

The Effects of Yaji Extract On Liver Enzymes of Carbon Tetrachloride Induced Hepatotoxicity In Adult Wistar Rats

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Abstract: - Effects of yaji extract on liver enzymes of carbon tetrachloride induced hepatotoxicity were studied. Twenty wistar rats weighing between 155-220g were used. They were allocated into four groups of five animals each. Group A animals served as the control and received 0.6ml of distilled water. Group B received 0.5ml of yaji extract, group C received 0.5ml of yaji extract plus 0.3ml of carbon tetrachloride while group D received 0.3ml of carbon tetrachloride. The oral administration lasted for twenty-eight days. Twenty-four hours after the last administration, the animals were weighed and sacrificed using chloroform. The Liver weight were recorded. The evaluation of the liver enzymes (AST, ALP,ALT) were carried out using randox kit method. The mean alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate aminotransferase (AST) levels of group D animals were significantly higher than the control.

Key words: - Yaji, Liver enzymes, Carbon tetrachloride, Body weight, Wistar rats.

I. INTRODUCTION

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' in Nigeria^[1].

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^[2].

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. It is a consumer fast food whose preparation and sales along the streets are usually not done under strict hygienic conditions because they are still done locally^[3].

It identified as a mass consumer fast food whose preparation and sales along streets are usually not done under strict hygienic environment and can serve as source of contaminants to the meat product^[4]. Suya as one of such intermediate moisture products that is easy to prepare and highly relished^[5].

Hepatotoxicity implies chemicals driven liver damage. Biochemical markers are often used to indicate liver damage. Liver damage is further characterized into hepatocellular and cholestatic types. Certain medicinal agents when taken in overdose and sometimes even when introduce within therapeutic range may cause liver injury. More than 900 drugs have been implicated in causing liver injury^[6], and it is the most common reason for drugs to be withdrawn from the market. Other chemical agents (hepatotoxins) such as those used in the laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals often cause sub-clinical injury to the liver which manifest only as abnormal liver enzyme tests. Carbon tetrachloride (CCl₄) is a well known hepato-destructive agent that is widely used to induce toxic liver injury in a range of laboratory animals^[7]. The hepatotoxicity of CCl₄ has been reported to be due to its biotransformation by cytochrome P450 system to produce trichloromethyl free radical (CCl₃) which readily reacts with molecular oxygen to form trichloromethyl peroxy radical^[8].

CCl₃OO which exert their action on lipids membrane of endoplasmic reticulum to evoke lipid per oxidation^[9]. Therefore, there is need to search, evaluate, and scientifically validate the activities of medicinal plants.

The aim of this study is to evaluate the effect of yaji extract on carbon tetrachloride induced hepatotoxicity in the liver enzymes (AST, ALP, and ALT) of adult wistar rats.

II. MATERIALS AND METHOD

PLANT COLLECTION

The yaji was prepared according to the method used by (2). 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 in Nnewi area of Anambra state in the month of June, 2013.

III. EXTRACTION OF PLANT MATERIAL

AQUEOUS EXTRACT

The constituents of yaji 649g was grinded using a ginger mill. The constituents were then macerated for 48hrs in 500ml of distilled water. The extract was strained through muslin and the filtrate then filtered through whatman No. 1 filter paper. The aqueous extract was concentrated on a rotary evaporator (Model type 349/2 Corning Ltd., England). The extractive value of the aqueous extract was 250mg/ml.

ANIMALS

Adult male wistar rats weighing 150-210g were obtained from the animal farm house, Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus. They were maintained under standard housing conditions and fed with standard rat chow (Growers mash) and provided with water ad libitum during the experiment. They were acclimatized for two weeks before the experiment.

EXPERIMENTAL DESIGN

Twenty (20) albino rats were divided into four (4) groups (A-D) of five animals each. Group A received 0.6ml of distilled water, Group B received 0.5ml of yaji extract, Group C received 0.3ml of CCl₄, Group D received 0.5ml of yaji extract plus 0.3ml of CCl₄ and administration lasted for 28 days. All administration was by oral route. Twenty-four hours after the last administration, the animals were weighed and sacrificed using chloroform. Liver tissue was collected, weighed and fixed in zenker's fluid for histological studies.

Twenty-four hours post CCl₄ administration, about 5ml of blood was collected from animals in all the groups by cardiac puncture under the ether anesthesia into a dry glass container. The blood was allowed to clot and sera separated from the cells and stored frozen until analyzed for liver enzymes. The animals were euthanized while still under anesthesia and their liver excised, washed in cold saline and fixed with 10% formal saline for histopathological studies.

MORPHOMETRIC ANALYSIS OF LIVER WEIGHTS

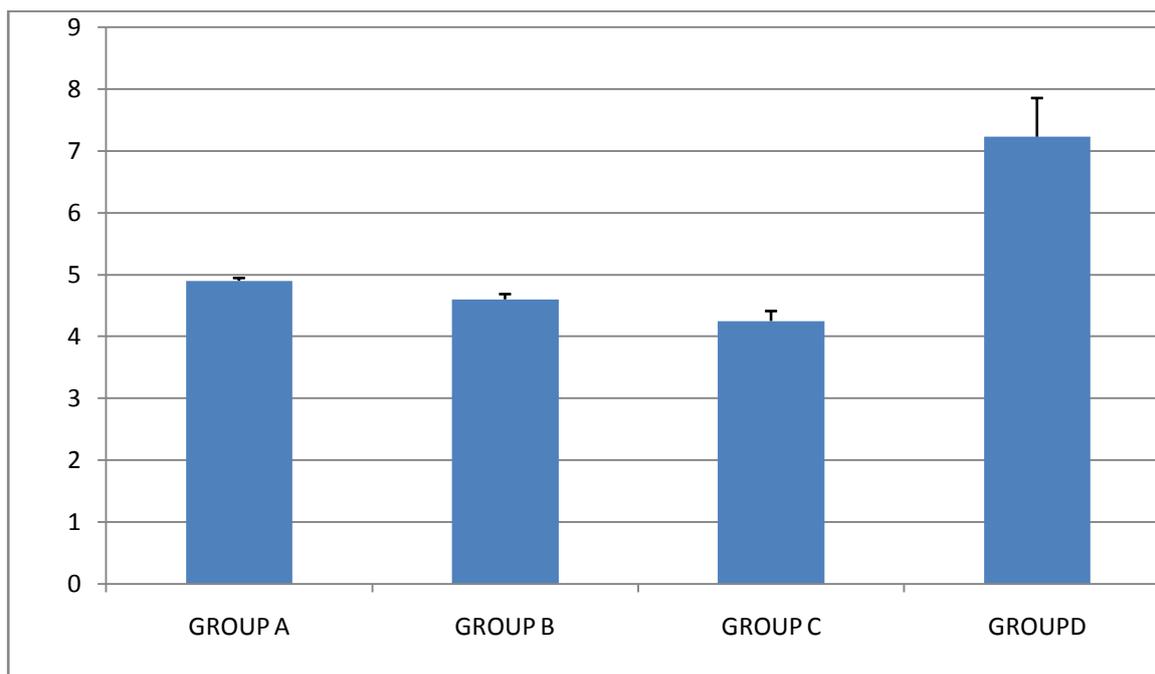
The results obtained from calculations of relative liver weight of the various groups are presented in table 1. The relative liver weight for group D (carbon tetrachloride administered) were significantly higher ($P < 0.001$) than that of the group A (control) and other experimental groups (B and C). The values for groups B and C were similar to the group A, (control).

Comparison of mean relative liver weight for group A, (control) and experimental groups (B, C and D)

Table 1 (Mean \pm SEM given for each measurement)

	GROUP A	GROUP B	GROUP C	GROUP D
LIVER WEIGHT	4.90 \pm 0.045	4.60 \pm 0.085	4.25 \pm 0.161	7.23 \pm 0.625

The bar chart representation of the relative liver weight of the various groups. The group D (carbon tetrachloride administered) were significantly higher ($P < 0.001$) than the control group (A) and groups B and C as shown in Figure 1.



Relative liver weight of different groups

Activities Of Serum Levels Of Aspartate Aminotransferase (Ast), Alanine Aminotransferase (Alt) And Alkaline Phosphotase (Alp)

Table 2 (Mean \pm SEM given for each measurement)

	G.P.A (CONTROL)	GP.B 0.55ML OF EXTRACT	GP.C 0.3ML OF CAROTENOI D	GP.D 0.41ML OF CCL ₄	F- RATIO	SIGNIFIC ANCE
AST	76.60 \pm 28.50	71.31 \pm 10.34	67.55 \pm 11.51	243.64 \pm 5.72	58.04	<0.0001
ALT	48.00 \pm 13.54	47.00 \pm 3.39	44.50 \pm 10.12	85.33 \pm 7.01	11.20	<0.0001
ALP	461.19 \pm 30.22	320.84 \pm 84.63	377.88 \pm 36.81	668 \pm 45.55	6.38	< 0.0012

From the results obtained from calculations of aspartate aminotrasferase (AST), alanine aminptransferase (ALT) and alkaline phosphotase (ALP), there were a significant decrease ($P < 0.001$) in the AST activity levels at all doses of the drugs relative to the control (A) except in group D treated with carbon tetrachloride (CCL₄). The group D activity level statistically were significantly higher ($P < 0.001$) than the control (A) and groups B and C.

The alanine aminotransferase (ALT) activity levels showed a significant decrease ($P < 0.001$) in groups B and C relative to the control (A) except in group D treated with carbon tetrachloride (CCL₄). The alkaline phosphotase (ALP) level in group D were significantly higher than the control group (A) and groups B and C.

The alkaline phosphotase (ALP) activity levels in groups B and C were significantly lower ($P < 0.001$) than the control (A). The alkaline phosphatase activity levels in group D were significantly higher ($P < 0.001$) than the control (A) and groups B and C.

IV. DISCUSSION

Yaji is composed of the spices- ginger, clove, red pepper, black pepper, white maggi (Ajinomoto) and salt ^[2] which on individual basis are known to be harmful. Some of the constituent exhibit antioxidant and hypolipidemic properties ^[10]. Others exhibit chemical, physiological and pharmacological properties ^[11], and are also capable of inducing tissue damage.

Our findings show that the extract has the potency to normalize the elevated liver marker enzyme levels and maintain the synthetic function of the when compared with the control.

This indicates restoration of the normal functional status of the liver. The significant elevations in the liver marker enzymes such as ALT, AST and ALP, as well as decrease in albumin levels in CCl₄ control when

compared with normal, suggest liver injury, since these are reliable indices of liver toxicity^[12]. Albumin is produced entirely by the liver and constitutes about 60% of the total serum protein. In liver damage, the synthetic capacity of the liver is reduced and consequently, syntheses of albumin, clotting factors and so on are affected. The mechanism of CCl₄ induced liver injury involves oxidative stress. Injury is through the free radical (CCl₃ and CCl₃ O₂) of its metabolism which may cause lipid peroxidation and subsequent injury^[13]. Several studies on extraranal lesions, such as hepatic cirrhosis and myelofibrosis have reported a close correlation between mast cells and fibrosis^[14].

The result of the study are summarized in table 2. The activities of aspartate aminotranferase (AST) and alanine aminotranferase (ALT) were determined in serum because analysis of liver function enzymes are used as indicators of biomedical changes in tissues in response to treatment^[15]. This result indicates a significant decrease in the aspartate to the control. This means that the group is not affected by the yaji received compared to the control. Therefore, extract of yaji combined with carbon tetrachloride suppressed the toxic effect of carbon tetrachloride on the liver tissues. Also, there was significant increase in alanine aminotranferase (ALT) activities which was similar to the control signify that the carotenoid was not toxic to the ALT enzyme. This decrease in the serum aspartate aminotranferase (AST) activity level observed in this study indicated that at the subcellular level, the liver cells may have been affected by the yaji since AST is found in the mitochondria, and cytoplasm. Also, the increase in serum alanine aminotranferase (ALT) level observed in this study indicated that the cytoplasm of the liver cells may have been affected by the drugs since ALT is found in the cytoplasm.

From the present study, the mean alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate aminotranferase (AST) levels of group D animals were significantly higher than the control. It is therefore recommended that further studies be carried out on the spleen and the kidney.

V. REFERENCES

- [1] Nwaopara, A.O., C.I.P. Anibeze and F.C. Akpuaka, (2010). Histological signs of neurodegeneration in the cerebrum of rats fed with diet containing *Yaji*: The complex Nigerian suya meat sauce. *Asian J. Med. Sci.*, 2(1): 16 -21.
- [2] Nwaopara A.O, L.C. Anyanwu, C.A. Oyinbo, I.C. Anaikot, (2004). The histological changes in pancreas of wister rats fed with diets containing *Yaji* (Local meat sauce) .*J. Exp. Clin. Anat.*, 3(2):44-47.
- [3] Igene JO, Mohammed ID (1983). Consumers' attitudes towards 'suya' meat product. *Ann. Borno*. 1. Retrieved 20th October, 2009 from <http://www.pjbs.org/pjnonline/fin512.pdf>.
- [4] Uzeh, R.E., R.E. Ohenhen and O.O. Adeniji, (2006). Bacterial contamination of tsire-suya, a nigerian meat product. *Pak. J. Nutr.*, 5(5): 458-460.
- [5] Omojola AB (2008). Yield and organoleptic characteristics of Suya (an intermediate moisture meat) prepared from three different muscles of a matured bull African *J. Biotech.* 7(13): 2254-2257.
- [6] Friedman SE, Grendell JH, McQuaid KR (2003): Current diagnosis and treatment gastroenterology. Lang medical Books/ McGraw-Hill New York pp: 664-679.
- [7] Reyes-Gordillo K, Segovia J, Shibayama, M, Vergara, P, Moreno MG, Muriel P (2007): Curriculum protects against acute liver damage in the rats by inhibiting NF-KB, proinflammatory cytokines production and oxidative stress. *Biochem Biophysics Acta.* 1770: 989-996.
- [8] Raucy JL, Kraner JC, Lasker (1993) : Bioactivation of halogenated hydrocarbons by cytochrome P450. *EI. Grit Review of Toxicology* 23:1-20.
- [9] Recknagel RO, Glende, EA, Dolk JA, Waller RL (1989):Mechanism of carbontetrachloride toxicity. *Pharmacological Therapy* 43: 139-54.
- [10] Manjunatha H, Srinivasan K (2008). Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental Rats. *Afr. J. Food Sci.* 2: 1-6.
- [11] Govindarajan VS, Sathyanarayana MN (1991).Capsicum production, Technology, Chemistry and quality. Part V. Impact on physiology, pharmacology, nutrition and metabolism: structure, pungency, pain and desensitization sequences. *Crit. Rev. Food Sci. Nutr.* 29: 435-474.
- [12] Omoniyi KY, Matthew CI (2006): Protective effects of *Zingiber officinale* against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats. *Phytotherapeutic Research.* 20:997-1002.
- [13] Sies H (1997) : Oxidative stress: Oxidants and antioxidants. *Experimental Physiology* 82 (2): 291-295.
- [14] Eddy, A.A., (2001a). Mast cells find their way to the kidney.*Kidney Int.*, 60: 375-377.
- [15] Key A., Anderson J.T., Grande F. (1957). Prediction of serum cholesterol responses of man to changes in fats in the diet, *lancet* 11. 959-966.