conclusion, 2.5 Gy whole body irradiation of pregnant rats induced oxidative stress in the developing rat cerebellum. Treatment, with aqueous extract of CP reduced the damage caused by irradiation when compared with vitamins C and E.

Keywords: *Oxidative stress, gamma- radiation, Carica papaya, developing cerebellum*

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INTRODUCTION

Radiation is a highly effective anticancer therapy, used in the treatment of most malignancies. The therapeutic effect of radiation, however, is limited by the tolerance of normal tissues. Although, radiation damages both normal cells as well as cancer cells, the goal of radiation therapy is to maximize the radiation dose to abnormal cancer cells while minimizing exposure to normal cells, which is adjacent to cancer cells or in the path of radiation (Begg *et al*., 2011) Radiation fields and dose fractions are designed to minimize damage to normal structures, but even with advances in radiation techniques, there is risk of adverse effects from radiation (Cross and Glantz, 2003). Central nervous system (CNS) toxicity is a significant source of morbidity in the treatment of patients with cancer. Radiation, traditional cytotoxic chemotherapy, and novel biologic and targeted therapies all have recognized CNS side effects; and the risks of neurotoxicity can increase with combination therapy (Soussain *et al.*, 2009). Some CNS complications appear during treatment, while others present months or even years later (Hall, 2000; Soussain *et al.*, 2009). High doses ionizing radiation can lead to various effects, such as skin burns, hair loss, birth defects, CNS dysfunction

especially of the cerebrum, cerebellum and hippocampus, cancer, and death (Fang *et al.*, 2002; Kamat *et al.*, 2000).

The cerebellum phylogenetically is derived from the metencephalon, a subdivision of the rhombencephalon and develops embryologically from the dorsal thicknenings of the alar plates (Langman *et al*., 1972). It is located in the posterior cranial fossa behind the pons and medulla and partially separated from them by the cavity of the fourth ventricle (Singh, 1997). It functions in controlling various motor activities in the brain including voluntary muscle activities (DeMyers, 1988), assists in regulating autonomic activities such as respiration, cardiovascular function, control of pupillary size, learning and classical conditioning, as well as cognitive processing and sensory discrimination (West, 1995; Chizhikov and Millen, 2003). Radiation has been reported to cause loss of precursor cells of oligodendrocytes, resulting in demyelination. Malomo *et al*. (2006) reported distortion of the monolayer of the Purkinje cells, astrogliosis and reduction in the thickness of the molecular and granule layers of the cerebellar cortex of irradiated neonatal rats. Despite precautions by radiation oncologists, radiation side effects may still occur after radiation therapy and this is due largely to radiation's non-discrimination between cancer and normal tissue adjacent to the cancer tissue destined for elimination (Neider *et al.,* 2007). Normal host tissue may be protected from the toxic actions of radiation by radio-protectors, which may be beneficial during radiotherapeutic procedures (Adaramoye *et al.*, 2008).

Radiation toxicity was reported to be mediated by the mechanism of free radical injury of tissues thereby causing oxidative damage (Aruoma 1998, Lee *et al.*, 2006, Farombi *et al.,* 2008). Antioxidants reduce the toxicity associated with radiation damage by mopping up the free radicals. Antioxidant activities reported in some plants have been studied for their radioprotective properties as part of prevention strategies. Studies on the antioxidant contents of fruits and vegetables are increasing because natural antioxidant consumption has been found to be related with decreased risk for cancer and heart diseases (Temple, 2000). It is recognized that normal tissue protection in radiotherapy is as important as the destruction of the cancer cells. Since herbal drugs being either non-toxic or less toxic have been found to offer an alternative to synthetic compounds known to have cytotoxic effects, research has intensified on protection modalities (Jagetia *et al.,* 2002), hence, the use of CP fruit extract.

Pawpaw fruit (*Carica Papaya* L.) belongs to the family of Caricaceae and several species of Caricaceae have been used as remedy against a variety of diseases (Mello *et al.*, 2008; Munoz *et al.*, 2000). *Papaya* was originally derived from the southern part of Mexico; *Papaya* is a perennial plant which is distributed over the whole tropical and subtropical area. It is one of the most consumed fruits and a good natural source of macronutrients (carbohydrates and proteins), macronutrients and antioxidant (vitamin A and vitamin C) (Peterson *et al*., 1982).

Carica Papaya has also been reported to have high antioxidant activity and blood lipid peroxidation decreases and blood antioxidant power increased significantly after oral administration of pawpaw fruit juice to rats at different doses 100, 200 300 and 400 mg/kg when compared with vitamin E. Studies have indicated the safety and anti-oxidative stress potential of the juice of CP when compared to the standard antioxidant compound, vitamin E (α-tocopherol) (Mehdipour, Yasa *et al.*, 2006).

Vitamin E, a fat-soluble vitamin and Vitamin C, a water-soluble vitamin are potent antioxidant and radical scavenger in chemical and biological systems, and functions mainly in maintenance of membrane integrity, thereby protecting the membrane from injury through its ability to prevent oxidation of unsaturated fatty acid (Cerecetto and Lopez, 2007). Vitamins C and E have been reported to inhibit ROS generation and lipid peroxidation by chelating free transition metals such as copper and iron (Zablocka and Janusz, 2008). This research was therefore designed to investigate the protective effect of CP fruit extract, and vitamins C and E against gamma radiation-induced oxidative damage on postnatal developing cerebellum of Wistar rat.

MATERIALS AND METHODS

Plant Materials: Fresh, ripe mature fruits of CP were purchased from Bodija market in Ibadan North L.G.A, Oyo State, identified and authenticated by Mr Shasarya O.S. at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria, with a Forest Herbarium Identification (FHI) number: 109722.

Animals: Forty-two sexually matured female rats of Wistar strain weighing between 175-220 g, obtained from breeders in College of Medicine central animal house, University of Ibadan. The animals were acclimatized for two weeks in a freely ventilated and naturally illuminated (12 hour light/dark cycle) animal house of the Department of Veterinary Pharmacology, University of Ibadan. The rats were divided in six groups of seven animals per group, fed with standard rat pellet and water provided *ad libitum*. They were mated, pregnancy confirmed by the presence of vaginal plug and smear, and taken as the first day of conception.

All animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals (prepared by the National Academy of Science and published by the National Institutes of Health)

Grouping of Animals: The pregnant rats were grouped and interventions administered orally as follows;
Group A animals received water and served as the control
Group B animals were exposed to 2.5 Gy (single dose) gamma irradiation only (IRR only).
Group C animals received 200 mg/kg CP fruit extract only.
Group D animals were exposed to 2.5 Gy gamma irradiation + 200 mg/kg CP (CP+IRR).
Group E animals were exposed to 2.5 Gy gamma irradiation + 500 mg/kg Vitamin E (VE+IRR).
Group F animals were exposed to 2.5 Gy gamma irradiation + 200 mg/kg Vitamin C (VC+IRR)

Extraction of juice from fresh samples: Fresh fruit of ripe CP was peeled, seeds were removed and the pulp then cut into pieces. Five hundred grams (500 g) of the fruits was weighed and blended into a beaker and 1.5 L of water was used to soak

the peeled and diced CP overnight. The juice was filtered using a Whatmann filter paper 125 mm and concentrated using a rotary evaporator (Josiah *et al.,* 2011). The filtrate was then oven-dried at 40^{o}C and the dried extract was used for the study (Nayak *et al.,* 2007).

Preparation and administration of vitamin E (α-tocopherol): Each soft gelatine capsule containing 100 mg of DL- α-tocopheryl acetate as 100 mg vitamin E acetate will be punctured with a new size 21G needle attached to a new 1 mL hypothermic syringe (Becton Dickinson, La Portde- Clair, France). The oily formulation of vitamin E will then neatly and completely aspirate out with the syringe. Each aspirate measured approximately 0.2 mL containing 100 mg of DL- α-tocopherol. The insulin syringe will be thereafter attached to a clean intra-gastric gavage through which each rat was administered orally the measured dose of 500 mg/kg/daily throughout gestation and breast feeding.

Preparation and administration of Vitamin C: Each tablet containing 100 mg Ascorbic acid B.P, manufactured by Kunimed Pharmachem Ltd, Lagos, Nigeria was administered orally to each rat at a dose of 500 mg/kg/daily, dissolved in distilled water throughout gestation and weaning.

Irradiation Procedures of Experimental Animals

Twenty eight experimental rats (Group B, D, E and F) were taken to Radiotherapy Department, University College Hospital, Ibadan for irradiation. The irradiation procedure was done as described by Owoeye, *et al.* (2010), they were anesthesize with ketamine (75 mg/kg body weight of rats) intramuscularly (right thigh) under aseptic conditions and each rat was stationed in a prone position. Gamma irradiation (2.5 Gy) obtained from Cobalt 60 Teletherapy AECL machine was delivered at a dose rate of 239.148cGy/min for 1.19 minutes. With a field size of 24cm by 33cm, source surface distance of 80cm and depth of 3.5cm which gave us a percentage depth dose of 88.01%. There was no shielding of any part of the rat's body. After irradiation each animal was allowed to recover from anesthesia. They were then transported back to Department of Anatomy, College of Medicine central animal house, University of Ibadan. All the animals were returned to their respective cages and given feeds and water *ad libitum.*

Sample collection and Sacrifice: The pups of all the groups were weighed, behavioural assessment done on day 21 and blood sample collected on day 21 for hematology (full blood count). The rats were killed at different stages after birth on day 1, 7, 14, 21 and 28, their brain dissected, weighed and preserved. Brains of day 21 pups were preserved in phosphate buffered saline at 4^{O}C and a pH 7.4 for biochemical analysis, while cerebellar tissues for histological and histomorphometric analysis were fixed in 10% formol-saline.

Behavioural tests: Behavioural tests were performed on 8 pups in all the groups on day 21 to evaluate exploratory movement and anxiety (Open field test) and motor function (Forelimb grip strength test).

Open field test: In this test, rats were taken to the test room in their home cages and were handled by the base of their tails at all times. Rats were placed in the centre of the open field and allowed to explore the apparatus for 5 minutes after which rats were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and allowed to dry between tests. A video camera connected to a computer was used to analyze the open field images. This test evaluates exploratory movement, exploratory behaviour and anxiety in the rats

Forelimb grip strength test: This test involves the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2 mm in diameter and 1 m in length), placed one meter above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire is recorded. A maximum time of 2 minutes is given to each rat after which it will be removed. This test reflects muscular strength in the animals (Tamashiro *et al.,* 2000).

Haematological analysis: Blood sample was collected from five pups at day 21 from each group into Ethylene Di-amine Tetra Acetic (EDTA) acid treated sample bottles for the determination of blood count which include; Packed Cell Volume (PCV) and Haemoglobin count (Hb). Blood was obtained from the retro-ocular plexus of the animals using heparinized capillary tubes. The procedure was performed at the Veterinary Pathology Laboratory of the University of Ibadan.

Biochemical assays: Brain samples from five animals in each group were taken for biochemical assays to determine oxidative stress. The assays include; Hydrogen peroxide (H_2O_2) (Wolff, 1994), glutathione peroxidase (GPx) (Rotruck *et al*., 1973), lipid peroxidation (LPO) (Varshney and Kale, 1990) and reduced glutathione (GSH) (Beutler *et al*., 1963).

Histological preparations: Cerebellar tissues from the pups of all groups were fixed in 10% formo-saline, processed employing routine paraffin embedding and stained with Haematoxylin and Eosin for histological and histomorphometric evaluation. They were examined and then evaluated under the light microscope.

Histomorphometry: The thicknesss of the external granular layer (EGL) and molecular layer (ML) of the cerebellar cortex was done with a graticle attached to the eye piece of a binocular light microscope, while the density of the Purkinje cells of the cerebellar cortex was done using the computer software, image-j.

Statistical analysis: Data obtained were expressed as mean ± S.D. Data and further analysed employing one-way ANOVA, followed by Dunnet's post-test for multiple comparisons using GraphPad Prism California, USA, version 5.0 for Windows, and the level of statistical significance set at $p< 0.05$.

RESULTS

The control and CP rats remained active throughout the experimental period, while, the irradiated rats appeared physically inactive. However, some of the irradiated rats returned to normal activities after 7 days, while inactivity of

others (three from irradiation only group) was prolonged. Four animals from irradiation only group had abortion 4 days after exposure to gamma radiation.

Body weight: An increased body weight was observed in the pups of the treated groups on days 7, 21 and 28 compared with the control and irradiated groups at $p<0.05$ (Table 1).

Brain weight: A significant increase in brain weight was seen in the pups of the treated groups on days 1, 21 and 28 compared with the control pups at $p<0.05$ (Table 2).

Behavioural assessment: Behavioural assessment on day 21 pups included open field test (line crossing and centre square) and forelimb grip strength test. There was significant increase in exploratory (horizontal and vertical) movements in the IRR and VC+ IRR pups in the open field compared with the control, CP, CP+IRR and VE+IRR pups at $p<0.05$. A significant increase in the time spent at the centre square was seen in the CP+IRR pups compared with the control and other treated groups at $p<0.05$. There was a shorter drop off time in the forelimb grip strength test in the IRR group compared with the CP±IRR groups at $p<0.05$ (Table 3).

Table 1:
Mean body weight (g) of the control and treated pups on days 1, 7, 14, 21 and 28

Group	Days post-partum				
	1	**7**	**14**	**21**	**28**
Control	6.30 ±0.39	10.26 ±0.75	13.12 ±0.19	24.98 ±0.35	37.52 ±0.8
IRR	6.22 ±0.19	9.28 ±0.24	17.58 ±0.47	26.86 ±1.18	37.74 ±1.31
CP	5.78 ±0.14	11.26 ±0.75	18.56 ±0.35	34.32 ±0.65[a]	42.56 ±0.46[a,b]
CP+IRR	6.96± 0.25[a,b]	19.10± 0.57[a,b]	28.02± 0.39[a,b]	39.08± 2.77[a,b]	45.14± 0.81[a]
VE+IRR	7.22± 0.29[a,b]	12.18± 0.49[a,b]	20.64± 1.60[a]	30.62± 2.90[a,b]	41.20± 0.31
VC+IRR	4.6± 0.56[a,b]	10.14 ±0.69	15.38 ±0.40	33.14 ±0.38[a]	42.08± 0.77[a,b]

Table 2:
Mean brain weight (g) of the control and treated pups on days 1, 7, 14, 21 and 28

Group	Days post-partum				
	1	**7**	**14**	**21**	**28**
Control	0.16 ±0.04	0.80 ±0.00	0.94 ±0.08	1.12 ±0.04	1.36 ±0.05
IRR	0.36 ±0.06[a]	0.72 ±0.08	1.00 ±0.71	1.30 ±0.00[a]	1.52 ±0.04[a]
CP	0.20 ±0.00	0.60 ±0.07[a]	1.22 ±0.08[a]	1.30 ±0.07[a]	1.44 ±0.06
CP+IRR	0.24 ±0.06	0.76 ±0.05	1.36 ±0.05[a]	1.68 ±0.08[a]	1.88 ±0.13[a]
VE+IRR	0.32 ±0.05[a]	0.58 ±0.04	1.10 ±0.00	1.34 ±0.09[a]	1.40 ±0.00
VC+IRR	0.26 ±0.05	0.68 ±0.08	0.88 ±0.04	1.40 ±0.00[a]	1.40 ±0.00

Table 3:
Behavioural assessment of the control and treated pups

Group	**Line crossing**	**Centre square (s)**	**Forelimb grip strength (s)**
Control	5.31±3.10	5.10±0.1	7.35±4.12
IRR	15.02±3.12[a]	15.07±6.15	2.20±0.67
CP	7.43±4.20	5.21±0.52	6.10±3.37
CP+IRR	1.82±0.17	155.20±60.18[a,b]	22.48±9.10[b]
VE+IRR	3.22±2.15	70.12±60.16	4.42±2.01
VC+IRR	17.66±4.06[a]	13.30±5.42	9.25±5.60

Table 4:
Mean Packed cell volume (PCV) and Haemoglobin concentration (Hb) in the control and treated groups on day 21 pups

	C	**IRR**	**CP**	**CP +IRR**	**VE +IRR**	**VC +IRR**
PCV (%)	24.4 ±2.6	26.6± 2.4	19.6± 3.2[a]	26.8 ±1.3	26.0 ±3.8	26.4 ±1.5
Hb (%)	8.22± 0.93	4.61± 0.30	6.06 ± 0.96	8.74± 0.56[b]	8.44± 1.07[b]	8.52± 0.54[b]

Table 5:
Mean levels of lipid peroxidation (LPO), glutathione peroxidase (GPx), reduced glutathione (GSH) and hydrogen peroxide (H_2O_2) in the brain of control and treated groups on day 21 pups

Group	**LPO µM/mg**	**GPx µg/mg**	**GSH µg/ml/mg**	**H_2O_2 µM**
Control	10.88 ±1.71	342.46 ±13.43	61.55 ±0.21	8.25 ±0.29
IRR	23.96 ±2.02a	356.69 ±2.87	63.15 ±0.29	9.95 ±0.55
CP	9.68 ±2.03[b]	388.04 ±28.43	62.25 ±0.64	8.30 ±0.42
CP+IRR	17.10 ±4.18[b]	391.90 ±9.58[a]	62.50 ±0.46	8.30 ±0.51
VE+IRR	19.82 ±2.76[a]	397.81 ±17.26[a]	62.75 ±0.25	8.25 ±0.25
VC+IRR	23.22 ±4.11[a]	381.99 ±4.57	62.20 ±0.27	8.45 ±1.30

Haematology: A significant decrease in the PCV was seen in the CP treated pups and in the Hb concentration of the IRR group on day 21 compared with the control at $p<0.05$. Administration of CP, VE and VC to irradiated rats significantly increased the Hb content of the irradiated pups at $p<0.05$ (Table 4).

Biochemical analysis: A significant increase in LPO was observed in the IRR, VE+IRR and VC+IRR groups compared with the control and CP groups on the pups of day 21 at $p<0.05$. Administration of CP extracts to IRR rats, significantly decreased the LPO when compared with the IRR group at $p<0.05$ (Table 5).

Histological and histomorphometric studies: Histologically, cerebellar cortex of the pups of day 21 showed the normal three layers, namely, molecular layer (ML), Purkinje layer (PL) and granule layer (GL) in all the groups, except the IRR group which showed a persistent external granular layer of about three cell layer thick (Plate 1).

Thickness of the Molecular layer: A significantly decreased thickness of the ML was seen in the IRR and VE+IRR groups compared with the control group in the pups of day 28 at $p<0.05$. Administration of CP extract and VC to IRR rats significantly improved the ML thickness compared with the IRR rats at $p<0.05$ (Table 6, Figure 2).

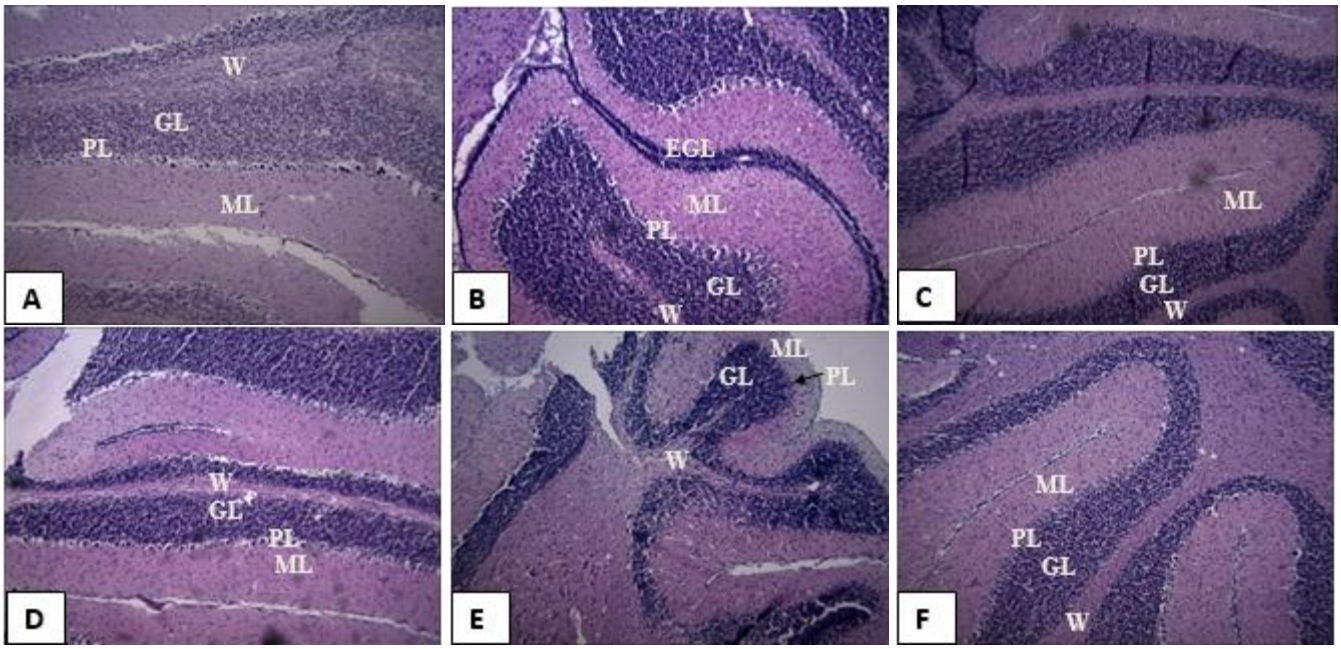

Plate 1
Photomicrograph the cerebellar cortex of day 21 pup, a) control, b) IRR group with persistent EGL, c) CP, d) CP+IRR, e) VE+IRR and f) VC+IRR. External granular layer (EGL), Molecular Layer (ML), Purkinje Layer (PL), Granular Layer (GL), White matter (W). H&E. X 100.

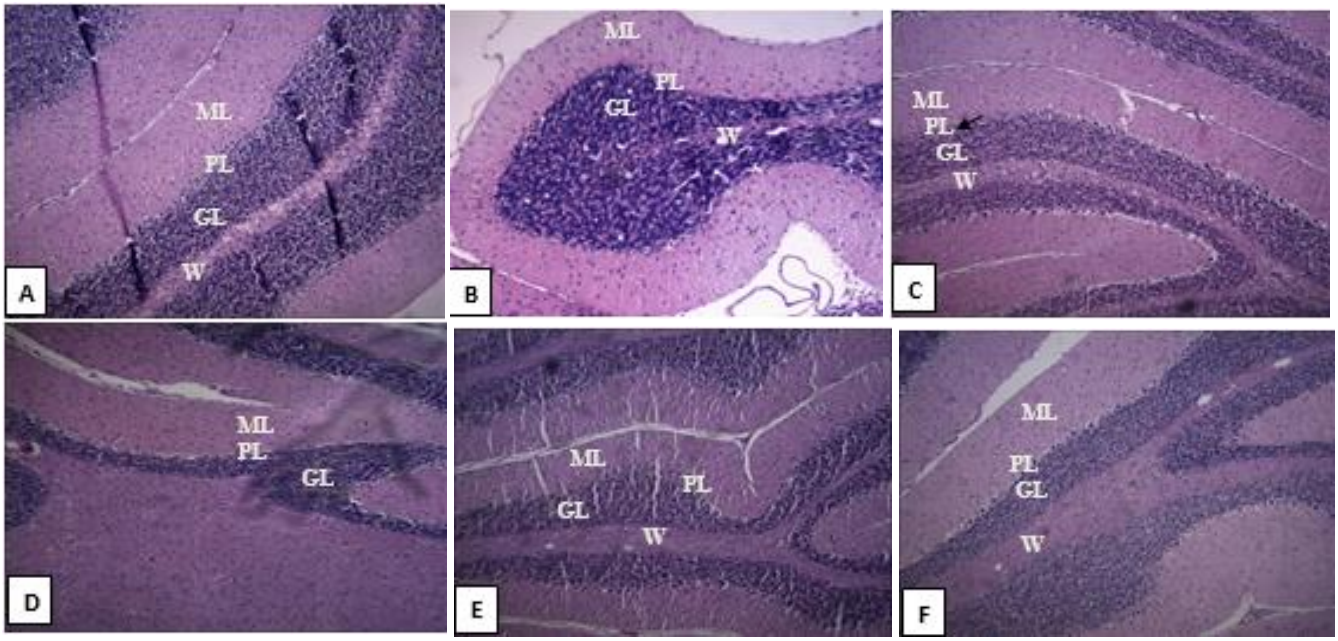

Plate 2
Photomicrograph the cerebellar cortex of day 2 pup, a) control, b) IRR group with decreased ML thickness, c) CP d) CP+IRR, e) VE+IRR with decreased ML thickness and f) VC+IRR. Molecular Layer (ML), Purkinje Layer (PL), Granular Layer (GL), White matter (W). H&E. X 100

Table 6:
Mean thickness of the ML (µm) of the cerebellar cortex of the control and treated pups on day 28

	C	IRR	CP	CP +IRR	VE +IRR	VC +IRR
Thickness ml	38.65 ±0.21	32.15 ±0.71[a]	33.65 ±0.71	39.50 ±0.36[b]	27.70 ±0.23[a]	35.60 ±0.85

Values are expressed as mean±SD, (n=5). ML thickness expressed in (µm). IRR-Irradiation, CP-Carica papaya, VE-Vitamin E, VC-Vitamin C. [a]p<0.05 versus control, [b]p<0.05 versus IRR.

DISCUSSION

In this study, the body and brain weight of the treated post weaned pups (days 7, 14, 21 and 28) showed significant increase compared with control and IRR pups. Although, radiation has been reported to induce cell death and reduce the size of the body and brain weight if given in excess of acute radiation dose of about 0.1 Gy, the rats used in this study were irradiated with 2.5 Gy gamma radiations (Ferrer, 1996). The biological effects of DNA damage in radiation injury have been reported by Ferrer (1996) to depend on two distinct factors: the greater efficacy of the DNA repair at low dose rates, and the probability of damaged cells to be eliminated by death. In this study, the rats were exposed prenatally, thus allowing much time for repairs. This might have been the reason for increase brain and body weight of the CP+IRR and VE+IRR groups. The significant increase seen in the treated pups however, might be suggestive of the antioxidant activity of CP, VE and VC which helps in mopping up excess free radicals generated by radiation and repair of DNA, thus preventing cell death.

The open field test was performed to evaluate the exploratory movement and anxiety-related behaviour of the pups. The increased total distance covered and decreased frequency of entries into and duration of time spent in the center zone by the pups in the IRR and VC+IRR are indication of increased in the anxiety-related behaviour in the pups. This result is consistent with the findings of Zhang *et al*. (2015) that microwave radiation *in utero* increased the anxiety-related behavior of mice. The forelimb grip test, which measures the muscular strength of rat, supports the observation in the open field. The IRR pups have reduced muscular strength which might be due to the reduction in size of layers of cerebellar cortex as a result of oxidative stress, affecting the motor coordination by the cerebellum. The control and other treated groups showed increased muscular strength. This result is in contrast with the findings of Zhou *et al*. (2011) in which they reported that there was no reduction of forelimb or hind limb grip strength in rats that were irradiated with 2.25 Gy on embryonic day17.

Haematologic parameters obtained from this study indicate that whole body irradiation caused a decreased heamoglobin content of the blood, which may be due to alteration in bone marrow as well as haemopoietic system of the animals. Similar observation was reported by Dixit *et al*., (2012). Whole body gamma-irradiation induced direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and reduced cell production (Casarett, 1968). The cellular elements of the blood are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids (PUFA) (Chew and Park, 2004). The decrease in hemoglobin content could be attributed to the decline in the number of red blood cells (Malhotra and Srivastava, 1978). Administration of CP extract, vitamin E and vitamin C to radiated rats, improved the Hb concentration in rat when compared with the corresponding radiation group. Oladunmoye and Osho (2007) suggested that CP possesses protective action on the haemopoitic system. This probably might have been due to the presence of flavonoids in CP, vitamin E and vitamin C. Changes in blood are observed to determine the extent of the injury on exposure to radiation.

In this study, the pups of day 21 IRR only showed elevated levels of lipid peroxidation, (monitored by the level of malondialdehyde (MDA)) in the brain homogenates and hydrogen peroxide (H_2O_2). The CP alone and CP+IRR rats, reduced the level of lipid peroxidation and H_2O_2 as compared with irradiation only, better than the VE+IRR and VC+IRR rats This reduction may be due to the antioxidant property of CP. Addai *et al*. (20132) reported that CP extract has high antioxidant activity because it was an important source of dietary source of vitamin C, vitamin E, mineral salts, amino acids, flavonoids, phenolic compounds and tannins. Flavonoids present in CP fruit exhibit strong antioxidant activity which helps protect cells against damaging effect of reactive oxygen species such as singlet oxygen, superoxide and peroxyl radicals. This flavonoid has been able to prevent an imbalance between antioxidants and reactive oxygen species which may result in oxidative stress and leading to cellular damage (Mirandal, 2000). Previous studies have shown that increase lipid peroxidation has a role in pathogenesis of several pathologies as neurodegenerative diseases (Dominguez *et al*., 2008).

The histological changes observed in this study was the persistent external granular layer (EGL) in the pups exposed to 2.5 Gy radiation on day 21. Hatten *et al*. (1995) reported that the EGL disappeared on day 20 after birth in rats. The EGL is the most metabolically active part of the developing cerebellum and its proliferation, migration and differentiation requires energy usually derived from body metabolism to produce the granule, outer stellate, basket and Golgi cells. Disruption of the energy generating pathways of the developing brain by any agent will ultimately affect cell differentiation (Malomo *et al*., 2004; Malomo *et al*., 2006). The persistent EGL may be due to delay in migration and differentiation of the cells of the EGL probably as a result of oxidative stress induced by radiation. The ML becomes the most superficial layer of the cerebellar cortex after the complete disappearance of the EGL (Altman and Bayer, 1978; Marzban et al., 2014) and its thickness is determined by the amount of cells and fibres present (Rakic and Sidman, 1970) but mainly by accretion of new parallel fibres (Rakic, 1971). In the study, there was reduction in the thickness of the molecular layer in the irradiated post weaned (day 28) pups.

The mechanism involved in the reduction of the thickness of the ML in the irradiated animals is not very clear but neuronal cell death induced by oxidative stress in the GL or delayed parallel fibre formation caused by delayed granule cell formation could have affected the density of unmyelinated parallel fibres in the ML and hence, the reduction. Ferrer (1996) and Zhou *et al.* (2017) reported that irradiation could cause cell death in the brain of young rats and induced apoptosis. The density of the Purkinje cells (only efferent neurons) of the cerebellar cortex was not changed in all the groups. Administration of CP, VE and VC to irradiated rats appeared to repair the damage induced by radiation in the EGL and ML probable by their antioxidant and anti-inflammatory actions on disrupted cell membranes. Aravind *et al* (2013) reported the anti-inflammatory activities of CP, which also contains vitamins E and C that play a role in anti-inflammation.

The question whether *papaya* is safe during pregnancy has also been reported by Aravind *et al* (2013) that *papaya* in unripe state contains high concentration of latex which its main constituents papain and chymopapain have teratogenic (abnormalities of physiological development) and abortifacient (can induce abortion) effects and this latex concentration reduces upon ripening and completely ripe has no latex left. In this present study, a completely ripe papaya was used it was significant in the reduction of radiation injury when compared with other treated groups Vitamin E and C.

From the results obtained, radiation induced oxidative stress in the developing cerebellum of rats via generation of oxygen free radicals as evidenced by, increased exploratory movement, decreased exploratory behavior, decreased haemoglobin content of blood, increased lipid peroxidation and hydrogen peroxide, delayed maturation of the EGL and reduction of the thickness of the ML. Administration of CP to radiated rats protected the developing cerebellum from radiation irregularities probably by its antioxidant property better than vitamins E and C.

Conflict of interest statement

The authors declared that they have no conflicts of interest.

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