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Effect of Yaji on the Anterior Pituitary Gland

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Abstract
The aim of this histological study is to determine the effect of yaji (a Nigerian meat sauce that is indiscriminately consumed) on the anterior pituitary gland based on the fact that there is evidence that some of its constituents have excitotoxic and neurotoxic potentials. Eighteen weeks old white albino rats of an average weight of 225g were used for this study. The animals were acclimatized for two weeks and the test substance was administered for eight weeks and weighed on weekly basis. Twenty male albino rats were used and were divided into four groups (A-D) of five animals in each group. Group A served as control and were fed with normal feed (growers mesh) only while group B-D served as the test group and were fed with normal feed plus graded levels of yaji (B: 5g; C: 10g; D: 15g). The photomicrographs showed no observable histological changes in the tissue for all the groups. Analysis of variance (ANOVA) showed significant differences ($P<0.05$) in weight between the groups for the period of eight weeks. The findings therefore suggest that yaji has no capacity to induce any histological changes on the anterior pituitary gland.
Keywords: Yaji, Suya, Spices, Anterior Pituitary Gland.

1. Introduction

In Nigeria, there is the growing concern about the excessive consumption of a meat sauce called 'yaji' which is used to serve the meat delicacy called suya (Nwaopara et al, 2010). Yaji is a complex mixture of spices and additives; its constituents are ginger, cloves, red pepper, black pepper, salt, white maggi (Ajinomoto) and groundnut powder; and their active ingredients on individual basis are known to be harmful if consumed in excess (Nwaopara et al, 2004). The active ingredient in ginger is gingerol (Witchtl, 2004); in cloves are eugenol, which has made it the subject of numerous health studies (Krishnaswamy and Raghuramulu, 1998); in red pepper are capsaicin (Collier et al, 1965) and steroidal saponins known as capsicidins found in the seed and root, and in black pepper are piperine, essential oil like terpenes (pinene, sabinene, limonene, caryophillene and linalool) that contribute to its aroma (McGee, 2004). Of interest is the fact that this sauce is produced without a standardized production format. 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 institutions. Uzeh et al (2006) identified it as a mass consumer fast food whose preparation and sales along streets are usually not done under strict hygienic surrounding environment and can serve as source of contaminants to the meat product. Omojola (2008) described suya as one of such intermediate moisture products that is easy to prepare and highly relished.

Although there are on-going histological investigations, one still wonders what the effect of yaji might be on the endocrine system, since the excessive consumption of each of the contained active ingredients alone is potentially harmful. The anterior pituitary gland regulates several physiological processes including stress, growth, reproduction and lactation. Its regulatory function is achieved through the secretion of various peptides hormones that act on the target organs including the adrenals, liver, bone, thyroid gland and gonads. The anterior pituitary gland itself is regulated by the hypothalamus and by negative feedback from these target organs. Considering the mass consumption rate of yaji (Uzeh et al, 2006) and its complexity, coupled with the available scientific evidence that excessive consumption of yaji is capable of inducing pancreatic, liver, kidney and brain tissue damage (Nwaopara et al, 2004; Nwaopara et al, 2007a; Nwaopara et al, 2008a; Nwaopara et al, 2008b; Nwaopara et al, 2009a; Nwaopara et al, 2010a; Nwaopara et al, 2010b) but has no effect on the heart, and taking into consideration that the pituitary gland is a master gland that controls the activities of other glands, it is important to state that however, no study on yaji has x-rayed its effect on any endocrine organ. As such, it is therefore necessary to investigate the effect of yaji on the anterior pituitary gland.

2. Materials and Method

2.1 Location and duration of study

The rats were allowed to acclimatize for two weeks. The actual administration of test samples to the test animals lasted for eight weeks, from June to July, 2012. The Guide for the Use of Laboratory Animals (1996) was followed.

2.2 The substance of study

Normally, the production of yaji is not standardized as regards what quantities in combination it should be. In this study however, all the constituents were measured to determine the quantities in a given measure of yaji. An electronic weighing balance (210/0.1mg digital balance ESJ-210-4) manufactured by Napco Precision Instrument Company Limited, Guangdong in China was used for the measurement. The constituents of yaji were purchased at the Nkwo market, Anambra state, Nigeria, and subsequently mixed together in powdery forms according to the method used by Nwaopara et al (2010). The measured quantities include: Ajinomoto (150g), Black pepper (30g), Clove (39), Ginger (78g) and Groundnut cake powder (230g), Red pepper (22g) and Salt (100g). The total weight of these constituents summed up to 649g.

2.3 The subject/substance administration

Twenty male albino rats of 18 weeks old were used for this study. They were purchased from the animal farm house of the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, and moved to the site of the experiment, the histology laboratory where they were allowed to acclimatize for two weeks and weighed using the analytical weighing balance manufactured by Yongkang Zhezhong weighing apparatus factory in China. They were maintained under standard housing conditions and fed with standard rat chow (Growers mesh) and provided with water ad libitum during the experiment. The animals were divided into four groups (A-D) by random selection. Five rats each were housed in four big cases. Group A served as control, while group B-D served as the test groups. For the whole eight weeks, the control group was fed with the normal feed (growers mash) only. The feed (Top feed) was purchased from Nkwo market, Anambra state, manufactured by Premier Feed Mills Company Limited (A subsidiary of flour mills Nigeria PLC) in Sapele, Delta state, Nigeria. For the eight weeks also, the test rats (group B-D) were fed with growers mash from the same source with graded quantities of yaji (B=5g, C=10g, D=15g). The total feeding allowance for each experimental group was 30g while the feeding allowance per rat is 6g. The control group was fed with a measured quantity of feed (30g) without yaji, group B were fed with 30g of feed plus 5g of yaji, group C were fed with 30g of feed with 10g of yaji and finally group D were fed with 30g of feed and 15g of yaji was administered.

2.4 Cervical dislocation

The animals were sacrificed by cervical dislocation which involves the separation of the cervical bone from the base of the brain or within the cervical spine area (the upper third of the neck).The rats were held at the cervical region by the left hand while the tail was drawn with the right hand dislocating the spinal column from the skull.

2.5 Extraction of the pituitary gland

The animals were placed in the anatomical position; the pituitary gland was then approached through the anterior cranial fossa, by breaking the bone of the skull around the orbit. The brain was assessed and the frontal lobe of the brain raised. The pituitary gland was located below the brain between the optic nerves, lying on a bony coverage known as the sella turcica. The pituitary gland was then removed and fixed in Bouin's fluid.

2.6 Tissue processing

Tissue processing was done according to the standard procedures (fixation, dehydration, clearing, impregnation, embedding, and sectioning, staining and final mounting) (David, 2004).

3. Results

3.1 Statistical Analysis

The mean and standard deviation was generated using the SPSS (Version 16) software package and the one way ANOVA test was determined at $P<0.05$. The means of all the test groups and the control (group A) were compared for eight weeks. From the first to the eight weeks, there was significant difference between groups ($P<0.05$). The result of this study shows that it is duration-dependent and dosage-dependent.

Table 1: The mean, standard deviation and ANOVA of different doses of yaji and the control group for eight weeks.

Different Weeks	Different Groups Of Yaji	Mean±SD.	F	Sig.
Wk 1	Control	111.00±7.42	55.933	0.000
	Group B	217.00±33.28		
	Group C	247.00±21.68		
	Group D	286.00±19.49		
Wk 2	Control	140.00±14.14	38.416	0.000
	Group B	216.00±30.50		
	Group C	246.00±21.91		
	Group D	284.00±18.17		
Wk 3	Control	148.00±14.83	29.465	0.000
	Group B	216.00±24.85		
	Group C	248.00±34.21		
	Group D	288.00±19.24		
Wk 4	Control	162.00±14.83	21.262	0.000
	Group B	210.00±22.36		
	Group C	236.00±28.81		
	Group D	270.00±20.00		
Wk 5	Control	174.00±15.17	15.868	0.000
	Group B	212.00±26.83		
	Group C	232.00±25.88		
	Group D	270.00±20.00		
Wk 6	Control	190.00±12.25	21.631	0.000
	Group B	222.00±27.75		
	Group C	254.00±23.02		
	Group D	290.00±15.81		
Wk 7	Control	202.00±8.37	15.246	0.000
	Group B	224.00±26.08		
	Group C	254.00±25.10		
	Group D	282.00±14.83		
Wk 8	Control	198.00±13.04	14.826	0.000
	Group B	220.00±24.49		
	Group C	232.00±20.49		
	Group D	275.00±15.00		

Data are means and standard deviation. Analysis of variance (ANOVA) was used to compare the means of all the groups. There was significant difference ($P<0.05$) between the groups for the eight weeks.

3.2 Physical Observation

The control group showed no changes in physical appearance throughout the duration of the experiment, however, the treated group showed less active behavior.

3.3 Histopathological Studies

The micrographs of relevant stained sections were subsequently taken with the aid of a light microscope at magnification of X200.

- *Group 1 (Control):* Showed no significant histopathological change. All parts of the anterior pituitary gland were intact and all the cells were also clearly seen as shown in Figure 1.
- *Group 2 (5g of yaji):* There was no observable change however; all parts of the anterior pituitary gland were also clearly seen and intact as shown in Figure 2.
- *Group 3 (10g of yaji):* There was no observable change; all parts of the anterior pituitary gland and the cells were also clearly seen and intact as shown in Figure 3.
- *Group 4 (15g of yaji):* There was no observable change, all parts of the anterior pituitary gland and the cells were also seen and intact as shown in Figure 4.

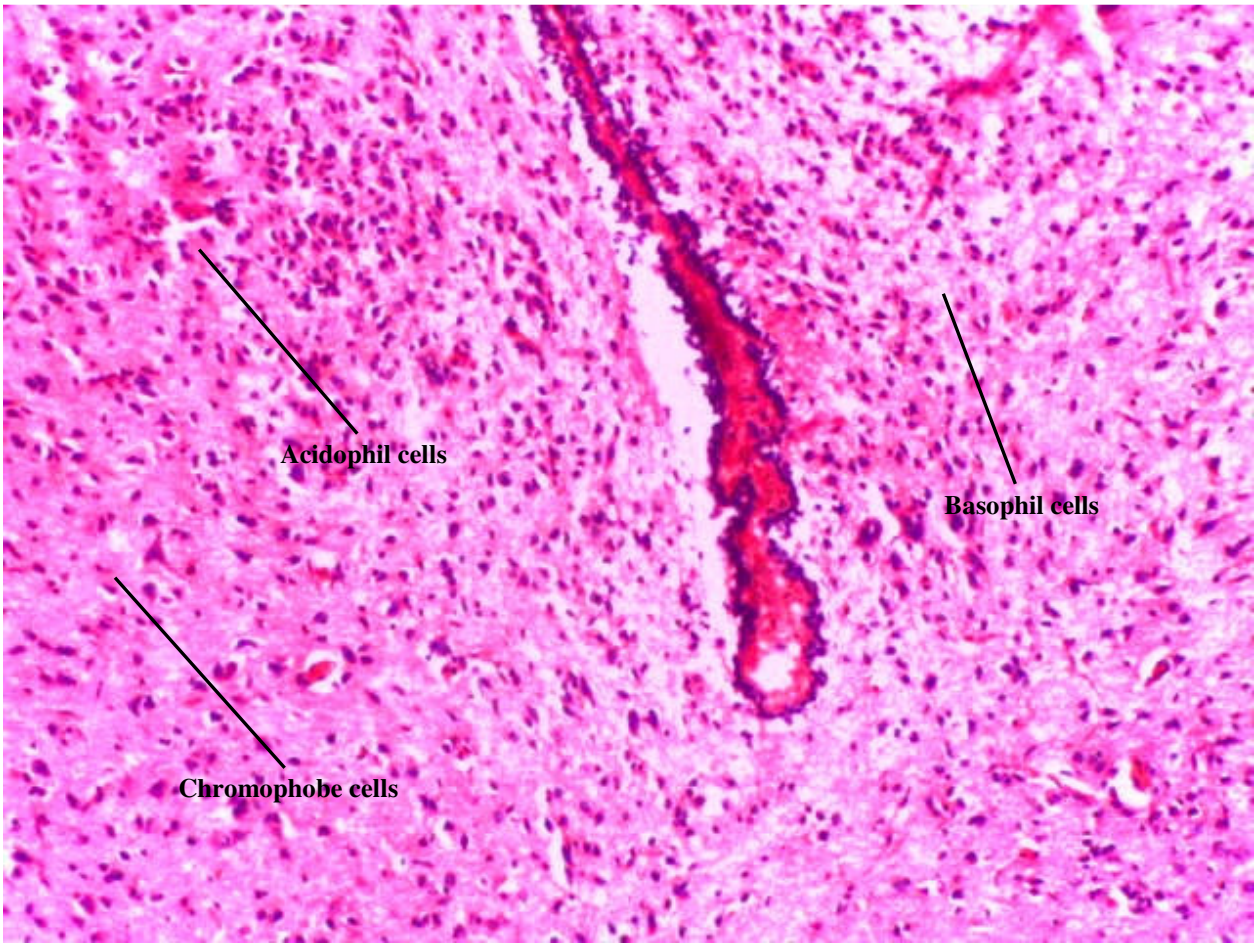

Figure 1: Control (H & E; ×200), no significant histopathological change

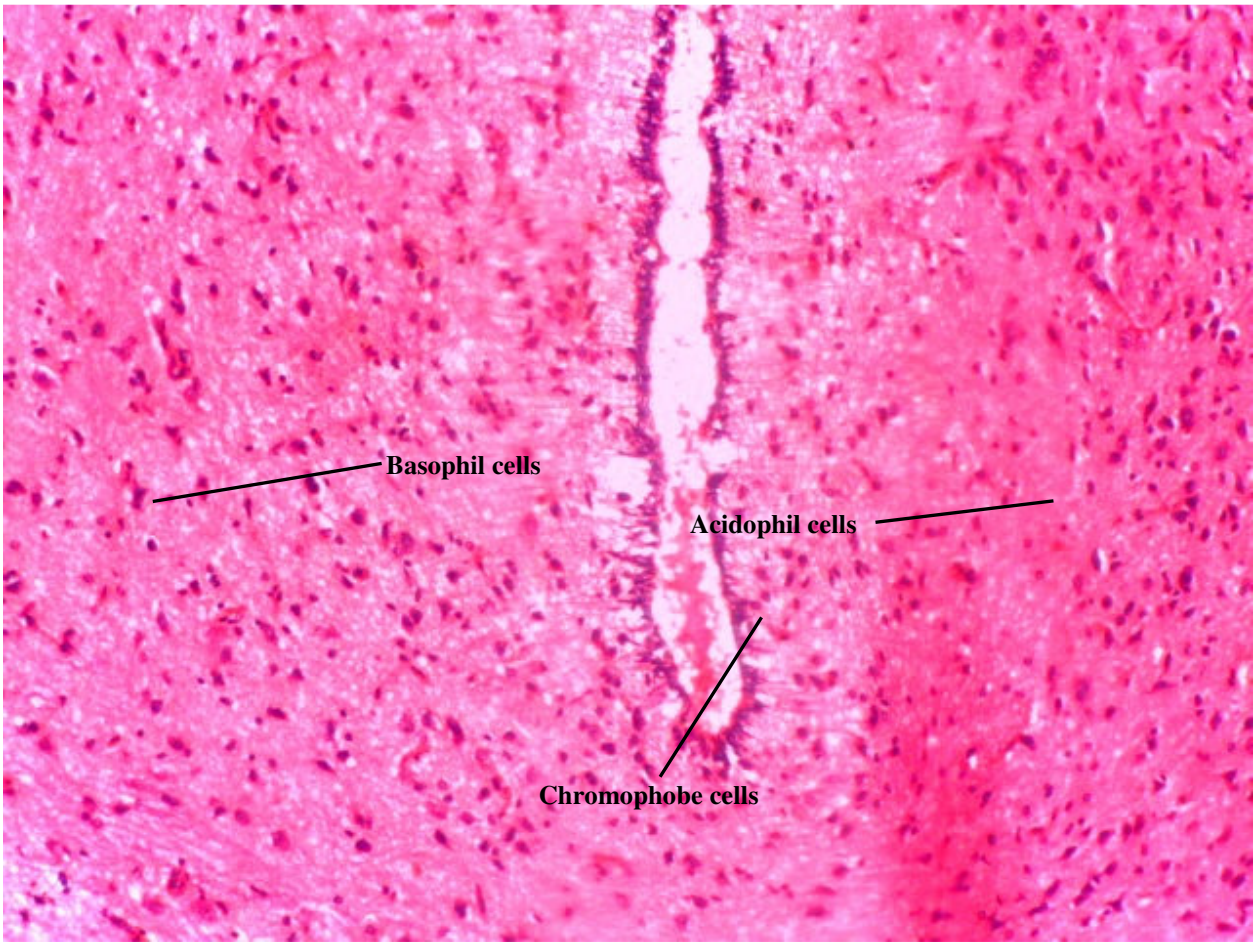

Figure 2: Group B (H & E; ×200); no observable change

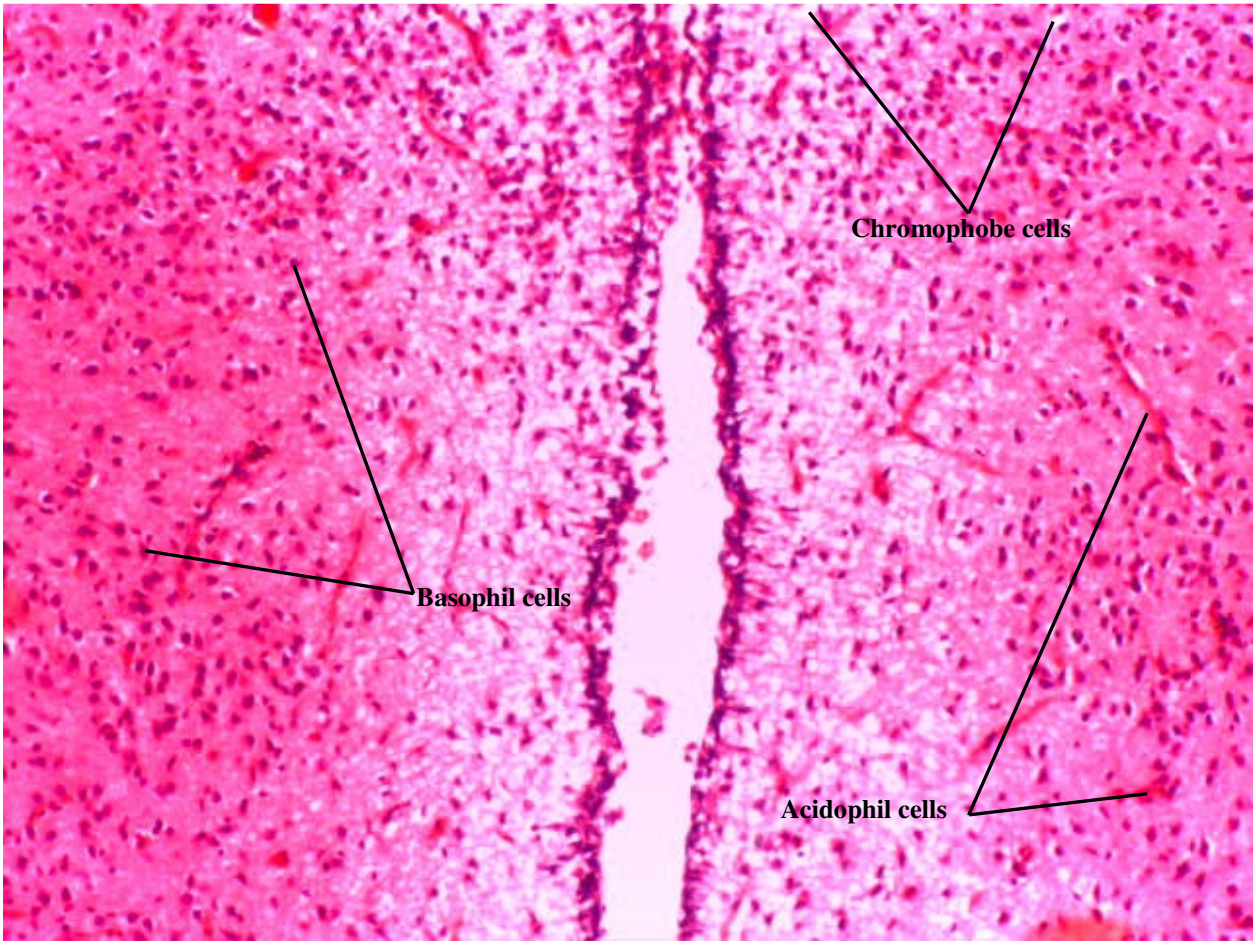

Figure 3: Group C (H & E; ×200); no observable change

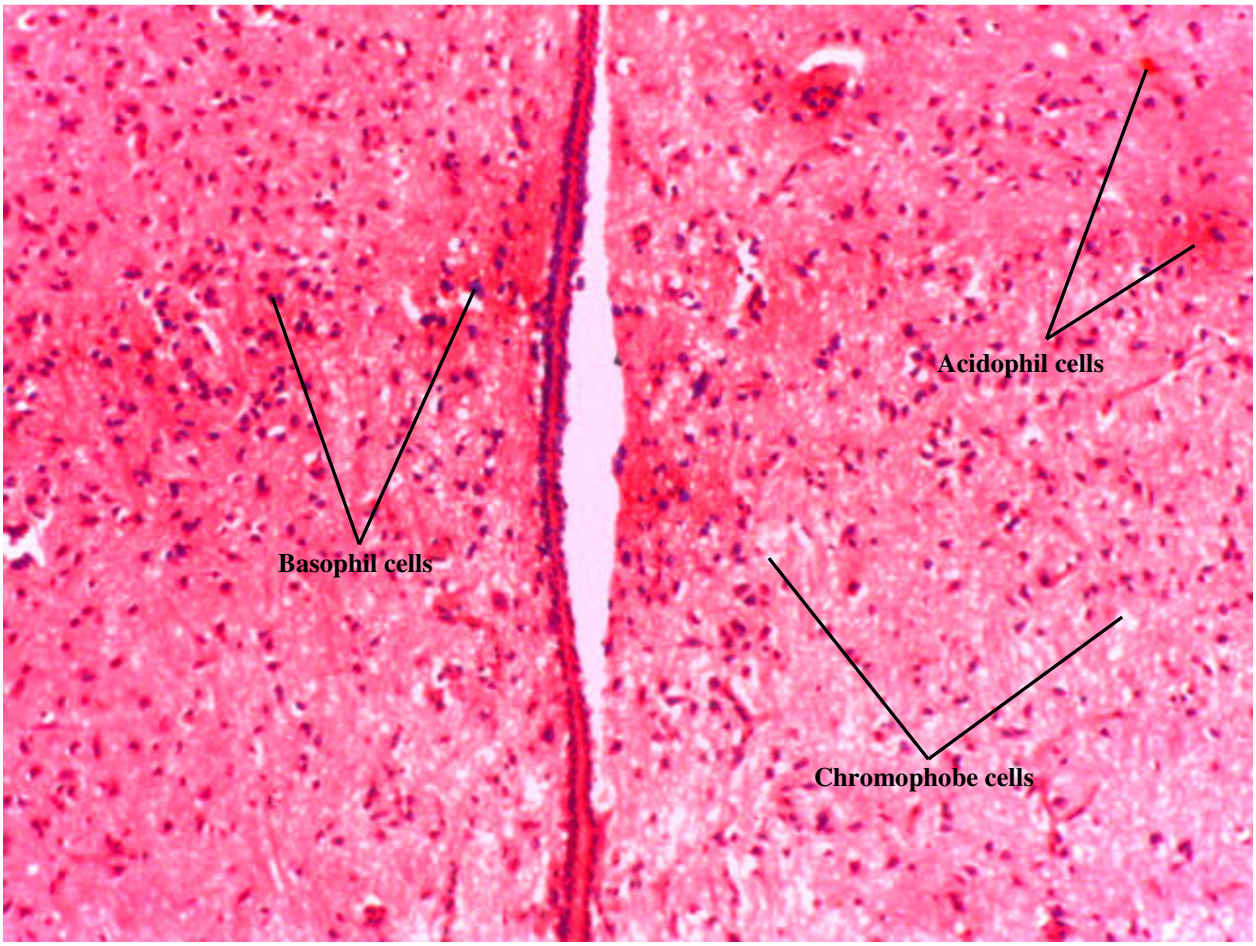

Figure 4: Group D (H & E; ×200); no observable change

4. Discussion

The spices in *Yaji* – ginger, cloves, red pepper and black pepper, contain gingerol (Witchtl, 2004), eugenol, capsaicin (Lejeune, 2003), and piperine (McGee, 2004) as active ingredients respectively, while the additives - ajinomoto, salt and groundnut cake powder, contain monosodium glutamate (Omojola, 2008), sodium chloride and oil (Fageria et al, 1997) as active ingredients respectively. The result of this research work, showed that there were no observable changes in all the groups as shown from the photomicrographs, however all parts of the anterior pituitary gland were all intact and all the cells (the acidophils, basophils, and chromophobes) were also clearly seen. However, irrespective of the possible influence of the active ingredients in *Yaji*, one should not lose sight of the other possibilities, which might have also contributed to the observed weight (gain). The observed weight gain is attributable to the established appetite enhancing potentials of monosodium glutamate (Bellisle, 2008), which might have increased the feeding rates of the animals in that group. The observed changes in weight were duration dependent judging by the determined weekly mean values. Interestingly, substance P in capsaicin has been shown to reverse diabetes in mice (Tsui et al, 2007). The study by Hui *et al* (2008) also reported that diets with different salt content fed to mature cats over a 6 month period produced no effect on food intake and body weight. High sodium diet have however, been shown to enhance water intake and urine output (Hui et al, 2008). As such, the weight gain caused by salt ingestion compared with the control in this study may be temporary as salt causes the body to retain water. Chronic dietary sodium restriction, an important non-pharmacological approach for the prevention and treatment of hypertension, has been linked with an increased white adipose tissue mass in rats (Prada et al, 2005). Furthermore, the influence of MSG on weight gain is in line with the reports of Tsang (2008) that MSG improves palatability of meals and influences appetite positively, hence the resultant increase in body weight. Although MSG improves taste stimulation and enhances appetite, available reports indicate that it is damaging to different organs (Vinodini et al, 2010). Moreover, the safety of MSG has generated much controversy. Research findings have shown that this flavor enhancer, found in many popular foods, causes weight gain and obesity in laboratory animals by damaging the appetite regulation center in the area of the brain known as the hypothalamus, causing leptin resistance (Barbara, 2009). Previous studies on MSG and obesity have been conducted on human and weight gain was significantly greater amongst those that consume MSG than in those who do not, even when they were given similar portions of food (Tsang, 2008).

A possible explanation for this is the fact that MSG alters the regulatory mechanisms that affects fat metabolism (Parker, 2008).

Relevant agencies involved in ensuring healthy nutrition in communities as well as food safety, should consider funding further research on this complex combination of active ingredients whose potentials are yet to be fully understood.

5. Conclusion

Findings on the effect of yaji on the anterior pituitary gland therefore shows that yaji has no effect on the anterior pituitary gland because there were no observable changes in all the test groups but progressively increase the weight.

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