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Histological Signs of Neurodegeneration in the Cerebrum of Rats Fed with Diet Containing Yaji: The Complex Nigerian Suya Meat Sauce

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Abstract: The aim of this histological study is to determine the neurodegenerative changes that might be associated with an indiscriminate consumption of a Nigerian meat sauce called *Yaji*, based on the fact that there is evidence that some of its constituents have excitotoxic potentials. Eighteen weeks old white albino rats of an average weight of 170 g were used for this study that lasted for two weeks. They were divided into eight groups (A-H). Group A rats served as control and were fed with normal feed (growers mash) only, while groups B-H served as the test groups and were fed with normal feed plus graded levels of *Yaji* (B, 10; C, 20; D, 30; E, 40; F, 50; G, 60 and H, 70%). The stained brain tissue micrographs showed neurogenerative changes (vacuolations, pyknosis and cavitations) in the cerebrum of the test rats irrespective of the dosage. Our findings suggest that an excessive consumption *Yaji* is capable of inducing brain tissue damage and this is likely dependent upon the concentration of those ingredients with excitotoxic potentials in a given measure of *Yaji*. It is our opinion that there is a need to regulate the production and consumption of *Yaji*.

Key words: Brain, cerebrum, excitotoxicity, neurodegeneration, *Suya* and *Yaji*

INTRODUCTION

Yaji is the meat sauce for a Nigerian meat delicacy called *Suya.* It is a complex mixture of groundnut cake powder, additives, spices and salt (Okonkwo, 1987). According to Igene and Mohammed (1983), *Suya* is a popular, traditionally processed, ready to eat Nigerian meat product, which may be served or sold along streets, in club houses, at picnics, parties, restaurants and within institutions. Omojola (2008) described it as one of such intermediate moisture products that is easy to prepare and highly relished, while Uzeh *et al.* (2006) identified it as a mass consumer fast food whose preparation and sales along the streets, are usually not done under strict hygienic condition.

Historically, *Yaji* was named after a 14th century Hausa ruler called Yaji (meaning the 'hot one') (Betumi, 2006). The spices in it are ginger, cloves, red pepper and black pepper (Nwaopara *et al.*, 2004). These spices contain gingerol (Witchtl, 2004), eugenol (Krishnaswamy and Raghuramulu, 1998), capsaicin (Collier *et al.*, 1965), and piperine (McGee, 2004) as active principle respectively. The other three constituents –white maggi (or Ajinomoto), salt and groundnut cake powder, contain monosodium glutamate (Omojola, 2008), sodium chloride (Carson *et al.*, 1998) and oil (Fageria *et al.*, 1997) as active principle respectively. This indicates that *Yaji* is a complex combination of ingredients with active principles that are potentially harmful when consumed in excess (Southgate, 1993).

Unfortunately, the production and consumption of *Yaji* is yet to be regulated and this has been the basis for several scientific investigations aimed at determining the effect of *Yaji* on body organs (Nwaopara *et al.*, 2004; 2007a, b; 2008a, b; 2009). Some of the histological findings on the Pancreas (Nwaopara *et al.*, 2004), Liver (Nwaopara *et al.*, 2007b) and Kidney (Nwaopara *et al.*, 2008), suggest that an excessive consumption *Yaji* can induce pancreatic, liver and kidney damage.

The aims and objectives of this study therefore, is to determine the neurodegenerative changes that might be associated with an indiscriminate consumption of *Yaji* since there are existing reports that some its active principles like capsaicin, piperine and monosodium glutamate are excitotoxic (Choi, 1988; Blaylock, 1997; Lipton and Rosenberg, 1994; Whetsell and Shapira, 1993; Olney, 1989, 1997; Sugimoto *et al.*, 1998; Ankarcrona *et al.*, 1998; Martin *et al.*, 2000).

MATERIALS AND METHODS

Location and Duration of Study: This study was conducted at the histology laboratory of Anthonio Research Center, Ekpoma, Edo State, Nigeria. The preliminary studies, animal acclimatization, ingredients procurement/*Yaji* production, actual animal experiment, histological processing, microscopy/micrography and evaluation of results, lasted for a period of seven months (July 2008 to January 2009). However, the actual administration of *Yaji* to the test animals lasted for two weeks.

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The Substance of Study: Normally, the production of *Yaji* is not standardized as regards what the quantities in combination should be. In this study however, all the constituents were measured to determine the quantities in a given measure of *Yaji*. A weighing balance manufactured by Denver Company USA (Model 200398.1REV.CXP-3000) was used for the measurements. The constituents were purchased at Aduwawa Cattle market, Benin City, Edo State, Nigeria, and subsequently mixed together in powdery forms as directed by the dealers. The measured quantities include: Ajinomoto (150 g), black pepper (30 g), clove (39 g), ginger (78 g) and groundnut cake powder (230 g), red pepper (22 g) and salt (100 g). The total weight of these constituents summed up to 649 g.

The Subjects/ Substance Administration: Eighteen weeks old white albino rats of an average weight of 170 g were used for this study. They were divided into eight groups (A-H) of five rats each. Group A served as the control, while groups B-H served as the test groups. For two weeks, the control rats (group A) were fed with normal feed (growers mash) only. The feed was purchased from Bendel Feeds and Flour Mills (BFFM), Ewu, Edo State, Nigeria. For two weeks also, the test rats (group B- H) were fed with growers mash from the same source plus graded quantities of *Yaji* (B, 10; C, 20; D, 30; E, 40; F, 50; G, 60 and H, 70%).

The total daily feeding allowance for each experimental group was 30 g while the feeding allowance per rat was 6 g. Test group B (10%) received 3 g of *Yaji* daily (0.6g per rat), C (20%) received 6g of *Yaji* daily (1.2 g per rat), D (30%) received 9 g of *Yaji* daily (1.8g per rat), E (40%) received 12 g of *Yaji* daily (2.4 g per rat), F (50%) received 15 g of *Yaji* daily (3 g per rat), G (60%) received 18 g of *Yaji* daily (3.6 g per rat) and H (70%) received 21 g of *Yaji* daily (4.2 g per rat).

Feeding pellets were produced by mixing the appropriate quantities of *Yaji* and feed with sprinkles of water to form a paste that was then split into bits and allowed to dry under the sun.

Tissue Processing: The animals were sacrificed after two weeks. The brain tissues harvested were immediately fixed in formaldehyde to prevent autolysis and putrefaction. Tissue processing was done according to standard procedures (fixation, dehydration, impregnation, embedding, sectioning and staining with Haematoxylin and Eosin) as described by David (2004). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope (at magnification x40).

RESULTS

The stained tissue micrographs show that the control rat's cerebrum (Fig. 1) presented normal pictures. On the contrary, test group B cerebrum (Fig. 2; 10%) presented

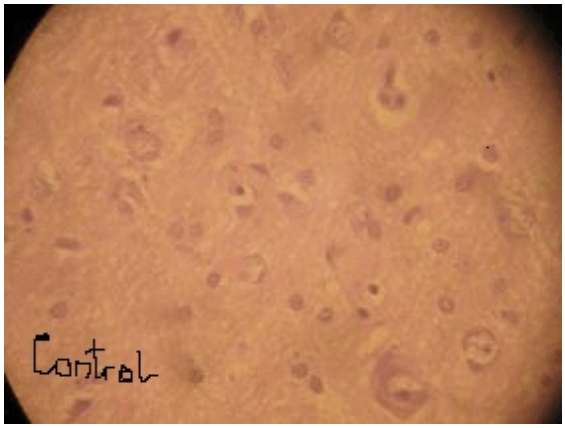

Fig. 1: (Control Cerebrum; H and E x40) showing normal histological features

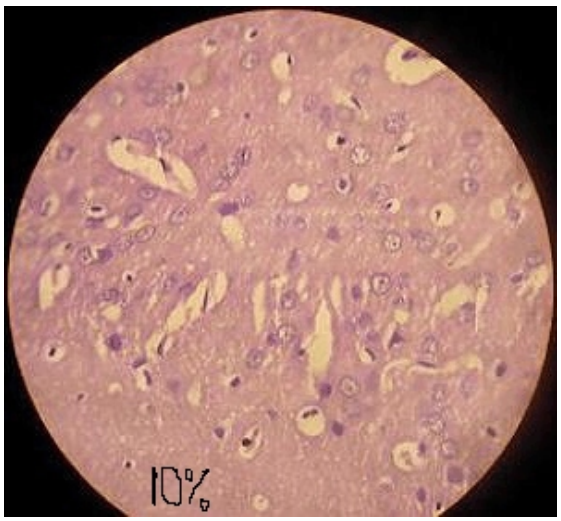

Fig. 2: (10% Cerebrum; H and E x40) showing severe neurodegenerative changes.with pyknosis and vacuolations.

severe signs of neurodegeneration with pyknosis and vacuolations. Test group C cerebrum (Fig 3; 20%) also presented severe signs of neurodegeneration with vacuolations and cavitations. Test group D cerebrum (Fig. 4; 30%) presented spongy degenerative changes with vacuolations and cavitations. Test group E cerebrum (Fig 5; 40%) presented neurodegenerative changes with eosinophilic cells, pyknosis and vacuolations. Test group F cerebrum (Fig. 6; 50%) presented severe neurodegenerative changes with several dark staining pyknotic bodies and vacuolations. Test group G cerebrum (Fig. 7; 60%) presented cloudy degenerative changes with pyknosis and vacuolations, while test group H cerebrum (Fig. 8; 70%) presented severe neurodegenerative changes with eosinophilic cells, pyknosis, vacuolations and cavitations. These neurodegenerative changes occurred in all the test groups irrespective of the dosage.

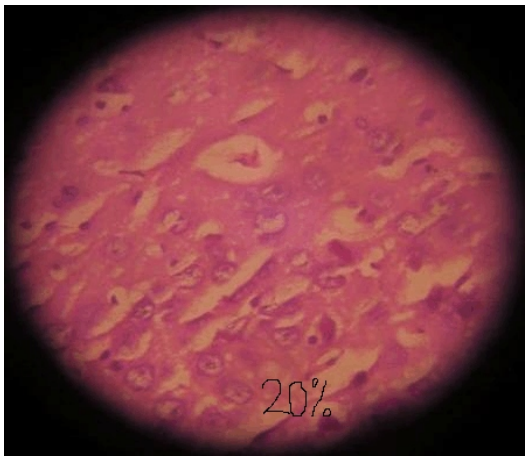

Fig. 3: (20% Cerebrum; H and E x40) showing severe degenerative changes with vacuolations and cavitations.

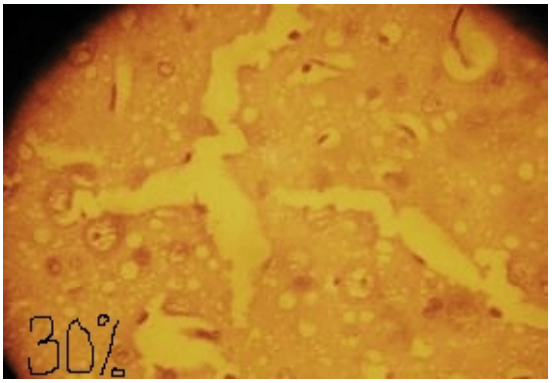

Fig. 4: (30% Cerebrum; H and E x 40) showing spongy degenerative changes with vacuolations and cavitations

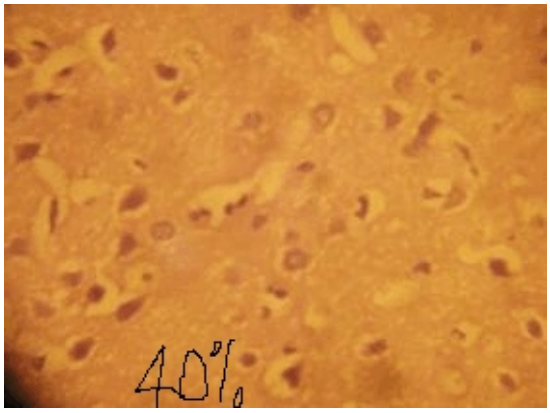

Fig. 5: (40% Cerebrum; H and E x40) showing neurodegeneration with eosinophilic cells, pyknosis and vacuolations.

DISCUSSION

The histological alterations observed in this study are suggestive of toxicity and justifies the fact that chemically

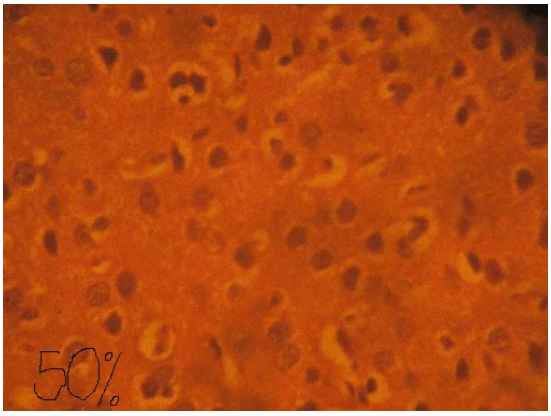

Fig. 6: (50% Cerebrum; H and E x40) showing severe neurodegenerative changes. Note the numerous dark pyknotic bodies with vacuolations.

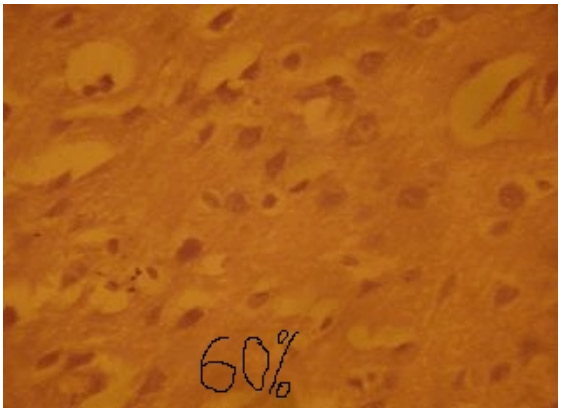

Fig. 7: (60% Cerebrum; H and E x40) showing cloudy neurodegenerative changes with pyknosis and vacuolations.

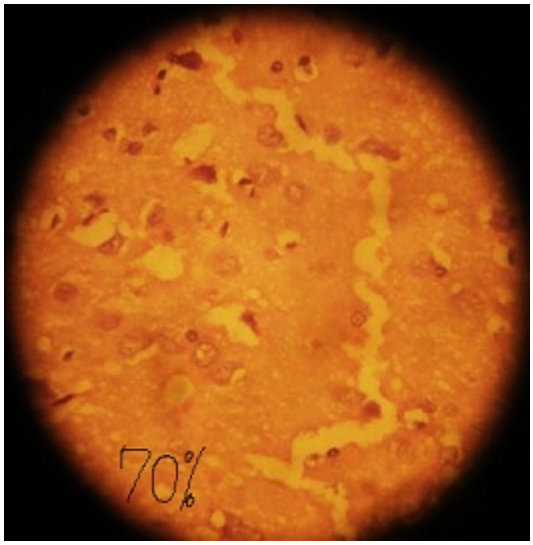

Fig. 8: (60% Cerebrum; H and E x40) showing severe neurodegeneration, pyknosis vacuolations and cavitations.

induced neurodegeneration is characterized by different patterns of neuronal cell death, gliosis, swollen or destroyed axons, or destruction of myelin sheath (Cavanagh, 1984). Comparatively also, one can say that the findings of this study are in line with those of Varner *et al*. (1998) who identified chromatin clumping, enhanced protein staining, pyknosis, vacuolation, presence of ghost-like cells and decreased neuronal density in adult rats that were exposed to chronic intake of 1 ppm fluoride in drinking water.

Our findings seem to implicate the active principles in *Yaji*, as there is evidence that MSG is excitotoxic (Espinar *et al*., 2000; Rothstein and Brem, 2001; Urena-Guerrero *et al*., 2003) and capsaicin administration causes degeneration of neurons (Jancso *et al*., 1997; Ritter and Dinh, 1993; Chard *et al*., 1995; Wood, 1993). Also, piperine in black pepper has been identified to be cytotoxic and its cytotoxicity is enhanced by the presence of tocopherol, suggesting a mechanism of lipid peroxidation (Unchern *et al*., 1998). Piperine also promotes DNA damage (Piychaturawat *et al*., 1995), which is itself, a significant trigger for apoptosis (selective cell damage).

One can as well say that the groundnut cake in *Yaji* might have contributed to the observed cytoachitectural changes. The basis for this assertion is the report by Florence and Adewale (2004) that diets containing oxidized groundnut oil have deleterious effects on the architecture of tissues. It is interesting to note that dietary oil rich in polyunsaturated fatty acids is susceptible to oxidative changes during use like frying (Ologan, 2002). In fact, during frying (as it is for groundnut cake), unhealthy peroxides, aldehydes, ketones, aldehydroesters and ozonides (Frankel, 1980; Kubow, 1992; Odutuga *et al*., 1997) are formed. The consumption of such peroxidized lipids has been reported to be injurious to health (Frankel, 1980; Halliwell and Gutteridge, 1984; Addis, 1986; Kubow, 1992).

Most importantly, the dose levels of all the excitotoxic elements (MSG, black pepper and red pepper) in *Yaji* as administered to the test rats, far exceeds what the normal daily values would have been for their weight (170 g). This assertion is based upon the comparison (as calculated for rat weight's equivalent) with the acceptable daily doses for a man of 70 kg, which are MSG (3 g) (Giacometti, 1979), black pepper (359 mg) (Kindell, 1984) and red pepper (30-120 mg) (Vitamin Supplements Guide, 2006). The import of this is that at high doses, *Yaji* is capable of inducing brain tissue damage and this is likely dependent upon the concentration of those ingredients with excitotoxic potentials in a given measure of *Yaji*. It is our opinion that there is a need to regulate the production and consumption of *Yaji*.

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