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Induced Histological Features of Hypoxia-Ischaemia in the Brain of Rats Fed with Diet Containing *Yaji*: The Complex Nigerian Meat Sauce

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Abstract: *Yaji* is a complex mixture of groundnut cake powder, additives, spices and salt. The production and consumption of *Yaji* is not regulated despite the potential health hazards of its numerous active principles. This fact has been the basis for several scientific investigations aimed at determining the effect of *Yaji* on body organs. The present histological study is intended to determine the effects of *Yaji* consumption on the blood supply of the brain. Eighteen weeks old white albino rats of an average weight of 170g were used for this study. They were divided into eight groups (A – H) of three subgroups each. Subgroup 1, 2 and 3 represents experimental periods of 2 weeks, 4weeks, and 6 weeks respectively. 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%). At the end of the respective experimental periods, test group rats were sacrificed in order to harvest the brain tissues for tissue processing. The stained brain tissue micrographs showed vascular changes (fat embolism and thrombosis) and cerebral infarction in the brain of test rats in group B2 (4weeks; 10%), B3 (6weeks; 10%) and F3 (6 weeks; 50%). Our findings suggest that at sustained high dose consumption, *Yaji* has the capacity to induce cerebrovascular changes with its attendant consequences such as hypoxia – ischaemia and subsequently, brain tissue necrosis.

Key words: Brain, cerebral infarction, fat embolism *Suya*, thrombosis and *Yaji*

INTRODUCTION

Yaji is the 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 *et al.* (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; 2007b; 2008a; 2008b; 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 present histological study, is intended to determine the effects of *Yaji* on the blood supply to the brain as there are reports that some the active principles like oil, salt and MSG, have potentials to induce respiratory, cardiac or generalised blood-vascular changes (Jimoh and Odutuga, 2002; Osfor *et al.*, 1997; Betran *et al.*, 1992; Diniz *et al.*, 2005 Jiang *et al.*, 1991; Kostić-banović *et al.*, 2005; Michael and Jocelyn, 1997; Palevsky *et al.*, 1996; Mocharla *et al.*, 1997; Young and Truax, 1979).

MATERIALS AND METHODS

The Substance of Study: Normally, the production of *Yaji* is not standardized as regards what the quantities in

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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 (150g), black pepper (30g), clove (39g), ginger (78g), and groundnut cake powder (230g), red pepper (22g), and salt (100g). The total weight of these constituents summed up to 649g.

The Subjects/ Substance Administration: Eighteen weeks old white albino rats of an average weight of 170g were used for this study. They were divided into eight groups (A – H) of three subgroups (n = 5) each. Subgroup 1, 2 and 3 represents experimental durations of 2 weeks, 4 weeks, and 6weeks respectively. Group A (A1, A2 and A3) served as the control, while the subgroups of B – H (B1 – H1; B2 - H2; and B3 – H3) served as the test groups. Group A rats were fed with normal feed (growers mash) only. The feed was purchased from Bendel Feeds and Flour Mills (BFFM), Ewu, Edo State, Nigeria. Test groups B1 – H1, B2 - H2 and B3 – H3 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%) for 2 weeks, 4 weeks and 6 weeks respectively.

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

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

Tissue Processing: The animals in subgroups 1, 2 and 3 were sacrificed after two weeks, four weeks and six weeks respectively. The brain tissues harvested from the groups 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) described by David (2004). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope (at magnification x40).

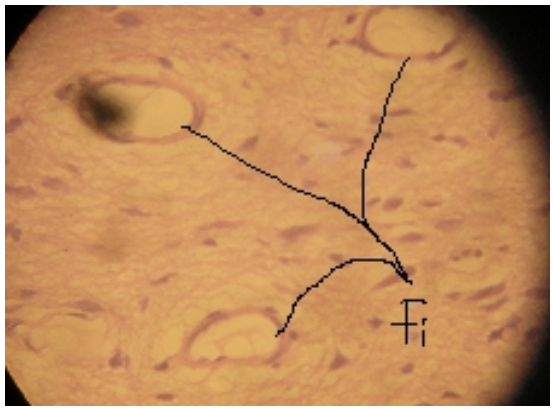

Fig 1: (Brain H&E X40) showing vessels (Fi) with fatty infiltrations (fat embolism) in the brain of rats within test group B2 (4wks; 10%). Note the membrane bound vacuoles (intravascular fat globules)

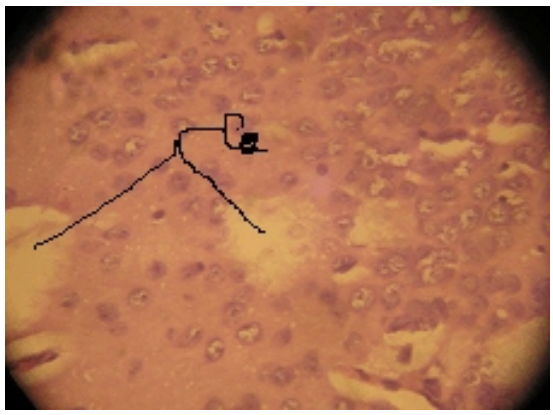

Fig 2: (Cerebrum H & E x40) showing histological signs of gliosis and cerebral infarction in the brain of rats within test group B3 (6wks; 10%). Note the pale regions (as marked Ca) in the micrograph.

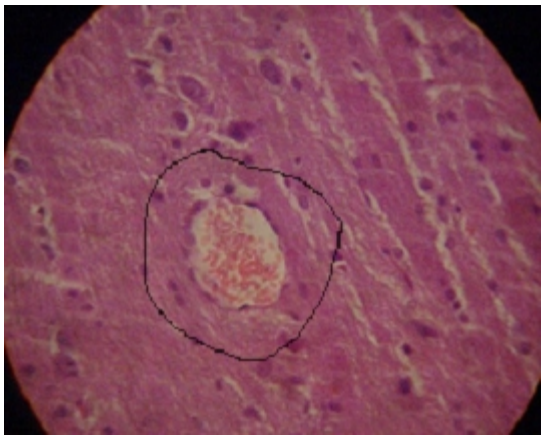

Fig 3: (Brain H&E X40) showing a centrally placed large thrombotic vessel (as encircled) in the brain of rats within test group F3 (6wks; 50%)

RESULTS

The stained brain tissue micrographs showed vascular changes (fat embolism and thrombosis), cerebral infarction and signs of neurodegeneration (gliosis) as represented by Fig. 1, 2, 3, 4 and 5. Fig 1 shows a vessel with membrane bound vacuoles (fat globules) consistent with fatty infiltration (fat embolism) as marked 'Fi' in the brain of rats within test group B2 (4wks; 10%). Fig 2 shows histological signs of gliosis and cerebral infarction (as marked Ca) in the brain of rats within test group B3 (6wks; 10%). Fig 3 shows a centrally placed large thrombotic vessel (as encircled) while Fig. 4 and 5 show thrombotic vessels tagged 'Ta' and 'Tb' respectively. These changes are conditions associated with hypoxia-Ischaemia.

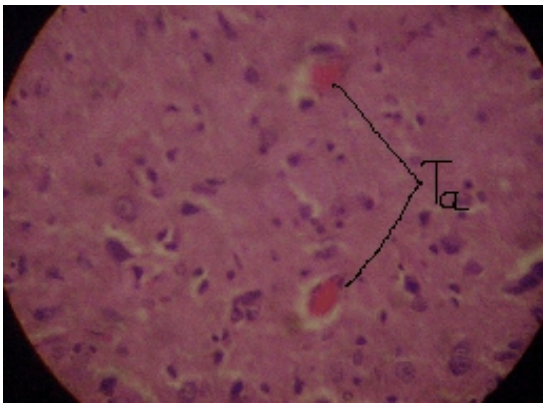

Fig 4: (Brain H&E X40) showing thrombotic vessels (Ta) in the brain of rats within test group F3 (6wks; 50%)

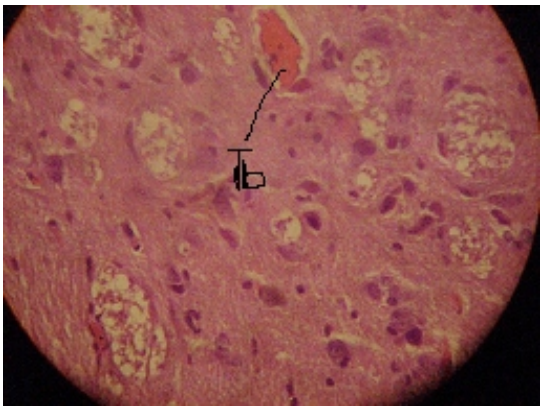

Fig 5: (Brain H&E X40) showing a thrombotic vessel (Tb) in the brain of rats within test group F3 (6wks; 50%)

DISCUSSION

Our finding on vascular fatty infiltration corresponds with those of 'fat embolism', which has been described as the blockage of blood vessels by intravascular fat globules ranging from 10-40µm in diameter (Weisz, 1977). This incidence seems to implicate the MSG in *Yaji,* as there are reports that MSG increases serum total proteins, cholesterol and blood glucose levels in mice (Osfor *et al*., 1997) and in rats (Betran *et al*., 1992). It has also been reported that MSG treated animals have increased triacylglycerol levels (Diniz *et al*., 2005), lipoperoxidation and alteration in markers of oxidative stress lipoperoxidation (Jiang *et al*., 1991). In more than 90% of cases, fat embolism is associated with accidental trauma to long bones or pelvis, or during surgical trauma and in up to 5% of the cases, atraumatic causes like bone marrow transplantation, pancreatitis, sickle cell disease, burns, prolonged high-dose corticosteroid therapy and diabetes mellitus might be responsible, while other rare causes include hepatic trauma, liposuction, lipectomy, external cardiac compression, gas gangrene, decompression sickness and lipid infusions (Levy, 1990; Dudney and Elliott, 1994). Of greater significance is the inclusion of pancreatitis and hepatic trauma in the list of causes of fat embolism, which can be correlated with the previous histological findings that *Yaji* can induce pancreatic and liver damage in experimental rats (Nwaopara *et al*., 2004; 2007).

Also, the oil in fried groundnut cake is implicated as well because there are reports that dietary oil rich in polyunsaturated fatty acids is susceptible to oxidative changes during use like frying (Ologan, 2002). The reason is that the polyunsaturated fatty acid constituents of these oils readily undergo oxidation resulting in the formation of peroxides, aldehydes, ketones, aldehydroesters and ozonides (Frankel, 1980; Kubow, 1992, Odutuga *et al.,* 1997). The consumption of such peroxidized lipids have been shown to be injurious to health (Frankel, 1980; Halliwell and Gutteridge, 1984; Addis, 1986; Kubow, 1992). In one report, Jimoh and Odutuga (2002) revealed that oxidised groundnut oil can induce the disintegration of alveoli membrane and collapse of alveoli spaces in the lungs (signifying an impairment in the oxygenation of blood) as well as a disorganization of the spatial arrangement of the cells and widening of the fibres of the heart, which might lead to weakness of the cardiac muscle and consequently, cardiac enlargement and cardiac failure. Of course, these conditions can induce cerebral hypoxia and ischaemia respectively.

Specifically, cerebral ischaemia connotes insufficient blood supply to the brain and can be viewed as hypoxia plus hypoglycemia (Summers *et al*., 1995a; Auer and Benveniste, 1997). It is well known that cells in the central nervous system (CNS) show selective vulnerability to the effects of ischaemia and hypoxia (often considered together as hypoxia-ischaemia) and due to high oxygen demand, neurons are first affected by hypoxia-ischaemia followed by oligodendrocytes, then astrocytes, and finally vascular cells (Summers *et al*., 1995a; Auer and Benveniste, 1997). In fact, infarction or necrosis (malacia) of the CNS parenchyma may result from cerebrospinal vascular occlusion associated with an embolus (Braund, 2003). The embolic material may

represent white platelet-fibrin and red erythrocyte-fibrin thrombi, cholesterol crystals, fragments of atherosclerotic plaques, calcified fragments of valves and plaques, air, fat, myxomatous tumor fragments, or bacterial vegetations or other foreign bodies including parasites (Chung and Caplan, 1999).

Therefore, the observed incidence of gliosis and cerebral infarction are indicative of brain damage because gliosis or astrocytosis is the brain's way of reacting to injury, insult, or "something" that should not be there (e.g., a tumor) (Prayson and Cohen, 2000). This may account for the presence of vessels with histological features of thrombosis because an injury to a blood vessel results in the release of platelets and fibrin to form a blood clot to prevent loss of blood. If that mechanism causes too much clotting and the clot breaks free, an embolus is formed (Furie and Furie, 2008; Handin, 2005).

In such a situation, heamorrhage into the brain substance (intraparenchymal) from damaged vessels may occur and quickly become space-occupying masses (hematomas), which, like brain tumors, compress brain parenchyma, and if unchecked, may lead to widespread brain edema, brain herniation, mid-line shifts, ischemia, brainstem compression and development of deep pontine hemorrhages (Summers *et al*., 1995b).

In addition, a comparison between the findings of this study and existing scientific data on excessive salt intake, reveals that the salt in *Yaji* is a likely 'suspect' as autopsy reports on cases of fatal sodium chloride poisoning have shown that brain edema, venous and capillary congestion, cortical venous thromboses and venous brain infarcts are more predominant (Kostić-banović *et al*., 2005). Several other scientific reports have also implicated hypernatremia in brain cells dehydration, brain volume decrease, mechanical damage of small blood vessels and subarachnoid and intra-cerebral hemorrhages (Michael and Jocelyn, 1997; Palevsky *et al*., 1996; Mocharla *et al*., 1997; Young and Truax, 1979). Hypernatremia occurs when there is a deficiency of water in the body compared to solute sodium chloride ions (Kostić-banović *et al*., 2005). This state can occur as a consequence of insufficient liquid intake or its extreme loss or increased exogenic sodium chloride intake (Michael and Jocelyn, 1997; Stefanović, 1994; Adrogue and Madias, 2000).

Generally, one can say that the observed histological changes appears to be dosage-duration dependent as the observed changes became obvious in the 4 weeks and 6 weeks text periods and at 10% and 50% dose levels of administration. In fact, we observed that the dose levels of the ingredients in *Yaji* as administered to the test rats, far exceed what the normal daily values should have been for a rat of an average weight of 170g. This assertion is hinged upon a comparison with the acceptable daily doses for man of 70kg, which, for example, is 3g for MSG, 259mg for black pepper, and 120mg for red pepper (Giacometti, 1979; Kindell, 1984; Vitamin Supplements Guide, 2006).

Finally, it is important to note that neurovascular disorders encompasses those conditions that results in cerebrospinal ischaemia, infarction and hemorrhage as various vascular and parenchymatous changes have been associated with vascular anomalies, cerebral arteriosclerosis, mineral and pigment deposition, malacias and necrosis, cerebral infarction, thrombosis, embolism, inflammatory processes, cerebral hemorrhage, and vascular neoplasms, including intravascular lymphoma (Fankhauser *et al*., 1965; McDonough *et al*., 2002). Our findings therefore, suggest that at sustained high dose consumption, *Yaji* has the capacity to induce cerebrovascular changes with its attendant consequences such as hypoxia – ischaemia and subsequently, brain tissue necrosis.

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