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# *In vivo* effects of dietary intake of *Yaji* (a complex Nigerian meat sauce) on some serum enzymes of Wistar albino rats

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**Abstract**

This study designed to determine the potential synergistic and independent effects of *Yaji* (a complex Nigerian meat sauce) and its constituent spices (clove, ginger, garlic and red pepper) on the serum levels of aspartate transaminase, alanine transaminase and alkaline phosphatase, involved male albino Wistar rats of an average weight of 74 g. The administration of spices was performed over a 21-day period via mixing with feeds. The amount of spices administered to the experimental animals in the various test groups was body weight-dependent. Blood samples were obtained from sacrificed animals at zero, 14$^{th}$- and 21$^{st}$-day and levels of plasma enzymes determined by standard methods. The test results showed no significant ($p>0.05$) changes in body weight of the experimental animals in all the groups on the 14$^{th}$ and 21$^{st}$ day as compared to day-0 values for individual groups. Similarly, there were no significant changes ($p>0.05$) in the plasma levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) (IU/L) respectively, in any of the test groups as compared with that of the control. Our findings suggest that the spices, within the administered doses administered, are not capable of causing hepatocellular damage or inducing enzyme activity.

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## Introduction

The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. The diagnostic use of enzymes in veterinary and human clinical pathology is mostly aimed at detecting, evaluating and monitoring organ damage based on the increase in organ-specific enzymes. However, enzymes are also used to evaluate the synthetic capacity of an organ, to diagnose the adverse effects of toxic compounds which are enzyme inhibitors, and to monitor the inductive activity of exogenous compounds or enzyme activation by minerals or vitamins (Braun *et al.,* 2008). Aspartate aminotransferase (AST, also sometimes termed SGOT) and alanine aminotransferase (ALT, also sometimes termed SGPT) are widely distributed in cells throughout the body. Aspartate aminotransferase catalyses the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. Alanine aminotransferase catalyses the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and glutamate in the cytoplasm of the cell while alkaline phosphates are a group of isoforms which hydrolyse many types of phosphate esters, whose natural substrate or substrates are unknown ((Bittinger *et al.*, 2003; Lone *et al.*, 2003). AST is found primarily in heart, liver, skeletal muscle, and kidney, while ALT is found primarily in liver and kidney, with lesser amounts in heart and skeletal muscle. Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity (Dufour *et al.*, 2000). Alkaline phosphatases were the earliest serum enzymes to be recognized to have clinical significance, when in the 1920s, it was discovered that they increase in bone and liver diseases. Since then, they have been the subject of more publications than any other enzyme (Lone *et al.*, 2003).

Available results from a number of studies have shown that an excessive consumption of *Yaji*-spices especially in combination is capable of inducing pancreatic, liver and kidney tissue damage (Nwaopara *et al.*, 2004, Nwaopara *et al.*, 2007b; Nwaopara *et al.*, 2008a). *Yaji* is a complex Nigerian meat Sauce. It is a sauce for the meat delicacy called *Suya*; a boneless lean meat of mutton, beef or goat stated on sticks, coated with its sauce, oiled and then roasted around a burning fire. *Yaji* is a complex mixture of groundnut cake powder, additives, spices (cloves, ginger, garlic, red pepper, and black pepper), salt and maggi cubes (Nwaopara *et. al.*, 2004).

The *Yaji*-spices exhibit several beneficial properties due to the presence of potential phytochemicals in them (Bhattacharjee and Segupta, 2009; Lampe, 2003). These phytochemicals can have complementary and overlapping actions, including antioxidant, antimutagenic and anti-inflammatory effects, modulation of detoxification enzymes, and induction of apoptotic activity and so on (Bhattacharjee and Segupta, 2009). However, Nwaopara *et al.* (2009) reported that the moderation of Yaji is preferable and safer.

The aim of this study, therefore, is to ascertain the effects of dietary intake of *yaji* which consists of several spices on some plasma enzymes (aspartate transaminase, alanine transaminase and alkaline phosphatase) of Wistar albino rats. The findings would help to ascertain the safe use or otherwise of *Yaji* meat sauce.

## Materials and methods

*Enzyme kits*

Enzyme kits for AST and ALP were obtained from Randox Laboratory Ltd., San Francisco, U. S. A.

*The spices*

The local meat sauce and its constituent spices - clove, ginger, garlic and red pepper were purchased from Slaughter market, Trans-Woji road, Port Harcourt, Nigeria. A hand-grinding machine was used to grind the spices into powdered form.

*Animals and Substance administration*

A total of thirty seven (37) wistar albino rats, average weight of 74 g, obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria were used for this study. The rats

were allowed to acclimatize for 3 weeks. Their normal feeds were purchased from Moboh Gospel farms Limited, Choba, Port Harcourt, Nigeria. The experimental animals were divided into seven groups (A-G). The various groups involved in the study are defined in Table 1. The groups were: A (normal feed), B (Mixture of clove, ginger, garlic and red pepper), C (*Yaji*), D (Clove), E (Ginger), F (Garlic) and G (Red pepper). Group A had seven rats and served as control while groups B-G had five rats each and constituted the test groups. The amount of spices administered to each of the test groups was determined by the average body weight of the experimental animals. A dose of 4 g of spices for a 70 kg adult as recommended by the Organic Herbs Incorporated (2012) was adjusted to suit the average weight of the experimental animals in the various test groups. The administration of spices was performed via mixing with feeds. Two rats (A*) from the control group were sacrificed at day-0 while two rats from each group were sacrificed at the 14$^{th}$ and 21$^{st}$ day respectively.

*Sample collection*

The rats were anesthetized with chloroform and dissected for blood collection. The blood was collected into lithium heparin bottles and EDTA bottles and analysis performed within one (1) h of collection. Before assays, the blood samples were centrifuged for 5 min using a bench-top centrifuge (MSE-Minor) and the supernatant plasma was then used for the determinations.

*Enzyme assays*

Aspartate amino transferase activity of the plasma was determined at 37 $^{o}$C using the Randox kit by monitoring the amount of oxaloacetate hydrazone formed in the presence of L-aspartate, α-oxoglutarate and 2,4-dinitro phenyl hydrazine as described by Reitman and Frankel (1957). Absorbance measurement was at 520 nm and the calculation of enzyme activity followed the directive of the kit manufacturer. Alanine transaminase (ALT) was assayed for using the Reflotron Dry Chemistry Analyser as described by Estridge *et al*., 2000. Alkaline phosphatase activity was measured at 37 $^{o}$C using the Randox kit as reported by Haussament (1977). The absorbance of p-nitrophenol formed from p-nitrophenyl phosphate was determined at 405 nm and calculation of enzyme unit followed as described by the kit manufacturer.

*Statistical analysis*

Results of all the studies are expressed as mean± standard deviation. Statistical analysis was carried out using analysis of variance (ANOVA). Data between groups were analysed using SPSS®: Version 16.0. $P<0.05$ versus respective control was taken as significant.

**Results**

The results for the effects of dietary intake of *Yaji* on average weights of the Wistar albino rats are shown in Table 2. No Significant ($p>0.05$) changes were observed in the body weight of the experimental animals in all the test groups at day-14 and day-21 as compared to day-0 values for each group. The results for plasma aspartate transaminase (AST) are presented in Table 3. Significant changes in AST were not observed in any of the test groups at days 14 and 21 respectively. For plasma alanine transaminase (ALT), the result as presented in Table 4 shows non-significant ($p>0.05$) changes all through the experimental period. Similarly, findings for plasma alkaline phosphatase (ALP) enzyme shown in Table 5 indicates no significant ($p>0.05$) changes in any of the test groups. Non-significant ($p>0.05$) increases were recorded in all of the test groups at day-14. As compared to control values at day-21, non-significant increases were also recorded in all other test groups with the exception of the garlic and red pepper-fed group.

**Discussion**

*Body weight of test animals*

The experimental rats in all the groups were observed to show no significant ($p>0.05$) increases in body–weight on the 14$^{th}$ and 21$^{st}$ day as compared to day-0 values for each of the experimental groups. This observation contrasted an earlier report by Nwaopara

*et al.* (2007b), where changes in weight were recorded in experimental rats fed with diets containing *Yaji*. However, findings in the present study seemed similar to the study by Nwaichi and Igbinobaro (2012) where the weight of test animals after administration of *Tetrapleura tetraptera*, *Piper guineense*, and *Xylopia aethiopica* showed no significant difference between the mean of the experimental and control groups.

**Table 1.** The Experimental groups.

| GROUP | SPICE(S) IN FEEDS RECEIVED (for 21 days) |
|---|---|
| A (CONTROL) | Normal feed |
| B | Mixture of clove, ginger, garlic and red pepper |
| C | Yaji |
| D | Clove |
| E | Ginger |
| F | Garlic |
| G | Red pepper |

**Table 2.** Average weights (g) of the experimental animals.

| Group | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 0-Day | 56.00±8.22 | 115.00±13.69 | 100.0±0.00 | 80.00±11.18 | 58.00±4.47 | 66.00±10.25 | 63.00±12.55 |
| 14th-day | 91.70±14.40 | 120.00±11.18 | 114.00±15.17 | 106.20±12.50 | 105.00±11.18 | 106.20±12.50 | 93.75±23.94 |
| 21st day | 112.50±17.68 | 141.70±14.43 | 117.30±13.28 | 125.00±0.00 | 116.70±14.43 | 125.00±0.00 | 112.50±17.68 |

Values are Mean ± SD.

*Plasma enzymes of experimental animals*

The findings of non-significant ($p>0.05$) changes in the plasma enzymes - AST, ALT and ALP in any of the test groups is in agreement with an earlier study on hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract by Oduola *et al.* (2001) who noted that values of ALT, AST, and ALP obtained for the study groups showed no statistical significant differences ($p>0.05$) between the study and control animals. The present report, however, contrasted an earlier report by Ajayi *et al.* (2009) who established that treatment with *Allium Sativum* (garlic) produced significant increase in plasma AST level.

**Table 3.** Activity levels of serum aspartate transaminase (AST) (IU/L).

| Group | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 0-Day | 12.00±1.41 | | | | | | |
| 14th-day | 11.00±2.83 | 10.50±4.95 | 11.50±2.12 | 13.00±2.83 | 11.50±2.12 | 11.00±0.00 | 10.00±0.00 |
| 21st-day | 10.00±1.41 | 8.500±0.71 | 8.500±0.71 | 8.500±0.71 | 10.00±4.24 | 7.50±0.71 | 9.50±2.12 |

Values are mean ± SD.

According to Stockham (2002), mechanisms of increased activity of ALP in serum include enzyme release from damaged cells or induction of enzyme activity (increase enzyme synthesis) from drug administration. Incidentally, the spices under study have been found to have similar properties with certain drugs, for example, steroidal contents as found in red pepper and black pepper (Wesołowska *et al*, 2011); analgesic properties as found in ginger (Raji *et al.*, 2002); and anti-inflammatory properties as found in cloves (Agbaje, 2009). Since no significant ($p>0.05$) increases were noticed in the plasma enzyme activities, it shows that the spices, within the administered doses administered, are not capable of causing hepatocellular damage or inducing enzyme activity.

**Table 4.** Activity levels of serum alanine transaminase (ALT) (IU/L).

| Group | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 0-Day | 10.00 ±1.41 | | | | | | |
| 14th-day | 9.50±2.12 | 10.00±2.83 | 11.50±0.71 | 10.00±1.41 | 9.50±2.12 | 9.00±0.00 | 10.5±0.71 |
| 21st day | 9.00±1.41 | 13.00±0.00 | 9.00±2.83 | 8.50±0.71 | 9.50±2.12 | 8.50±0.71 | 8.00±0.00 |

Values are mean ± SD.

**Table 5.** Activity levels of serum alkaline phosphatase (ALP) (iu/l).

| Group | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 0-Day | 9.35 ±4.12 | | | | | | |
| 14th-day | 9.20±0.00 | 9.35±0.65 | 10.27±0.66 | 9.97±1.07 | 10.43±0.44 | 9.66±1.53 | 9.66±1.53 |
| 21st day | 9.20±0.44 | 11.04±0.44 | 9.97±1.09 | 9.97±0.21 | 9.35±0.21 | 8.58±0.00 | 7.98±0.42 |

Values are Mean ± SD.

More interestingly, the dose level of the constituent spices of *Yaji* as administered to the Spice Mixture–fed group far exceeds what the normal daily values would have been, as well as, the amount usually consumed as *Yaji* . The significance of this is that the use of *Yaji* (a formulation from clove, ginger, garlic and red pepper) is safe for consumers.

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