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Toxicity of Prepared Fermented Soybean Condiments from Indigenious Fermenters

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Several studies have shown that most condiments consumed in Nigeria today are fortified with chemicals that alter the nature, and negatively affect organs and cells. Several condiments have been developed from fermented foods in order to control this ugly situation and to produce a non-toxic condiment with improve health qualities. The aim of this study was to evaluate the toxicity of the fermented soybean condiments produced using indigenous fermenters. Soybean (*Glycine max*) sample was fermented with indigenous microorganisms isolated from 7 days old fermented soybean sample; this was oven-dried, pulverized and packaged in a cleaned sterile screw capped container. The toxicity was evaluated using *in vivo* technique. *Lactobacillus plantarum* strain ZS 2058 (L), *Bacillus subtilis* strain 168 (B) and *Saccharomyces cerevisiae* strain YJM555 (Y) were the indigenous microbes used singly and in consortium for the production of light to dark brown condiments with water activity ranging from 0.27 – 0.37 for the fermented soybean in the plate and

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0.22 - 0.36 for the fermented soybean wrapped with *Thaumatococcus danielli* leaves (called *Uma* in Igbo and *Ewe eran* in Yoruba). No death was recorded from the experimental rats.The experimented rats showed significant (P<0.05) increased in body weight, no evidence of severe deformities in the organs and cells, slight or no increased in organ weights and these were statistically non-significant (P>0.05). Therefore condiments produced from fermentation of soybean using indigenous B, L, Y, BL and BLY are recommended safe and non-toxic and those fermented in plastic plates using BLY were most efficient, preferable and acceptable.

Keywords: Soybean condiments; fermentation; toxicity.

1. INTRODUCTION

"The origin and history of soybean dates back to 2838BC in China, and the emperor sheng-Nung of China named soybean as one of the five scared grain" [1]. "Soybean contains approximately 35%, protein, 31% carbohydrate, 17% fats, 5% mineral and 12% moisture" [1]. "The soybean protein contains acceptable amount of essential amino acids visa viz histidine, isoleucine, leucine, lysine, phenylalanine, tryrosine, tryptophan and valine which is recommended for daily intake as a balanced diet" [1]. "In addition to essential nutrients, soybean products, especially fermented soybean products contain various functional components including peptides, isoflavonoids and saponin" [2]. "Soybean has been reported to improve cancer, improvement in bone mineral density and provide protection against bowel and kidney disease" [2,3,4]. "These health benefits are caused by the presence of isoflavone, saponins, protein and peptide in soybean" [4,5,6,7,8]. "Soybean as a food have many different formulations such as soymilk, soyflour, soy oil, feed for livestock and poultry, soy concentrate, protein isolates, soy yoghurt, tofu and fermented foods such as Tempeh, soy sauce, *misco,* Natto and *sufu"* [9]. Although soybean has high protein content, minerals, vitamins and bio-actives [7] it has little direct use because of high oil content, poor digestibility, green beany taste, long cooking time and persistent bitterness. "Fermentation is a proven method used to improve flavor, texture and nutritional quality of soybean. Besides bringing physicochemical and sensory quality changes, fermentation also helps towards the preservation of food due to release of metabolites that discourage the growth of pathogenic bacteria in foods" [9]. "These fermented soybeans are used as condiments to flavor foods such as stir-fries, stew, and soups" [2]. "Fermentation involves a range of microorganisms such as lactic acid bacteria, acetic acid bacteria, yeasts, mould and *Bacillus subtilis"* [10].

"One of the most reported health benefits of *B. subtilis* fermentation is almost complete removal of indigestible oligosaccharides (stachyose) which are responsible for indigestion and flatulence in humans and manogastric animals" [11]. "They have also shown to reduce the activity of anti-nutrients that hinders availability of proteins and phytochemicals present in soybeans, also they completely remove the beany odour of raw soybeans and increases sensory quality of the product" (Shrestha and Noomhorm, 2001; Hu et al*.,* [12] Palai et al. [10] suggested that "probiotics from strains of lactic acid bacteria and *Bacillus* spp could be used as remove 1996 and 1999 references alternative feed additives to piglets so as to replace antibiotic and antimicrobial compounds in their feeds which may contribute to antibiotic resistance in human". Shrestha et al. [9] also claimed that "*Rhizopus* fermented soybeans (Tempeh) reduces the duration of diarrhea when added to the diet of malnourished children". Kiers et al*.,* [13] showed that "while fermented soybeans inhibits adhesion of ETEC K88 in *Rhizopus* fermented soybeans, *Bacillus* which acts as probiotics allows *Bacillus* fermented products to inhibit the proliferation of pathogens in gut". Wei et al. [14] reported that "isoflavone which acts like oestrogen is able to reduce risks related to cardiovascular diseases, lower rate of prostrate, breast and colon cancers and improve health benefits". Similarly, Kon et al*.* [15] linked "consumption of fermented soybeans with reduction of diabetes type -2 which improved glucose control and insulin resistance. Other studies have investigated the toxicity of some fermented soybean condiments but its toxicity against target organs of mice has not been investigated". Therefore, in this study, we investigated the toxicity of the fermented condiments produced from soybean seeds.

2. MATERIALS AND METHODS

2.1 Preparation and Local Fermentation of the Soybean

Two hundred and fifty grams of cleaned soybean seeds were weighed using an analytical weighing balance and steeped in 500ml bucket of water overnight, after which the seed coat was removed by rubbing between the palms and then the chaff were removed using sieve. The soybean seed were then thoroughly washed and placed inside cleaned *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) and wrapped properly and then kept inside 500ml bucket that was well covered with the lid for fermentation to take place for 7 days at room temperature.

2.2 Processing of the Fermented Soybean

After the fermentation the fermented soybean were prepared for culturing and the diluents used was peptone (BIOTECH) water which was prepared according to the manufacturers instruction, then was sterilized by autoclaving at121ºC for 15min at 15psi. "Ten grams of the fermented soybean was aseptically weighed using analytical weighing balance into a 200 ml beaker (G.G) and little amount of the diluent was added and homogenized and then make upto 100 ml, part of these preparations was transferred into 100 ml beaker (G.G) and boiled for 10-15 min using a pressure pot" [16].

2.3 Isolation of the Test Sample

"The media used for this isolation includes Sabourand dextrose agar (SDA), de Man Rogosa and Sharpe broth (MRS) and Nutrient agar (BIOTECH). A 0.1ml of the preparation/inoculum collected using a sterile pipette and aseptically plated onto solidified Sabouraud dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in [17] supplemented with chloramphenicol (0.05%) and spread using a spreading rod, 0.1 ml of the boiled preparation/inoculums was collected and plated unto solidified nutrient agar plate also 1 ml of the inoculums was collected using sterile pipette and aseptically inoculated into sterile 100 ml conical flask (Glassco) containing MRS broth (BIOTECH) which was prepared according to the manufacturers instruction and the conical flask were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon iv oxide). The incubation was done for 24 – 72 h at (30±2ºC). The SDA and NA were incubated in an inverted position for 24 h at 35±2ºC (for NA) and 30±2ºC (for SDA) in an incubator (STXB128)" [16]. The plate that showed discrete colonies were selected after 24 h and each colony was aseptically streaked using a sterile wireloop on a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. Similar procedure was repeated on SDA plate (90 mm x15 mm) for the yeast and also on DeMan Rogosa Sharpe (MRS) agar plate (90mm x 15mm) that was prepared according to the manufacturers instruction after which it was incubated at their required growth conditions. The pure bacterial isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere et al. [18]. The fungal isolates were identified to the genus/species level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures as described in the study published by Iheukwumere et al. [19].

2.4 Preparation of Soybean Condiments

2.4.1 Processing of soybean for fermentation

This was carried out using the modified method of [20]. "One kilogram of soybean were carefully picked and weighed using analytical weighing balance and steeped in 200ml bucket of water overnight for fermentation to take place, after the soybean were dehaulled by rubbing between the hands to remove seed coat, after the chaff/seed coat were properly removed using a clean sieve, the soybean was then properly washed and placed inside a beaker and then autoclaved at 121ºC for 15 min at 15psi" [16]

2.4.2 Fermentation process

This was carried out using the modified method of [12,21]. After autoclaving the soybean, a 100g of soybean was weighed using analytical weighing balance and placed inside 6 different *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) which was properly sterilized using electric oven at 180^0C for 2 h, each of the leaves containing the soybean were inoculated with the fermenters prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by

mixing 0.6mL of 1% $BaCl_2$. $2H_2O$ and 99.4 mL of 1% Conc. H_2SO_4, 10ml of suspension *Bacillus* was added and labeled as "B" , 10ml of suspension of *Lactobacillus* was added and labeled as "L", 10ml of suspension of yeast was added and labeled as "Y" , consortium of suspensions 5ml of Bacillus and 5ml of Lactobacillus was added and labeled as "BL", consortium of suspensions of 3ml of Bacillus,3ml of Lactobacillus and 4ml of yeast were added and labeled as "BLY" consecutively and one of the leaves containing only soybean was set aside as the control. These leaves were carefully wrapped. This same method was repeated using sterile plates. The wrapped leaves and the plates containing the soybean were kept at room temperature for fermentation to take place for 7days.

2.4.3 Storage and packaging

"After fermentation, the fermented samples were aseptically dried using an electric oven at 80ºC for 7days**.** After drying water activity of the fermented samples was determined, after which it was grinded into powder and stored in a sterile screw capped container for subsequent analysis" [16].

2.4.4 Albino wistar rats

The albino Wistar rats were purchased at animal house, Zoology Department, University of Nigeria, Nsukka (UNN). The rats were transported to the animal house at Department of Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University (NAU), Awka. The rats were critically examined for their weights and experimented for their suitability for the study. The rats were selected and grouped based on their weights and experimented design.

2.4.5 *In vivo* study

A total of 52 albino Wistar rats were used for this study. The rats were grouped into 13 groups. Each group contained 4 rats each. The rats were orally administered 1.0 g/ kg (tenfold of normal administration) of the prepared condiments except the last group that was giving ordinary distilled water as normal control. The rats in each group were monitored for 21 days during which the acute toxicity was determined after 72 h, liver enzymes, Kidney (creatinine, urea) and heart (Lactate dehydogenase LDH) monitoring parameters and effects on the cells (histopathology study) were checked and recorded as described in the work published by Iheukwumere et al. [18], Nwobodo et al. [22] and Lai et al. [23].

3. RESULTS

The study revealed the acute toxicity of the sample. No death rate was recorded after 24, 48 and 72 h as shown in Table 1. The study revealed the effect of the samples on the body weight of albino Wistar rats (Table 2). The body weight was taken at Day O, and after Day 7, Day 14 and Day 21 there were pronounced increase in the body weight of the albino. The highest weight was recorded from sample BLYL and the least weight from Cp after 7 days. BLYL also recorded the highest weight after 14 days while Cp recorded the least weight. After 21 days sample BLYL showed the highest weight while sample Cp.The study revealed the effects of the samples on the organ weights of the albino Wistarrats (Table 3). Sample Cp showed the highest organ weight on the liver and sample BLYL showed the least. Sample Cp showed the highest organ weight on kidney while the least weight was seen on BLY. Sample Cp Showed the highest organ weight on the heart while LL, BLL and BLYL showed the least. Sample Cp showed the highest organ weight on the lungs while samples BLP, BLYP, LL, YL and BLYL showed the least. Sample Cp showed the highest organ weight on the spleen while samples LL and BLYL showed the least organ weight. Sample BLYL showed the highest organ weight on the intestine while sample Cp showed the least organ weight. The study revealed that the prepared condiments had no negative effect on the heart, kidney and liver of the experimented rats as shown in Table 4. The Lactate dehydrogenenase (LDH) value as the marker for monitoring the activity of the heart were slightly the same when compared to the group of the rats that were fed with the condiments and the control group, and the values were statistically non – significant ($P > 0.05$), but a slight increase was observed among the groups that were fed with the condiments prepared with Cp and Cl. The values of urea and creatinine which serve as markers for monitoring kidney function were slightly equivalent when compared to the groups fed with the condiments and the control group. Although slight increase was observed among the rats fed with the condiments prepared with Cp and Cl, but these were not statistically significant ($P> 0.05$). Similar trends were observed in the values of aspartate aminotransferease (AST) and alanine aminotransfereases (ALT) which serve as

Table 1. Acute toxicity of the studied samples

Sample	Total Number of Rat	Number of Death Recorded After			Number survived after 72h
		24h	48h	72h	
BP	6	0	0	0	6
LP	6	0	0	0	6
YP	6	0	0	0	6
BLP	6	0	0	0	6
BLYP	6	0	0	0	6
CP	6	0	0	0	6
BL	6	0	0	0	6
LL	6	0	0	0	6
YL	6	0	0	0	6
BLL	6	0	0	0	6
BLYL	6	0	0	0	6
CL	6	0	0	0	6

Table 2. Effect of the samples on the body weights of albino wistar rats

Sample	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
BP	123.66±1.21	128.22±1.42	138.11±1.22	142.17±1.27
LL	123.11±1.36	127.68±1.17	135.26±1.89	138.62±1.47
YP	122.96±1.96	127.37±1.21	134.17±1.91	137.28±1.31
BLP	122.87±1.67	129.06±1.33	139.88±1.41	1.44.14±1.21
BLYP	123.14±1.22	133.17±1.24	141.36±1.22	147.25±1.41
CP	123.22±1.41	126.77±1.67	131.12±1.52	134.11±1.09
BL	122.97±1.43	129.12±1.18	139.77±1.61	144.22±1.12
LL	123.21±1.09	128.44±1.29	136.12±1.24	139.17±1.37
YL	122.89±1.31	128.06±1.26	134.92±1.71	138.23±1.19
BL	122.92±1.47	130.81±1.41	140.31±1.21	145.29±1.19
BLYL	123.13±1.51	133.92±1.28	143.21±1.28	149.11±1.22
CL	122.94±1.25	127.22±1.08	133.92±1.19	136.48±1.62
Control	123.17±1.19	127.11±1.21	133.02±1.22	136.22±1.51

markers for monitoring the liver function. Also the AST/ALT ratio showed the safety nature of the condiments in the liver of the experimented rats when compared to the control group.

The histopathological features of the internal organs harvested after sacrificing those albino Wistar rats fed with condiments 'okpeyi', maggi(star) and distilled water (normal control) are shown in Plates 1-9. The study revealed that the organs (heart, lung, kidney, spleen, stomach mucosa) harvested from those sacrificing rats fed with the condiments showed normal liver, kidney, stomach mucosa and heart when compared with the control groups. Their lungs and spleens were also normal except the lungs of those rats fed with condiment prepared with Bl and maggi, which showed evidence of intestinal pneumonia in the lungs tissue. Also the spleen of those rats fed with condiments prepared with Yl and maggi showed reactive follicular hyperplasia. The overall results showed that the condiments were safe and had no negative impacts in the cells and tissues of the visceral organs.

4. DISCUSSION

Determining the toxic effect of condiments to organs in the body is paramount in safeguarding the overall body physiology. The significant ($P < 0.05$) increase in the body weight of Wistar rats administered BLYL could be ascribed to high nutritious value, especially protein. Protein had been shown to be responsible for an increase in body tissues and repair due to its active amino acids. This finding corroborates with the observation made by Friedman and Brandon [4] who investigated the nutritious value of soybean. The study also revealed the administration of the prepared condiments had no significant alteration in the normal organ weight of the experimented Wistar rat. This indicates that the condiments have no negative impact to the physiology and

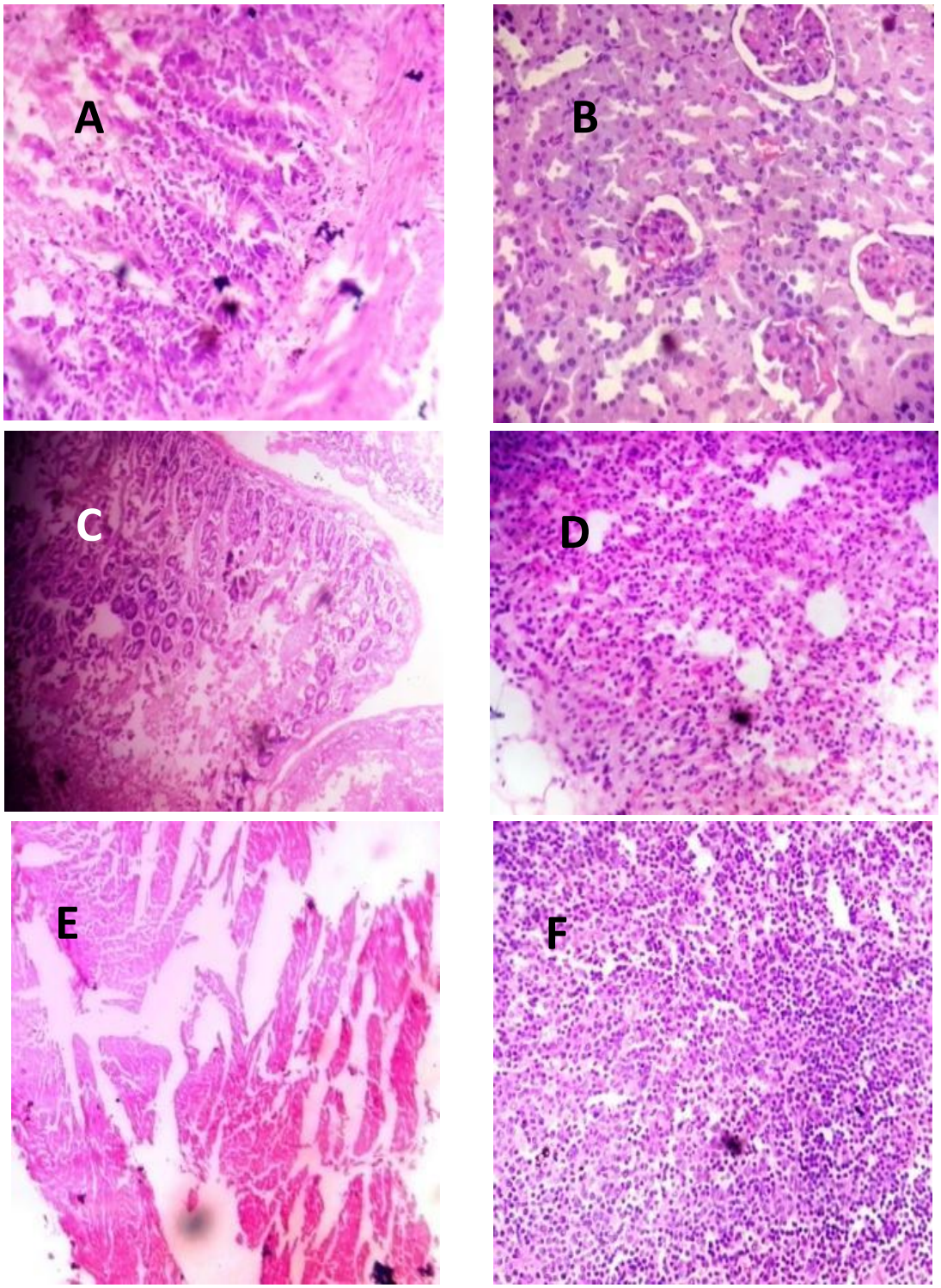

Plate 1. This represents control. A, normal kidney at x 40 objective lens magnification
B, Normal Liver at x 40 magnification (Objective lens). C, Normal Stomach Mucosa at x 40 magnification (Objective lens). D, Lung tissue with evidence of interstitial pneumonia at x 40 magnification (Objective lens). E, Normal Heart Muscles at x 40 magnification (Objective lens). F, Spleen with reactive follicular hyperplasia at x 40 magnification (Objective lens)

metabolic processes that occur in the organs as supported by Nwobodo et al. [22]. The slight increase in LDH in Wistar rats that were administered condiments prepared with CP and CL was not significant ($P > 0.05$) which indicates that the condiments do not interfere with the

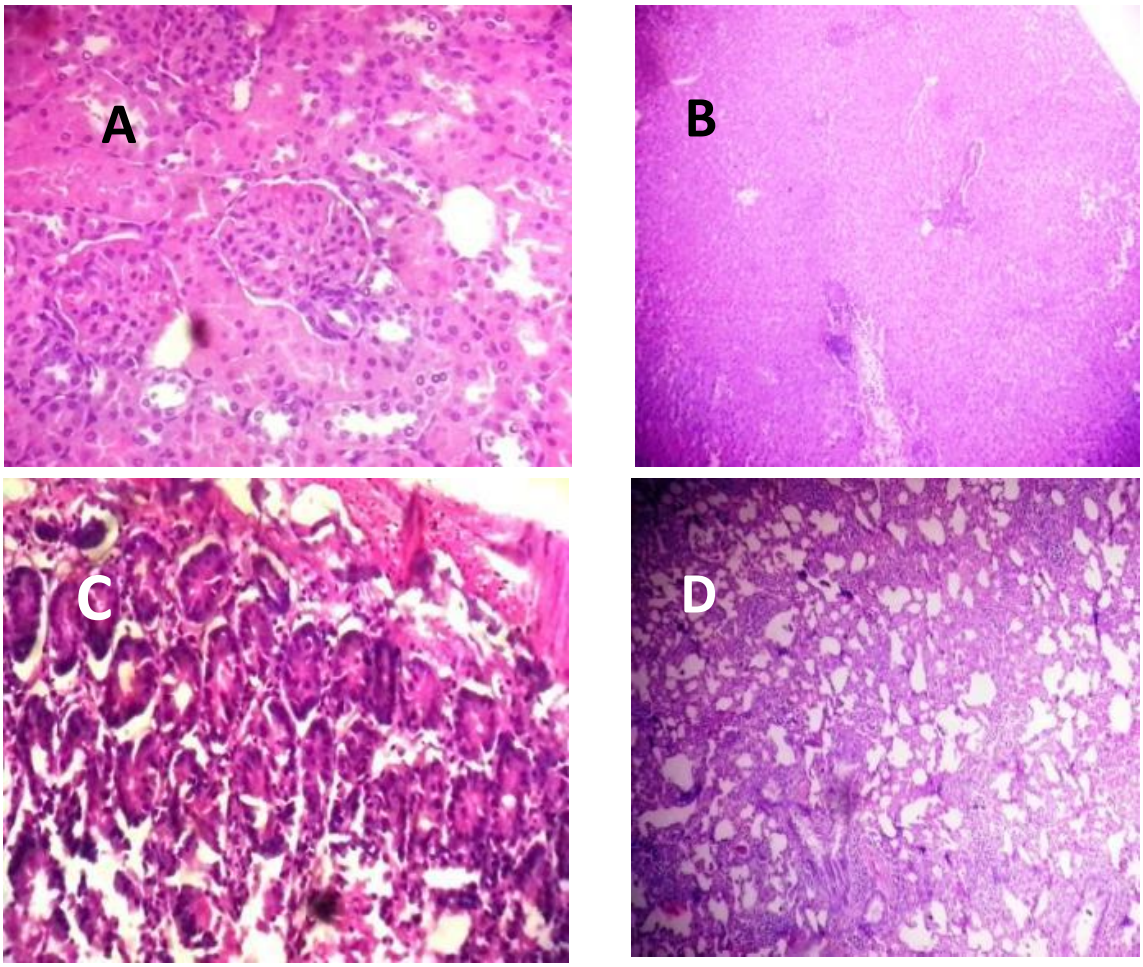

Plate 2. This represents BL.A, normal kidney at x 40 objective lens magnification

B, Low power photomicrograph of liver parenchyma showing normal features. ***C,*** *Normal Stomach Mucosa at x 40 magnification (Objective lens). D, Lung tissue with evidence of interstitial pneumonia at x 40 magnification (Objective lens)*

Table 3. Effects of the samples on the organ weights of the albino wistar rats

Sample	Liver (g)	Kidney (g)	Hearts (g)	Lungs (g)	Spleen (g)	Intestine (g)
BP	642±0.07	0.51±0.00	0.44±0.00	1.11±0.00	1.21±0.01	13.26±1.14
LP	6.37±0.03	0.55±0.00	0.42±0.00	1.02±0.00	1.10±0.01	15.21±1.08
YP	6.47±0.11	0.57±0.00	0.43±0.00	1.04±0.00	1.15±0.00	13.92±0.41
BLP	6.35±0.01	0.52±0.00	0.44±0.00	1.01±0.00	1.12±0.00	15.42±0.58
BLYP	6.33±0.10	0.48±0.00	0.43±0.00	1.01±0.00	1.11±0.01	15.93±0.42
CP	6.58±0.14	0.58±0.00	0.48±0.00	1.18±0.00	1.19±0.01	12.91±0.87
BL	6.46±0.03	0.52±0.00	0.44±0.00	1.10±0.00	1.17±0.01	14.11±0.41
LL	6.36±0.11	0.53±0.00	0.41±0.00	1.01±0.00	1.08±0.01	15.81±0.58
YL	6.47±0.03	0.55±0.00	0.43±0.00	1.01±0.00	1.10±0.01	14.77±1.01
BLL	6.33±0.07	0.51±0.00	0.41±0.00	1.02±0.00	1.10±0.01	15.86±0.82
BLYL	6.32±0.11	0.51±0.00	0.41±0.00	1.01±0.00	1.08±0.01	16.12±0.41
CL	6.56±0.03	0.53±0.00	0.46±0.00	1.16±0.00	1.14±0.01	13.01±0.14
Control	6.30±0.01	0.48±0.00	0.41±0.00	1.01±0.00	1.08±0.01	13.55±0.14

normal heart function. It is worthy to note that the enzyme (LDH) is released into the bloodstream in excess when there is an abnormality in the normal heart function [23]. Similar observations were recorded in the value of urea and creatinine, AST/ALT, which are biomarkers for

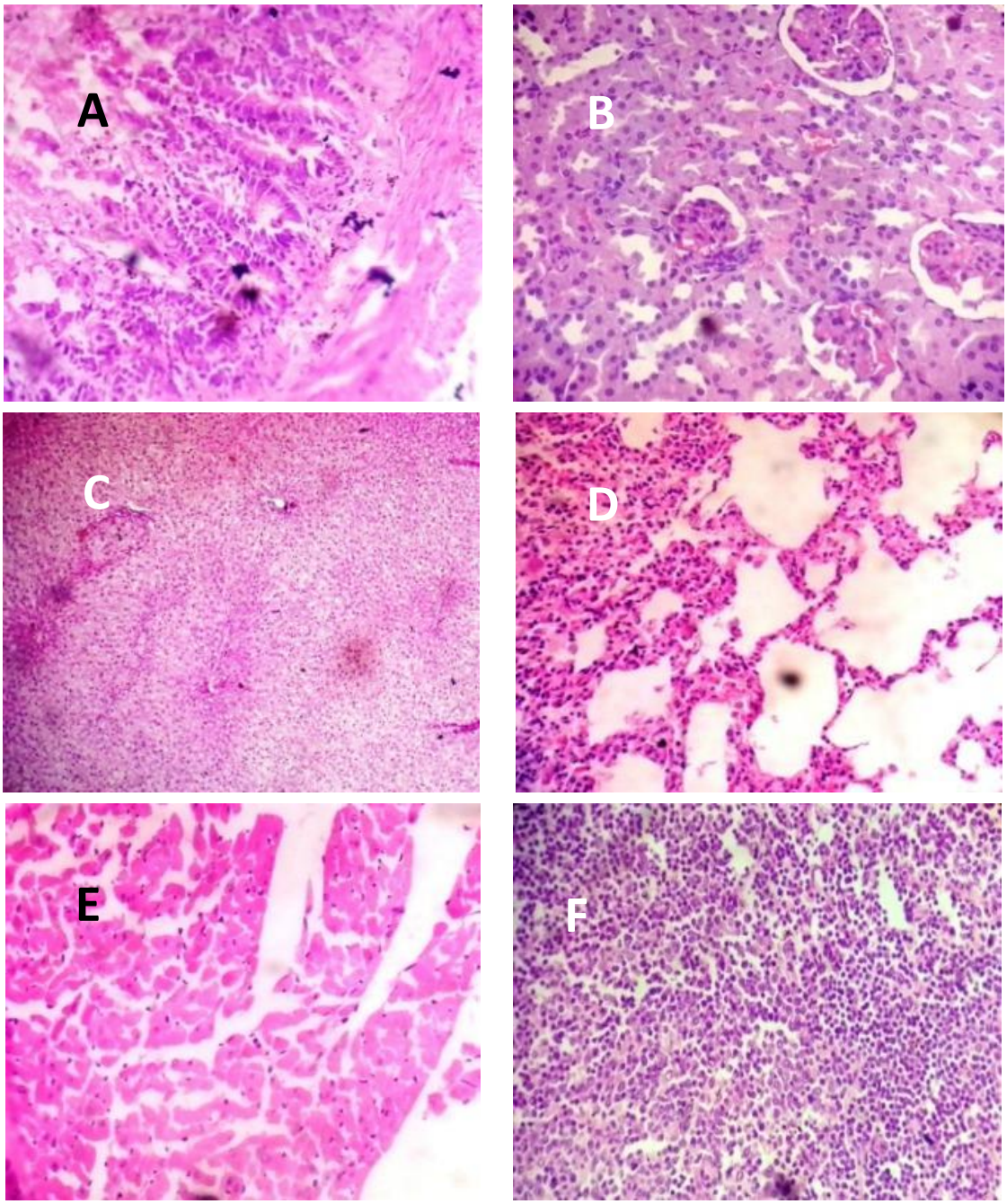

Plate 3. This represents YL.A, normal stomach mucosa at x 40 magnification (Objective lens)
B, Normal Kidney at x 40 magnification (Objective lens). C, Normal Liver at x 4 magnification (Objective lens). D, Lung tissue with evidence of interstitial pneumonia at x 40 magnification (Objective lens). E, Normal Heart Muscles at x 40 magnification (Objective lens). F, Spleen with reactive follicular hyperplasia at x 40 magnification (Objective lens).

determining kidney and liver function, respectively, of which the normal concentrations of the biomarkers were maintained after administration of the condiments. Similar findings were documented by several researchers (Lai et al., [23] Iheukwumere et al., [18] Nwobodo et al., 2019; Ademiluyi et al., [24]. The normal histopathological features of the organs investigated further provethat the condiments are non-toxic. However, the intestinal pneumonia and hyperplasia observed in the spleen of Wistar rats that were administered with condiments prepared with BI, YI, and maggi suggest that the chemical constituent in maggi could be responsible for such alteration, which are highly detrimental to humans. This

alteration in the spleen due to the effect of maggi could affect the function of the spleen in eliminating worn out red cells [25,26,27,28].

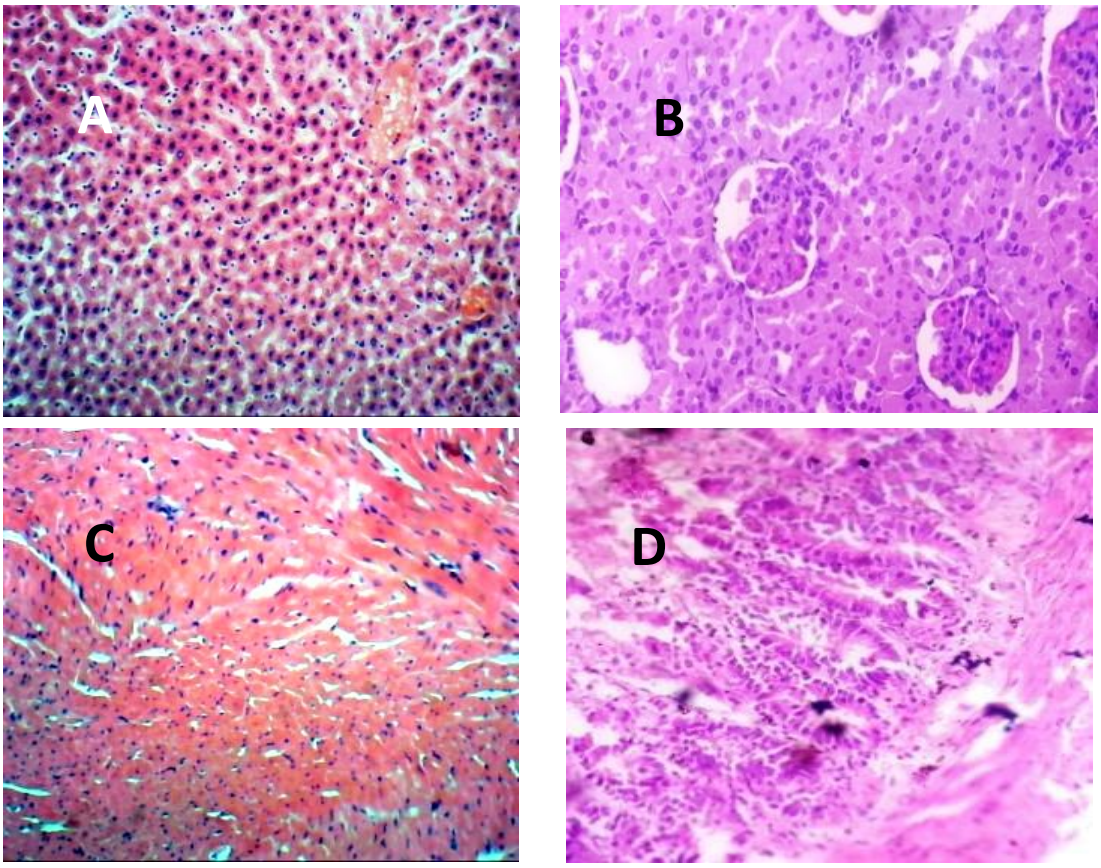

Plate 4. This represents Bp, A, Normal Heart at x 40 Objective lens magnification

B, Normal Stomach Mucosa at x 40 magnification (Objective lens). C, Normal Kidney at x 40 Objective lens magnification with vascular congestion. D, Liver at x 40 Objective lens magnification with mild vesicular steatosis

Table 4. Effects of the prepared condiments on the organs functions of the experimented rats

Sample	Heart	Kidney		Liver		
	LDH (U/L)	urea (mg/dl)	creatinine (mg/dl)	ALT (U/L)	AST (U/L)	AST/ALT
Bp	13.847	8.8120	0.4341	17.880	27.535	1.54
Lp	12.782	7.9620	0.3964	17.220	26.002	1.51
Yp	13.492	8.1408	0.4103	17.460	26.539	1.52
BLP	13.612	8.3405	0.4310	17.470	26.554	1.52
BLYP	12.754	7.9505	0.3920	17.180	25.180	1.51
Cp	16.714	9.2040	0.4658	18.640	34.484	1.85
BL	13.814	8.7404	0.4310	17.720	27.466	1.55
LL	12.688	7.9660	0.3952	17.190	25.957	1.51
YL	13.474	8.0802	0.4018	17.380	26.244	1.51
BLL	13.419	8.1820	0.4290	17.440	26.509	1.52
BLYL	12.712	7.9480	0.3908	17.140	25.710	1.50
CL	16.576	9.1904	0.4622	18.610	33.126	1.78
Control	12.816	7.9468	0.3902	17.120	25.680	1.50

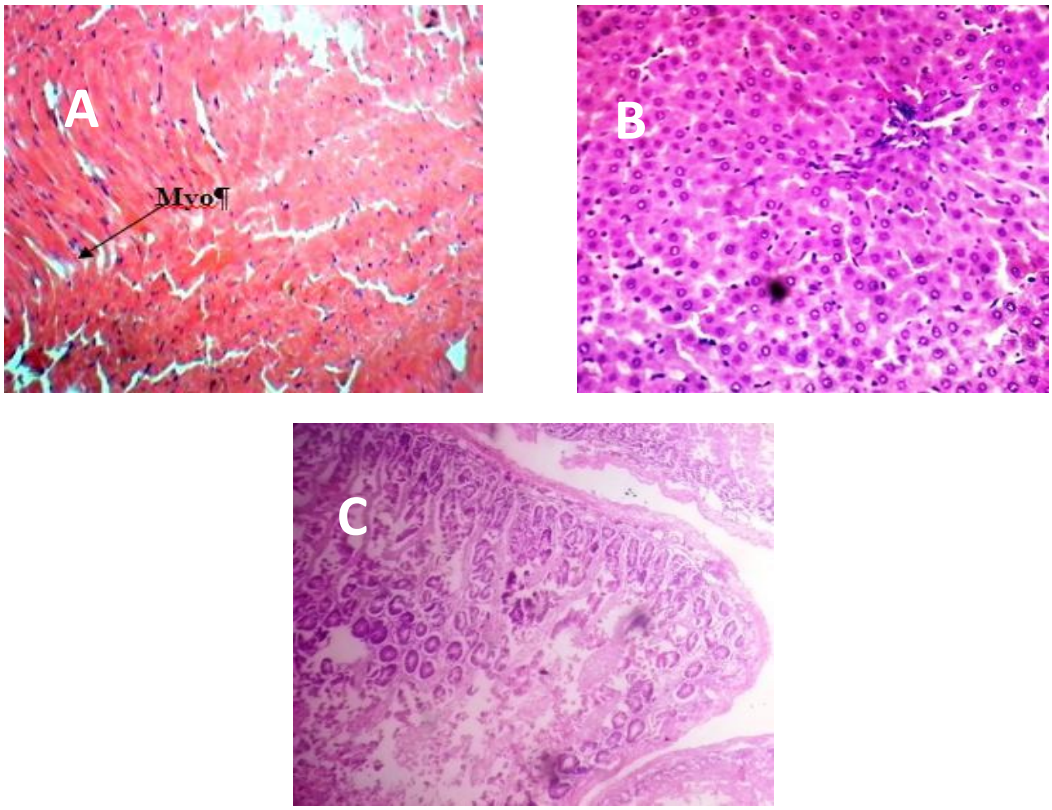

Plate 5. This represents BLP.A, normal heart at x 40 objective lens magnification
B, *Normal Liver at x 40 Objective lens magnification.****C,*** *Normal Stomach Mucosa at x 40 magnification (Objective lens).*

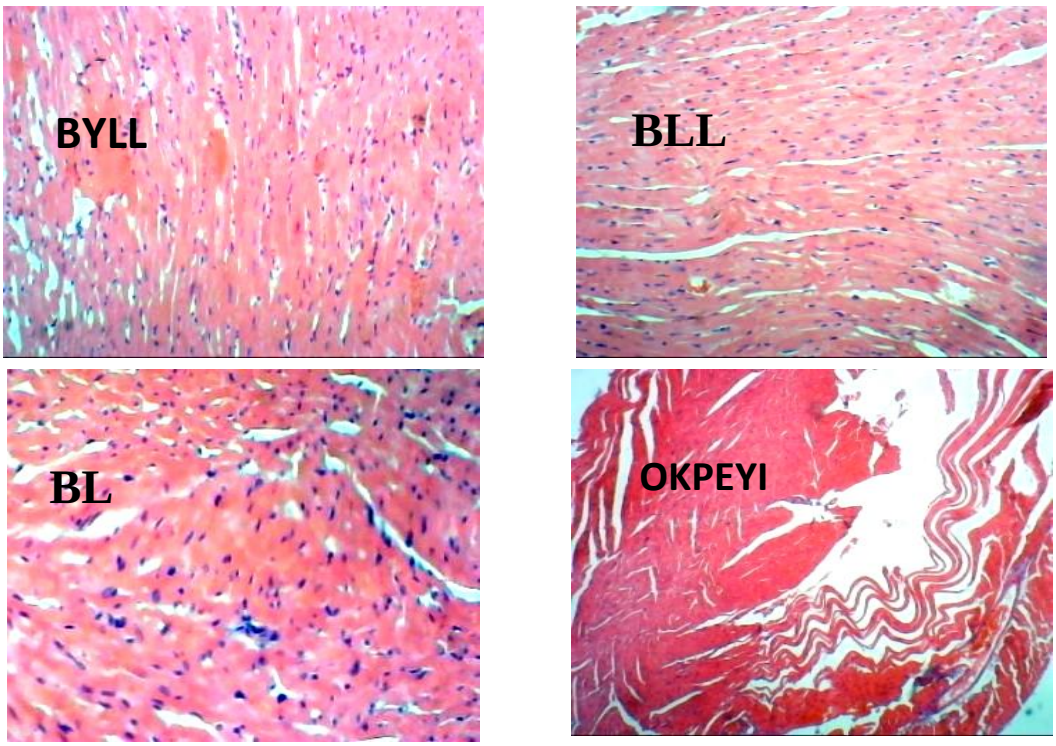

Plate 6. These represent BYLL, BLL, BL, OKPEYI, normal heart at x 40 objective lens magnification

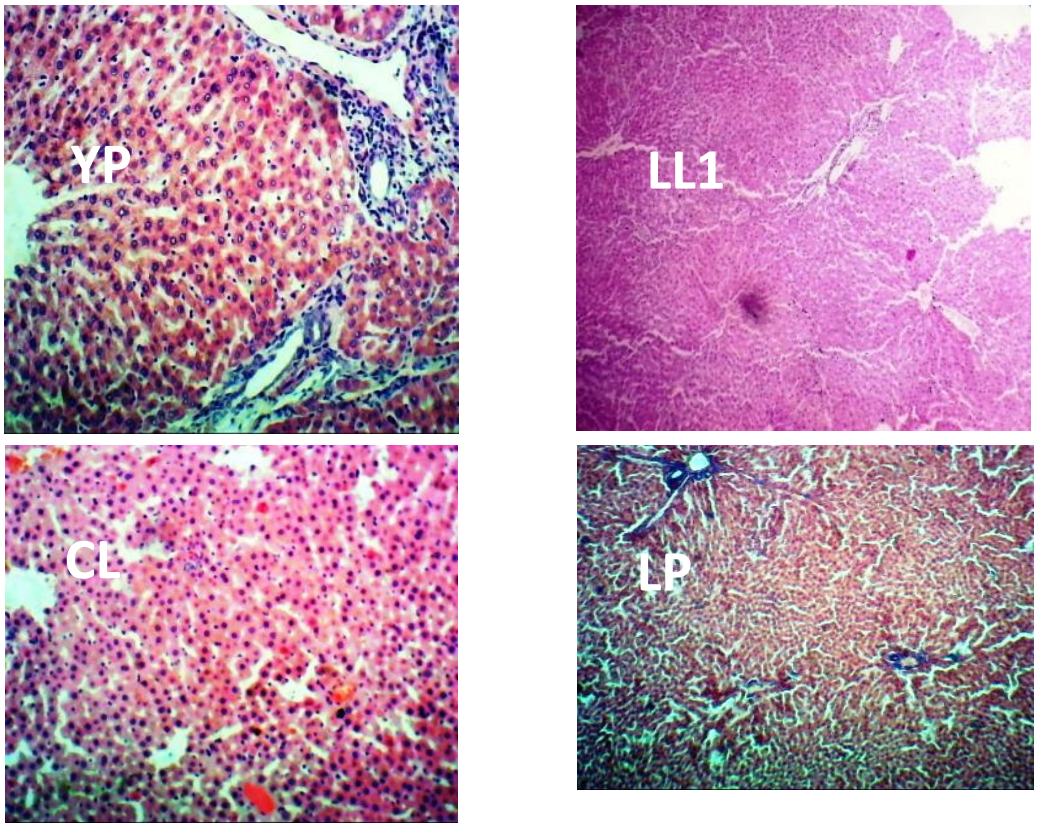

Plate 7. These represent YP, LL1, CL, LP, normal liver at x 40 objective lens magnification

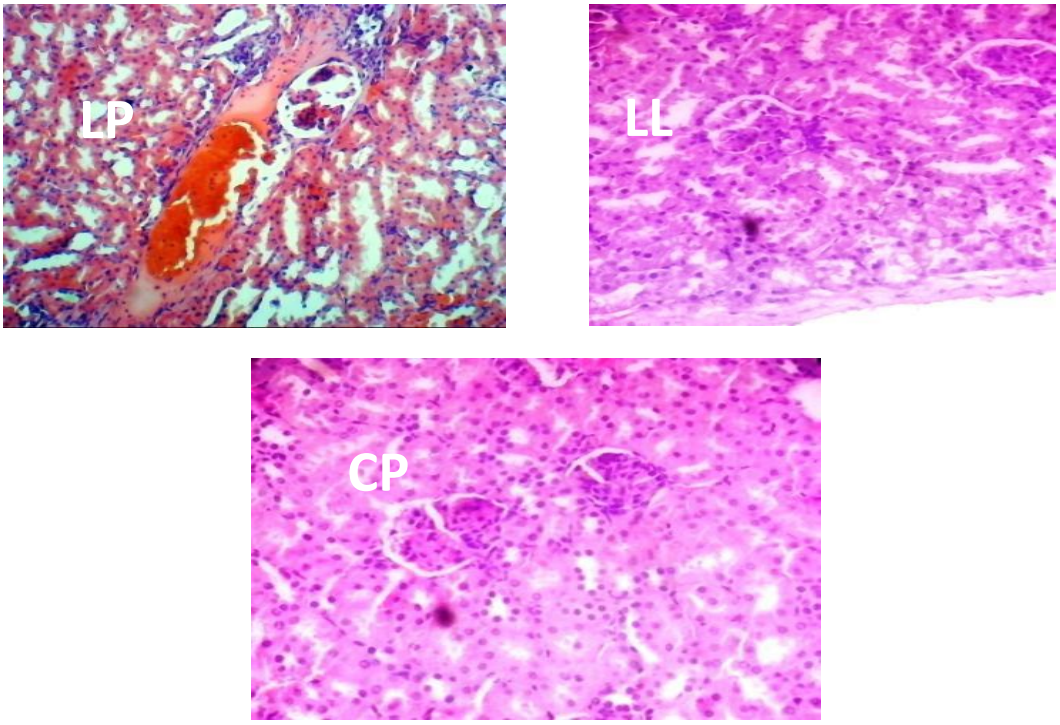

Plate 8. These represent LP, LL, CP, normal kidney at x 40 objective lens magnification

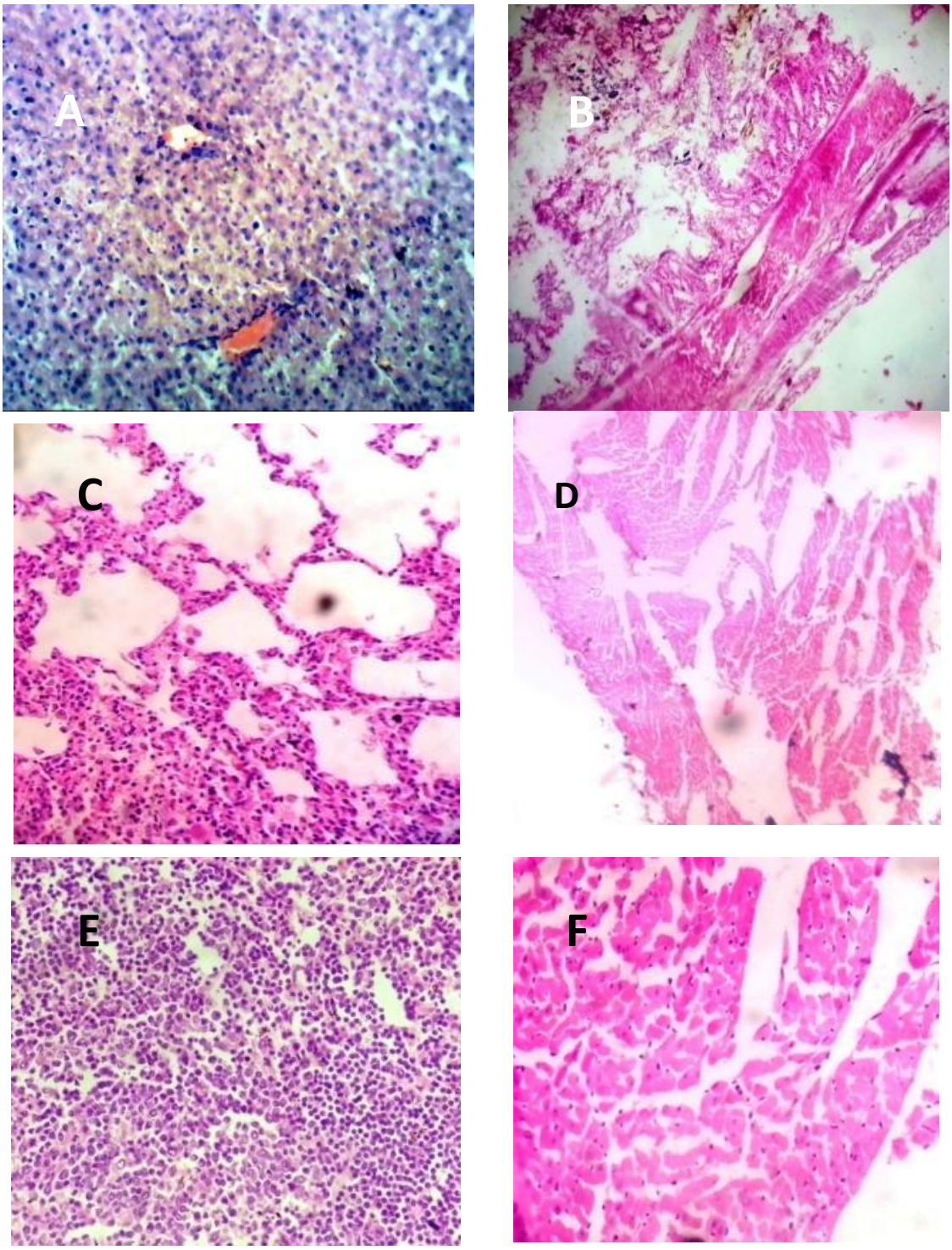

Plate 9. This represents maggi. A, normal kidney at x 40 objective lens magnification
B, Normal Liver at x 40 magnification (Objective lens). C, Normal Stomach Mucosa at x 40 magnification (Objective lens). D, Lung tissue with evidence of interstitial pneumonia at x 40 magnification (Objective lens). E, Normal Heart Muscles at x 40 magnification (Objective lens). F, Spleen with reactive follicular hyperplasia at x 40 magnification (Objective lens)

5. CONCLUSION

This study has shown that condiment produced from fermentation of soybean using indigenous *Bacillus subtilis* strain 168 (B), *Lactobacillus plantarum* strain ZS2058 (L) and *Saccharomyces cerevisiae* strain YJM555 (Y) were safe and had both hepatoprotective and renal protective properties. Any formulation of bacterial cells or raw pure culture cell load indicate

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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