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RESEARCH PAPER

A RELATIVE STUDY ON WEIGHT CHANGES AND NOTABLE PHYSICAL OBSERVATIONS IN ADULTS ALBINO WISTAR RATS FED WITH *YAJI* (A COMPLEX MEAT SAUCE)

[1,2]Agbo E.G., [1,3]Nwaopara A.O., [2]Festus O.O., [4]Odike M.A.C. and [5]Nosakhare, I.O.

[1]Anthonio Research Center, Ekpoma, Nigeria; Department of [2]Medical Laboratory Science; [3]Human Anatomy, [4]Histopathology, Ambrose Alli University Ekpoma, Edo State, Nigeria; [5] Department of Medical Microbiology, University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.
*Corresponding author: a.glowresearch@gmail.com

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ABSTRACT

This 6-week study investigates the effect of *Yaji* on body and organ weights of rats averagely weighing 229g. The rats (n=32) were divided into 4 groups (A-D) with 2 subgroups (A_1-D_1 and A_2-D_2) respectively. Group A served as the control, while B-D served as tests. B_1, C_1, and D_1 (n=8 each), received 80g/20g, 60g/40g and 40g/60g of feed/*Yaji* respectively for a period of 3 weeks (acute period), while B_2, C_2 and D_2 (n=4 each) received 40g/10g, 30g/20g and 20g/30g of feed/*Yaji* respectively for the remaining 3 weeks (chronic period). Control subgroups A_1 (n=8) and A_2 (n=4) received normal feed. Throughout the study, the physical and behavioral changes of the rats were recorded as well as the body and organ weights of the rats before and after the rats were sacrificed. Results showed statistically significant weight changes (weight loss in group B during the acute and chronic periods and in C and D during the acute period only) and weight gain in C and D during the chronic period only. Although human data remains yet undetermined, our findings however, supports earlier claims that *Yaji* has potentials for weight management, while suggesting a working reference weight reduction *rat*-dose of 20g.

Keywords: Yaji, Addictives, Spices, Nutrition, Weight changes, Physical observations.

INTRODUCTION

Over the years, researchers have maintained that insufficient nutrients to maintain bodily functions are associated with malnutrition, which may result from faulty nutrition, poor diet, or excessive consumption of particular foods or spices (Bender, 1975). Such foods can cause increased body fat and weight gain, with the development of certain age related chronic diseases -cardiovascular disease, diabetes and obesity (David *et al.,* 2009; Keith, 2007; Kushner, 2007). Though fat buildup is essential in prolonged starvation (Keith, 2007), several health complications however, have been associated with excessive weight gain (Akpamu *et al.,* 2011), and this remains a leading cause of preventable deaths worldwide (WHO, 2000). More so, despite the controversies about their risks and benefits, herbs, additives and spices have remained a major constituent of our diet (Milner, 1989; Gaby, 2005; McCann *et al.,* 2007; Moore, 2003).

Of interest in this study is *Yaji*- a complex mixture of spices and additives including ginger, cloves, red pepper, black pepper, white-magi (Ajinomoto), salt, and groundnut cake powder, used as a sauce for the popular Nigeria meat product called "*Suya*" (Nwaopara et al., 2004). The active ingredients include gingerol (Witchtl, 2004), eugenol (Krishnaswamy and Raghuramulu, 1998), capsaicin (Collier *et al.,* 1965), piperine (McGee, 2004), monosodium glutamate (MSG) (Omojola, 2008), sodium chloride (Carson *et al.,* 1998) and oil (Fageria *et al.,* 1997). Interestingly, several scientific investigations have exposed the inherent dangers in *Yaji* consumption, which have over the years raised concerns about its complexity, mass-consumption and the indiscriminate, unregulated and 'non-standardized' production pattern (Nwaopara *et al.,* 2004, 2011).Until date, several questions on the effect of *Yaji* are still been raised as new lines of thought are develop. This study therefore, investigates the effect of *Yaji* on both body and organ weight of rats, while observing notable physical changes.

MATERIALS AND METHOD

Study Area: This study was carried out at the Experimental Laboratory of Anthonio Research Center Ekpoma, Edo State, Edo State, Nigeria. Edo State lies roughly between longitude $06^{o}04^{1}$E and $06^{o}43^{1}$E and latitude $05^{0}44^{1}$N and $07^{0}34^{1}$N with a land mass of 17,450sqkm and located in the South-South geopolitical zone of Nigeria.

Experimental Animals/Housing Conditions: Adult Albino Wistar rats (n=32) of average weight (150-250g) were bought from the animal farm of Anthonio Services Nigeria (ASN), and moved to the experimental Laboratory of ASN at No. 40 Ujoelen Extension, Ekpoma, Edo State, Nigeria, where they were allowed to acclimatize for three weeks. During this period of acclimatization, the rats were fed with growers mash (25kg) daily from Grand Cereals limited, Km 17, Zawan Roundabout Jos, Plateau State, Nigeria and water was given *ad libitum*. The animals were kept in wire-cages designed to separate the animals from faeces and urine, and thus, avoid infections. Overall, the animals were handled in accordance with the standard guide for the care and use of laboratory animals (Richard and Crawford, 2012).

Substance of Study: The substance combination used by Nwaopara *et al.* (2009) was adopted for this study. The measured quantities included Ajinomoto (150g), black pepper (30g), clove (39g), ginger (78g), and groundnut cake powder (230g), red pepper (22g), and salt (100g); totaling 649g. The constituents were purchased at the Hausa quarters market, Ekpoma, Edo State and at Nkpor Market, Onitsha, Anambra Sate, Nigeria. They were subsequently mixed together in powdery forms as directed by the dealers.

Groupings/ Substance Administration: The experimental animals were divided into four groups (A, B, C and D) using 4 big cages. Each group has 2 subgroups: group A (subgroup A_1 and A_2), B (subgroup B_1 and B_2), C (subgroup C_1 and C_2) and group D (subgroup D_1 and D_2). Group A (A_1 and A_2) served as the control, while groups B-D (B_1, C_1, D_1, and B_2, C_2, D_2) served as the test groups. The first subgroups (A_1, B_1, C_1 and D_1) (n=8) represent the acute treatment period (3 weeks), while the second subgroups (A_2, B_2, C_2 and D_2) (n=4) continued treatment for the remaining 3 weeks as the chronic treatment group (6 weeks). The rats were weighed after acclimatization (i.e. before the administration of the *Yaji* spices) and after the experiment. All passed through the acute treatment period after which 4 rats from each of the groups were picked while the remaining 4 rats in each groups continued as the chronic treatment period. The spices were given orally each day between 8am – 10am and the total feed per day during the acute and chronic treatment periods was 100g and 50g respectively. Water was also given *ad libitum* during the period of the experiment. Test feed was produced by mixing appropriate quantities of *Yaji* (20g, 40g and 60g for B1, C1 and D1; 10g, 20g and 30g for B2, C2 and D2 respectively) and feed (80g, 60g and 40g for B1, C1 and D1; 40g, 30g and 20g for B2, C2 and D2 respectively) with sprinkles of water to form a paste.

Study Duration: The preliminary studies, animal acclimatization, ingredients procurement/*Yaji* production and actual animal experiment, lasted for a period of five months.

Data collection: At the end of the acute and chronic treatment period respectively, the rats were selected for weighing using the manual weighing balance and the average weight recorded accordingly, the weighed rats were then taken to a separate non-observatory cage. After which the rats were sacrificed and their organs weighed using an electronic weighing balance by Denver Company USA (200398. IREV CXP-3000), after which the average weights of the test group were compared with that of the control.

Statistical analysis: The results obtained were expressed as Mean ± SD. The statistical analysis was performed using the one way ANOVA (LSD) of SPSS version 17. The comparison was done at 95% confidence level and value $p<0.05$ were considered statistically significant.

RESULTS

Physical observations showed fur color changes from normal to dirty brown as observed in groups B, C and D during the chronic treatment period. Feed rejection and increased water intake were noticeable in all the test groups. Litter delivery was observed in all the groups except in groups B and D during the chronic treatment periods. The animals suffered diarrhea and appeared very weak resulting in two deaths during the acute treatment period and one death during the chronic period in group D. All other observations and behavioral changes were noted and tabulated (see table 1). Also, the feed intake was observed to be higher only in the acute treatment period in all the groups and the comparable differences were statistically significant (p<0.05) except for the control groups (see table 1).

Table 1: Observations and average feed consumption rate of rat fed with graded doses of *Yaji*

Observations	Group A		Group B		Group C		Group D	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Fur colour change	-	-	-	+	-	+	-	+
Behavioral changes	-	-	-	+	+	+	+	+
Skin changes	-	-	-	-	-	+	+	+
Eye colour changes	-	-	-	-	+	+	+	+
Diarrhea/weakness	-	-	-	+	-	+	+	+
Deaths	-	-	-	-	-	-	+	+
Quick response to Water	-	-	+	+	+	+	+	+
Feed rejection	-	-	-	+	+	+	+	+
Birth	+	+	+	-	+	+	+	-
Physical agility	**Active**	**Active**	**Active**	**Weak**	**weak**	**weak**	**Weak**	**Weak**
Inability to move/seizure	-	-	-	-	-	-	-	+
Altered respiratory rate	-	-	-	-	-	-	-	+
Feed intake (day^{-1} Gp^{-1})	100.00 ±0.00 ^{a}	50.00± 0.00 ^{b}	98.55± 3.99 ^{a}	49.95± 0.13 ^{b}	87.53± 8.10 ^{a}	45.36± 1.71 ^{b}	65.12± 26.31 ^{a}	17.77± 1.89 ^{c}

Key: + = present; - = absent. Values are Mean ± Standard deviation. Values with different superscript are statistically significant at p≤0.05 compared to control.

The body weight changes recorded for control and test groups after acclimatization and after the experiment (acute and chronic treatment periods) are shown in table 2. There was weight loss in group B while weight gain was observed in groups C and D. Body weight changes of all the test groups were statistical significant (p≤0.05) when compared with the values obtained after acclimatization. Table 3 however, shows the growth performance and feed conversion rate (FCR). Weight difference, percentage weight difference and the average daily growth (ADG) were also documented. While control (group A) showed a slight body weight gain and decreased ADG throughout the period of the experiment, test group B suffered weight loss a percentage of -24.69% and -8.20% during the acute and chronic periods respectively. The ADG of all the test groups showed slight but insignificant increases compared to the control.

As regards the feed conversion rate, feed quantity adjustments was effected for group D bearing in mind the death of one rat on the 16th day of the experiment (acute period). As such, the total feed for the remaining rats in group D was reduced by 3 given a total of 33.3g (that is *Yaji,* 20g and growers mesh, 13.3g). Following the death of another rat in group D two days later, the feed was further reduced by 2, given a total of 16.7g (10g of *Yaji* and 6.7g of growers mesh). During the chronic period, the total feed for group D was 25g since the rats were reduced to two as a result of deaths. Five days into the experiment, one rat died causing the total feed for the remaining rats to be 12.5g. Therefore, the FCR decreased slightly in the control (group A) and group B, during the acute and chronic treatment periods; in group C and D however, there was an increase.

The organ-weight values are as presented in Tables 4, 5, and 6. Organ-weight values measured after the experiment showed statistically significant changes (p≤0.05) only in groups A and B (Brain and left kidney respectively), while in the other groups, there was no significant changes. However, a gradual but steady weight increase in a dose related fashion was observed for the heart, liver, left lung, spleen and intestine while a decrease was observed in the weights of the brain and right lung. On the contrary, irregularities in the weights of the right and left kidneys were registered.

Table 2: Body weight changes of rats fed with graded doses of *Yaji* at various treatment periods.

Parameter	Body weights in gram at different treatment period			
	Group A	Group B	Group C	Group D
After Acclimatization	181.25±37.20[a]	253.13±63.30[a]	193.75±29.12[a]	290.63±59.67[a]
After Experiment				
Acute period	190.63±49.89[a]	190.63±42.13[b]	129.38±22.27[b]	130.83±38.78[b]
Chronic period	200.00±40.83[a]	175.00±0.00[b]	131.25±12.50[b]	150.00±0.00[b]

Values were Mean ± Standard deviation. Values in a column with different superscript are statistically significant ($p<0.05$)

Table 3: Growth performance and feed conversion rate of rats fed with graded doses of *Yaji*.

Parameters	treatment periods	Control	Group B	Group C	Group D
Weight difference (ED) (g)	Acute period	9.38	-62.50	-64.37	-159.80
	Chronic period	9.37	-15.63	1.87	19.17
% Weight difference (%WD) (g)	Acute period	5.18	-24.69	-33.22	-54.98
	Chronic period	4.92	-8.20	1.45	14.65
Average Daily Growth (ADG) (g)	Acute period	0.45	-2.98	-3.07	-7.61
	Chronic period	0.22	-0.37	0.04	0.46
Feed Conversion Rate (FCR) (g)	Acute period	10.66	-1.58	-1.36	-0.41
	Chronic period	5.34	-3.20	24.26	0.93

Key: WD = final weight – initial weight; **%WD** = (final weight – initial weight ⁄ initial weight) x 100; **ADG** = final weight – initial weight / number of days; **FCR** = feed intake (g) / body weight difference (g).

Table 4: Organ weight differences in gram of rats fed with *Yaji*, during acute and chronic treatment periods

Groups	Brain (g)		Heart (g)		Liver (g)	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
A	1.75±0.39[a]	1.64±0.07[b]	0.50±0.13	0.60±0.12	5.01±0.78	7.83±0.79
B	1.76±0.07	1.69±0.11	0.72±0.81	0.74±0.15	5.98±0.85	6.90±0.72
C	1.72±0.06	1.51±0.25	0.52±0.98	0.52±0.11	4.31±0.62	5.13±1.29
D	1.77	1.73	0.60	0.65	3.90	7.24

Key: Rt =Right, Lt =Left. Values are Mean ± Standard deviation. Values with different superscript are statistically significant ($p<0.05$).

Table 5: Organ weight differences in gram of rats fed with *Yaji*, during acute and chronic treatment periods

Groups	Spleen (g)		Rt. Kidney (g)		Lt. Kidney (g)	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
A	0.53±0.29	0.83±0.22	0.53±0.09	0.46±0.03	0.54±0.10	0.56±0.13
B	0.56±0.21	0.66±0.26	0.66±0.63	0.71±0.11	0.67±0.05[a]	0.63±0.18[b]
C	0.29±0.09	0.49±0.10	0.44±0.18	0.59±0.16	0.50±0.12	0.54±0.09
D	0.47	0.96	0.59	0.58	0.42	0.71

Key: Rt =Right, Lt =Left. Values are Mean ± Standard deviation. Values with different superscript are statistically significant ($p<0.05$).

Table 6: Organ weight differences in gram of rats fed with *Yaji*, during acute and chronic treatment periods

Groups	Rt. Lung (g)		Lt. Lung (g)		Intestine (g)	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
A	0.77±0.36	0.49±0.20	15.84±3.09	18.24±1.41	0.48±0.22	0.56±0.22
B	0.74±0.14	0.59±0.20	12.72±3.35	14.6±2.64	0.50±0.07	0.66±0.32
C	0.65±0.19	0.56±0.21	8.51±1.26	14.3±4.02	0.40±0.53	0.44±0.15
D	0.68	0.31	8.29	19.16	0.34	0.71

Key: Rt =Right, Lt =Left. Values are Mean ± Standard deviation. Statistically significant ($p<0.05$).

DISCUSSION

The obvious changes observed in this study such as colour changes (fur, eye and skin), diarrhea, feed rejection, seizure, altered respiratory rate, reduced animal activity and death, emphatically suggests that *Yaji* has potentials to alter physiological processes that may result in undesirable conditions. In fact, the assertions by Nwaopara et al. (2011) that the contents of *Yaji* can induce neurodegenerative changes may explain the seizures observed. This was earlier acknowledged by Eweka and Om'Iniabohs (2007) that MSG consumption may have some deleterious effects on the cerebellum of adult wistar rats at higher doses. This is predicated upon the fact that MSG may act as a toxin to cerebellar neurons, affecting its cellular integrity, and causing defects in membrane permeability as well as cell volume homeostasis (Espinar *et al*., 2000; Urena-Guerrero *et al*., 2003); leading to tremor, unstable and uncoordinated movement or ataxia that are associated with seizure (Eweka and Om'Iniabohs, 2007).

Similarly, the incidence of diarrhea in the test animals might be linked to the potentials of some of the active ingredients in *Yaji* as identified by Desai et al. (1977), Nopanitaya (1973), Kawada et al. (1986), and Srinivasan and Satyanarayana (1987) that capsaicin in red pepper has been shown to cause dyspepsia in patients with or without ulcer, gastric mucosal edema, and decrease in the gastric acid output; and can also affect carbohydrate absorptive ability of the duodenal epithelial cells. Bhatia (2000) also noted that black and red pepper may induce epigastric pain by removing the stomach's hydrophobic lining and activating intramucosal pain receptors. In addition, the observed altered respiratory rate is in line with the suggestions made by Malson (2003) that certain spices and addictives like clove, can influence on the respiratory system; noting that an excessive consumption of clove can result in allergic reactions including difficulty in breathing, throat obstruction, and inflammation of the lips, tongue or face. Also, the observed food rejection affirmed by the low feed intake and diarrhea might explain the resultant weakness and reduced activities due to dehydration and reduction in ATP, which is usually managed in children by the administration of Oral Rehydration therapy (ORT), to replace the lost water and electrolytes (Strazzullo *et al.*, 2009). The state of stooling and vomiting in the presence of little or no food might also explain the number of deaths recoreded in the course of the experiment.

Furthermore, the comparative lower body weight in the *Yaji* treated group B as compared to the relatively higher values for the control might be due to the repulsive effect of the pungent nature of the spices such as red pepper, which usually leads to reduced feeding (Oyewole *et al*., 2007). According to the findings by Akpamu et al. (2011), this observation can induce weight loss as affirmed by the report of Choi and Suh (2004). It has also been reported that piperine (a constituent of black pepper) can stimulate metabolism and binds to the so-called Transient Receptor Potential Vanilloid (TRPV1) receptors in the brain and other parts of the nervous system; and as TRPV1, works as the body's thermometer which, once activated, turns up the heat by boosting heat production by the body (Food Navigator, 2006). Also, substance P in capsaicin has been shown to reverse diabetes in mice (Motluk and Geddes, 2005; Tsui *et al.*, 2007). Available reports have shown that capsaicin has the capacity to reduce adiposity in rats, which can be partly explained by its enhancing effects on energy and lipid metabolism via catecholamine secretion from the adrenal medulla through sympathetic activation of the central nervous system (Lejeune *et al.*, 2003). In addition, an increase in diet-induced thermogenesis immediately after a meal containing capsaicin, has been reported in humans (Yoshioka *et al.*, 1999; 2001), implying a shift in substrate oxidation from carbohydrate to fat oxidation.

Although MSG enhances taste stimulation and appetite, available reports indicate that it is damaging to different organs (Moses and Sefcikova, 2004; Farombi, 2006; Eweka, 2007; Vinodini *et al.*, 2010). Moreover, the safety of MSG has generated much controversy as research findings have shown that this flavor enhancer, found in many popular foods, causes weight gain, obesity (in lab animals by damaging the appetite regulation center in the hypothalamus) and leptin resistance (Barbara, 2009). Akpamu et al. (2011) reported that previous studies on MSG and obesity showed that weight gain was significantly greater amongst those that consumed MSG than in those who did not; even when they were given similar portions of food (Tsang, 2008; Parker, 2008). This is possibly linked to the potential of MSG to alter the regulatory mechanisms that influences fat metabolism (Tsang, 2008).

Another line of thought considers the influence of the groundnut in *Yaji*. Alper and Mattes (2002) states that the incorporation of whole peanuts to the habitual diet of healthy adults for 8 weeks resulted in a 1kg increase in body weight. Akuamoah-Boateng *et al.* (2007) also reported that there was a slight but significant increase in the body weight of participants consuming nuts oil within an 8-week intervention period. Fortunately, spices have established properties that make them efficient tools in the management of weight. According to Kalpana and Srinivasan (2004), spices have long been recognized for their digestive stimulant action. Animal studies have shown that many spices induce higher secretion of bile acids which play a vital role in fat digestion and absorption. When consumed through diets, spices also produce significant stimulation of the activities of pancreatic lipase, amylase and proteases. Thus, the digestive stimulant action of spices seems to be mediated through the stimulation of the liver to secrete bile rich in bile acids -components that are vital for fat digestion and absorption.

Interestingly, the results from group B suggest that *Yaji* can induce weight loss probably due to the combined influence of the active ingredients in clove, ginger, red pepper and black pepper, which have potentials to induce body weight reduction. On the other hand, *Yaji* can as well induce weight gain based on the requisite potentials of salt, MSG and groundnut. However, the capacities of salt, MSG and groundnut to induce weight gain is likely counteracted by the opposing influences of clove, ginger, red pepper and black pepper to induce weight loss. The homeostatic reality of these so-called opposing influences as regards *Yaji,* remains to be determined. Although human data remains yet to be determined, our findings support earlier claims that *Yaji* has potentials for weight management, while also suggesting a working reference weight reduction *rat*-dose of 20g. In fact, this potential in *Yaji*-spices can be harnessed especially in the management of obesity, which has become a community health challenge requiring urgent attention.

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Agbo, G. coordinated the animal experiment, while Nwaopara A.O., Festus O.O., Odike M.A.C. and Nosakhare, I., provided all the needed technical input, especially in the documentation and presentation of this paper.