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STUDY OF THE HISTOLOGICAL AND BIOCHEMICAL EFFECT OF INSTANT NOODLES ON THE KIDNEY AND LIVER ON RATS MODEL

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ABSTRACT

Instant noodle is produced from flour, and sold in dried form in packets. The noodles are easy and quick in preparation to eat, it can also eat as snacks or as main food. The aim of this research is to investigate the effects of noodle with and without seasoning on the histology as well as biochemical parameters of liver and kidney on rats' model. Three groups of 5 Wistar rats were used, they were fed with noodle with and without seasoning for the period of 28 days. After 28 days the blood of rats was collected for biochemical analysis, the rats were also sacrificed, liver and kidney were removed for histological analysis. Histological results show only a little change in the architecture of the liver and kidney, but only small amount of fat droplet in liver section. The Liver and Kidney tests didn't show any changes in their parameters, only little increase in urea level of the kidney. Conclusively from this research, it was revealed no much effect of consuming instant noodle in rat model.

Keywords: Noodle, Wistar rat, Seasoning, Liver, Kidney

INTRODUCTION

Instant Noodles is a food product produced from flour of either wheat or rice or any other flours as well as starch as the major ingredient, together with other ingredient or without any ingredients. In its preparation it may be mixed by alkaline agents; it is prepared by the use of pre-gelatinization method as well as dehydration through frying or any other means of preparation (WINA, 2020). Noodles is crafted by unleavened dough (from any flour), it produced in different brands and flavours of seasoning powder that prepared from different components (Sikander et al., 2017). Dried noodles can be eaten after being cooked or soaked in warm water, precooked may be reheated or eaten directly from the packet base on the choice of the consumers. Most of the sales of noodles covers many countries of the world such as Indonesia, Australia, America, Nigeria and more countries, though it was revealed that it first consumed in Japan (Benson, 2018). Because of its easy in preparation, many people are eaten it as snacks and major food. Noodles are versatile this makes it more patronage to most consumers from different categories of work life. In recent life, Nigerian market has become more with different varieties noodle's brands. The association on noodles in the world ranks Nigeria as 11th among the highest country of noodles consumers (Benson, 2018).

The consumption of Instant noodles has increased massively over the years in Nigeria as well as world at large. With the exception of bread, no any other simple and convenient food consumed other than instant noodles. Base on the report by Alcaide et al., (2019), Nigeria is one of the countries with the largest market of instant noodles in the world, with 1.76 billion of local consumers of noodles annually. Currently in Nigerian, markets there were about 16 brands on noodles in circulation, noodles lead the Nigerian market with a dominance of about 74% (Alcaide et al., 2019).

A big debate came about over the years on the safety of consuming noodles, a concern about health safety have been raised on different brands of noodles, especially the nutritional on health, due its high content of fats, sodium as well as carbohydrates contained in the seasoning, because it was found out that it contained no or little additional fortification like vegetables, meats and eggs, making the proteins and dietary fibre very low. Another factor of consideration is the oil used in the production of noodles, if care is not taken much accumulation of the oil may produce oxidation products that may results to very serious problems to the health of consumers (Gotoh et al., 2005, Gotoh and Wada, 2006). A study revealed that long consumption of instant noodles may have a toxic effect through promoting oxidative stress in the liver and kidney tissue of Wistar rats (Saleh et al., 2019).

A research conducted by Daniel et al (2014), revealed that 120 g pack of noodles contained 60.01 % carbohydrates, 12.69 % proteins, 24.00 g fats, 2.60 g of moisture, 0.20 g crude fiber and 0.50 g of ash per serving (Daniel et al., 2014). Also, there is present of tartrazine (E102) as a synthetic lemon-yellow azo dye which is used as a dye for colouring food. This is water soluble that has the absorbance in water solution. Tartrazine possessed an azo (-N=N-) group, which is known to be very toxic to life (Wan Ngah et al., 2010). It has been shown that the constituents of noodles have possibility of causing carcinogenic or teratogenic changes in experimental rats (Moutinho et al., 2007). Monosodium glutamate (MSG) is a flavour enhancer due to its ability to rounds, blends and balances the complete perception of many tastes (Löliger, 2000), MSG may be use in preparation of noodles which is very harmful to health (Löliger, 2000). Mostly noodles are manufactured using application of different substances such as oxidizing agent and oil (during frying), sodium, which might be very toxic to health. Previous report revealed that MSG have results to different health hazard, such as damage of kidney (Sharma, 2015), liver damage (Onyema et al., 2006) as well as harmful effect to testis; the harmful effect to testis is as a result of producing a great oligozoospermia as well as increases the abnormal morphology of the sperm in a dose dependant on experimental rats (Onakewhor et al., 2017). It was also documented that it has effect in male infertility by causing changes of sperm cell population, degeneration and haemorrhage on testicular cells (Oforofuo et al., 1997). Low sperm count was also observed as a result of MSG in experimental rats (Nayanatara et al., 2008). In the current study we evaluate histological and biochemical effect of instant noodles consumption on the liver and kidney of Wistar rats (with and without seasoning).

MATERIALS AND METHODS

Preparation Pellets from Instant Noodles

Instant noodles were bought from Sokoto Central Market, Sokoto State, Nigeria. Only the instant noodles were used in this study; it was removed from its sealed wrapping and milled with mortar and pestle into granules. Afterwards, it was mixed with water (with or without seasoning), before it was then moulded into a hand-sized mould, allowed to dry and then grinded again. The prepared noodles were weighed before placed in their respective cages. Moulded rat pellets without instant noodles were used as control (group C).

Animals Handling

Apparently healthy Wistar rats weighing an average of 110g were obtained from the National Animal Production Research Institute ABU, Zaria (NAPRI). The animals were housed in metal cages and maintained under standard laboratory conditions with free access to instant noodles (with or without seasoning) or rat Pellets and tap water *ad libitum*. The research adhered to the Laboratory Animal Care National Institute of Health (NIH) publication No. 88-2959.

Experimental Design

Fifteen adult Wistar rats weighing averagely 110g were used for this study. They were divided into three groups of five rats each.

i. Group 1(Control group): Daily consumption of 50g normal rat pallet for 28 days.
ii. Group 2 (Experimental group): Daily consumption of 50g of instant noodles grinded into granules with seasoning for 28days.
iii. Group 3 (Experimental group): Daily consumption of 50g of instant noodles grinded into granules without seasoning for 28days.

Animal Sacrifice and Tissue Processing

After 28days of consumption, the blood sample was collected through the cardiac puncture under chloroform anaesthesia from each rat of the three groups respectively, and was used for kidney and liver function tests. the rats were sacrificed by cervical dislocation and the kidney and liver were then collected and fixed immediately in 10% buffered formal saline. The tissues were processed accordingly using automatic tissue processor for histological studies.

Tissue Microtomy

Rotary microtome was used to trim the tissue at 10µ, placed downward in an ice bath before it was cut at 5µ. Two sections were cut for each group; for haematoxylin and eosin. A slide was used to place the cut section in 20% alcohol, then transferred to a water bath after which it was picked up with a glass slide coated with albumin to enhance adhesion of the tissue on the glass slide. The slide with the section was placed at a standing position to drain the excess water and then it was transferred to a hot plate to melt the wax, further dry the section and adhere the section to the glass slide.

Haematoxylin and Eosin Staining Technique

Slides were dewaxed in two changes of xylene for 10 minutes each. Then dehydrated in descending grades of alcohol (absolute, 90% and 70% respectively). Harris Haematoxylin was used to stain the slides for 10minutes, and washed afterwards. 1% acid alcohol was used to briefly differentiate section and Scott's tap water was used to blue section for 5minutes. Counter stain was carried out with eosin for 3minutes. Running tap water was used to wash off excess stain. Sections were dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX (Drury and Wallington, 1980).

Estimation of Urea (DAM method)

Sample was pipetted into clean dry test tubes labelled as blank (B), standard(S) and test (T):

Table 1: Protocol of Urea Estimation

Addition sequence	B (ml)	S (ml)	T (ml)
Urea reagent (L1)	1.0	1.0	1.0
Acid reagent (L2)	1.0	1.0	1.0
DAM reagent (L3)	1.0	1.0	1.0
Distilled water	0.01	-	-
Urea standard (S)	-	0.01	-
Sample	-	-	0.01

Sample was mixed well and kept in boiling water at 100°c for 10 minutes. Sample was allowed to cool under running tap water and absorbance of the standard (Abs.S) was measured and that of the Sample (Abs. T) against the blank using a colorimeter at a wavelength of 520nm.

Calculation: $\text{Urea in mg/dl} = \frac{\text{Abs.S}}{\text{Abs.T}} \times 40$

Estimation of Serum Bicarbonate Concentration (modified van slyke's method)

Using a universal container 2ml of distilled water was dispensed into it. 100µl of sample was added to a test tube. Then1ml of 0.01N hydrochloric acid was added to the sample. Two drops of indicator were added to the mixture producing a pink solution. The solution was mixed properly to expel the CO_2 generated. 1ml of 0.01N of NaOH was titrated against the acid mixture with the sample to give an end point of salmon yellow, the titer was recorded.

Electrolyte estimation

Electrolytes present in the sample (Na^+, K^+ and Cl^-) was quantitatively estimated using ion selective electrode method (ISE) which is a semi- automated machine (Abraham et al., 2008).

After the machine was switched on, activation of the electrodes was done within 60 seconds. The calibration of standard A and standard B reagent were made after which the machine was expected to be ready for use provide the slope was passed. The sample was placed in the machine, the probe aspirated 160ml of the sample and it was taken to the electrode where the ionic activity was measured and the result was displayed.

Determination of Serum Creatinine

Serum creatinine was determined using Jaffe's method (Hawk, 1914).

Stage 1: Two test tubes labelled test and blank were set, 1.5 ml of distilled water was added to each of the tubes, 0.5 ml of

the sample was added to the tube labelled test, 0.5 ml of 2/3N H_2SO_4 was added to both test and blank, followed by 0.5ml of 10% Na_2WO_4. The content of both tubes was mixed and centrifuged at 4000 rpm for 10 minutes.

Stage 2: Three test tubes were labelled test, standard and blank, 1.5ml of supernatant from test and blank from stage 1 were transferred into the tubes labelled test and blank respectively. 1.5ml of creatinine working standard was transferred into the test tube labelled standard, 0.5ml of 0.75N NaOH and picric acid were added into each test tubes. The content of the test tubes was mixed properly and allowed to stand at room temperature for 15 minutes, the absorbance was read spectrophotometrically at 520 nm.

Calculation

$$\text{Creatinine concentration } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard (3.0)}$$

Determination of Serum Total and Direct Bilirubin

Serum total and direct bilirubin was determined using Malloy and Evelyn Method (1937)(Malloy and Evelyn, 1937).

Procedure for Total Bilirubin

One hundred microlitres (100 µl) of sulphanilic acid was added to both test and blank test-tubes and a drop of nitrite added into test test-tube only. 500 microlitre of caffeine and 100 of plasma is added to test and blank tubes. The tubes were then being mixed and incubated at room temperature in the dark for 10 minutes. 500µl of alkaline tartrate is added into test and blank tubes, mixed and allowed to stand at room temperature for 5 minutes. The absorbance of the test is read against the sample blank at 580nm spectrophotometrically.

*Calculation***:** Total bilirubin (mg/dl) = OD x 185

Procedure for Direct bilirubin

One hundred microlitre (100µL) of sulphanilic acid was added into both test and blank test-tubes followed by the addition of nitrite into the test test-tube only. 1mL of normal saline will then be added to both test-tubes. 100 L of the sample was added into both test-tubes. The tubes were mixed well and incubated at room temperature for 10 minutes after which the absorbance of the sample was read against the blank spectrophothometrically at 540nm.

*Calculation***:** Direct bilirubin = O.D x 246

Determination of Serum Total Protein

Serum total protein was determined using Biuret method (Henry, 1974). Twenty microlitre (20µL) each of sample and the protein standard was added to 1mL of total protein reagent in test-tubes labelled test and standard respectively. It was mixed and incubated at room temperature for 10 minutes. Absorbance of both test and standard was read spectrophotometrically at 540nm.

Calculation:

Serum Total Protein (g/l) = OD of Sample/OD of Standard X Concentration of Standard (60)

Determination of Serum Albumin

Serum albumin will be determined using Bromocresol green (BCG) method (Doumas et al., 1971). 10 microlitre (10µL) each of sample and standard was added to 1mL of albumin reagent in the test-tubes labelled test and standard respectively. The tubes were mixed and incubated at 37ºC for 5 minutes, after which the absorbance was measured spectrophotometrically at 546nm.

*Calculation***:**

Serum Albumin (g/l) = OD of Sample/OD of Standard X Concentration of Standard (40)

Determination of Serum Aspartate Transaminase (AST)

Serum AST was determined using the method of Reitman and Frankel, (1957). 500 microlitre (500µL) of reagent containing buffer was to 100 microlitre (100µL) of the sample. The mixture was then incubated for 30 minutes at 37ºC. 2, 4-dinitrophenyl hydrazine wasn added to the mixture after which it was incubated for 20 minutes at 20ºC followed by the addition of 5mL of sodium hydroxide (NaOH) and it was incubated again for 5 minutes. The absorbance was measured at 546nm spectrophotometrically (Reitman and Frankel, 1957).

*Calculation***:** AST activity (U/L) = (OD/min) x 1768

Determination of Serum Alanine Transaminase (ALT)

Serum ALT was determined according to the method of Reitman and Frankel, (1957). 100 microlitre of sample was added to 500 microlitre of the buffer. The mixture was incubated at 37ºC for 30 minutes, after which 500 microlitre of dinitrophenyl hydrazine was added. The mixture was further incubated at room temperature for 20 minutes. 5mL of 0.4N sodium hydroxide was then added to the mixture and allowed to stand for 5 minutes. The absorbance was read at 546nm spectrophotometrically (Reitman and Frankel, 1957).

Calculation: ALT activity (U/L) = (OD/min) x 1768

Statistical Analysis

The statistical analysis was performed using statistical package for social sciences (SPSS) version 20 (SPSS Inc., Chicago, II, USA). The data was presented as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) test was used to determine the significances among different groups. P value < 0.05 was taken as statistically significant.

RESULTS AND DISCUSSION

Histological Results

The stained sections were examined using a microscope to determine the effect of the consumption of instant noodles on the normal histology of the kidney. The glomeruli were well preserved with presence of the bowman's capsular space, proximal convoluted tubules and distal convoluted tubules. The histological result showed no significant changes in the histology of the kidney in group 2 and 3 compared with control group.

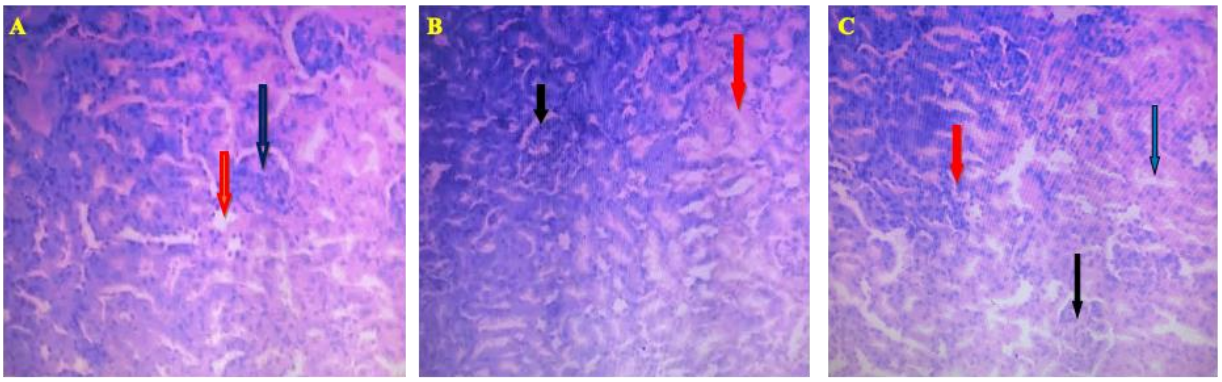

Plate 1: Kidney section stained with H and E (X 400). **(A)** control group 1 fed with pallet for 28 days showing normal kidney architecture with normal glomerular capsular space (black arrow) renal tubules (red arrow). **(B)** group 2 fed with instant noodles with seasoning for 28 days, showing no changes in the histological architecture of the kidney with well-defined bowman's capsule (black arrow) renal tubule (red arrow). **(C)** group 3 fed with instant noodles without seasoning, showing no histological changes to the architecture of the kidney, distal convoluted tubule (red arrow), the proximal convoluted tubule (blue arrow and Bowman's capsule (black arrow).

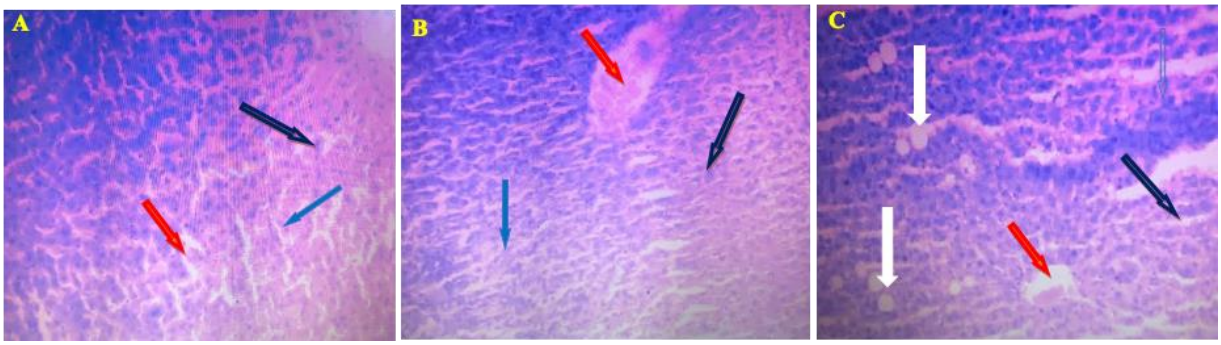

Plate 2: Liver section stained with H and E (X400). **(A)** Control group 1, showing normal architecture of liver, (Black arrow) hepatocyte, (Red arrow) central vein, (green arrow) hepatocyte. **(B)** Group 2 fed with seasoning showing normal architecture of liver, (Black arrow) hepatocyte, (Red arrow) central vein, (green arrow) hepatocyte. **(C)** Group 3 fed with noodles without seasoning, Some areas had hepatocellular steatosis (White arrow) showing some droplets of fats, (Black arrow) normal hepatocyte, (Red arrow) normal central vein.

Biochemical Result

Renal integrity is determined by urea, creatinine and electrolytes (sodium ion, potassium ion, chloride ion and bicarbonate) levels in serum. From the renal function results, no significant increase or decrease of the parameters were observed except in urea which showed a statistical significance with the p value of 0.00 which is less than 0.05 (Table 2).

Table 2: Effect of instant noodles on renal function parameters (mmol/L)

PARAMETERS (mmol/L)	GROUP 1 (CONTROL)	GROUP 2 (SEASONING)	GROUP 3 (WITHOUT SEASONING)	P-VALUE	P-VALUE
SODIUM	150.00±0.55	150.80 ±1.28	150.40±1.17	0.87	0.15
POTASSIUM	5.64±0.45	5.90±0.38	4.90±0.32	0.21	1.76
CHLORIDE	110.6±1.29	113.00±1.41	112.20±1.37	0.47	0.81
BICARBONATE	17.80±1.11	16.80±1.98	19.20±1.39	0.37	1.12
UREA	8.84±0.36	5.06±0.42	5.04±0.39	0.00	31.58
CREATININE	1.04±0.17	1.30±0.06	0.94±0.12	0.14	2.29

Data are presented as mean ± SE

Discussion

Histological analysis shows that there was no effect on the architecture of the kidney from the rats that were fed instant noodles with seasoning (group 2) for 28 days, had normal kidney architecture with well-defined bowman's capsules, distal and proximal convoluted tubule and those that were fed instant noodles without seasoning (group 3), also for 28 days had normal kidney architecture with well-defined bowman's capsules, distal and proximal convoluted tubule. Also kidney sections from the rats that were fed normal Pellet (control group 1) had normal kidney architecture with well-defined bowman's capsules, distal and proximal convoluted tubule, Eze et al., (2017), revealed that the frequent and prolonged consumption of instant noodles may affect the vital function of glomerular filtration, thereby resulting to morphological changes in the kidney followed by severe histopathological changes (Eze et al., 2017), this is not in conformity with the current study, it could be as a result of methods employed. The Rats were fed with instant noodles at a longer duration of time there might be morphological changes in the architecture of the kidney. It has been reported that Instant noodle and its flavouring sauce base contain huge amount of monosodium glutamate (MSG), which causes neuroplacental neurotoxic effect. MSG also reportedly causes cataract, causes induced retinal lesions and genotoxicity (Kim et al., 2000). It was shown that from previous researches that the noodle with

seasoning in combination results to severe damage to the blood vessels wall of the kidney, leading to narrowing of the lumen of blood capillaries to greater extend of even blocking it, hence result to renal arteriosclerosis, that causes kidney damage as well as Ischemia. Ischemic damage to kidney may result to kidney failure as well increase blood pressure to the kidney (Eze et al., 2017, Hostetter, 2003).

From the current study we observed some fats deposition on the liver of adult rats that received instant noodles without seasoning, this is slightly contrary to Eweka and Adjene (2007) that reported that chronic consumption of Monosodium glutamate a major constituents of instant noodles seasoning had more marked necrotic and cellular degenerative changes in rats consuming noodles with seasoning, than those without (Eweka and Adjene, 2007), this might be from duration of feeding which is 28days, which may not be enough to be chronic.

The condition most commonly associated with fatty liver disease is metabolic syndrome, this includes conditions such as type II diabetes, obesity, and hypertriglyceridemia (Tommolino, 2018). Other factors, such as drugs (e.g., amiodarone, tamoxifen, methotrexate), alcohol, metabolic abnormalities (example; galactosemia, glycogen storage diseases, homocystinuria, and tyrosinemia), nutritional status (example: over nutrition, severe malnutrition, total parenteral nutrition [TPN], or starvation diet), or other health problems (example: celiac sprue and Wilson disease) may contribute to fatty liver disease (Tommolino, 2018), from the etiologic factors listed by Tommolino, 2018, it can be suspected from the current study that the deposition of fats in the liver of rats that consumed instant noodles without seasoning might be due to under-nutrition; as the rats in group 2 were feeding on instant noodles without spices (poor-fortification).

The biochemical analysis of the kidney in the current study showed no significant value in all parameters except in urea (p value= 0.00) which is lower than 0.05 when the parameters were compared among the groups (normal p value < 0.05), this is in agreement with research conducted by Khudhur et al. (2021), that revealed a blood urea shows a significant value on a rats fed with instant noodles with or without seasoning (Khudhur et al., 2021). This can be seen in biochemical results from group fed with instant noodles (with or without seasoning). A p value less than 0.05 is statistically significant, it indicates strong evidence against the null hypothesis, as there is less than 5% probability the null is correct. Therefore, we reject the null hypothesis and accept the alternative hypothesis. For results obtained in urea levels, it was noticed that addition of spice caused a statistically significant increase in serum urea levels, this increase could be traceable to high protein breakdown and could be implicated in passage of excess protein in urine (proteinuria). The possible cause of this is likely due to the disruption and distortion of the renal filtration membrane as a result of the active ingredients (Monosodium glutamate, Sodium and Fats) in the added spice as against those of control animals. This finding is supported by a study conducted by Saleh et al., (2019) that previously reported a distortion as well as disruption of the morphology of the kidney and urea levels caused by long consumption of instant noodles (Saleh et al., 2019). Also, the rate of urea production is influenced by protein content of diet; low protein diet is related with reduced urea formation while high protein diet is associated with increased in production of urea (Higgins, 2016). The limitation of urea analysis in renal function is due to reduced sensitivity as well as specificity, therefore normal urea does not necessarily exclude renal disorder and slight to moderate high in urea might not assumed to be as a result of renal disease (Higgins, 2016).

In the current study the results of liver function indices after feeding rats with noodles, the P-value of rats feeding with and without seasoning showed non-significant change ($p>0.05$) in the activity of alanine transaminase (ALT) compared to control. The activity of aspartate transaminase (AST) was also non-significant ($p>0.05$) in the groups 1 and 2 when compared to control. There was a no significant increase in concentration of albumin and total protein in Group 1 and 2 compared to control ($p>0.05$). Also, no significant change ($p>0.05$) seen in the concentration of conjugate and total bilirubin in Group 1 and 2 compared to control. Activities of AST and ALT in the serum are recognized as the markers of liver injury (Milinković-Tur et al., 2005). Changes in the levels of these enzymes shows an injury to some cell organelles like mitochondria resulting to release of some soluble enzymes such as AST (Dahiru et al., 2003). But in the current study there was no increase in level of such enzymes. High level of total Protein, Albumin as well as Bilirubin in the blood is an indication of functional state of the liver (Shahin, 2017). Albumin and plasma proteins bring about colloidal osmotic pressure that functioned to maintain a normal blood volume. Thus, the low level in serum protein and serum albumin might be as a result of diminished synthetic liver function (Adebayo et al., 2009). The result of the current study showed no significant difference in all parameters between test and control groups. A research conducted by Osuchukwu et al., (2021) revealed that there was no significant difference in the level of ALP and ALT in the serum of that rats fed with noodles with and without seasoning, results show normal function of the liver of all the group of the rats (Osuchukwu et al., 2021), this is in conformity with the current research in which we observed the normal liver function in all groups with and without seasoning.

CONCLUSION

From the outcome of this research, it was concluded that consuming Indomie Instant Noodles doesn't have much effect on histology of kidney and liver as well as the Biochemical parameters of kidney and liver, the effect is less by formation of fats droplets in the liver histology as well as small increase in urea level in kidney function.

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