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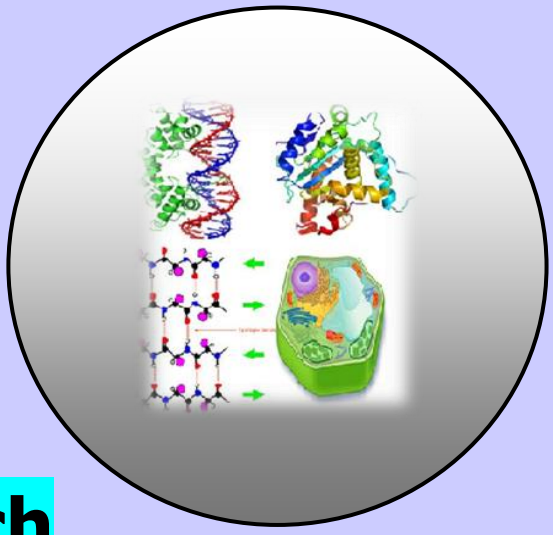

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RESEARCH PAPER

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Effect of Formalin in Local and Foreign Instant Noodles on Haematological and Biochemical Parameters of Male Albino Rats

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ABSTRACT

In recent days, use of formalin as food preservative has alarmingly increased specifically in fishes, noodles, fruits and many other food items exposing the large majority of population to severe health risk. This research was undertaken to evaluate the toxicity of formalin on blood, liver and kidney in rat's animal models. A total of 36 rats were divided in 6 groups as control and treatment. The animals of the treatment groups were given a compounded feed of 70% normal rat ration and 30% noodles both foreign and local with a group treated specially with formalin at a concentration of 60mg/kg feed. Results showed the presence of formalin in all the samples, the raw samples had average formalin contents of 4.5±1.0mg/kg and 9.0±1.0mg/kg for both local and foreign respectively. Haemoglobin and Packed cell volume of the treatment groups (D, E, F) were significantly ($p<0.05$) increased compared to the control. There were also significant ($p<0.05$) increases in creatinine, urea, ALT, AST levels of the treatment groups compared to the control. These findings clearly suggest that the consumption of formalin in food causes liver and kidney toxicity.

Keywords: Male Albino Rats, Formalin, Toxicity, Haematological and Biochemical Parameters.

INTRODUCTION

Noodles are very popular wheat foods made from common wheat flour, water and some additives (Sui *et al*., 2006). During the last 20 years, the annual consumption of noodles has increased because of consumers change in perception about noodles (Wang *et al*., 2010).In Nigeria, instant noodles are well consumed by almost every category of people. According to world Instant Noodles Association, Nigeria was the 13th largest consumer of instant noodles in the world and over 100 billion packs of instant noodles were consumed worldwide in 2012. However, there is a dark side to instant noodles. Since they are made to

bear a longer shelf life, they are highly processed and are laced with artificial colors, preservatives, additives and flavorings. Regular consumption of instant noodles leads to multiple nutrient deficiencies. Many essential nutrients are lost when wheat is processed into white flour; fiber: 95% lost, iron: 84% lost, folate: 59% lost, vitamin: E 95% lost and vitamin B6: 87% lost (Amin and Anwar, 2013). Noodles contain 11-15% protein (dry basis) but are deficient in lysine and threonine (the first and second limiting amino acid), common to most cereal products (Sisson *et al*., 2005). Thus, there is a need for nutritional enhancement of these noodles. There has been greater alarming increase in the concentration of Formaldehyde in food items which could result from packaging or other production procedures (Yao *et al*., 2005). Foods such as noodles, fruits, meatballs and salted fish commonly use formalin as preservative. Formalin not only extends the life of the food but also improves the external appearance of food and protects the food from the attack of fungi and bacteria. The use of formalin in diet can cause toxicity in a functional organ of the human body. It is characterized by symptoms such as difficulty of swallowing, poor appetite, nausea (as a reaction to the rejection of the stomach), acute abdominal pain (as a reaction to the rejection of the liver, stomach and colon), diarrhea and eventually accompanied by vomiting. At the worst level, symptoms may lead to severe depression in the nervous system or circulatory disorders. Based on formalin carcinogenic nature, if the body contains with a high concentration of formalin, the body will react with almost all the body cells, causing cell damage, and even cell mutations that leads to the development of cancer, after accumulated for relatively long time in the body. Formaldehyde (CH_2O) is the simplest aldehyde that shows high reactivity towards cellular macromolecules like DNA and proteins (Lu *et al*., 2010). Upon intraperitoneal, oral, or inhaler exposure, formalin quickly diffuses into many tissues including the brain, testis, and liver (Smith, 1992). Formaldehyde is quickly absorbed from gastrointestinal tract following ingestion and from respiratory tract following inhalation which makes it a dangerous chemical to be used as preservative. However, it is inadequately absorbed following dermal tract. Its main rote of exposure in our environment are air (through occupational exposure like formaldehyde and resin production), smoking, dermal exposure and also through water and food naturally and artificially when used as food preservative (Restani et al., 1992).Formaldehyde is not only ubiquitously present as an environmental pollutant but is also generated in substantial amounts in the human body by enzyme-catalyzed reactions (Cloos *et al*., 2008). However, owing to formaldehyde detoxification by cellular enzymes, a steady state balance is established between formaldehyde-generating and disposing a process that leads to a normal blood formaldehyde concentration of around 0.1mM (MacAllister *et al*., 2011). Although various country governments have issued a series of standards to regulate formaldehyde exposure, concentrations in homes, office buildings, workshops, public places and food often exceed the national standards. In addition, there have been numerous reports of formaldehyde-induced health problems, including poisoning and cancer (Neri *et al*., 2006).

MATERIAL AND METHODS

Sample Procurement

A total of 10 noodles of various net weights were bought from Shop Rite shopping mall, Onitsha and Radopin supermarket, Awka, Anambra State, Nigeria. Five of them were foreign noodles while five were locally made.

Reagents and Chemicals

All reagents and solvents used were of analytical quality.

Preparation of Samples

The noodles were cooked for 5minutes at 100^{o}C and dried under room temperature for four days. They were powdered and compounded with commercial rat feed in the ratio of (30%:70%) respectively. The compounded feed was used to form pellets which were fed to the rats for 21 days.

Animals

36(thirty six) male rats were purchased from Mr. Chris animal house at Mgbakwu, Anambra State. They were housed in a well standard animal cage in the department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria for a period of ten days for acclimatization. During this period the animals were given unrestricted access to water and commercial rat feed. After acclimatization, they were weighed and then grouped. The amounts of feed taken by the animals were monitored daily.

Animal Grouping

The animals were divided into six groups (A, B, C, D, E, and F)

Group A (Negative control): Were fed with normal commercial rat feed.

Group B: Received compounded feed composed of 70% commercial rat feed and 30% noodles containing 4.6mg/kg.

Group C: Received compounded feed composed of 70% commercial rat feed and 30% noodles containing 3.9mg/kg.

Group D: Received compounded feed composed of 70% commercial rat feed and 30% noodles containing 11.0mg/kg.

Group E: Received compounded feed composed of 70% commercial rat feed and 30% noodles containing 8.7mg/kg.

Group F: Received compounded feed composed of 70% commercial rat feed and 30% noodles treated with 60.0mg/kg of formalin.

Sacrifice and Blood Collections

At the end of the study period, the animals were anaesthetized using chloroform soaked in cotton wool. Blood were collected by heart puncture into anticoagulated and non anticoagulated universal sample tubes. Whole blood samples were used for haematological parameters determination while the serums from the centrifuged coagulated blood were used for biochemical parameters.

METHODS

Qualitative Test for Formaldehyde

Qualitative Determination

The method of Jinping *et al*., (1994) was used for the determination of the presence of formaldehyde in the both foreign and local noodles.

Quantitative Test for Formaldehyde

Quantitative Determination

The method of Altshuller *et al*.,(1961) was used for the quantitative determination of formaldehyde.

Creatinine Content Determination

Randox kits were used for creatinine determination.

Urea Content Determination

Randox kits were used for urea determination.

AST Activity Determination

Randox kit was used for AST activity determination.

ALT Activity Determination

The same procedures as for AST were used for ALT activity determination.

PCV Determination

Microhaematocrit method was used for PCV determination.

Hemoglobin Determination

This was determined using Sahli-Hellige method..

Statistical Analysis

Results of the study were expressed as mean+_ standard deviation. Differences between mean of the treated groups and their control for noodles and animal studies were analyzed using Analysis of Variance (ANOVA) of SPSS 16.0 spread sheet statistical package. Values were taken to be significant at $p<0.05$.

RESULTS

The results of the qualitative and quantitative analyses of the samples showed that the samples contained formalin in various concentrations. The foreign noodles contained higher amounts of formalin than the local noodles.

The results of the qualitative analysis of all the samples are shown in table.

Table 1. Qualitative analysis of samples.

Noodle	Inference
S_1	++
S_2	+++
S_3	++
S_4	++
S_5	+
S_6	++
S_7	++
S_8	++
S_9	+
S_{10}	+

Key

S/NO	Symbol	Meaning
1	+	Indicated
2	++	Strongly indicated
3	+++	Very strongly indicated
4	_	Not indicated

S Sample

Animal Study

The results of animal studies showed that liver and kidney function markers were more elevated in the treatment groups than the control. It was also observed that hematological parameters were higher in the treatment groups than the control.

Three animals from group F died before the end of the treatment period. At the commencement of the treatment, the animals showed aggressive behavior and movement while at the end of the treatment, the animals especially in group F were very calm, limited in their movements, in group F, they gathered at one corner of the cage probably to avoid the area of maximum formalin concentration.

The results of the Quantitative Analysis of all the Samples are shown in Table 2

Table 2. Quantitative Analysis of the Samples.

Samples	Concentration of Formalin(mg/kg)
Foreign noodles	
S_1	7.7
S_2	11.0
S_3	8.7
S_4	9.3
S_5	8.7
Mean	9.0±1.0
Local noodles	
S_6	4.6
S_7	6.2
S_8	3.9
S_9	3.9
S_{10}	3.9
Mean	4.5±1.0

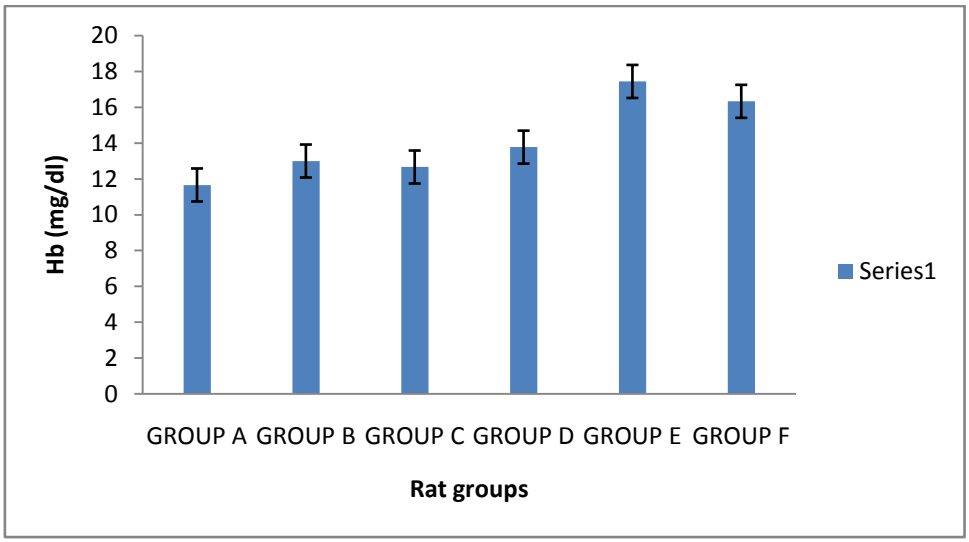

Figure 1. Average Haemoglobin level (mg/dl) in the various rat groups.

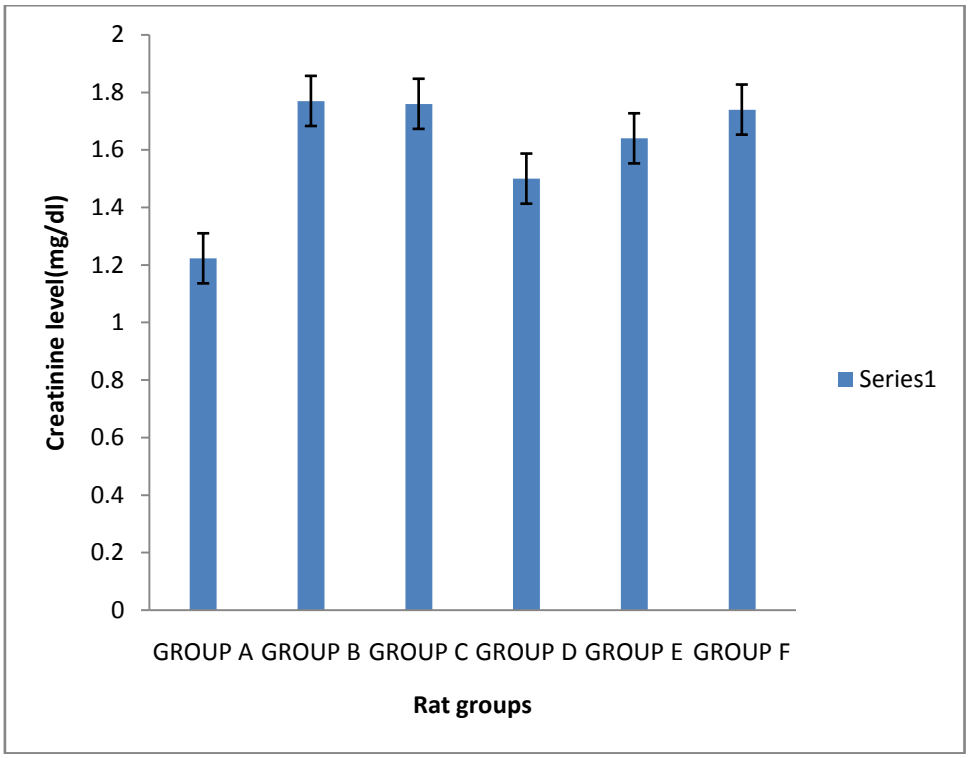

Figure 2. Average creatinine level (mg/dl) in the various rat groups.

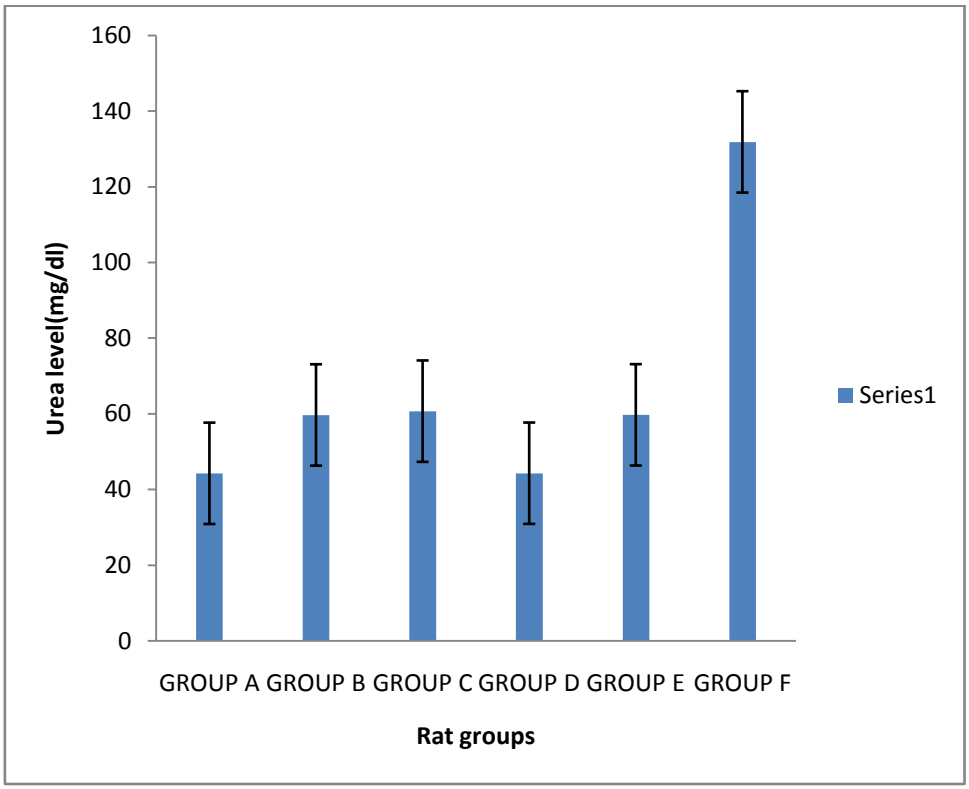

Figure 3. Average urea level (mg/dl) in the various rat groups.

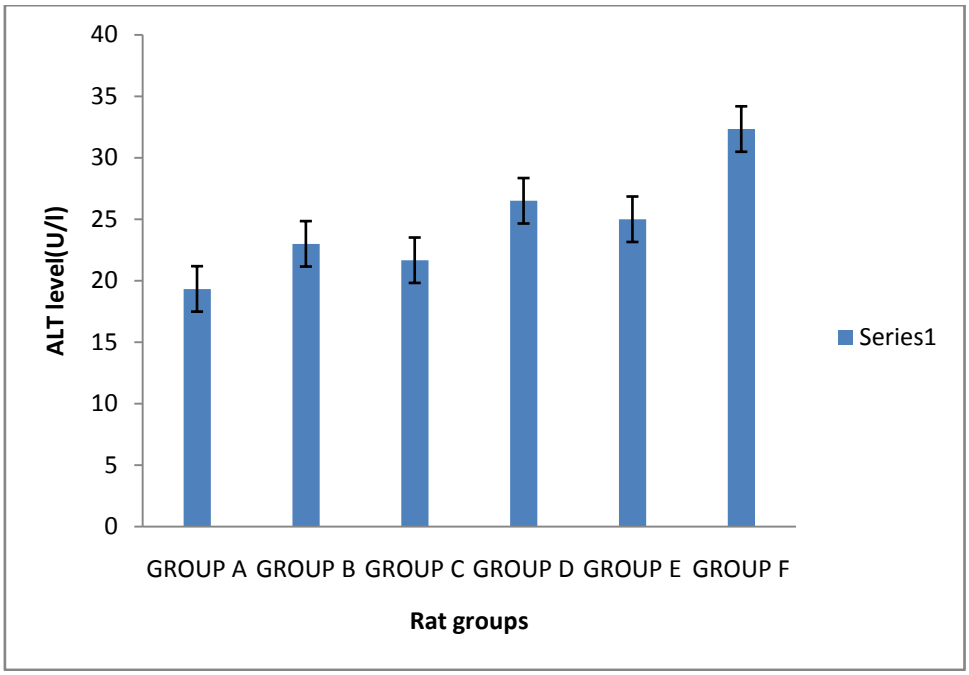

Figure 4. Average ALT level (U/l) in the various rat groups.

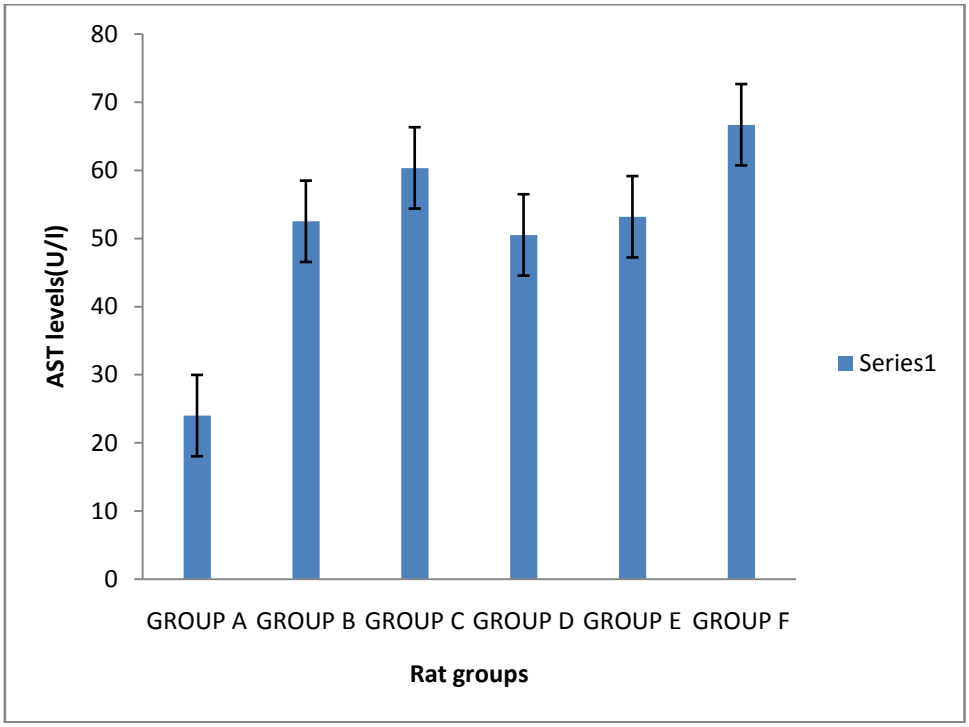

Figure 5. Average AST level (U/l) in the various rat groups.

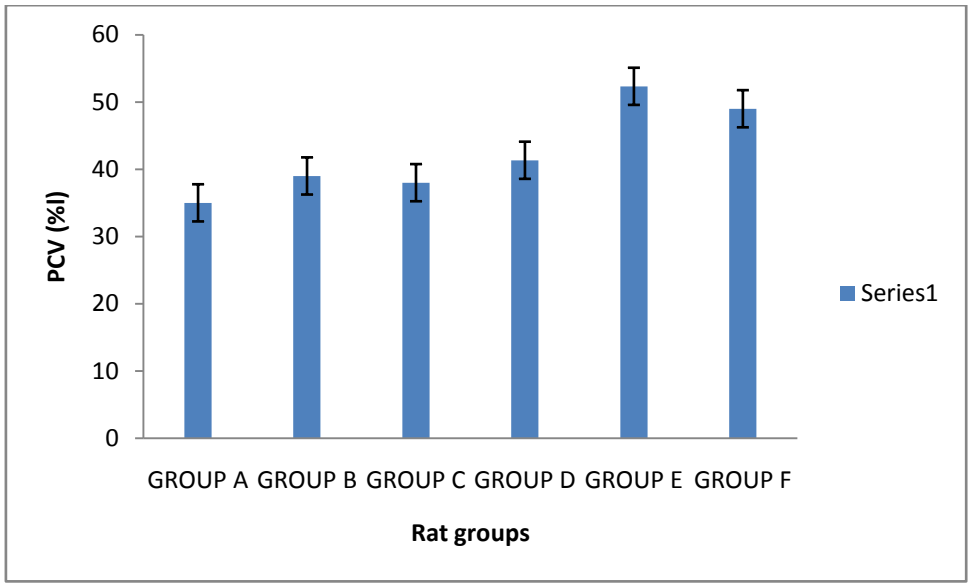

Figure 6. Average PCV level (%) in the various rat groups.

DISCUSSION

The results showed presence of formalin in all the samples with the highest concentrations found in the foreign noodles. It was observed that cooking significantly reduced the formalin contents of the sample ($p<0.05$).

The results of hematological parameters showed that there is significant increase ($p<0.05$) in hemoglobin and packed cell volume contents of the treatment groups (D, E, F)compared to control group A. Formalin has been shown to induce histotoxic hypoxia; this is inability of the cells to utilize oxygen. It is possible that the system is responding to this stress by increasing hemoglobin level in the system. Vargova *et al* (1993) observed that in male rats exposed to a high dose of formalin, increased monocytes, red blood cells and hemoglobin were detected but lymphocyte counts were decreased.

The present work noticed lower food and water intake with a significant decrease in weight with rats fed with 60mg/kg formalin treated commercial feed (group F) which might have caused the significant decrease in the body weight as observed in group F. This result is in agreement with that reported by (Kamata *et al*., 1997).

The creatinine levels of all the treatment groups (B, C, D, E, F) were significantly increased ($p<0.05$) compared to control.

The urea levels of the treatment groups (B, C, E, F were significantly increased ($p<0.05$) compared to control. This is suggestive of kidney damage.

Significantly higher levels of blood urea and serum creatinine observed in birds given higher levels of formalin could be ascribed to decreased fluid intake and urine production and consequently, the retention of urea and creatinine in the blood ultimately resulting in the increased concentration of the parameters in the blood. Thus, an elevated concentration of urea and creatinine in the blood could be associated with impaired renal function at high formalin doses (Til *et al*., 1988, 1989) and (Tobe *et al*., 1989) reported that rats given higher levels of formalin in drinking water showed increased levels of urea and creatinine in the blood.

The ALT results of the treatment groups (B, D, E, F) showed significant increase ($p<0.05$) compared to the group A. In 2000, a 32 year old male experienced abdominal pain, healthy stool, hematemesis and a high serum Alanine Amino Transferase (ALT) level of 105ug/l after imbibing 300ml of formaldehyde contaminated water (Liu and Song, 2001).

Also AST results of the treatment groups showed a significant increase ($p<0.05$) compared to control. Benjamin (1978) reported a significant increase in AST and ALT activities of cockerels fed with formalin treated feed. High levels of AST and ALT in the blood is indicative of liver damage. An increase in the activity of these enzymes is a very sensitive index of hepatic damage (Benjamin, 1978).

CONCLUSION

It is obvious from the results of the study that some of the noodles both local and foreign consumed in Nigeria contain formalin, and that their consumption maybe injurious to health. The sources of contamination might be from the packaging materials, deliberate adulteration or treatment by the manufacturers or maybe a natural part of the wheat from which the noodles are produced. Regulatory authority should strictly monitor the safety of the noodles sold in Nigerian markets. Attempts should also be made to find out the sources of the contamination.

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