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Morpho-functional Changes in Liver due to Long Term Consumption of Instant Noodles in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author IS conducted the bench work. Authors AJO and IEO supervised and designed the experimental protocol. Author OMO wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Food is a basic requirement for the survival of living organisms, and is also necessary for growth, development, replacement and repairs of worn-out tissues, as well as energy production for various body functions, among others.
Aim: Current study investigated the histo-architectural and biochemical changes in the liver due to prolonged consumption of instant noodles by Wistar rats.
Methods: Fifty (50) adult Wistar rats of an average weight of 200 g were procured for the study. Following two weeks of acclimatization, the animals were randomly assigned into five (5) groups (A, B, C, D and E) of ten (10) rats each. Group A received cooked instant noodle (Type A) only; whereas, group B rats were fed with cooked instant noodles with its spice. Group C received

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cooked instant noodles (type B) only. Group D received cooked instant noodles type B with its spice while group E were fed with grower marsh (control). After thirty (30) days of administration of test substances, rats were sacrifice by cervical dislocation and liver tissue obtained for histological technique and bioassay of selected antioxidant enzyme levels [Catalase, Malonyldialdehyde (MDA), Glutathione Peroxidase (GPx) and superoxide Dismutase (SOD)]. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS version 21) to evaluate the result obtained. Histological section of liver of the treated animals showed cellular degenerative changes, hypertrophy, hepatic steatosis and mild fibrosis which were sparsely distributed as compared to the control group.

Results: Biochemical assay of oxidative stress markers revealed that MDA levels were significantly ($p < 0.05$) increased in all treated liver groups as compared with the parallel control animals. Concomitantly, the levels of SOD levels were found to be significantly ($p < 0.05$) decreased in the treated liver groups as compared with the control.

Conclusion: Thus, long consumption of instant noodles exert toxic effects by promoting oxidative stress in the liver tissue of adult Wistar rats.

Recommendation: Further study aimed at corroborating these findings should be carried out.

Keywords: Liver; instant noodles; histo-architecture; biochemical enzymes.

1. INTRODUCTION

Over the last two decades, noodles have become one of the most common and convenient foods in Nigeria. They are reportedly high in calorie (60%), with a market size of about 250,000t as of 2012 [1,2]. With just four brands released in 2006, today, the Nigerian market now boasts of up to 16 competing brands [3], and has hitherto become a house hold diet across Nigerian homes [4].

Available reports have shown the instant noodles to constitute primarily of wheat flour, vegetables oil, iodized salt, sodium polyphosphate, sodium carbonate, potassium carbonate, guargum, tartrazine and antioxidant tert-Butylhydroquinone (TBHQ) [5]. Its seasoning powder (spices) contains more of iodized salt, monosodium glutamate (MSG), sugar, hydrolysed vegetable protein, soy powder, pepper, garlic powder, chicken flavour and chili powder [5].

Recently, the MSG, which is a constituent of the flavour in noodles has been implicated to be teratogenic (carcinogenic) in rats [6]; inducing physiological and nutritional roles via initiation of food digestion in the stomach and liver [7]. The high-calorie content and concentration of refined carbohydrates, fats, and sodium. In instant noodles, contribute to an increased risk of metabolic disease [8,9,10]. A single serving of instant noodles is high in carbohydrates and fat, but low in fiber, vitamins and minerals [11].

In animals, the liver, being a major metabolic site of ingested food items also regulates the availability of most chemical substances in the blood, including those obtained as bye- products due to consumption of instant noodles. In real life scenarios, most of the blood that leaves the stomach and intestine often go through the liver for processing this balances and creates the nutrient and also metabolizes them into usable forms for the body [12].

Overtime, studies on the over-consumption of instant noodles has received a number of attention, possibly due to reports on its association with obesity and cardio metabolic syndrome among adults [9]. For instance, as at 2010, approximately 95 million humans reportedly consume instant noodles across the globe, with China accounting for about 42 (44%) billion consumption of packs per year [4]. Albeit, Nigeria is currently ranked to be 13th largest consumer of instant noodles in the world [4].

Recently, the MSG, which is a constituent of the flavour in noodles has been implicated to be teratogenic (carcinogenic) in rats [5,6]; inducing physiological and nutritional roles via initiation of food digestion in the stomach and liver [13]. The high-calorie content and concentration of refined carbohydrates, fats, and sodium in instant noodles, contribute to an increased risk of metabolic disease [14,15]. A single serving of instant noodles is high in carbohydrates and fat, but low in fiber, vitamins and minerals, Chicken flavour and chili powder [16,17]. Being a major fast food that are commonly and conveniently consumed by all classes of people in global scale, instant noodle consumption may presumably contribute some risk to metabolic diseases. Hitherto was this study designed.

The liver being the second largest (after the skin) organ in the human body weighs an average of 1500 g, and it lies inferior to the thoracic diaphragm in the right hypochondriac region of the abdomen. The liver is cone shaped, dark reddish brown in colour, and vascularized with oxygenated blood from the hepatic artery and nutrient rich blood flows from hepatic portal vein. The liver holds about one pint (13%) of the body's blood supply at any moment, and consists of two main lobes of eight segments and 1000 lobules (small lobes) each. It is the primary homeostatic site of most metabolic processes and excretes a product called bile. The liver processes blood, breakdown, balances and creates the nutrient, metabolizing drugs into usable, nontoxic forms by the cells of the body [12]. As a commonly consumed, major fast food of people in global scale, instant noodle consumption may presumably contribute some risk to the metabolic activities of the liver; being a major site of food breakdowns and hence, this study was undertaken.

1.1 Aim of Study

This study investigated the effects of long term consumption of instant noodle on the histo-architecture and selected biochemical variables of adult Wistar rats. Specifically, this study investigated;

i. The histological changes associated with the liver tissue due to long term consumption of instant noodles in adult Wistar rats
ii. The changes that long term consumption of instant noodles may have on selected oxidative stress markers of the liver in adult Wistar rats.

2. MATERIALS AND METHODS

2.1 Animals

Fifty (50) adult Wistar rats of an average weight of 200 g, were procured from the animal holdings of the Department of Anatomy and Cell Biology, Delta State University, Abraka. The rats were then maintained in the Animal holding of the Department of Public and Community Health, Novena University, Ogume, Delta State, Nigeria.

2.2 Study Design

After two weeks period of acclimatization, the animals were randomly assigned into five (5) groups: with A, B, C and D being the treatment groups, while group E acting as control. Each group contained ten rats each. The treatment groups (Group A) received cooked instant noodle (Type A) only; whereas, those in group B received cooked instant noodles with its spice. Group C received cooked instant noodles type B only. Group D received cooked instant noodles type B with its spice while rats in the control group E were fed with grower marsh obtained from Animal care services, Konsult (Nig Ltd), Asaba, Delta State for 30 days. The animals were given water liberally.

2.3 Procedure

2.3.1 Sample preparation

After bulk purchase from local markets, Noodle samples were taken randomly from the composite pack and re-dried at 105ºC for 24 hours, following which it was grinded to powder form using mortar. The ground solid samples (5 g each) were placed in crucibles and then made into various samples; for subsequent addition to rat feeds based on study design

2.3.2 Sacrifice and tissue extraction

Twelve (12) hours following the last treatment (31st day), the animals (per group) were re-weighed and immediately sacrificed via cervical dislocation. Thereafter, the liver was carefully removed and some fixed in a 10% formal-saline for histological technique after the method of Drury, et al. (1976) while other some liver samples were quickly homogenized in a mortar and pestle with a pinch of acid washed sand and a total of 5 mls of normal saline (0.9%) added sequentially during the homogenization process. The homogenates were centrifuged at 3500 rpm for five minutes with the aid of, centrifuge and keep in refrigerator for biochemical analysis on selected oxidative stress markers. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated till needed for biochemical assay.

2.3.3 Histological study

The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with Hematoxylin and Eosin. Photomicrographs of the results were obtained using research photographic microscope in the

Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

2.4 Bio-Assay of Oxidative Stress Markers

2.4.1 Superoxide Dismutase (SOD)

The SOD concentrations in these tissues were determined by the method of Misra and Fridovich [17]. An aliquot (0.04 mls) of the supernatant was added to 5 mls of 0.05 m carbonate buffer (Ph 10.2) equilibrated in the spectrophotometer for 2-3 minutes. The reaction was then initiated by the addition of 0.6 mls of freshly prepared adrenaline as substrate to the buffer-supernatant mixture which was quickly mixed by inversion and the absorbance taken. The reference cuvette contained 5 ml of the carbonate buffer, 0.6 m of the substrate and 0.4 ml of distilled water. The increase in absorbance of 420 nm due to the adenochrome formed was monitored every 30 seconds for 120 seconds. 1 unit of SOD concentration was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adenochrome during 120 seconds.

2.4.2 Catalase assay

The method of Cohen, et al. (1970) was adopted [18, 19]. Aliquots of the homogenate supernatant (0.05 ml) were added into ice cold test tubes while the blank contained 0.05 ml distilled water. The reaction were initiated by adding sequentially, at fixed interval, 5 ml of cold 30 nM hydrogen peroxide and was mixed thoroughly by inversion. The test samples and the blank were taken one at a time, and 7 ml of 0.01 M potassium permanganate was added which was mixed twice by inversion and absorbance at 480 nm. It was read within 30-60 seconds. The spectrophotometer standard was prepared by adding 7 ml of 0.01 M phosphate buffer with pH 7.0 and 1 ml of 6M - Tetraoxosulphate (VI) acid solution. The spectrophotometer was zeroed with distilled water and the concentration of enzymes was estimated.

2.4.3 Peroxidase assay

The assay was based on the method of Junqueira, and Carneiro (2004) in which 0.4 ml of the sample homogenate was added into clean test tubes, follow the addition of 5 ml phosphate buffer and then 5 ml hydrogen peroxide which was subsequently followed by 3 ml of distilled water [20]. Finally the addition of 5 ml of pyrogallol and the absorbance was taken at 330 nm. The blank was prepared by the addition 0.5 ml of phosphate buffer, follow by 5 ml of hydrogen peroxide. 3 ml of distilled water was then added and finally, pyrogallol which was used to zero the spectrophotometer before taking the absorbance of the test.

2.4.5 Malonyldialdehyde (MDA) Assay

Lipid peroxidation was estimated in terms of thiobarbiturate acid reactive species using malonlydialdehyde (MDA) as standard by the method of Beuge and Aust, [21]. 1.0 ml of a sample extract was added with 2 ml of the TCB-TBA reagent (15% w/v TCA, 0.375% (W/V) TBA and 0.25 N HCl). The contents were boiled for 15 minutes, cooled and centrifuge at 10,000 rpm to removed precipitate. The absorbance was read at 535 nm and the Malonlydialdehyde coefficient of 1.56×10^5 M^{-1} Cm^{-1}.

2.4.6 Reduced glutathione assay

This method was based on the development of a relatively stable (yellow) Color when 5′, 5′– dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of DTNB with the reduced glutathione, 2 – nitro-5-thiobenzoic acid possesses a characteristic absorbance at 412 nm and the amount of reduced glutathione in the sample is proportional to the absorbance at this wavelength [7].

2.5 Statistical Analysis

Results obtained from the study were recorded and compared statistically using the unpaired sample t-Test and symmetric measured t -Test of the Statistical Package for Social Sciences (SPSS). The results from the various assay were also analysed and taken the significant level of ($p < 0.05$) as taken below variables.

3. RESULTS

Obtained result is presented bellow after data sorting and careful analysis. Photomicrograph of sampled tissues are also shown in the figures.

4. DISCUSSION

The results (H&E) of this study revealed some cellular degenerative changes on the liver of adult Wistar rats that received instant noodles but with the one that received instant noodles and spiced more marked as compared to control.

This work is in line with Eweka and Adjene, (2007) report which found that chronic consumption of MSG affect the geniculate bodies of the treated animals. Their study also revealed some necrotic and cellular degenerative changes as compared to the control group [22].

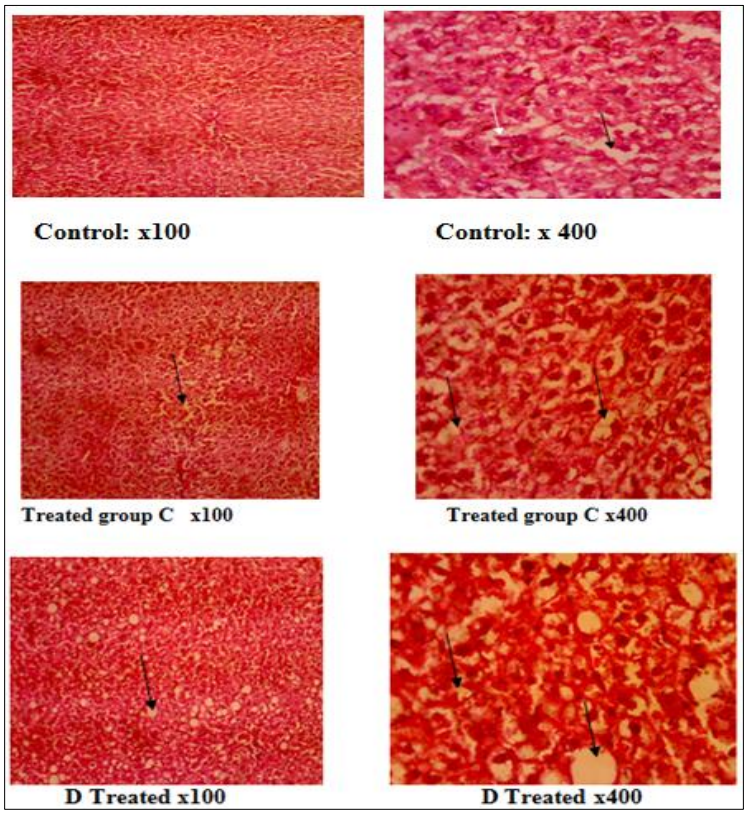

Fig. 1. Comparison of histological changes between control and instant noodles groups

The desired section of liver from the control animals showed a normal histological features with the hepatocyte appearing distinct and intact. The section of the treated liver group D revealed several cellular degenerative change, hypertrophy, hepatic steatosis and mild fibrosis (fatty liver) as compared to the control group

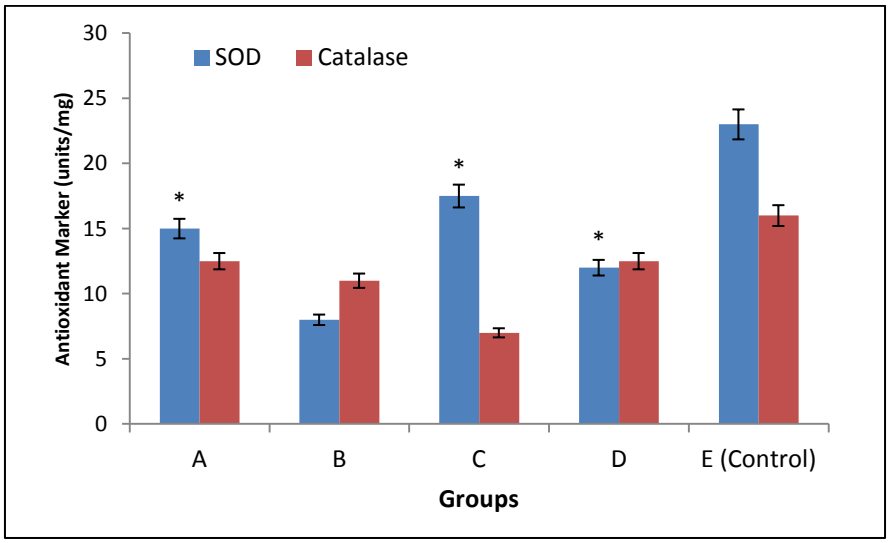

Fig. 2. Comparative effects of instant noodles consumption on Catalase and SOD marker Levels

*SOD = Superoxide Dismutase, * = significant decrease compared with control*

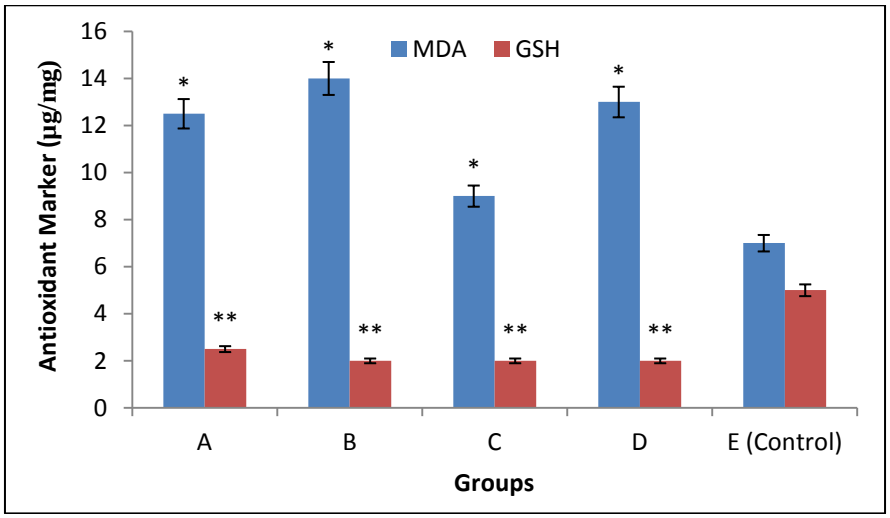

Fig. 3. Comparative effects of instant noodles consumption on MDA and GSH marker Levels
**= Significant increase compared with control; ** = insignificant increase compared to control group*

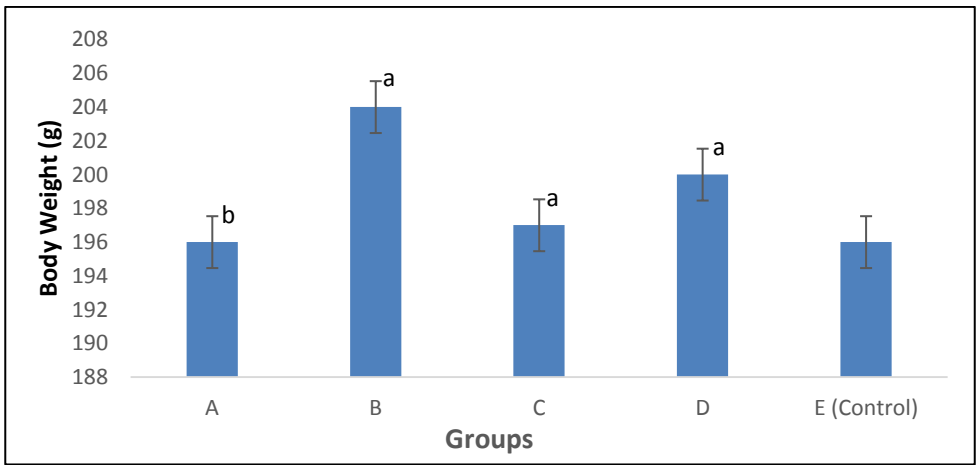

Fig. 4. Comparative effect of instant noodles consumption on body weight (g)
a = significant increase, b = significant decrease compared with control

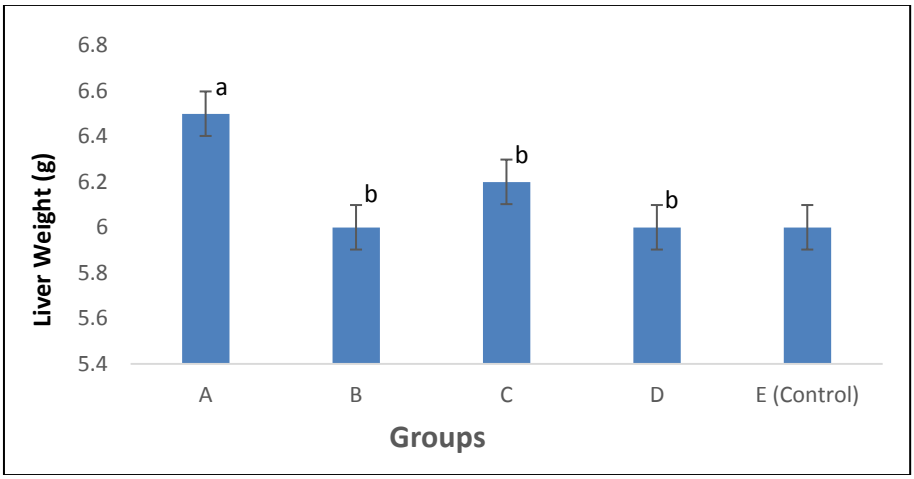

Fig. 5. Comparative effect of instant noodles consumption on liver weights (g)
a = significant increase, b = insignificant decrease compared with control

Table 1. Comparative changes in body and liver weight due to instant noodles consumption

	A	B	C	D	E (Control)	P
Body weight	196.0±1.9[a]	204.0±2.4[b]	198.0±1.2[a]	201.0±2.4[a]	196.0±1.9	0.047
Liver	6.5±0.5[a]	6.0±0.2[a]	6.3±0.4[a]	6.1±0.5[a]	6.1±0.3	0.908

Values are expressed as Mean ± SEM. a = significant increase, b = insignificant increase as compared to control

Monosodium glutamate consumption may have some deleterious effect on the liver of adult Wistar rats at higher doses, by extension may affect the functions of the liver. Histological studies of the teratogenic effects of MSG on the developing liver of Wistar rats revealed that there was a dilatation of the central veins which containing lysed red cells, cyto-architectural distortions of the hepatocytes and other possible abnormalities that could result in a new born when a pregnant animal is exposed to MSG in the first trimester [23].

Many essential bimolecular synthesis and detoxification of molecules may also occur in this organ. The smooth functioning of liver is very vital for healthy performance and composition of food, nutrients and drugs result in mediation of these functions. The modern diet composition is resulting in liver damage and metabolic diseases. The most of liver damage like fibrosis, necrosis and hepatitis is associated with food derived peroxidation and low level of glutathione. Reactive oxygen species and reactive nitrogen species derived from the consumption of lipid and proteins leading to hepatoxicity required more attention to address the liver damage problems. Similarly the free radicals generation from drug uses induces toxicities of liver cells.

A variety of proteins, peptide hormones, plasma proteins and blood cells are captured by hepatocytes. In next step, hepatocytes decompose them to amino acids for new synthesis. Liver only syntheses enzymes and receptors proteins in deficient state of proteins and amino acids from diet. Liver synthesizes all albumins, most of α1-globulines, 75% of α2-globulines and 50% of β-globulins, blood lipoproteins, fibrinogen, blood clotting factors and cholinesterase. Those excess amino acids which are not further used for protein synthesis are transformed into different substances. In normal conditions, main source of energy for liver is oxidative decomposition of amino acids [24].

All the studies found in the literature employed ligature of the common hepatic duct. Other studies with 80 Wistar rats demonstrating that after 30 days of common hepatic duct ligature, canicular proliferation and portal fibrosis around hepatic lobes took place [25]. Biochemical analyses indicated an increase of bilirubin, alkaline phosphatase and transaminases with a decrease in albumin levels. Other causes of secondary biliary cirrhosis include prolonged mechanical obstruction, sclerosing cholangitis, cystic fibrosis, and congenital biliary cysts [26].

Regardless of the causing agent of the hepatic lesion, the liver will apparently react in five ways: 1. Necrosis. 2. Degeneration. 3. Inflammation. 4. Regeneration and 5. Fibrosis. Necrosis may follow practically any lesion whose changes are significant, taking a toll on hepatocytes. However, before it becomes characteristically necrotic, hepotocytes may become swollen and edematous, with irregularly compact cytoplasm and great clear spaces. Retained biliary material may have a swollen, frothy, and diffuse aspect. These characteristics are related to degeneration.

The increased in the body weight observed in the treated groups that received instant noodles and spices may be due to the noodles and the spices, which lead to significant ($p < 0.05$) increase due to the long term consumption of instant noodles on the morphology/ body weight of the adult Wistar rats. However, there was no significant difference in the effects of the long term consumption of instant noodles on the liver weight of the rats ($p < 0.05$). This is in line with study conducted by Adjene, et al. 2017 on the effects of long-term consumption of instant noodles on the body and brain weights of adult Wistar rats [27].

Long term consumption of instant noodles on the oxidative stress parameters of the liver tissue found that MDA as a non-enzyme biomarker of oxidative stress was significantly ($p < 0.05$) higher on the treated groups with that of the spiced as compared to their corresponding control group. Significant ($p < 0.05$) decrease in anti-oxidative enzyme catalase which is an enzyme responsible for breaking down hydrogen peroxide thereby detoxified substances and GSH which is a major and most abundant antioxidant which protect liver cell against toxicity. Therefore it could be inferred that there is a potential of long term instant noodles consumption in adult Wistar rats. There is also significantly ($p < 0.05$) decreased in SOD which is responsible for the

free flow of bile into the intestine which suggested liver dysfunctions which is in line with the work reported bt Adjene and Enaibe (2002) on histological effects of camphor on the liver of adult Wistar rats [23].

The oxidative stress is the major reason of liver injuries. The metabolism of lipids, proteins and carbohydrates generates free radicals that are unstable and reactive to interact with livers cells. There is oxygen and nitrogen based radicals produced from lipid, carbohydrates and proteins molecules. Oxidative free radical species are hydroxyl, superoxide and peroxyl and non-radicals hydrogen peroxide while nitrogenous species include nitric oxide and superoxide. The radicals induced from oxygen species are known as reactive oxygen species (ROS) and free radicals induced from nitrogenous molecules are known as reactive nitrogen species (RNS). These ROS and RNS inherited as characteristics of initiating peroxidation of lipid and oxidizing the deoxyribonucleic acid and ribonucleic acid of cell membranes and tissues [28].

The oxidative stress not only produces liver injuries but also alters the signal pathways of biological functions. Since proteins and gene expression is directed by these signals suggesting oxidative stress a pathological mechanism of initiation and progression of hepatic diseases. It also indicates the cross linkage among pathological factors, free radicals, inflammation, and immune responses [11].

5. CONCLUSION

From the current study, long term consumption of instant noodles was seen to damage the liver of adult Wistar rats. Histological section of liver of the treated animals showed cellular degenerative changes, hypertrophy, hepatic steatosis and mild fibrosis which were sparsely distributed as compared to the control group. Biochemical assay of oxidative stress markers revealed that MDA levels was significantly ($p < 0.05$) increased in all treated liver groups as compared with the parallel control animals. Concomitantly, the levels of SOD was found to be significantly ($P < 0.05$) decreased in the treated liver groups as compared with the control. Thus, long consumption of instant noodles exert toxic effects by promoting oxidative stress in the liver tissue of adult Wistar rats.

ETHICAL APPROVAL

Ethical approval was obtained from the Research and Ethics committee of the College of Health Sciences, Novena University, Ogume, Delta State. Guidelines on use and handling of laboratory animals were also strictly followed [5], ensuring that no animal live was unduely wasted without actual need for it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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