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Oral consumption of unripe pulp and seed of *Carica papaya*: Implication on the cerebrum and cerebellum of rats

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Abstract

Oral consumption of many natural products (such as plant, leaf, stem, bark) may or may not trigger deleterious responses in many organs of the body at cellular levels. *Carica papaya* has been reported in folklore medicine to be effective in treating blindness. The aim of this study was to observe the implication of oral consumption of the pulp and seeds of unripe *C. papaya* on the cerebrum and cerebellum of rats using histological protocols. Rats were fed with 8.50 g of rat chow, 8.50 g of 1:1 mixture of *C. papaya* pulp and the rat chow, and 8.50 g of 1:1 mixture of *C. papaya* seeds and rats' chow, twice daily respectively. Twenty-four hours after the last treatment, the rats were sacrificed by cervical dislocation; the head and cranium of the rats were carefully removed, and processed for histological procedures using routine staining techniques. The histological outcome revealed that oral consumption of unripe *C. papaya* pulp and seeds conferred neuronal degeneration in the cerebrum and cerebellum of the treated rats in when compared with sections of the same organs in the control rats.

Keywords: Rat, *Carica papaya*, cerebrum, cerebellum, neurons.

INTRODUCTION

In the long history of the world, plants have been used medicinally. A large and increasing number of individuals use medicinal herbs and/or plants or seek the advice of their physician regarding their use (Hara et al., 1998). According to Chang (1987), it has been estimated roughly, that presently more than half of the total population of the world use plant based drugs. Increasing interest in medicinal plants has increased scientific examination and investigations of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use (Hara et al., 1998).

Carica papaya Linn. (Family: *Caricaceae*) is a widely grown, perennial tropical tree, which grows up to about 8 - 10 m in height with an erect trunk. Its leaves are large, measuring about 50 - 70 cm in diameter, deeply palmately lobed with seven lobes (Duke, 1984). Its fruit (*papaya*) is known by different names in different parts of the world and these include *fruta bomba* (in Cuba), *lechoza* (in Venezuela, Puerto Rico, the Philippines and the Dominican Republic) and *papaw* (Sri Lankan) (Lohiya, 200). In Nigeria, it is also known by different local names depending on the tribe. For example, among the Yoruba (South-West Nigeria) it is known as "Ibepe" and "gwanda" among the Hausa (Northern Nigeria), "ojo" and "okwere" among the Igbo (South-East Nigeria), "etihi-mbakara" among the Efik (South-South Nigeria).The ripe fruit is edible and is usually eaten raw, without the skin or seeds. The unripe green fruit (which is a rich source of vitamin A) can be eaten cooked, usually in curries, salads and stews as used in Thai cuisine (Lohiya, 200).

Different parts of the plant are attributed with different medicinal values. For instance, in African folklore medi-

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cine, the hot infusion from the boiled green leaves of *papaya* combined with leaves of *Azadirachta indica*, *Cymbopogon citratus*, *Psidium guajava* and stem bark of *Alstonia boonei* is drunk as one wine glass full three times daily in the treatment of malaria (Gill, 1992). According to Gill (1992), the fresh leaves *C. papaya* is also efficacious in the treatment of gonorrhea, syphilis and amoebic dysentery. The whitish sap of the unripe fruit is a potent abortificant, anti-helminthic for roundworms, stomach disorders and enlargement of liver and spleen. The seeds are also effective as a vermifuge and are very useful in the treatment of hypertension, diabetes mellitus and hypercholesterolemia (Gill, 1992).

Results from investigations on the biological activities of *Carica papaya* parts, extracts and isolated compounds showed that the latex and root extracts inhibited *Candida albicans* while extracts of pulp and seeds showed bacteriostatic properties against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Entamoeba histolytica*, in vitro (Emeruwa, 1982). Its root aqueous extract has equally been shown to have purgatory effect (Akah et al., 1997).

Papaya (*Carica papaya*) is a major fruit crop in many tropical parts of the world. It has been ranked first amongst 38 common fruits based on its accordance to the United States Recommended Daily Allowance for many vitamins, and consumption of papaya has been recommended for preventing vitamin A deficiency (Gouado et al., 2007) which causes childhood blindness in many tropical and subtropical countries.

The fruits, leaves, seeds and latex are used medicinally (Beckstrom et al., 1994). The folklore medicinal use of *C. papaya* is as a digestive agent. The latex from the trunk of the tree is also applied externally to enhance 'quick' healing of wounds, ulcers, boils and warts. The seed is used to expel worm, the flower may be taken in an infusion to induce menstruation (Reed, 1976; Morton, 1977; Duke, 1984). *Annonaceous acetogenins* derived from the extracts of the twigs of the *C. papaya* tree may be good chemotherapeutic agents for cancer as these compounds inhibit enzymes necessary for metabolism in tumor cells (Rupprecht et al., 1986; Hui et al., 1989a; Hui et al., 1989b; Zhao et al., 1992; Reiser et al., 1992; Zhao et al., 1995).

Despite the folkloric, wide and historical use of *Carica papaya* in the traditional management of many diseases, the scientific validation of its implication on the cerebrum and cerebellum is lacking. In view of this, the current preliminary study was designed to evaluate the seed and pulp of *Carica papaya* on the cerebrum and cerebellum in rats.

MATERIALS AND METHOD

Collection of plant and preparation of plant extracts

Unripe fruit of *Carica papaya* (Honey dew variety) were obtained from Imam Ahmad Garden, Asa-Dam, Ilorin. It was taken to the Department of Plant Science, University of Ilorin, Nigeria for identification and authentication. The pulp was sliced, seeds removed and both air-dried separately. The dried samples were pulverized separately into powdery form with a Maxwell blender (Model W55110V).

Animal treatment and management

Animal treatment

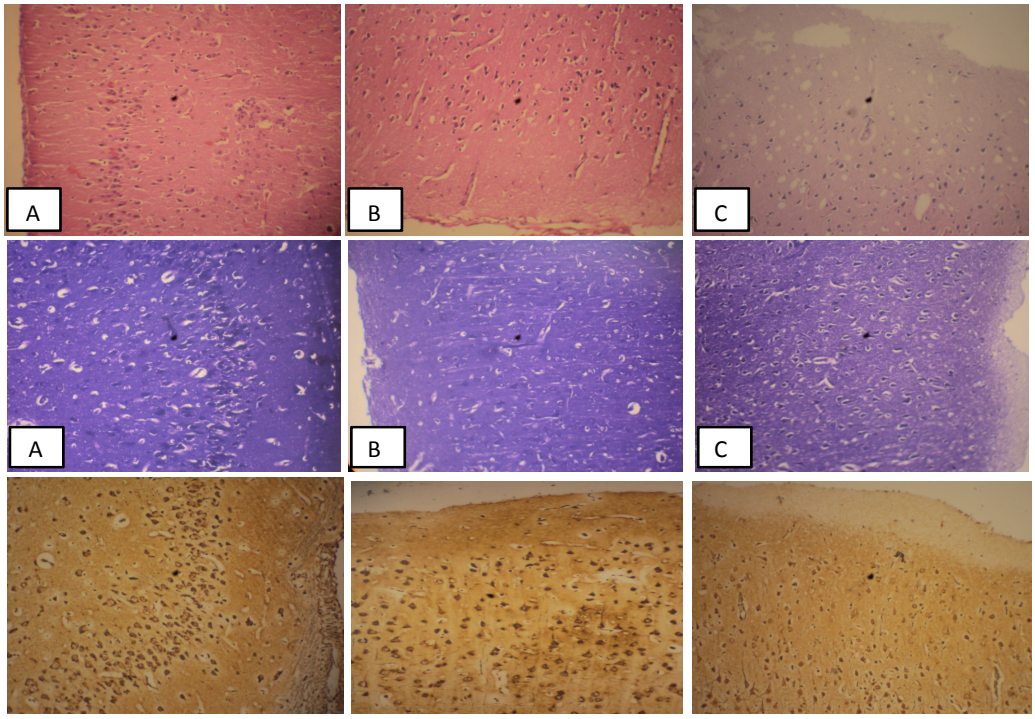
Twenty rats (Wistar strain) of both sexes 6-7 weeks old were used for this study. The rats were purchased from the pharmacy department of the Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The rats were acclimatized for 24 hours in the Animal Holding of Anatomy Department, University of Ilorin. Ethical approval was obtained from the Ethical Committee of the College of Health Sciences of the University of Ilorin on the use of animals for scientific studies and was strictly adhered to. All rats were allowed free access to water *ad libitum*. The rats were randomly assigned into three groups designated as A, B and C. The duration of treatment was for 30 days and the following is the protocol of treatment;

- Group A: each rat was fed with 8.50 g of standard rat chow purchased from Bethel Feed Mill, Sawmill, Ilorin, twice daily.
- Group B: each rat was fed with 8.50 g of 1:1 mixture of *Carica papaya* pulp and standard rat chow, twice daily.
- Group C: each rat was fed with 8.50 g of 1:1 mixture *Carica papaya* seeds and standard rat chow, twice daily.

Histopathological study

Twenty-four hours after the last administration, the rats were sacrificed by cervical dislocation; the head and cranium were carefully removed, avoiding pressure on the underlying brain. The head and exposed brain were immersion-fixed in 10% formol calcium fixative solution. The brain remained *in situ* for seven days before its removal from the skull to avoid the development of neuronal hyperchromatosis. Whole brains were then removed and immersed in fresh 10% formol calcium fixative. A rotary microtome was used to cut and collect 3 µm serial sections, three sections per slide. Serial sections sets of the cerebrum and cerebellum were stained with conventional hematoxylin and eosin (H and E) and the following special staining techniques: Cresyl fast violet (CFV) for the demonstration of Nissl bodies and Marsland, Glees and Erikson's (MGE) method for the demonstration of nerve cells and axons. Photomicro-

HISTOLOGICAL ILLUSTRATIONS



Panel 1: H and E stained section of the cerebrum of representative rats in groups A, B and C respectively (**10x**)

Panel 2: CFV stained section of the cerebrum of representative rats in groups A, B and C respectively (**10x**)

Panel 3: MGE stained section of the cerebrum of representative rats in groups A, B and C respectively (**10x**)

Figure 1. Panels (Figures A-C) showing representative micrographs of the cerebrum of rats in groups A, B and C stained with H and E, CFV and MGE respectively.

graphs of the corresponding region of interest in serial sections were obtained using an Olympus (XSZ-107BN, No. 071771) binocular light microscope with 10× objective and a DXM1200 digital camera for histological analysis.

RESULTS

Routine observations

During acclimatization, all rats appeared presumably healthy, with smoothly laid hairs on their skin, pinkish eyes, normal skin color and unimpaired locomotion. Groups A and B rat maintained this pre-administration status throughout the period of the treatment. Group C rat did not eat well, however, each rat ate less than 6.0 g daily, their hairs appeared erect, skin appeared pale and locomotor activities were significantly impaired from the 7th day of treatment.

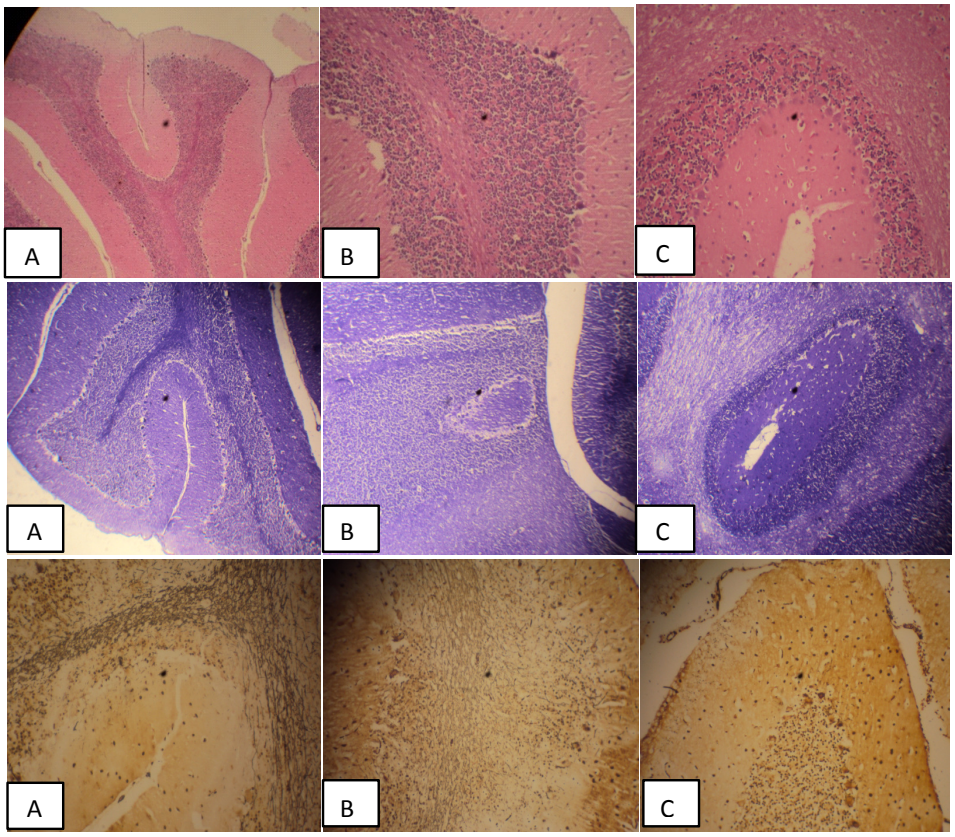
Histopathological observations

Histopathologic examination of the cerebrum and cerebellum was performed for the *C. papaya* seed and pulp and control rats after 30d of treatment.

The histological profile of the three layers of the cerebellar cortex of the representative rat in the control group was well preserved. The outer molecular layer, granular layer and Purkinje cell layer appeared normal devoid of abnormally shaped neurons, necrotic or apoptotic bodies. The neurons were intact with no perineuronal vacuolation or cavitations. The Purkinje cell pool were well surrounded by basket cell axons, the Nissl substances were anatomically placed within the neurons and the neurons were intact with their cytoplasmic contents (Panels 1A, 2A and 3A in Figure 1).

The characteristic histological profile of the rats in group B was slightly preserved with few insignificant degenerating neurons in the Purkinje cell layer. In few of the cells in the Purkinje cell layers, there is progressive loss of cytoplasmic content and loss of the nucleus with progressive vacuolation. The Nissl bodies are eccentrically placed signifying chromatolysis. There was reduction in the population of the basket cell axon pool surrounding the Purkinje cells in the Purkinje cell layer (Panels 1B, 2B and 3B in Figure 2).

There were severe neuropathologic alterations in the cerebellum of the rats in group C. These alterations include: marked vacuolation and cavitations in the three cell layers with eccentrically placed nucleus and loss of cytoplasmic content, chromatolysis of the Nissl bodies in the molecular and Purkinje cell layers and significant



Panel 1: H and E stained section of the cerebellum of representative rats in groups A, B and C respectively (**10x**)

Panel 2: CFV stained section of the cerebellum of representative rats in groups A, B and C respectively (**10x**)

Panel 3: MGE stained section of the cerebellum of representative rats in groups A, B and C respectively (**10x**)

Figure 2. Panels (Figures A-C) showing representative micrographs of the cerebellum of rats in groups A, B and C stained with H and E, CFV and MGE respectively.

neuronal degeneration and margination of the nucleus of the Purkinje cells with significantly scanty population of basket cell pool surrounding the Purkinje cell (Panels 1C, 2C and 3C in Figure 2).

DISCUSSION AND CONCLUSION

There is dearth of literatures on the effect of *Carica papaya* on the cerebral and cerebellar cortices. Though there were lots of phytochemical studies done in different parts of the world, which revealed that the plant pulp contains various beneficial elements (Oloyede, 2005) and that seeds are toxic to some body organs of various experimental animals in dose dependent manner (Chinoy et al., 2000), yet none was found on the cerebrum and cerebellum in rats or other laboratory animal models.

It has been reported that the safety assessment in experimental animals of both medicinal and non-medicinal biologically active chemicals (be it plant based or synthetic) has been very successful in predicting toxicity in humans (Zhao et al., 1995). It has also been documented that the major advantages of preclinical safety assessment studies are the known responses of experimental species, the controlled conditions under which they can be maintained and the establishment of appropriate metrics, such as tissue volume rates, which can be applied to extrapolation of findings in laboratory animals to assessment of possible side effects in human.

In general, the response(s) of humans is similar to that of experimental animals, with notable exceptions, such as peroxisome proliferators (Avwioro, 2002) and α2u globulin nephropathy inducers (Olson et al., 2000) that do not elicit in humans the same effects as in rodents. The cerebellum and cerebrum cortices are important regions in the brain. The cerebellum coordinates muscular activity and maintains posture and equilibrium (Young et al., 2006) while the cerebrum is involved in many of the higher function such as storage of information, processing of intellectual functions of the brain, characteristic learning and memory (Guyton and Hall, 1996).

It was observed from the histological observations of the cerebrum and cerebellum of the rats in the treated groups B and C that the administration of *C. papaya* confers neuropathological derangements on the histological profiles of the cerebrum and cerebellum compared with that of the rats in the control groups.

The physically observed changes in group C rats during administration could be as a result of the phyto-

chemical composition of the seeds, which has been shown to induce variable responses depending on the dose, duration, and route of administration in laboratory animals (Udoh and Kehinde, 1999).

There was marked disruption in the histological outline of the cerebrum and cerebellum of rats in group C. these disruptions include; the presence of numerous vacuolations (degeneration) with loss of cytoplasmic contents and chromatolysis of Nissl's substances. The marked chromatolysis observed in the distribution of Nissl's substances in the neurons of group C rats provides a valuable microscopic assessment of the condition of the cells.

It is observed from this research finding that unripe *Carica papaya* pulp and seed conferred deleterious characteristics on the histological profiles of the cerebrum and cerebellum of the treated rats.

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