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Assessment of the effect of Yaji on the liver profile of Adult Albino Wistar Rats

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Abstract

The excessive consumption of *Yaji* and its additives in Nigeria has raised a growing concern especially as it remains the sauce for the meat delicacy called *'Suya'*, leaving the liver, the main organ of metabolism at risk.This six-week study was designed to assess the effects of Yaji and its additives on Liver Profile of adult albino Wistar rats. The animal subjects(40) were divided into four groups (A-D) of two (2) subgroups (n=5). The first three weeks served as the acute treatment period (B1 D1), while the first and second three weeks (six weeks) served as the chronic treatment period (B2 D2).At the end of each three weeks, blood samples were collected and enzyme activities of serum AST, ALT, ALP, and GGT, levels of TP, ALB and GLO were assayed by colorimetric methods. The results showed that in the acute treatment period (3 weeks duration), serum AST, ALP, GGT activities were significantly increased ($p< 0.05$) in group D1 (AST and GGT), and in all the groups (ALP) while there was a non-significant increase ($p>0.05$) in serum ALT activity of the other groups, also the total protein, albumin and globulin levels were significantly increased ($p< 0.05$) in all the groups when compared with the respective controls. Furthermore, the chronic treatment period (6 weeks duration), the serum AST and ALT activities were significantly increased ($p< 0.05$) in group C2 and D2 (AST) and in group D2 (ALT). The ALP activity was significant ($p< 0.05$) in all the groups, while the GGT was increased significantly ($p< 0.05$) in group B2. However, no significant changes were observed in the total protein, albumin and globulin levels. It was concluded from this study that *Yaji* is dosage and duration dependent (3 and 6 weeks duration), has the potentials to induce liver damage considering the changes in serum activities of the liver enzymes. Therefore, its consumption should be controlled.

INTRODUCTION

For decades, food additives have remained a major constituent of our diets, despite the controversies about the risks and benefits of additives coupled with their known hazardous effects [1,2] . In Nigeria, one of such commonly consumed food items with several spices and additives is the *Suya* meat sauce called [3].

Yaji is a complex mixture of groundnut cake powder, additives, spices and salt [4] . The spices in it are ginger, cloves, red pepper, and black pepper [5] . These spices contain gingerol [6] , eugenol [7] , capsaicin and piperine [8] as active components respectively. The other three constituents: white magi (or Ajinomoto), salt and groundnut cake powder, contain monosodium glutamate [9] , sodium chloride [10] and oil [11] as active components respectively.Historically, the name *'Yaji'* was an adaptation of the name of a 14th century Hausa ruler called "Yaji (meaning the 'hot one')" [3] . Considering the chemical potentials of its constituents, particularly in combination, there is indeed a growing concern about its excessive consumption especially as it remains the sauce for the meat delicacy called *'Suya'* [5]. [12] described *Suya* as a mass consumer fast food whose preparation and sales along streets are usually not done under strict hygienic conditions because they are still done locally. Moreover, many young men and ladies including children show great appetite for *Suya* and *Yaji* due to its pleasant taste and they strive hard to buy *Suya*. This trend amongst the young, agrees with the findings of [13] that: "habits are generally strong for animal foods but are more flexible for other foods". Thus, fears have been expressed with regard to the excessive consumption of *Yaji*, especially, the non-standardized mode of production, the high consumption rate, the large consuming population, and the possibility of contaminants like aflatoxin [5].

In fact, there is documented evidence that these active components have excitotoxic, apoptotic and tumourigenic potentials [14- 24].Also the effects of *Yaji* spices on the histology of the liver of adult rabbits have been reported. The histological observations presented necrosis of the liver hepatocytes and therefore initiates acute hepatitis [25] .Along this line of thought therefore, the aim of this study is to assess the effect of *Yaji* on liver profile of adult albino Wistar rats as research models.

MATERIALS AND METHODS

Experimental Animals/Housing Conditions:40 Adult Albino Wistar rats of comparable weight (150 to 300g) were bought from the animal farm, Anthonio Services Nigeria, Ekpoma, Edo State and moved to the experimental Laboratory Anthonio Research Center at No. 40, Ujoelen Extension, Ekpoma, where they were allowed to acclimatize for three weeks. During the period of acclimatization, the rats were fed with growers mash (25kg) daily from Grand Cereals limited, a subsidiary of UAC of Nigeria Plc, km 17, Zawan Roundabout Jos, Plateau State, and water *ad libitum*. The animals were kept in wire cages with tripod that separates the animals from its faeces to prevent contamination and were also maintained and utilized in accordance with the standard guide for the care and use of laboratory animals [26] .

Groupings/Substance Administration: The experimental animals were divided into four groups (A, B, C and D), using 4 big cages, (N=40) each group has 2 subgroup, that is group A (subgroup A1 and A2), group B (subgroup B1 and B2), group C

(subgroup C1 and C2) and group D (subgroup D1 and D2), each group has a total of 10 rats, with n=5 in each subgroup. The first subgroup (A1, B1, C1 and D1) represent the acute treatment period (3 weeks), while the second subgroup (A2, B2, C2 and D2) continued for the remaining 3 weeks as the chronic treatment period (6 weeks). Group A (A1 and A2) served as the control, while groups B-D (B1, C1, D1and B2, C2, D2) served as the test groups for the 3 and 6 weeks respectively.

The rats were weighed before the administration of the spices and just before they were sacrificed. The spices were given orally each day between 8am 10am at different dosage and duration as follows:

Group A (n=10)

- Subgroup A1 (n=5) received 100g of growers' mash only for 3 weeks.
- Subgroup A2 (n=5) received 50g of growers' mash only for the remaining 3 weeks (6 weeks).

Group B (n=10)

- Subgroup B1 (n=5) received 20g of *Yaji* plus 80g of growers' mash for 3 weeks.
- Subgroup B2 (n=5) received 10g of *Yaji* plus 40g of growers' mash for the remaining 3 weeks (6 weeks).

Group C (n=10)

- Subgroup C1 (n=5) received 40g of *Yaji* plus 60g of growers' mash for 3 weeks
- Subgroup C2 (n=5) received 20g of *Yaji* plus 30g of growers' mash for the remaining 3 weeks (6 weeks).

Group D (n=10)

- Subgroup D1 (n=5) received 60g of *Yaji* plus 40g of growers' mash for 3 weeks
- Subgroup D2 (n=5) received 30g of *Yaji* plus 20g of growers' mash for the remaining 3 weeks (6 weeks).

Water was also given *ad libitum* during the period. Test feed was produced by mixing appropriate quantities of *Yaji* species and feed with sprinkles of water to form a paste.

Study Duration: The animal's acclimatization, ingredients procurement/*Yaji* production, actual animal experiment and chemical analysis lasted for a period of five months. The *Yaji* sample was administered to the test animals for periods of 3 weeks and 6 weeks, respectively.

Substance of study: Normally, the production of *Yaji* is not standardized as regard the quantities of each ingredient used. However, for this study the mixture preparation was as represented by [27] . The measured quantities include: Ajinomoto (150 g), black pepper (30 g), clove (39g), ginger (78 g), and groundnut cake powder (230 g), red pepper (22 g) and salt (100 g). The total weight of these constituents summed up to 649 g. The constituents were purchased at Hausa quarters, Ekpoma Market, Edo State, and at Nkpor Market, Onitsha, Anambra Sate, Nigeria, and subsequently mixed together in powdery forms as directed by the dealers.

Sample collection: Five milliliters of blood samples were collected from the rats at the end of 3 and 6 weeks under mild anesthesia using chloroform, from the jugular vein and dispensed into lithium heparin and EDTA containers labelled appropriately. This was centrifuged at 3000rpm for 5minutes and the plasma separated and stored at -20°C for subsequent analysis.

Sample analysis/Methods: The aminotransferases (AST and ALT), alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) activities in the samples were estimated by the method described by [28] , and IFCC. Total protein level was estimated using the Biuret method by [28] , Albumin level using the Bromocresol green (BCG) method described by [28] , while Globulin level was estimated indirectly by subtracting the albumin concentration from total protein concentration [29] .

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 values $p<0.05$ were considered statistically significant.

RESULTS

The results for the acute treatment period (3 weeks duration) showed a progressive increase in the serum AST activity, however, was significant ($p<0.05$) only in group D. Also, there was a significant ($P<0.05$) progressive increase in serum ALP activity in all the groups, while GGT activity increases but was significant ($p<0.05$) in group D. Furthermore, levels of TP increased significantly ($P<0.05$) in all the groups,except for group C that posted a non-significant decrease in ALB level ($p>0.05$), groups B and D posted a significant increase ($p<0.05$).Globulin (GLO) level was found to increase progressively, but was significant ($p<0.05$) in groups C and D. However, *Yaji* did not cause any significant change in the activity of serum ALT even though there was a progressive increase as compared to controls (see Table 1).

In the chronic treatment period (6 weeks duration), the results showed a progressive increase in serum AST and ALT activities which was significant ($p< 0.05$) in group D respectively. In addition, serum ALP activity was significantly increased ($P<0.05$) in all the groups, while serum GGT activity was significantly increased ($p<0.05$) only in group B. Finally, *Yaji* did not cause any statistically significant difference in the levels of TP, ALB and GLO in this stage but there were progressive high levels except for groups B and C that showed a decrease in GLO level (Table 2).

DISCUSSION

The results of this study showed *Yaji* to induce variations in liver enzymes activities and by implication liver function in a dosage and duration fashion.

In the acute treatment period (3 weeks duration), the observed significant difference in the AST activity as compared with control suggests liver injury, increase serum activity generally indicates enzyme leakage from the cytoplasm and mitochondria as a result of tissue damage [30] ,and possible cardiac insult, since AST was defined as a bio-chemical marker for the diagnosis of acute myocardial infarction [31] .

This contradicts the earlier report by [27] , considering the carcinogenic and neurotoxic effect of groundnut cake; which is predicated upon the fact that dietary oil rich in polyunsaturated fatty acids is susceptible to oxidative changes during use like frying as in fried ground cake powder in *Yaji* [32] resulting in the

Table 1. Serum AST, ALT, ALP, GGT activities, levels of TP, ALB, and GLO in tests of the acute treatment period (3weeks duration) compared with controls.

		TEST GROUPS		
PARAMETERS	**CONTROL (n=5)**	**B (n=5)**	**C (n=5)**	**D (n=5)**
AST (U/L)	45.50±16.96	49.40±23.83	57.29±9.85	71.55±15.29[a]
ALT (U/L)	12.44±5.01	13.56±4.68	22.37±18.87	22.89±10.58
ALP (U/L)	73.00±15.13	116.6±10.81[a]	132.40±73.93[a]	133.80±15.88[a]
GGT (U/L)	2.22±1.55	4.22±1.39	5.50±3.59	6.80±4.40[a]
TP(g/dl)	67.83±4.84	75.01±3.80[a]	75.37±3.74[a]	79.71±7.53[a]
ALB(g/dl)	36.08±2.96	41.16±1.93[a]	36.90±1.77	41.14±3.56[a]
GLO(g/dl)	31.75±3.39	33.85±1.58	38.47±5.10[a]	38.57±5.42[a]

Values of the test groups with superscript (a) are significant ($p<0.05$) as compared with control

Table 2. SSerum AST, ALT, ALP, GGT activities, levels of TP, ALB, and GLO in tests of the chronic treatment period (6weeks duration) compared with controls.

		TEST GROUPS		
PARAMETERS	**CONTROL (n=5)**	**B (n=5)**	**C (n=5)**	**D (n=5)**
AST (U/L)	50.51±14.44	65.80±23.79	72.36±16.75[a]	79.94±17.40[a]
ALT (U/L)	15.45±6.55	20.80±7.76	22.67±8.41	28.79±7.29[a]
ALP (U/L)	80.80±32.81	120.20±18.42[a]	136.00±20.49[a]	40.08±16.36[a]
GGT (U/L)	2.70±2.24	6.68±2.63[a]	3.92±1.76	3.38±0.91
TP(g/dl)	76.59±4.68	75.21±1.47	74.64±12.04	84.17±10.17
ALB(g/dl)	40.72±1.83	42.95±4.56	40.94±6.39	38.59±5.50
GLO(g/dl)	35.87±4.85	32.26±4.87	33.70±6.70	43.58±10.47

Values of the test groups with superscript (a) are significant ($p<0.05$) as compared with control

formation of peroxides, aldehydes, ketones, aldehydroesters and ozonides known to be injurious to the cardiac cells [33-37] ,also black pepper and monosodium glutamate in Ajinomoto in agreement with the earlier report of [38] and [3] , the high salt intake increases the risk of high blood pressure [39] ,

The significant difference in the levels of ALP of all the groups as compared with the control indicated liver and bone diseases. [45] reported that an elevation in serum ALP activity commonly originate from the liver and bone, in liver disease involving cholestasis of both intra and extra hepatic origin, an increase ALP is observed, and also in other causes of hepatic infiltration. Moderate elevation in liver enzymes is usually an indication of liver cirrhosis with some hepatitis as a result of toxic substances [41] .Also, the significant difference in the GGT level of group D as compared with control suggests hepatobiliary disease [42] . GGT is the most sensitive enzymatic indicator of hepatobiliary disease available. It is highest in cases of intrahepatic or posthepatic biliary obstruction [42] .Furthermore, the observed significant changes of TP in all the groups, ALB levels in groups B and D and GLO levels in groups C and D as compared with their respective controls are indications of a non-prolonged dehydration as stated by [43] that hyperalbuminaemia, an indicative of high levels of total protein is almost always caused by dehydration. The results obtained in the chronic treatment period (6 weeks duration) showed a significant

difference in the AST activity of groups C and D which confirm the assertion of liver and cardiac assaults in the acute treatment period. This period likewise showed a significant difference in the ALT activity of group D as compared with control which also points to liver damage [44] .ALT rises dramatically in acute liver damage, such as viral hepatitis or paracetamol (acetaminophen) overdose [45] . Several drugs elevate ALT levels; for example, [46] . This implicates ginger [47] , red pepper [48] , and clove [49]; [7]; [50] that possess properties as these drugs. This was not seen in the acute treatment period probably due to the study duration and dosage.

The ALP activity showed also a significant increase in all the groups, a confirmation of hepatic and bone disorder in the acute treatment period which progresses for a prolonged duration and dosage. However, the decrease in the levels of TP, ALB and GLO in this period is an indication of liver injury when the fact of [51] , is considered, that in cirrhosis, hepatic synthesis of albumin decreases. By implication, the reduction in the levels of albumin, total proteins and globulin with chronic ingestion of *Yaji* may signify cirrhosis of the liver.

These changes and variations in liver enzymes activities are not unexpected considering the fact that the liver is involved in the metabolism of substances ingested by man [52] . Considering the changes in enzyme markers and liver proteins, this study therefore agrees with the earlier reports by [5,25] , and [53] , that the excessive consumption of *Yaji* induce some lymphoid follicular aggregation in portal tracts, ballooning degeneration of hepatocytes and patchy necrosis of hepatocytes which are symptoms of acute hepatitis, such occurrence is accompanied by marked elevation of AST and ALT [54] as observed in this study. The broad groups of drugs and other substances that are capable of inducing such a liver damage includes: ethanol, steroids, psychotropic agents, analgesic, anti-inflammatory agents, anti-convulsants, anti-metabolites and immunosuppressive drugs, antibiotics, anesthetic agents, drugs used in the treatment of diabetes mellitus, endocrine disorders and cardiovascular disorders, industrial agents and toxins [55] . Incidentally, the spices under study have been found to have steroidal contents as found in red pepper and black pepper [56] ; as anti-inflammatory agent as found in clove [7] ; as an antibiotic as found in red pepper [56] ; with antitumorigenic, hypoglycaemic effects, analgesic properties and in the treatment of cardiovascular disorders as found in ginger [57,47,58,59] for prevention of toxicity from environmental pollutants like carbon tetrachloride, digestive tracts cancer and joint inflammation as in the case of eugenol of cloves [7] . These scientific evidences shows that the spices under study possess some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing liver damage and thus, explains their capability to effect the histological changes observed. Hence, the biochemical results of this study are highly justified.

Worthy of note in this study, is the dose and duration dependent effect of *Yaji* considering the changes in the acute and chronic treatment periods which are in line with previous studies by [5,25,27,60] and [61-63] that the effects of *Yaji* are dosage and duration dependent.

CONCLUSION

Conclusively, the findings of this study suggest that *Yaji* has the potentials to induce liver damage in a dose and duration dependent fashion, considering the changes in serum activities of AST, ALT, ALP, GGT, and levels of TP, ALB and GLO during the 3 and 6 weeks durations.

REFERENCES

1. Moore, L.K.,: Developing Human. 2nd. Philadelphia. W.B. Saunders co. Ltd. 2003; Pp. 173-183.

2. McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E., Lok, K., Porteous, L., Prince, E., Sonuga-Barke, E., Warner, O.J. and Stevenson, J. : Food additives hyperactive behaviour. *Lancet.*5. 2007

3. Betumiblog , Kuli-Kuli is and buy. Retrieved from *www.betumi.com.* 2006

4. Okonkwo, T.M. About Suya and Yaji.*J. Food Agric.* 19871(1) 51.

5. Nwaopara, A.O., Anyanwu, L.C., Oyinbo, C.A. and Anaikot, I.C. The Histological Changes in Pancreas of Wister Rats Fed with Diets containing *Yaji* (Local meat Sauce). *J. Expt. Clin. Anat.* 2004;3(2): 44- 47.

6. Witchtl, M. : Herbal Drugs and Phytopharmaceuticals 3rd ed. Boca Raton FL: CRC press pp. 2004;653-656.

7. Krishnaswamy, K. and Raghuramulu, N. : Bioactive phytochemicals with emphasis on dietary practices. *Indian J. Med. Res*. 1998; 108:167-81.

8. McGee H : On food and Cooking: The Science and Lore of the Kitchen. New York, Scribner. 2004; Pp. 427-429.

9. Omojola, A.B. : Yield and organoleptic characteristics of *Suya* (an intermediate moisture meat) prepared from three different muscles of a matured bull. *African J. Biotech*. 2008 7(13): 2254-2257.

10. Carson, S.H., Osborn, J.W. and Wyss, J.M. : Hepatic innervations chronically elevate arterial pressure in wistar-Kyoto rats. *Hyperten.* 1998; 32: 46-51.

11. Fageria, N.K., Balgar, V.C. and Jones, C. : Growth and mineral nutrition of field crop. Marcel Dekker, Inc, New York. 1997;Pp. 494.

12. Uzeh, R.E., Ohenhen, R.E. and Adeniji, O.O. : Bacterial Contamination of Tsire-Suya, a Nigerian Meat Product. *Pak. J. Nutr*. 2006; 5(5): 458-460.

13. Parry, E.H.O. Principle of Medicine for Africa. Ibadan, Oxford University Press1978;. Pp: 36.

14. Choi, D. : Glutamate neurotoxicity and diseases of the nervous System. *Neuron* 1988;1: 623-634.

15. Olney, J. : Glutamate, a neurotoxic transmitter. J. *ChildNeurol.* 1989; 4:218-226.

16. Whetsell, W. and Shapira, N. : Biology of disease, neuroexcitation, excitotoxicity and human neurological disease. *Lab Invest*. 1993;68: 372-387.

17. Lipton, S. and Rosenberg, P. Excitatory amino acids as a final common pathway for neurologic disorders. 1994;*NEJM.* 330: 613-622.

18. Blaylock, L.R. : Excitotoxins, the taste that kills. Santa Fe, N.M., Health press1997; Pp. 248- 254.

19. Olney, J.W., ozniak, D.F. and Farber, N.B. : Excitotoxicneurodegeneration in Alzheimer's disease. *Arch.Neurol*. 1997;54:1234-1240.

20. Ankarcrona, M., Dypbukt, J.M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S.A. and Nicotera, P. : Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. *Neuron*1998;. 15: 961-973.

21. Sugimoto, T., Xiao, C. and Ichikawa, H. : Neonatal primary neuronal death induced by capsaicin and axotomy involves an apoptotic mechanism. *Brain Res*1998;. 807(1-2): 147-154.

22. Martin, L.J., Sieber, F.E. and Traystman, R.J. : Apoptosis and necrosis occur in separate neuronal populations in hippocampus and cerebellum after ischemia and are associated with differential alterations in metabotropic glutamate receptor signaling pathways. *J. Cereb Blood Flow Metab*. 2000;20: 153-167.

23. Rothstein, J.D. and Brem, H. Excitotoxic destruction facilitates brain tumor growth. 2001; *Nat. Med*. 7: 994-995.

24. Bellamy, M. (2008): Eliminate MSG to greatly improve your health. Retrieved 10th October, 2009 from *http://nursinglink.monster.com/news/articles/6427022013.*

25. Nwaopara, A.O., Odike, M.A.C., Inegbenebor, U. and Adoye, M.I. The Combined Effects of Excessive Consumption of Ginger, Clove, Red Pepper and Black Pepper on the Histology of the Liver. *Pak. J. of Nutr*. 2007;6 (6): 524-527.

26. Richard, L and Crawford, D.V.M. (2012): Animal welfare. In: Animal Welfare Act Quick Reference Guides. Animal welfare information centre with Virginia-Maryland Regional College of Veterinary Medicine. Title 9 code of Federal Regulations (CFR), chapter-1, subchapter A.

27. Nwaopara, A. O., Anibeze, C. I. P., Akpuaka, F. C. : Histological Signs of Oligodendroglioma in the Brain of Rats Fed With Diet Containing Yaji: The Complex Nigerian Suya Meat Sauce. *J. Clin. Rev. Opin*. 2009; 1(2): 21-25.

28. Reitman, S. and Frankel, S. : Randox Laboratories Limited. *Amer. J. Clin. Path.* 1957; 28:56.

29. Ochei, J. and Kollhatkar, A. : Medical Laboratory Science: Theory and Practice. Tata McGraw-Hill Publishing Company, New Delhi. 2000; Pp.152-159.

30. Kuramitsu, S., Okuno, S., Ogawa, H. and Kagamiyama, H. : Aspartate aminotransferase of *Escherichia coli:* Nucleotide sequence of the asp. C gene.*J. Biochem.* 1985; 97(4): 1259-1262.

31. Gaze, D.C The role of existing and novel cardiac biomarkers for cardioproetction. *Curr.Opin. Invest. Drugs*2007; 8(9): 711-717.

32. Ologan, F.O. : Some physicochemical properties of thermally oxidized groundnut oil and their toxicological effects on selected rat tissues. Ph.D. Thesis, University of Ilorin, Ilorin, Nigeria. 2002

33. Frankel, E.N.,. Lipid Oxidation.Prog. Lipid Res., 1980; 19: 1-22.

34. Halliwell, B. and Gutteridge, J.M.C. : Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet, 1984; 1: 1396-1398.

35. Addis, P.B. Occurrence of lipid oxidation products in food. Food Chem. Toxic. 1986; 24: 1021-1030.

36. Kubow, S. : Route of formation and toxic consequences of lipid oxidation products in food. Free Rad. Bio. Med., 1992; 12: 63-81.

37. Odutuga, A. A., Obaleye, J.A. and Ologan, F.O. : Hermoxidied Soybean oil: Spectroscopic investigation and effects on selected rat tissues. Biokemistri, 1997; 1: 45-58.

38. Moses, S. and Sefeckora, Z. : Obesity and changes of alkaline phosphatase activity in the small intestine of 4080 days old rats subjected to early postnatal over feeding of monosodium glutamate. *Physiol. Res.* 2004;53 (2): 177-186.

39. Strazzullo, P., D'Elia, L., Kandala, N. B., Cappuccio, F.P. Salt intake, stroke and cardiovascular disease: Meta-analysis of prospective studies. *Biomedical J*2009;339:4567.

40. Donald, W., Moss, A., Ralph, H., John, F. and Kachmar, D. : Enzymes. In: Tietztextbook of Clinical Chemistry. 3rd Edition.Ashwood, J. and Burtis, C. Saunders. Philadelphia. 1987;Pp. 385-391.

41. Negredo, E., Mellon, L. and Carr, A. : Bone mineralization density (BMD) in HIV infected patients. *Retrovir.Human. Hel.* 2001; 13:232-245.

42. Berk, G. and Korenblat, A. : Plasma gamma-glutamyltranspeptidase elevation in patients receiving enzyme-inducing drugs. *Lancet.*2 (7720): 2007; 376-377.

43. Guall, H., Wright, C.E. and Gaull, G.E. : protective effect of taurine, zinc and tocopherol on retinol-induced damage in human lymphoblastoid cells. *J. Nutr.* 2004;114(12): 2256-2261.

44. Omata, M., Johnson, C.S., Tong, M.J., Simmons, J.F., Weiner, J. and Tatter, D. : The pathological spectrum of liver disease in sickle cell disease. *Dig. Dis. Sci.* 1986; 31: 247-257.

45. Schmidt, E.: Enzyme Biol. *Clin. Chem*1993;. 3:1

46. Paul, T. and Giboney, M.D. : Mildly elevated liver transaminase levels in the asymptomatic patient. *Am. Family Physician*2008;.32(1):33-37.

47. Metz, C. and Cupp, M. Toxicolgy and clinical pharmacology of Herbal products. Totowa, New Jersey: Humana press. 2000

48. Coleridge, H.M., Coleridge, J.C. and Luck, J.C. : Pulmonary afferent fibres of small diameter stimulated by Capsaicin and by hyperinflation of the lungs. Clin.Ter. 1977; 83: 71-83.

49. Fortin, F. (1996): Editorial Director, The visual foods Encyclopedia. New York, Macmillan.

50. Ghelardini, C., Galeotti, N. and Cesare, M. L. : Local anesthetic activity of beta-caryophyllene. Farmac O2001; 56: 387-9.

51. McGlynn, K., Rosvold, E., Lustbade, E., Hu, Y., Clapper, M. and Zhou, T. : Susceptibility to hepatocellular carcinoma is associated with genetic variation in the protein denaturation of aflation. *Proc. Natl. Acad. Sci.* USA. 1995; 92:2384-2387.

52. Guyton, A. C. and Hall, J. E. : The Liver as an organ. In: Textbook of Medical Physiology. 11th Edition.John F. K. Elsevier Saunders.Philadelphia. 2011; Pp. 859-864.

53. Kumar, V., Abbas A.K. and Fausto, N. : Robins and Contra, Pathologic Basis of Disease. 2004; Pp. 345-356.

54. Aronson, J.K., Grahame, D.G. and Smith, G. : Liver diseases. In: Text Book of Clinical Pharmacology and Drug

Therapy. Oxford University Press. 1984; Pp. 24-35.

55. Giarelli, L., M. and Antoutto, G. : Colour Atlas of Pathology. United Kingdom, Butterworths. 1987; Pp. 260-264.

56. Saber, M.S.: Antimicrobial substances in certain members of Solanaceae detection of active principles in pepper plant1982;. Pp. 5

57. Chevalier, A. (2000): Encyclopedia of Herbal Medicine Dorling-Kinderesly, London.

58. Ody, P. (2000): Complete Guide to Medicinal Herbs 2nd Ed. London. Dorling-kinderesly.

59. McCann, J. (2003): Herbal medicine Handbook 2nd Ed. Philadelphia: Linppincott.

60. Nwaopara, A.O., Odike, M.A.C., Inegbenebor, U., Nwaopara, S.O. and Ewere, G.I. : A comparative study on the effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the Kidney. *Pak. J. Nutr*. 2008; 7(2): 287-291.

61. Akpamu, U., Nwaopara, A. O., Izunya, A .M.,Oaikhena, G. A, Okhiai, O., Idonije, B.O. and Osifo, U.C. . A comparative study on weight changes in rats fed with diet containing *Yaji*, *Yaji*-additives and *Yaji*-spices. *Biol. and Med.* 2011;*3 (5): 06-15*

62. Akpamu, U., Nwaopara, A.O. and Oyadonghan G.P. (b): The effect of acute and chronic oral ingestion of yaji and yaji-additives on PCV, WBC and Differential WBC count. *Annals of boil. Res.* 2011; 2(6):9-15.

63. Akpamu, U., Nwaopara, A.O., Izunya, M.A., Oaikhena, A.G, Okhiai, O., Idonije, O.B., Osifo, U.C. : A Comparative study on the acute and chronic effect of oral administration of Yaji (a complex Nigerian meat sauce) on some haematological parameters. *British J. Pharm. Toxicol*2011;.3(2): 108-112.