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Effect of Instant Noodles Seasoning on Liver Enzyme Parameters Using Albino Rats

[*1]Arunsi, O.M., [1]Nnatuanya, I., & [2]Okwareke, K.N.
[1]Department of Medical Laboratory Science, Madonna University, Elele, Rivers State
[2]Department of Statistics, Abia State Polytechnic, Aba

[*]**Corresponding author email**: mbaarunsi3@gmail.com.

Abstract
This study was done to check for the potential toxic effect of instant noodles seasoning on liver enzyme parameters-Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatases (ALP) using Albino rats as a model for the study. Twelve adult female albino rats were divided into three groups and were fed for 28days. The control group (group 1) was fed with standard rat feed and water only, group 2 (low dose) was fed with rat feed and given 5g of seasoning daily, and group 3 (high dose) was fed with rat feed and given 10g of seasoning daily. The serum level was analysed for AST, ALT and ALP using standard biochemical methods. Data analysis was determined statistically using SPSS version 27, Windows 10. The result from the study showed that the control group exhibited mean AST levels of 8.750±0.50 U/L, ALT levels of 6.75±0.95 U/L, and ALP levels of 36.65±1.91 U/L. The low-dose group had AST levels of 9.750±0.95 U/L, ALT levels of 8.75±0.50 U/L, and ALP levels of 39.75±1.79 U/L. The high-dose group had AST levels of 12.00±0.816 U/L, ALT levels of 11.25±0.50 U/L, and ALP levels of 46.16±0.54 U/L, showing a dose-dependent increase in AST, ALT and ALP levels in the treated groups, with statistically significant differences compared to the control ($p<0.05$). These findings suggest that daily/regular consumption of these seasonings, which contain harmful additives such as monosodium glutamate (MSG), tertiary butylhydroquinone (TBHQ), and excess sodium, may lead to liver damage. The study shows the need for greater awareness of dietary risks associated with processed food additives and strongly supports the use of natural alternatives for safer liver health.

Keywords: Monosodium glutamate, Tertiary butylhydroquinone, Instant noodle seasoning, AST, ALT and ALP

Introduction

Depending on the brand, instant noodles are precooked, dry noodles made from wheat flour that are packaged with oil, seasoning, flavouring powder, and other components. (Amin & others, 2010). These noodles still exist today in many types of instant noodles, and some of their ingredients include refined flour made from weed, salt, and oil (Kim, 1996a). The great majority of supplements have been shown to work remarkably well with noodles. Most people call them junk food (Hope, 2009). According to Joseph et al. *(*2017), a specific meal consisting of these instant noodles is stated to be poor in vital nutrients, including vitamins, protein, fibre, and minerals and high in added sugar, sodium, and other chemicals. These noodles are particularly popular because they are readily available, inexpensive, and simple to prepare, as they only take two to three minutes. In recent times, there have been multiple brands of instant noodles available in the market. (Shin, 2003). Around the world, there are a plethora of distinct instant noodle options (Seller et al., 2007). Indofood's Indomie instant noodles were the first instant noodle brand to be introduced in Nigeria in 1988. Numerous other nations throughout the world also distribute instant noodles (Seller et al., 2007). Nigeria has been producing instant noodles since 1995; however Indonesia is still the world's largest producer of Indomie (World

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Instant Noodle Association (WINA, 2016). Ever since the first instant noodle brand was introduced into Nigeria, rich people, middle-class people, and the impoverished have accepted it into their homes in both urban and rural areas. Children and even some adults referred to all types of instant noodles as "INDOMIE" as the Indomie instant noodle developed a household word over time (Alabi et al.,2014). However, because of the intricate advertisements for many brands of these noodles, as well as their accessibility and ease of consumption, they have drawn people from a variety of social groups in both urban and rural areas, including children, adults, students, and the working class. 2010 saw Indofood's release. Instant noodles are a popular food item consumed worldwide, especially in Asian countries. They are well known for their convenience, affordability, and versatility. Instant noodles seasoning is a mixture of various flavouring agents, including monosodium glutamate (MSG), salt, and spices. However, the excessive consumption of instant noodles has been associated with a higher risk of liver damage. This study aims to investigate the effect of instant noodles seasoning on liver enzyme parameters (ALP, AST, ALT) using albino rats as an animal model.

Instant noodles have become a staple food in many households globally due to their convenience, affordability, and quick preparation time. However, several studies have identified the potential negative effects of instant noodles on human health, particularly on the liver. The liver is a vital organ that performs essential metabolic functions, including the regulation of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Recent studies have shown that the high sodium, fat, and preservative content in instant noodles seasoning can lead to liver damage and affect enzyme levels in the liver (Eteng et al., 2023). Several factors, such as brand image and advertising effectiveness, influence the purchasing decisions of instant noodles consumers. The study by Tripambudi and Purwanto (2023) revealed that brand image and advertising effectiveness significantly influence the purchasing decisions of consumers in the Gresik District of Indonesia. In contrast, Toiba et al. (2023) found that consumers' preference for organic instant noodles in Indonesia was driven by their health concerns and environmental consciousness. Nevertheless, the preference for organic instant noodles may not necessarily translate into lower liver enzyme levels, as the seasoning and preservatives used in organic instant noodles may still have negative health implications.

Low-sodium condiments and seasoning alternatives may be a healthier option for instant noodles seasoning. (Chailek et al.,2023) found that low-sodium seasoning alternatives were available in the Bangkok Metropolitan Region but were not widely used. The study highlights the need for increased awareness of the health implications of high-sodium seasoning and the benefits of using low-sodium seasoning alternatives. Furthermore, the addition of natural spices and seasoning to instant noodles may improve their nutritional value. The study by Eteng et al. (2023) found that instant noodles cooked with natural spices and seasoning significantly improved liver function in rats compared to those cooked with artificial seasoning.

Materials and Methods

Experimental Animals

Twelve adult female albino rats with weights ranging from 150-200g were purchased and allowed to acclimatise at Madonna University animal house for 2 weeks before the beginning of the experiment with access to standard chow and water. They were weighed using an electronic weighing balance before being randomly assigning by method of random sampling. Test animals were assigned into three groups composed of five rats each, where they were fed with the Indomie seasoning mixed with their feed. The cages were cleaned every 2 days to prevent infection of the animal, care and treatment will be conducted in compliance with the international guidelines of the National Institute of Health (NIH) (NIH Publication 86-23, 1985) for laboratory animals' care and use.

Reagent

The reagents that were used in this work were of analytical grade. They were purchased commercially, and the manufacturer's standard operating procedure (SOP) was strictly followed.

Indomie seasoning treatment

A carton of instant noodles was purchased commercially from a supermarket in Port Harcourt.

It contained 40 pieces of noodles. Each pieces contains 40 packets of noodles seasoning weighing 5g each.

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Experimental Design

The study was carried out with 3 groups of albino rats, four rats per group.

Control groups, which contain 4 albino rats, where given feed and water throughout the experiment.

High dose groups, which contain 4 female albino rats, were given feed and 10g of noodles seasoning diluted in 100ml of water.

Low dose groups, which contain 4 female albino 4 female albino rats, were given feed and 5g of noodles seasoning diluted in 100ml of water.

After a duration of 4weeks, blood samples were collected for analysis.

Ethical Approval

This study was conducted according to the rules and regulations of Madonna University, Elele campus, ethical committee on the use of experimental animals.

Sample Collection

After 4 weeks of the experiment process of feeding with indomie seasoning, the albino rats were anaesthetised with the aid of chloroform and blood samples were collected through cardiac puncture of each of the rats using a sterile needle and syringe and were stored in a plain container for AST, ALT, and ALP estimation.

Clinical Laboratory Assay

Measurement of Aspartate Aminotransferase (AST)

Method: using the Reitman-Frankel Method (1957)

Principle: AST catalyses the transfer of the amino group (Aspartate) to oxo-glutarate to form glutamic acid and oxaloacetic acid. Oxalo acetic acid formed reacts with 2,4 dinitrophenylhydraxine (2,4-DNPH) to form 2,4-dinitrophenylhydrazone, which in an alkaline solution forms a red-brown colored complex. The colour intensity formed is directly proportional to AST activities in the sample.

Procedure:

Test procedure;

1. Three test tubes were arranged and labelled blank, control and test.
2. 500 microlitres of R1 was added to three test tubes
3. It was incubated in a water bath at 37°C for 5minutes.
4. After incubation, 100 microlitres of the sample was added into the test tube labelled test and control.
5. It was mixed and incubated in a water bath at 37'C for 30minutes
6. 500microlitre of R2 (2, 4) dinitrophenylhydrazone) was added to three test tubes.
7. 5ml of 0.4N NaOH was added into the test tubes labelled test and control, and was mixed and incubated at room temperature for 5minutes
8. The spectrophotometer was set at 546nm wavelength, I blanked the spectrophotometer using the reagent blank and and the absorbance was taken.

Estimation of Alkaline Aminotransferase (ALT)

Method: using the Reitman-Frankel Method (1957)

Principle: ALT catalyses the transfer of the amino group (Alkaline) to keto-glutamate to form glutamic acid and pyruvic acid. Pyruvic acid formed reacts with 2,4 dinitrophenylhydraxine (2,4-DNPH) to form 2,4-

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dinitrophenylhydrazone , which in an alkaline solution forms a red-brown colored complex. The colour intensity formed is directly proportional to AST activities in the sample

Procedure:

1. Three test tubes were arranged and labelled blank, control and test.
2. 500 microlitres of R1 (substrate) was added to the test tubes.
3. It was incubated in a water bath at 37`C for 5minutes.
4. After incubation, 100 microlitres of the sample was added into the test tube labelled test and control.
5. It was mixed and incubated in a water bath at 37'C for 30minutes.
6. 500 microlitres of R2 (2,4 dinitrophenylhydraxine) was added to three test tubes.
7. 5ml of 0.4NNaOH was added into the test tubes labelled test and control, It was mixed and incubated at room temperature for 5minutes.

The spectrophotometer was set at 546nm wavelength, and blanked the spectrophotometer using the reagent blank and the absorbance was taken.

Test Procedure for Alanine Phosphatase (ALP)

Estimation of Alkaline phosphatase using the P-Nitrophenyl phosphate method

Principle: Alkaline phosphatase catalyses the hydrology P-Nitrophenylphospyin a highly alkanineph to form P-Nitrophenol and inorganic phosphate 9free phosphate). The reaction is monitored kinetically at 405nm wavelength by the rate of formation of P-Nitrophenol, which is proportional to the activity present in the sample.

Procedure;

- 1,000microlitre of working reagent (R1a and R1b) was pipetted into a test tube.
- The machine was set at 405nm wavelength, blanked with an empty covet.
- 20microlitre of the patient sample was added into the test tube.
- The reading was taken immediately at the zero mark.

Statistical Analysis

Data generated from this study will be analysed statistically using the Statistical Package for the Social Science (SPSS) version. The groups mean, standard deviation and standard error of chemical pathology parameters will be calculated and computed, and significant differences will be identified by Analysis of Variance (ANOVA). The mean of chemical pathology parameters between each pair of groups will be compared using Tukey`s Post-Hoc Test. And the correlation between chemical pathology parameters and indomie seasoning doses will be analysed.

Table 1: Showing the Aspartate Transaminase (AST), Alanine Transaminase and Alkaline Phosphatase levels among different groups fed with noodle seasoning.

GROUPS	AS**T (U/L)**	AL**T (U/L)**	**ALP (U/L)**
CONTROL	8.750 ± 0.50	6.75 ± 0.95	36.65 ± 1.91
LOW DOSE	9.750 ± 0.95	8.75 ± 0.50	39.75 ± 1.79
HIGH DOSE	12.00± 0.816	11.25 ± 0.50	46.16 ± 0.54
F-value	18.136	43.059	39.194
P-value	0.001	0.000	0.000

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Significant at $p < 0.05$;

Non-significant at $p > 0.05$.

Table 1: Shows the Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase levels among different groups fed with noodle seasoning. The control group exhibited mean AST levels of 8.750±0.50 U/L, ALT levels of 6.75±0.95 U/L, and ALP levels of 36.65±1.91 U/L. The low-dose group had AST levels of 9.750±0.95 U/L, ALT levels of 8.75±0.50 U/L, and ALP levels of 39.75±1.79 U/L. The high-dose group had AST levels of 12.00±0.816 U/L, ALT levels of 11.25±0.50 U/L, and ALP levels of 46.16±0.54 U/L. The F-value for AST was 18.136 with a p-value of 0.001, indicating a significant difference. The F-value for ALT was 43.059 with a p-value of 0.000, indicating a significant difference and for ALP F- F-value of 39.194 and a p-value of 0.000, indicating a significant differences.

Post Hoc Comparison

Table 2: Showing the comparisons of Alanine Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP)levels among different groups fed with noodle seasoning.

		AST	ALT	ALP
GROUP GROUP 2	1VS	0.221	0.007*	0.047*
GROUP GROUP 3	1VS	0.001*	0.000*	0.000*
GROUP GROUP1	2VS	0.221	0.007*	0.047*
GROUP GROUP 3	2VS	0.007*	0.002*	0.001*
GROUP GROUP 1	3VS	0.001*	0.000*	0.000*
GROUP GROUP 2	3VS	0.007*	0.002*	0.000*

P<0.05= Significant (*)

P>0.05= non-significant

Table 2 shows the multiple comparisons of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) levels among different groups fed with noodle seasoning. Values were considered significant at $p < 0.05$ and not- not-significant>0.05**.**

Discussion

The findings from this study showed a statistically significant increase ($p < 0.05$) in serum liver enzyme levels (AST, ALT, ALP) among albino rats fed with Indomie seasoning compared to the control group. Specifically, the enzyme levels rose progressively from the low-dose to and highest in the high-dose group. This result suggests that the consumption of instant noodles seasoning exerts a dose-dependent hepatotoxic effect.
The rise in AST and ALT in the treated groups is indicative of hepatocellular damage, potentially caused by constituents like Monosodium Glutamate (MSG) and Tertiary Butylhydroquinone (TBHQ) present in the seasoning. These findings align with several studies: Eweka et al. (2011) observed similar liver damage and enzyme elevation after prolonged MSG administration in rats. Sahin et al. (2023) reported increased ALT, AST, and ALP levels following MSG exposure, confirming oxidative stress-induced hepatic dysfunction.. Etim et al. (2006) demonstrated hepatotoxic effects associated with artificial food additives and preservatives.
Furthermore, the high-sodium content in the seasoning may contribute to hepatic metabolic dysfunction. Li et al. (2021) showed that high salt intake alters liver metabolic pathways, even without overt histological damage initially. The post hoc results emphasise that even a low dose of seasoning has a measurable impact, with high-dose intake

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exacerbating liver enzyme abnormalities. This supports the hypothesis that Indomie seasoning significantly alters liver function markers, thus rejecting the null hypothesis (H_0).

Conclusion

This study concludes that instant noodles seasoning significantly increase liver enzyme levels (AST, ALT, and ALP) in albino rats in a dose-dependent manner. The observed hepatic enzyme changes indicate potential hepatotoxicity associated with the chronic consumption of such seasoning. These effects are likely mediated by chemical additives like MSG, TBHQ, and excess sodium, which disrupt liver function and homeostasis. The implication is that frequent consumption of artificially seasoned noodles poses a risk of liver injury, especially with long-term use.

Recommendations

1. Public Awareness: Nutrition and health authorities should intensify public awareness campaigns about the risks of excessive consumption of processed seasoning.
2. Use of Natural Seasoning Alternatives: Consumers should go for natural spices over synthetic seasonings to minimize hepatotoxic risks.
3. Regulatory Oversight: Food regulatory bodies (e.g., NAFDAC) should enforce stricter regulation and labeling of food additives in instant noodles as toxic.
4. School and Youth Education: Target youth-focused health education to discourage the habitual intake of instant noodles among students.

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