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A Comparative Study on the Acute and Chronic Effect of Oral Administration of *Yaji* (A Complex Nigerian Meat Sauce) on Some Hematological Parameters

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Abstract: This comparative study to determine the acute and chronic effects of *Yaji* (a complex Nigerian meat sauce) on some hematological parameters involved Wistar rats of an average weight of 188 g. The Wister rats were randomly assigned into six groups (n = 24); group A rats served as the control while group B1-F1 and B2-F2 served as the test groups. Group A (control) received 300 g of growers mash (feed) only and B received 237 g of feed plus 9 g each of the combined constituents of *Yaji*, while group C, D, E and F, received 291g of feed plus 9 g of clove, ginger, red pepper and black pepper, respectively. At the end of each week, 3 rats were picked at random from the groups for blood sample collection. The collected blood samples were analysed to determine PCV, WBC and differential count; and the resultant average values were then recorded. The first four weeks served as the acute treatment period for test groups B1-F1, while the combination of the first and second four weeks (eight weeks) served as the chronic treatment period for test groups B2-F2. The test results showed a decrease in PCV as compared with that of the control (51.88 .36%). The observed differences in this regard was statistically significant ($p<0.05$) for the acute treatment group B1, D1 and F1; and for the chronic treatment group C2. However, the PCV value in the chronic treatment group B2, D2, E2 and F2 increased as compared with the acute treatment groups, though this was not statistically significant ($p>0.05$). Also, there was no significant difference ($p>0.05$) in the WBC count and differential count of the test groups as compared with group A. Our findings suggest therefore, that the changes observed in the test groups appear to be duration and dosage dependent and as such, indicates that an unregulated consumption of *Yaji* has its implications on health and wellbeing considering the clinical significance of PCV.

Key words: Acute, chronic, heamatological parameters, malnutrition, spices, *Yaji*

INTRODUCTION

For decades, nutrient-insufficiency to maintain healthy bodily functions has been known to be the basis of malnutrition, which according to Bender (1975), may result from faulty nutrition, poor diet, or excessive consumption of particular foods. Nwaopara *et al*. (2010a) reported a growing concern about the excessive consumption of a meat sauce called '*Yaji*', which is used to serve the meat delicacy called '*Suya*' in Nigeria. Of interest, is the fact that this sauce, which is a complex mixture of spices and additives (Clove, ginger, red pepper, black pepper, table salt, MSG and groundnut), is produced without a standardized production format. Moreover, spices are rarely consumed as a single flavouring agent in Nigeria, but rather as a complex of several spices and non-spice additives in dishes (Ugwuja *et al*., 2009). The spices in *Yaji* include ginger, clove, red pepper and black pepper (Nwaopara *et al*., 2004), with gingerol (Witchtl, 2004), eugenol (Krishnaswamy and Raghuramulu, 1998), capsaicin (Surh and Lee, 1995), and piperine (McGee, 2004) as their active principles respectively.

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, 2007a, b, 2008a, b, 2009a, 2010a, b), it becomes necessary therefore, to determine its effect on some haematological parameters as such provides important information about the internal environment of an organism (Masopust, 2000).

Although there are on-going histological investigations, one still wonders what the acute and chronic effects of *Yaji* might be on basic physiological

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and biochemical parameters, since the excessive consumption of each of the contained active ingredients alone, is potentially harmful. While haematological parameters have often been evaluated and are of diagnostic importance in relation to the state of health, it is important to state however, that no research has appraised the effect of *Yaji* on haematological parameters. This study therefore, is designed to determine and compare the acute and chronic effects of oral ingestion of *Yaji* with the respective effects of its spices on some hematological parameters in adult Wistar rats.

MATERIALS AND METHODS

Location and duration of study: This study was conducted in Ekpoma, Edo State, Nigeria with rats that were allowed to acclimatize for three weeks. 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 eleven months; while the actual administration of test samples to the test animals lasted for eight weeks.

Materials of study: Clove, ginger, red pepper, black pepper, table salt, MSG (white magi or Ajinomoto) and groundnut, were purchased dried from Aduwawa market, Benin City; Nigeria. The spices and feed (growers mash from Bendel Feeds and Flour Mills, Ewu, Edo State) were crushed separately using an electric blender. Measurement of spices was carried out using Electric Balance (by Denver Company USA -200398. 1REV.CXP-3000) in the diagnostic Laboratory of the Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma.

Experimental rats: Adult rats of an average weight of 188 g were procured from the animal farm house of the Department of Physiology, College of Medicine, Ambrose Alli University, Ekpoma, and moved to the site of the experiment at No. 5B Palmwell Street Ujemen, Ekpoma, were they were allowed to acclimatize for three weeks. The animals were separated into six groups (A-F) in 6 big cages (n = 24). Group A rats served as the control while group B-F served as the test groups. During the period of acclimatization, the rats were fed growers mash daily and water given *ad libitum*.

Administration of test sample: After acclimatization, the rats in each of the groups received as follows: Group A (control) received 300 g of feed; B received 237 g of feed plus 9 g of each of the respective constituents of *Yaji*; C received 291 g of feed plus 9 g of clove; D received 291 g of feed plus 9 g of ginger; E received 291 g of feed plus 9 g of red pepper; and F received 291 g of feed plus 9 g of black pepper daily. For the purpose of this study, pellets were produced by mixing respective *Yaji* constituents with the appropriate amount of feed (grower mash) via sprinkling water onto the mixture until a semisolid paste is formed. The resultant paste was then split into bits and allowed to dry under the sun. The total feeding period was eight weeks but on each day, it lasted between 9:00 am - 10:00 am.

Samples collection and analysis: At the end of each week and before the commencement of the next feeding week, 3 rats were selected at random for whole blood sample collection. This was done using the jugular vein. The collected blood samples were immediately stored in sterile bottles containing heparin (an anti-coagulant) pending when hematological analysis is done. The resultant mean values were then recorded. The first four weeks served as the acute treatment period, while the combination of the first and second four weeks (eight weeks) served as the chronic treatment period.

Packed Cell Volume (PCV) was estimated by the macro-hematocrit method, WBC counts by the visual means of the new improved Neubauer counting chamber using a diluting fluid (Turk's fluid) in a ratio of 1: 20, while differential white blood cell count was carried out using Leishman's stain as described by Dacie and Lewis (1991, 2001).

Data analysis: The mean±S.D was generated using SPSS (version 17) soft ware package and the one way ANOVA (LSD) test determined at $p<0.05$.

RESULTS

The test results showed a decrease in PCV as compared with that of the control (51.88±3.36%). The observed differences in this regard was statistically significant ($p<0.05$) for the acute treatment group B1, D1 and F1; and for the chronic treatment group C2. However, the PCV value in the chronic treatment group B2, D2, E2 and F2 increased slightly as compared with the acute treatment groups, though this was not statistically significant ($p>0.05$) (Table 1).

Comparing the mean value of group A ($4.58\pm2.48 \times 10^3/mm^3$) with the acute treatment test groups, it was observed that group B1 presented a non significant increase in WBC count, while C1, D1, E1 and F1 showed a statistically non significant reduction. On the other hand, the chronic treatment group (B2, C2, D2, E2 and F2) showed a non significant increase in WBC count as compared with the acute treatment except for group B2 which presented a non significant reduction. However, the observed changes in WBC count for the chronic treatment groups were statistically not significant when compared with the control value (Table 2).

Table 1: Effect of acute and chronic oral ingestion of Yaji and its spices on PCV of rats

PCV (%)	B	C	D	E	F
Control(A)	51.88±3.36	51.88±3.36	51.88±3.36	51.88±3.36	51.88±3.36
Acute	38.25±18.95*	45.50±18.08	40.75±9.78*	48.75±2.22	27.33±16.56*
Chronic	54.25±1.71	29.50±28.99*	52.50±2.12	49.25±2.99	48.00±1.63

A: control; B: Yaji; C: clove; D: ginger; E: red pepper; F: black pepper; Values are mean ± Standard deviation; *: represent $p<0.05$ compared with control

Table 2: Effect of acute and chronic oral ingestion of Yaji and its spices on WBC of rats

WBC ($\times 10^3/mm^3$)	B	C	D	E	F
Control(A)	4.58±2.48	4.58±2.48	4.58±2.48	4.58±2.48	4.58±2.48
Acute	5.18±0.44	3.86±2.44	3.65±1.10	2.38±1.70	4.38±2.73
Chronic	4.78±.24	5.36±0.42	4.43±0.60	4.13±0.30	4.78±0.45

A: control; B: Yaji; C: clove; D: ginger, E: red pepper; F: black pepper; Values are mean ± Standard deviation; *: represent $p<0.05$ compared with control

Table 3: Effect of acute and chronic oral ingestion of Yaji and its spices on differential count of rats

Neu (%)	B	C	D	E	F
Control(A)	42.50±6.14	42.50±6.14	42.50±6.14	42.50±6.14	42.50±6.14
Acute	45.50±4.20	45.00±7.02	41.00±5.29	44.00±3.65	42.50±5.07
Chronic	48.00±1.63	43.50±2.12	46.00±8.49	48.25±5.56	46.00±1.63
Lym (%)	**B**	**C**	**D**	**E**	**F**
Control(A)	49.75±8.36	49.75±8.36	49.75±8.36	49.75±8.36	49.75±8.36
Acute	49.75±5.06	46.25±9.78	52.25±5.19	51.50±1.73	52.25±1.71
Chronic	46.25±4.65	47.50±3.54	45.50±2.12	45.00±3.37	46.00±1.41
Mon (%)	**B**	**C**	**D**	**E**	**F**
Control(A)	7.50±3.59	7.50±3.59	7.50±3.59	7.50±3.59	7.50±3.59
Acute	4.50±3.79	6.75±3.95	6.75±6.18	4.50±2.08	5.00±4.76
Chronic	5.75±3.69	8.50±2.12	8.00±5.66	6.00±3.65	7.50±2.08

A: control; B: Yaji; C: clove, D: ginger, E: red pepper; F: black pepper; Values are mean ± Standard deviation; Neu: Neutrophils; Lym: Lymphocytes; Mon: Monocytes; *: represent $p<0.05$ compared with control

On the differential WBC count in both the acute and chronic treatment groups, inconsistent changes were observed but were not statistically significant ($p>0.05$) (Table 3).

DISCUSSION

The results of this study have shown that *Yaji* has the potential to alter the values of PCV. Specifically, group B1 results (acute treatment) indicated a statistically significant reduction ($p<0.05$) in the PCV, while group B2 results (chronic treatment) indicated a non-significant increase in the PCV. This acute response to the oral ingestion of *Yaji* is suggestive of the fact that the constituents of *Yaji* in combination can induce anemia since PCV is an important diagnostic tool used to determine blood loss, health status and anemia. On the other hand, the results obtained from the chronic treatment groups are indicative of the fact that the body might have responded to the prolonged effect of *Yaji* on PCV with the appropriate defensive physiological mechanisms. The question however, is how long would this seeming physiological line of defense be sustained as even the comparative difference between the chronic treatment values and those of the control were not statistically significant.

Another line of thought is the influence the individual spicy constituents of *Yaji* might have had as regards the outcome of this study. One can take a cue from the study on European catfish by Velisek *et al*. (2006), who reported a significant ($p<0.05$) decrease in hemoglobin and lymphocyte count immediately after clove anesthesia and a significantly ($p<0.05$) decreased in leukocyte count 24 h after anesthesia. Considering the fact that the level of hemoglobin is an important indicator for PCV status, our findings on clove ingestion as observed in group C2 therefore, agrees with the report by Velisek *et al*. (2006). However, Waterstrat (1999) and Small (2003) had reported that 100 mg/L clove oil is a safe concentration for anesthesia as shown in an experiment on channel catfish (*Ictalurus punctatus*). As contrary as this may appear in relation to our findings, it is our opinion that the seeming disagreement in this regard is suggestive of the fact that the observed difference is dosage dependent since 9 g of clove as applied in this study is definitely higher than 100 mg.

As regards the possible influence of ginger, we did observe that there are conflicting reports in the literature. On one hand, Ugwuja *et al*. (2009) reported that ginger had no significant effect on haematological parameters (PCV, Hb and WBC), but on the other, Micheal *et al*. (2009), reported that ginger induced a reduction in PCV. The later report however, corroborates our findings in the acute treatment group.

The result on red pepper was in line with the report by Okokon *et al*. (2004) who had reported a reduction in PCV with sings of anemia following the ingestion of red pepper. In fact, the report by Myers *et al*. (1987) that red

pepper consumption has the capacity to induce mucosal microbleeding can indeed offer an explanation for the observed chnges. Our results on black pepper however, contradicts the findings by Hassan *et al*. (2010), who had reported that black pepper induces an increase in value of PCV.

Furthermore, the observed non significant (p>0.05) changes in WBC and differential counts within the acute and chronic treatment groups were expected considering the antimicrobial potentials of *Yaji* (Nwaopara *et al*., 2009b). Specifically, group C (fed with clove) showed no significant changes in WBC and differential counts, and this agrees with the report by Velisek *et al*. (2005a, b) that that there were no changes in the haematological profile in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) following clove oil anesthesia. Also, the group D (fed with ginger) results on WBC were consistent with those of Hassan *et al*. (2010), Micheal *et al*. (2009) and Olayaki *et al*. (2007).

Our findings suggest therefore, that the changes observed in the test groups appear to be duration and dosage dependent and as such, indicates that an unregulated consumption of *Yaji* has its implications on health and well being considering the clinical significance of PCV. However, we do not advocate a ban on the ingestion of *Yaji*, but rather, the regulation of its production and consumption since this study has shown that *Yaji* and its spicy constituents, has the capacity to induce PCV reduction.

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