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Effect of Soy Flour Processing Methods on Weight Gain and Physiological Parameters in Wistar albino rats

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Abstract

Assessment on the effect of selected standard processing methods on body weight, biochemistry, haematological and histological profile of Wistar albino rats fed with soy flours was done. Three soy flour samples were prepared using three processing methods (PM1 –PM3). PM1 involves soaking of clean soy bean seeds, dehulling, sundrying (2-3 days), milling, cooling and packaging; PM2 involves soaking with (0.5% $NaHCO_3$), decanting, washing, dehulling, dehusking, sundrying (2-3 days), milling, cooling and packaging; PM3 involves soaking, blanching (100˚C for 5min), draining, cooling, dehulling, sundrying (2-3 days), milling sieving and packaging. Data of the body weight, biochemistry, haematological and histological indices were statistically analyzed. Weight gain in the test groups B (84.50±13.58 g) and C (51.37±12.46 g) differed and above value for D (35.17±6.17) and control (35.80 ± 4.68(g); AST, ALT, ALP, TP, ALB, GB, T.BILI, D.BILI, and LDH had range of values 37.24±0.01-41.90±0.17 u/l, 11.00±0.00-13.00±0.00 u/l, 21.99 ±0.01-34.21±0.30 u/l, 56.94±0.02-62.19±0.01g/l, 42.72±0.01-48.87±0.01 g/l, 11.54±0.00-28.74±0.16 g/l, 0.15±0.00-0.85±0.01, mg/dl, 0.14±0.00-0.61±0.01 mg/dl and 30.55±0.01-34.53±0.02 u/l, respectively. The body weight gain of Wistar Albino rats given soy flour from processing method one (PM1) had the highest weight, followed by that of processing method two (PM2). Liver enzymes and kidney parameters were however, within normal ranges, hence, no appreciable impact on the biochemistry profiles of the Wistar Albino rats fed soy flour from processing methods. The morphological shapes of liver and kidney of rats given soy flour from PM1 and PM3 were perfectly intact. However, the spleen of rats in group D, liver, spleen and kidney of rats in group C showed inflammatory cell within the tissue, loss of tissue (necrosis) while the spleen showed atrophy. The selected standard processing methods appreciably impacted positively on the weight gain of the Wistar Albino rats fed with soy flour from the different processing methods.

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Keywords: Nutritional properties, processed soyflour, processing methods, sensory properties,

INTRODUCTION

Research efforts in the area of health promotion with regards to nutrition has been on the increase in the last ten years. In this context, soybean and products developed from it have become of great interest in human and animal nutrition (Liu 2000). Soybean is regarded as one of the most nutritive food sources known to man. It is unique in its nutritional value because of its high content of protein (40%) and oil (20%) (Enwere1998; Fabiyi 2006). Soya bean is a widely used inexpensive, and nutritional source of dietary protein (McArthur *et al*.,1988). Its protein content (40%) is higher and more economical than that of beef (18%), chicken (20%), fish (18%) and groundnut (23%) (IITA, 1990).

For centuries, the Chinese and other oriental people, including Japanese, Korea and Southeast Asians have used the bean in various forms as one of the most important sources of dietary protein and oil (Liu, 2012). Soybean can be growing in a wide variety of soils and climatic conditions than any other major world crop. It is well known for its variation in physical properties as well as in its chemical composition (Osthoff *et al.,* 2010). Soy contains certain desirable nutritious protein such as niacin and phytochemical. It is used in a wide range of foods such as meat products, baked goods and infant foods (Liu, 2012). Not being of animal origin, it lacks lactose and cholesterol, and therefore is less of a problem with dietary disorders or intolerances (Liu, 2012).

The only drawback of soybean is the high content of anti-nutritional factors in the form of trypsin inhibitors (Brinda et al., 2017; Ibanez et al., 2020) which is almost completely removed during processing of soymilk using various heat treatments.

In Asian countries, soybean is processed into various products such as soymilk powder, soymilk, tofu, soy sauce, soy flour, soybean oil, tempeh etc. (Tyug *et al.,* 2010). Different processing methods including soaking and grinding, fermentation, pasteurization, thermal treatment, high pressure processing, traditional methods etc are used for the production of these products either to inactive spoilage microorganisms and extend the shelf-life of soymilk or decrease anti-nutritional factors that are found in soy.

According to Tyug *et al.* (2010) the processing of soybean into soy powder involves three major steps. The unit operations involved in the processing of soyabeans include soaking, dehusking, drying, grinding, sieving and boiling. The resulting slurry from boiling was now dried employing a vacuum dryer to produce soy milk that is grade A, while the waste could find application in animal feed production. However, in Malaysia, the wastes are processed to a lower-quality powder, called grade B soymilk powder (GBSP) (Tyug *et al.,* 2010).

Soy flour is now the alternative to the aqueous extract of soymilk which closely resembles cow milk in appearance and composition but with short shelf life. Soy flours are prepared using different processing methods to ensure availability of soy protein (soymilk). However, the effect of these processing methods on the body weight, biochemistry, haematological and histological indices of Wistar albino rats fed with soy flours needs be investigated. Therefore, this study was designed to investigate the effect of these selected standard processing methods on the body weight, biochemistry, haematological and histological indices of Wistar albino rats given soy flours.

MATERIALS AND METHODS

Materials

The variety of Soybean seeds used for the research is Var: 1904 - 6F, which was procured from National Cereal Research Institute (NCRI), Yandev Sub-Station, Box 454, Gboko, Benue state, Nigeria. All the chemicals and reagents ($NaHCO_3$, CCL_4, Olive oil etc) needed for use were made available at the University of Mkar, Mkar, Benue state for the preparation of soy flour samples.

Methods

Three (3) selected standard processing methods for processing soybean seeds into flour

The first processing methods (PM1) is a traditional soymilk processing method described by Iwe, (1991) but slightly modified to produce soy flour. The unit operations involved in this processing method are illustrated in Figure 1.

The second processing method (PM2) involves the use of sodium bicarbonate described by Afroz *et al*. (2016) but slightly modified to produce soy flour. In this method, the unit operations involved are shown in Figure 1.

The third processing method (PM3) is a soymilk powder processing method described by Cheryl (2021) but with some modifications to produce soy flour. The unit operations involved using this method are indicated in Figure 1.

Experimental animals

A total of sixteen Wistar Albino rats, with range of age between ten (10) to twelve (12) weeks old, with weight range of between 50.30 grams and 144 grams were used for the research. The acclimatization of the experimental animals was done for seven days (one week). During acclimatization the rats were fed normal commercial diet (Stock Finisher product by Feeds PLC Ikeja, Nigeria), purchased from Gboko Market, Benue State. The rats were housed in a wooden cage and were provided clean tap water ad libitum. Assessment using animal experiments was carried out following standard ethics on caring and usage of laboratory animals having gotten ethical approval from the committee of College of Health Science, Benue State University, Makurdi.

Feeding procedures

The design and layout for the experiment was randomized design (CRD). Sixteen (16) Wistar Albino rats of either sex was used. After seven days acclimatization period the rats were weighed using weighing balance LP4001A 4000g/0.1g (Scientific & Instrument Company, England) and randomly divided into four (4) groups of four rats each with group A as control, B, C and D as test groups (plate 5). The soy flour diet from PM1, PM2 and PM3 in aqueous solution was fed to each rat in the test groups in mg/kg body weight in addition to normal commercial diet on daily basis for 28 days. The designation used to identify the rats is as stated below:

Control A = fed normal commercial diet (NCD) only
Group B = fed soy flour diet from PM1+ NCD
Group C = fed soy flour diet from PM2 + NCD
Group D = fed soy flour diet from PM3 + NCD

NB: In the same way the rats in each group were given individual identification number, I, 11, 111, IV, respectively; NCD – Normal commercial diet (Stock finisher product by Feeds plc, Ikeja, Nigeria).

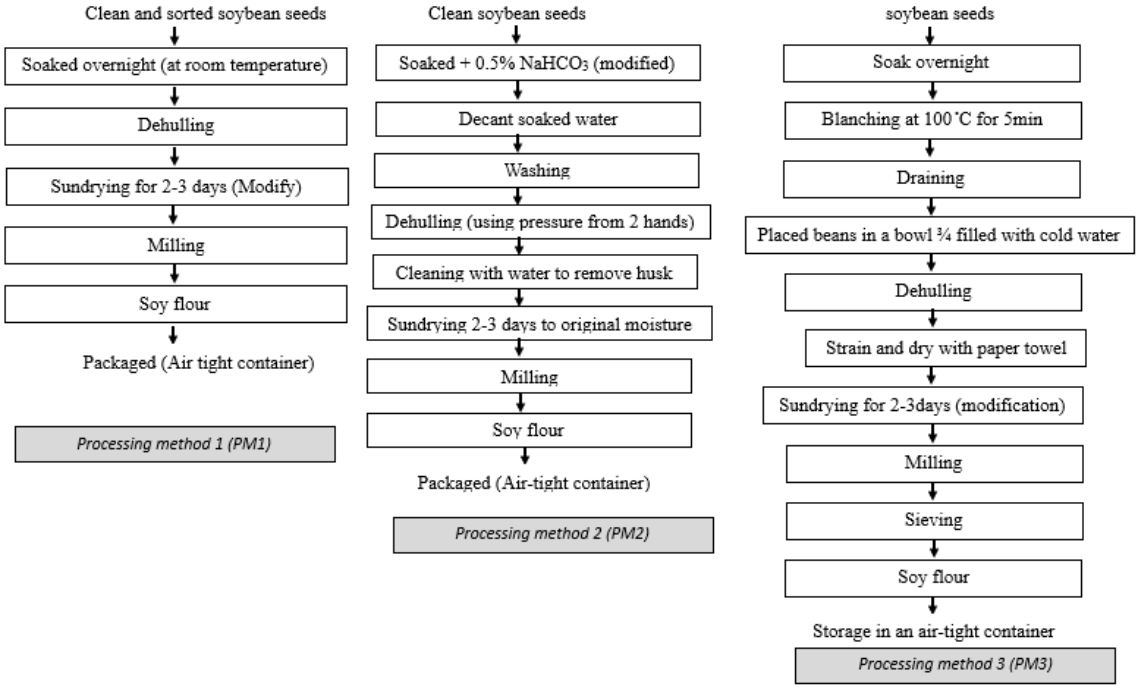


Figure 1. Flow chart showing the three processing methods used in the preparation of soy flour

Feeding experiment

Before feeding trial commenced, each test rat was induced with using single dose carbon tetrachloride (CCL_4), in mg/kg body weight. Carbon tetrachloride is a reagent that can cause acute liver injury (Hepatotoxicity) to the rats. A vehicle chosen to dissolve CCL_4 with ratio of 1:1(v/v) was olive oil. From the onset of experiment inducement was administered once. The doses of CCL_4 induced ranged from 0.46 to 0.84mL (approximated to 1.0 mL) based on mg/kg body weight of the rat according to Woumbo *et al* (2017). The soy flour diet from PM1, PM2 and PM3 in aqueous solution was fed to each rat in the test groups in mg/kg body weight in addition to normal commercial diet on daily basis for 28 days. This was achieved by oral administration using gauge 5mm syringe (plate 6). The aqueous solution of soy flour from PM1- PM3 was administered per rat weight. The solutions were prepared and dissolved in the ratio of 1:1 (w/v) i.e. one (1.0) gram of soy flour to one volume of distilled water. The rats were also fed normal commercial diet throughout the feeding trial. When the feeding reached 28th day (i.e. 4 weeks), fasting of the rats was done for one day so as to prepare them for sacrificing with the view to collecting blood samples for laboratory analyses such as biochemistry profiles, haematological indices and histopathology of tissues.

Analyses of feeding experiment

Body weight gains

The body weight gains of the Wistar albino rats fed soy flour diet evaluated using model by Ndatsu *et al*. (2013) given as;

Weight gain = Final weight – Initial weight (1)

Also, general observations of the experimental rats on daily basis for abnormal behaviour, physical appearance and health statuses were also carried out during feeding trial.
The level of SOD activity was determined following the method of Misra and Fridovich, (1972). Noticeable increase in absorbance at 480 nm was monitored.

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5} \quad (2)$$

Where A_0 = absorbance after 30 seconds
A_3=absorbance after 150 seconds

$$\%\text{Inhibition} = \frac{increase\ in\ absorbance\ for\ substrate}{increase\ in\ absorbance\ for\ balnk} \times 100 \quad (3)$$

Catalase activity was determined according to the method described by Claibornee (1985). The volume of reagent used is as indicated in Table 1.

Table 1. Catalase activity determination

Test	Blank	Sample
Phosphate buffer	3 mLs	2.95 mLs
Sample	-	50 µl
Total	3 mLs	3 mLs

Optical density was read at 240 nm at 1 min, 2, 3, 3: 30, 4, 4: 30, 5 mins

$$Catalase\ activity = \frac{(\Delta OD/min\ x\ volume\ of\ assay\ system)}{\mu mole\ H2O2/min/mg\ protein} \quad (4)$$

(0.0041 x Volume of Sample x mg protein)

Reduced glutathione was determined following the method of Beutler *et al.* (1963). The serial dilutions used is as indicated in Table 2.

Table 2. Preparation of serial dilutions of the GSH working Standard

Stock mL	Phosphate buffer	Ellman's reagent	Abs (412nm)	GSH Conc (µg/mL)
0.01	0.24	2.25	0.04	8
0.025	0.225	2.25	0.101	20
0.05	0.20	2.25	0.194	40
0.10	0.15	2.25	0.38	80
0.15	0.10	2.25	0.572	120
0.20	0.05	2.25	0.749	160

Total reaction mixture: 2.25mL

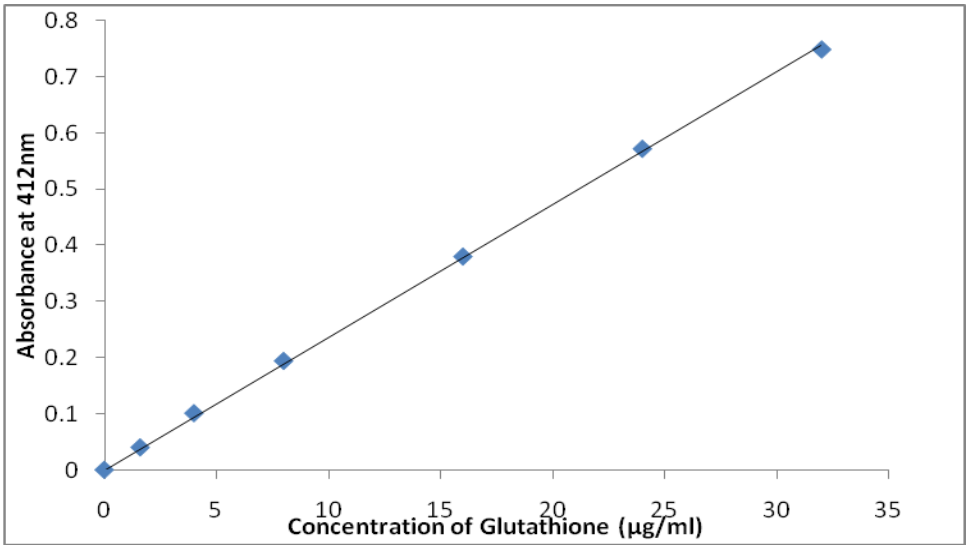


Figure 2. Standard curve for reduced glutathione

Lipid peroxidation was determined by measuring the levels of Malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale (1990). Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 10^5 M^{-1} Cm^{-1}

$$LPO\ (MDA\ formed/mg\ protein) = \frac{Absorbance\ x\ volume\ of\ mixture}{E532nm\ x\ volume\ of\ sample\ x\ mg\ protein} \quad (5)$$

MDA FORMED = mmol/mg protein (6)

The Glutathione peroxidase assay was carried out following the method described by Rotruck *et al.* (1973)**.** Glutathione peroxidase activity was observed by plotting the standard curve and the concentration of the remaining GSH was extrapolated from the curve.

GSH consumed = 245.34 – GSH remaining (7)

$$\text{Glutathione peroxidase activity} = \frac{H2O2}{mg\ Protein} \quad (8)$$

Biochemistry profiles (liver function tests)

The serum alanine amino transferase (ALT) was determined using colorimetric method described by Reitman and Frankel (1957). The sample blank and sample (2 test tube) were set up as indicated in Table 2.

Table 2: Reagent mixture for ALT assay

	Sample Blank	sample
Reagent A (mL)	0.5	0.5
Sample (mL)	0.5	0.1
The contents were mixed and incubated at 37^{0}C for 30 minutes		
Reagent B (mL)	0.5	0.5
Sample (mL)	0.1	

Aspartate amino transferase (AST) activity was assayed by the method of Reitman and Frankel (1957).

Table 3. Reagent mixture for AST assay

	Sample	Sample (Blank)
Reagent A (mL)	0.5	0.5
The contents were mixed and incubated at 37^{0}C for 30 minutes		
Reagent B (mL)	0.5	0.5
Sample (mL)		0.1
The contents were mixed and incubated at 25^{0}C for 20minute		Reagent
C	2.5	2.5

The alkaline phosphatase (ALP) activity was assessed according to the method described by Walter and Schutt, (1974). The relation in equation 9 was employed.

$$\text{Alkaline phosphatase activity } (\mu/L) = \frac{\text{optical density of SA}}{\text{optical density of ST}} \times 30 \quad (9)$$

The protein concentrations of the various samples were determined by means of the Biuret method as described by Gornall *et al.* (1949) with some modifications: potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. The dilutions used for the experiment is presented in Table 5.

Table 4. Reagent mixture for ALP assay

Parameter	SA	ST
Water (mL)	0.5	0.5
Substrate (mL)	0.01	0.01
The contents were mixed and incubated at 37^{0}C for 5 minutes		
Standard (mL)	--	0.05
Sample (mL)	0.05	-
The contents were mixed and in		
Incubated at 37^{0}C for 20 minutes		
Colour developer (mL)	2.5	2.5

Table 5. Assessment of Protein

Test tube No	**1**	**2**	**3**	**4**	**5**
Stock BSA (mL)	0.02	0.04	0.06	0.08	0.10
Distilled H_20 (mL)	0.18	0.16	0.14	0.12	0.10
BSA Concentration (mg/mL)	1	2	3	4	5
Biuret reagent (mLs)	0.80	0.80	0.80	0.80	0.80
Absorbance (540nm)	0.081	0.136	0.189	0.251	0.323

Source: Gornall et al, (1949)

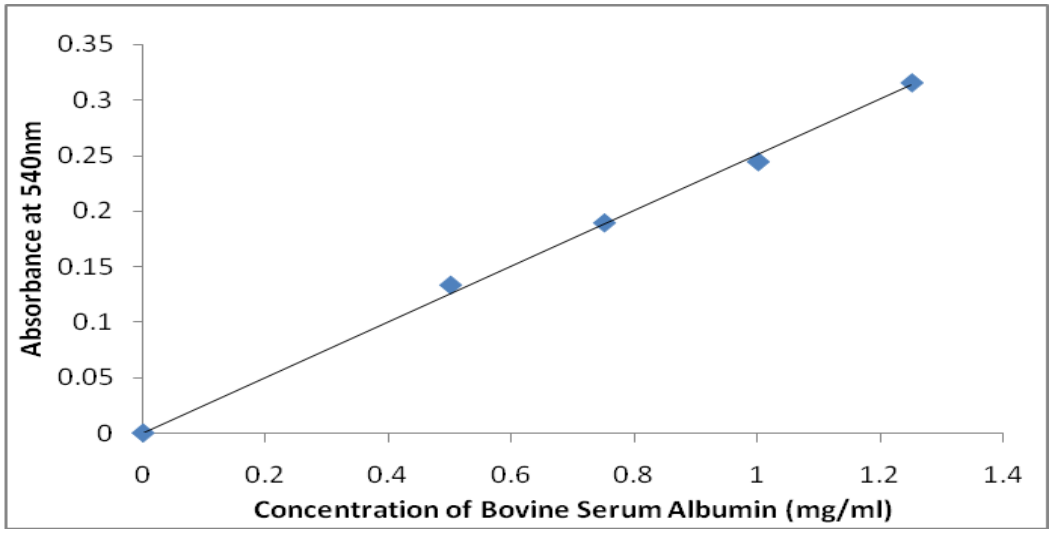


Figure 3. Standard curve for protein determination by Biuret's method
Source: Gornall *et al,* (1949)

The total protein in sample was estimated following Biuret method. The total protein was computed using the relation in equation (10).

$$\text{Total protein (g/dl)} = \frac{\text{A sample}}{\text{A standard}} \text{ x concentration of standard} \quad (10)$$

The Albumin concentration was determined by the Bromo Cresol Green method described by Spencer and Price, (1977). The albumen concentration was computed following the equation (11).

$$\text{Albumin conc. g/L or g/dL} = \frac{\text{A sample}}{\text{A standard}} \text{ x concentration of standard} \quad (11)$$

Bilirubin concentration was done according to the method described by Ochei and Kolthatkar, (2008). The volume of the reagents used in the assessment is presented in Table 6.

Table 6. Addition order for determination of total bilirubin

	Sample Blank (U/L)	Sample (U/L)
R1	200	200
R2	-	50
R3	1000	1000
Sample	200	200
The contents were mixed and incubated at 25^{0}C for 10 minutes		
R4	1000	1000

Source: Ochei and Kolthatkar, (2008)

The contents were mixed and incubated at 25°C for 10minutes and the absorbance at 578 nm was read. Thus Total bilirubin (U/L) = 185 x absorbance 578 nm (12)

In mg/dL = 10.8 x absorbance 578 nm (13)

Table 7. Addition order for determination of total bilirubin

	Sample Blank (U/L)	Sample (U/L)
R1	200	200
R2	-	50
0.9% NaCl	2000	2000
Sample	200	200

Source: Ochei and Kolthatkar, (2008).

The contents were mixed and incubated at 25°C for 10 minutes and the absorbance was read at 546 nm. The direct bilirubinn concentration is expressed thus:

Direct bilirubin (U/L) = 246 +Absorbance at 546 nm. (14)

Direct bilirubin measured in mg/dL = 144 x Absorbance at 546 nm (15)

The activity of lactate dehydrogenase (LDH) was determined using Sigma Aldrich kit (catalog No- MAk066) described by Ochei and Kolthatkar, (2008) in which LDH assay reduces NAD to NADH which is specifically detected by microplate colorimetric assay. The relation in equation 17 was used to estimate the LDH.

$$\Delta A_{450} = (\Delta A_{450})\text{final} - (\Delta A_{450})\text{initial} \quad (16)$$

$$\text{LDH activity} = \frac{\text{B x sample dillution facor}}{\text{Reaction time x volume}} \quad (17)$$

Biochemistry profiles (Kidney Function Tests)

The measurement of urea concentration was done using a method described by Widyastuti *et al.* (2019). The absorbance (at 340nm) of the first and second of the serum samples were computed with average alongside with the urea concentration.

Plasma creatinine concentration was determined using the method of Henry (1974). The volume of analytical grade reagent used is presented in Table 8. The relations used in the estimation of plasma creatinine is stated in equation (18).

$$\text{Creatinine Concentration} = \frac{\text{A sample}}{\text{A standard x conc. of standard } {}^{\text{mg}}/_{\text{dl}}} \quad (18)$$

Table 8. Reagent mixture for the determination of Creatinine

m/L	Blank (m/L)	Sample (m/L)	Sample
Distilled water	0.5	-	-
Standard solution	-	0.5	-
TCA	0.5	0.5	
Supernatant	-	-	1.0
Reagent mixture	1.0	1.0	1.0

Source: Henry (1974)

The uric acid level of the samples was estimated by applying the method described by Nugrahaningsih *et al*. (2021).

Plasma concentrations; sodium (Na and Na^+**),** potassium **(**K, and K^+**),** Calcium **(**Ca+**)** were determined using a flame photometer method described by Ojogbane, (2013).

Serum bicarbonate was determined by method reported by Van slyke *et al* (1924) was employed while the estimation of Serum Chloride (Cl-) was done using a method described by Mohammed and Inuwa (2012).

Haematological Indices

The PCV was determined by Micro- haematocrit method as described by Audu *et al.,* (2017) with some modifications. The PCV was determined instrumentally with the aid of hematocrit reader, and the result computed in percentage.

Total white blood cells were determined by hemo-cytometer method described by Al *et al.* (1991). Homogenous mixing of known volume precisely 2mL of the sample was achieved by easily inverting the test tube containing the sample. A Pasteur pipette was used to introduce 20µL of sample into the counting chamber, left for 2 min. Using the low power objective (x10), in all the 16 small square boxes of the 4 large outer squares

$$\text{WBC} = \frac{\text{N x DF x } 10^6}{\text{A x D}} \quad (19)$$

Where N = Number of cells counted
DF = Dilution factor
A = Area of chamber
D = Depth of chamber
10^6 = Conversion factor

Determination of red blood cell (RBC) was done as described by Tanvir *et al.* (1997). All the test tubes for the test(s) were labelled. The blood sample was gently mixed by repeated inversion. 4000µL of formol citrate solution was pipetted into the test tubes and allowed to stand on the bench for about 5 minutes. The chamber was filled by means of a pasteur pipette and allowed to settle for 2minutes.Using a low power objective, count the number of cells in the central squares (5 squares)

$$\text{RBC} = \frac{\text{N x DF x } 10^9}{\text{A x D}} \quad (20)$$

Where N = Number of cells counted
DF = Dilution factor
A = Area of chamber
D = Depth of chamber
10^9 = Conversion factor

The haemoglobin (HB) was determined with the application of spectrophotometric assay as described by Avwioro (2011) with some modifications. Haemoglobin was obtained spectrophotometrically.

The thin film making was done employing the thin and Leishman's staining procedures.

Histological parameters

Tissue processing was done by paraffin wax (conventional) method described by Avwioro (2011) and Choji *et al* (2015) with some modifications.

Tissues were embedded in molten paraffin wax. Sectioning of the tissues was done with the aid of an instrument known as microtome.

Haematoxylin and eosin staining technique was done as described by Avwioro (2011) and Choji *et al. (*2015). Stained slides were differentiated in 1% acid-alcohol and then washed well

in water, blued in Scott's tap water substitute for 5 minutes and rinsed briefly in distilled water, counterstained in 1% aqueous eosin for 2 minutes, washed well in water, dehydrated in descending grades of alcohol, cleared in xylene and mounted in DPX (Destrene, plasticiser and xylene).

Statistical analyses

Data generated from the experiment were analysed using Statistical Package for Social Sciences (SPSS version 21), means separated using Duncan's multiple range tests. The pairs of treatment were differentiated at 5% level of significance.

$$\text{Least significant difference (LSD)} = \sqrt[ta]{\frac{2S^2}{r}} \quad (21)$$

RESULTS AND DISCUSSION

Weight gains of Wistar Albino rats fed soy flour diets and normal commercial diet

The result of weight gains of Wistar Albino rats fed soy flour diets and normal commercial diet is presented in Table 9. The weight of Wistar albino rats after seven days acclimatization was considered as initial weight and was taken at wk_0 (week zero). Subsequently, weights were taken on weekly basis. Weight loss across the rats (B, C, D) induced CCL_4 during the first week of administration of aqueous soy flour from the three processing methods. (Appendix C, Figure 15 Wk1). As can be seen from appendix C figure 15, Observable weight increase of rats was noticed in during by second week. The weight gain in the test groups B (84.50±13.58 g) and C (51.37±12.46 g) differed and above value for D (35.17±6.17) and control (35.80 ± 4.68(g). However, in terms of weight gain group D and control were not different. General observation was also made of the dull appearance of the rats in group D from the day of induction of CCL_4 throughout experimental period.

Table 9. Weights of Wistar Albino rats fed Soy flours

Rat groups	Diet	Mean weight$_1$ (g)	Mean weight$_2$ (g)	Mean weight gain (g)
A Control	NCD	126.77±14.96a	162.57±10.29ab	35.80±4.68b
B	PM1 + NCD	109.10±12.30a	193.60±1.27a	84.50±13.58a
C	PM2 + NCD	116.5±35.33a	167.87±23.89ab	51.37±12.46b
D	PM3 + NCD	107.33±15.54a	142.50±10.42b	35.17±6.17b

Means are values of two replicates, then the standard deviation. (n=2.000).

Mean Weight$_1$: Initial mean weight; Mean Weight$_2$: Final mean weight
Control A = Fed normal commercial diet (NCD) only
Group B = Fed soy flour diet from processing method one + Stock finisher feeds
Group C = Fed soy flour diet from processing method two + Stock finisher feeds
Group D = Fed soy flour diet from processing method three + Stock finisher feeds
NCD = Normal commercial diet (Stock finisher feeds)

Biochemical Profiles of Wistar Albino rats fed soy flours

The results showing the biochemistry profile of Wistar Albino rats fed soy flours are presented in Tables 10, 11 and 12.

Anti-Oxidant/Oxidative stress biomarkers

Observable increase in SOD levels for rats in groups B and C fed soy flours from processing methods one and two (PM1 and PM2) was noted as compared to control A. This implies improved oxidative stress (Resim *et al.,* 2015). It has been reported that this increase in SOD

concentration may be linked with response to oxidative stress at cellular dimension. However, decrease SOD concentration for D relative to control sample implies high level of oxidative damage. Observable increase was noted for C and MDA for rats in groups B and D fed soy flour diets from processing methods one and three (PM1 and PM3). Increase in MDA according to Olatosin *et al*. (2014) is an indication of increase oxidative stress. The findings in this study is in consonance with the report of Ubhenin *et al* (2016).

These two markers indirectly reflect the extent of OS in the body (Bajpai *et al*., 2017). Group C and control A were not different with respect to MDA level. Adewale *et al*., (2019) reported that decreased level of MDA is an indication that lipid peroxidation rate was also decreased by the antioxidant properties of the diet. A decrease was observed in GSH and GPX level in all the rats induced with CCl_4 and fed soy flour from selected processing methods (PM1- PM3) when compared to control A.

Liver function status

The result of liver function test showed significant ($p<0.05$) increase in AST in the test groups B and D rats and ALT in B and C as compared to control A, which is an indication of acute liver injury or damage as reported by Okonkwo *et al.* (2012).

Table 10. Liver function test of Wistar Albino rats fed soy flours

Biochemical Profiles	NCD	PM1	PM2	PM3	SE	LSD
	Control A	B	C	D	-	
AST (u/l)	39.78±0.02^{c}	41.90±0.17a	37.24±0.01d	41.08±0.04b	1.81	0.25
ALT (u/l)	11.00±0.00^{c}	12.20±0.28b	13.00±0.00a	11.00±0.00c	0.32	0.39
ALP (u/l)	34.21±0.30^{a}	21.99 ±0.01d	22.90±0.15c	24.78±0.01b	1.84	0.22
TP (g/l)	62.19±0.01^{a}	56.94±0.02d	58.92±0.01c	59.76±0.48b	2.44	0.67
ALB (g/l)	48.87±0.01^{a}	46.35±0.35c	47.35±0.03b	42.72±0.01d	0.97	0.89
GB (g/l)	22.33±0.04^{b}	28.74±0.16a	11.54±0.00d	17.05±0.47c	2.60	0.70
T.BILI. (mg/dl)	0.19±0.01^{b}	0.16±0.01c	0.15±0.00c	0.85±0.01a	0.11	-
D.BILI. (mg/dl)	0.19±0.01^{c}	0.14±0.00d	0.55±0.01b	0.61±0.01a	0.01	-
LDH (u/l)	32.72±0.07^{b}	30.55±0.01d	31.30±0.01c	34.53±0.02a	0.57	0.09

Means are values of two replicates, then the standard deviation. (n=2.000).

The serum ALP decreased among test rats. Increased activities of the AST and ALT marker enzymes is severally reported in literature as indication of liver damage (Raju *et al.* 2015; Raju *et al.,* 2016; Belhadi *et al.,* 2018; Barsha *et al.,* 2018; Ghorbel *et al.,* 2016 and Gedik *et al.,* 2017). On the other hand, Ndatsu *et al.* (2013) reported that, decrease in serum marker enzymes indicate the ability of the food to protect the hepatocytes from oxidative damage hence, the decrease in ALP. This implies that the soy powder samples from the selected processing methods have been potent against severe oxidative injury caused by CCL_4. No observable difference with respect to level of ALT between D (11.00±0.00) and control A (Table 10).

Concentration of protein in total was observed to decreased in the rats fed soy powder from selected processing methods. Elevated total protein may indicate inflammation or infections such as viral hepatitis B or C or HIV, bone marrow disorders etc while low total protein may indicate bleeding, liver and kidney disorders, malnutrition (Cindie and Natalie, 2016). In this study protein level and albumin concentration decreased in the groups compared to control. Globulin level was inconsistent but was discovered to increase in B rats but decreased in C and D rats compared to control. Albumin +Globulin = Total protein (Glob +Alb = TP).

Table 11. Anti-Oxidants/Oxidative stress biomarkers

Group/Parameter		SOD	CAT	GSH	MDA	GPX
A control NCD		35.03 ±0.01c	7.23±0.01b	6.24±0.01a	1.60±0.01c	2.23±0.01a
B	PM1	37.62±0.01b	6.77±0.07d	5.24 ±0.02b	2.40±0.07b	2.21±0.01b
C	PM2	41.42±0.18a	7.83±0.05a	4.74±0.01d	1.56±0.07d	1.61±0.18c
D	PM3	33.21 ±0.04d	6.95±0.01c	5.01±0.01c	3.40±0.06a	1.30±.04c
SE		1.0	0.21	0.21	0.12	
LSD		6.28	1.83	3.65	2.61	2.71

Means are values of two replicates, then the standard deviation. (n=2.000).

KEY: Superoxide Dismutase(µmole/min); Catalase (µmoleH_2O_2/min/mg protein); Reduced Glutathione (µg/ml); malondialdehyde (mmol/mg protein);

Control A = Fed normal commercial diet (Stock finisher feeds) only
Group B = Fed soy flour diet from processing method one + Stock finisher feeds
Group C = Fed soy flour diet from processing method two + Stock finisher feeds
Group D = Fed soy flour diet from processing method three + Stock finisher feeds

In the study on biochemical indices of liver function tests of albino rats supplemented with three sources of vegetable oils; Imafidon and Okunrobo, (2012) reported that increased protein and albumin levels indicate impairment in the normal function of the liver.

Direct and Total bilirubin content was within normal range. It is used to help find the cause of health conditions like jaundice, anaemia and liver disease (Cleveland Clinic, Accessed 2021). Higher levels of bilirubin than normal, is a sign that either the red blood cell is breaking down at an unusual rate or that the liver is not breaking down waste properly and not clearing the bilirubin from blood (Cleveland Clinic, 2021). The bilirubin quantity did not show appreciable increase therefore, it is an indication that the liver was properly being cleared of bilirubin from the blood and may be due to the nutrient compositions of the soy powder from selected processing methods.

The level of lactate hydrogenase (LDH) reduced appreciably in B and C but high up for group D compared to control A. According to Ndatsu *et al.* (2013) increased level of LDH is an indication of abnormality in the liver functioning which may be due to the formation of highly reactive free radicals caused by the diet overdose. An enzyme found to be instrumental in the production of energy is the LDH. It is present in almost all of the tissues in the body and its level rises in response to cell damage (Ndatsu *et al.*, 2013). In this study there was however, no case of diet overdose rather the hepatotoxic effect of carbon tetrachloride (CCL_4) in the rats was observed. The LDH increase in group D rats was however within normal range (Appendix B).

Kidney function

Nitrogen of the blood urea, creatinine, uric acid and serum electrolytes were indices with which the biochemistry profiles (kidney function test) of CCL_4- induced Wistar Albino rats fed with soy flour from selected processing methods was assessed. The levels of these give information on the condition of the kidney. Urea is excreted from the catabolism of the amino acid as end product of protein catabolism; so high protein levels can cause high urea levels (Friedman *et al.*, 2012).

In this study, blood urea nitrogen reduced appreciably in B and C rats but increased in group D rats. This may be due to the level of globulin in D (Table 12). According to Ihemeje *et al.*

(2013) and Aderogba *et al.* (2011), decrease in urea concentration suggests either that, the urea cycle may have been affected by plant extract leading to reduction of urea, or alkaloids and saponins contained in the plant extract and has produced systemic toxicity invariably leading to reduced ability to excrete waste, and failure to maintain balance in body fluid and electrolytes. In support of the above statement, the quantity of saponins and alkaloids in the two soy flour samples from processing methods (PM1 and PM2) fed to B and C showed significance and may cause reduction in the blood urea nitrogen.

Creatinine, according Edurado *et al.* (2018) is an excretion product of muscle activity, which circulates in blood. Its elimination is exclusively renal, so there is a correlation between levels and renal function (Edurado *et al.*, 2018). Maintenance of creatinine in the appropriate proportion is primarily kidney's function. Comparatively, a slight decrease in creatinine amount was observed in test rats and control which is attributable to the diets. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidney (Charles 2022). Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys (Edurado *et al.*, 2018; Charles,2022). From above analogus, it showed that the creatinine levels were within normal range implying there was no severe renal malfunction probably due the effect of antioxidants in the soy flour samples.

Table 12. Kidney function status of Wistar Albino rats fed soy flours

Biochemical Profiles	**NCD**	**PM1**	**PM2**	**PM3**	
	A control	B	C	D	SE
Urea(mg/dl)	43.60±0.01b	42.41±0.02c	41.51±0.00d	45.72±0.01a	0.60
Creatine (mg/l)	0.30±0.01a	0.29±0.00ab	0.28±0.00b	0.27±0.01c	-
Uric acid (mg/l)	6.21±0.00d	7.46±0.01c	8.96 ±0.01a	8.41±0.01b	394.1
K^+ (mg/l)	0.32±0.02a	0.24±0.01d	0.28±0.00b	0.26±0.01c	0.00
Na^+ (mg/l)	138.15±0.07b	136.15±0.01d	139.51±0.07a	136.71±0.07c	0.49
Co^{-3} (mg/l)	23990.00±0.07a	21435.00±7.07c	20540.00±7.07d	21990.±0.01b	8.0
Ca^{2+}(mg/dl)	9.98±0.01a	8.44±0.01d	9.45±0.00b	9.35±0.01c	0.21
Cl^- (mg/l)	73.53±0.02b	76.01±0.01a	69.30±0.01c	63.96±0.01d	-

Means are values of two replicates, then the standard deviation. (n=2.000).

Control A = Fed normal commercial diet (NCD, Stock finisher feeds) only
Group B = Fed soy flour diet from processing method one + Stock finisher feeds
Group C = Fed soy flour diet from processing method two + Stock finisher feeds
Group D = Fed soy flour diet from processing method three + Stock finisher feeds

Uric acid test measures the amount of uric acid in blood or urine. Foods with high levels of purines include liver, anchovies' sardines, dried bean and beer (https://medlineplus.gov>labtest; KidsHealth, 2018 and Mayo Clinic, 2018). Appreciable increase in amount specifically for uric acid in rats in test groups B, C and D administered aqueous soy flour from PM1- PM3 as compared to control A (Table 12). The appreciable increase in uric acid quantity in test groups for rats could be attributed to soy flour from processing methods not being able to effectively protect the kidney against damage caused as a result effect brought about by CCL_4. The amount of uric acid for rats in B, C and D were appreciably high implying negative effect on the rat kidney. This negative effect was countered by presence of other serum electrolytes in the serum blood for rats.

Appreciable decrease for serum potassium ion (K^+) for rats in test groups B, C and D fed with soy flour from PM1- PM3 respectively. Significant ($p<0.05$) differences were obtained in sodium ion (Na^+) concentration for rats in the test groups as compared to the control A. Kang

et al. (2002) reported that higher concentration of sodium is rare but can occur when there is loss of body fluids containing less sodium than plasma along with water intake restriction or if there is excessive sodium intake with limited liquid intake. In his contribution, Nduka (1999a) stated that hypernatraemia almost always indicates water depletion. However, Significant ($P > 0.05$) differences were observed in the levels of bicarbonate (HCO_3^-) for rats in test groups compared to the control. The level of serum calcium (Ca^+) appreciably decreased for A, B and C) in comparison with the control. Conversely, B significantly increased in blood chlorine (Cl^-) while C and D decreased in contrast with control A. The changes observed with respect to increase and reduction in all these serum electrolytes are however, within normal ranges.

Haematological Indices

Haematological indices are indicative of blood status of the animal and are used to assess protein quality and utilization (Oloyede *et al.,*2004). The haematological indices of CCl_4-induced Wistar albino rats indicated appreciable decreases regarding amount for pack cell volume, the haemoglobin and for red blood cell. No appreciable difference was noticed for amount of white blood cell but observable increase in the quantity of lymphocytes for the rats when compared with the control. However, this increase observed falls within normal range (5.0 – 23.0). This result is in agreement with the findings of Asmaa *et al.* (2018) who reported depletion of red blood cells (RBC), decrease in pack cell volume (PCV) and haemoglobin (Hb) with elevation in the levels of white blood cell (WBC) caused by CCL_4 induction compared to control. Since the increase was found to be within normal range, it inferred there was no ($P>0.05$) significant effect.

In a study of serum electrolytes, creatinine (crt) & haematological indices of rats fed on processed atlantic horse mackerel, Olaolua *et al.* (2015) reported that low RBC is indicative of anemia caused by nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency); While significant increased ($p<0.05$) RBC and Hb levels indicates polycythemia, usually caused by; dehydration, smoking or genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release) Pagana and Pagana (2017). Also, an increase in RBC is said to be indicative of thallasaemia while decrease points to anaemia (Rao 2006; CBC 2012).

Appreciable nutrophil's increase signify haemorrhage and reduction is suggesting a viral infection and possibly a disfunction of bone marrow. Also, an increase in Hb is implicative of lung diseases and a decrease may result in anaemia as reported by CBC, (2012). Ruothalo (2008) and Agiang *et al.* (2017) reported that due to their role in defence of an organism against attack, increasing the number of lymphocytes through diet will result in boosting the immune system. The increased concentration of WBC implies the diet was capable of boosting the immune system thus supporting antibacterial, antifungal, antiviral and immune-stimulative properties of the diet (Morton, 1987).

lymphocytes quantity appreciably increased with percentages 78%, 81% and 83% for rats B, C and D, respectively in comparison to control A inferring improved immune system. Several studies have reported that a significant decrease ($p < 0.05$) in WBC of blood indicates a decline in the proportion of the defensive mechanism to combat infections, a situation which would naturally make the animal more susceptible to various physiological stresses resulting in diseases, greater mortality and poor growth as reported by Muhamad (2012). Appreciable percentage decrease in neutrophil, specifically 15% to 20% in rats in comparison with control.

Presence of monocytes in C and D administered with soy powder extracts from processing methods PM2 and PM3, respectively. Increase in Monocytes is indicative of Protozoan diseases, leukemia or malignant diseases as reported by Rao (2006); Ruothalo (2008).

Esinophil was not detected in group B but was significantly ($p < 0.05$) present in C and D as compared to control A, Esinophil, are types of WBCs that play important role in the immune system, particularly in the body's response to allergic reactions, asthma and in fighting parasitic infections. The overall result showed little or no impact regarding haematological indices for test animal fed with soy powder from selected processing methods as all the increases and decreases in the haematological parameters were within normal ranges.

Histological parameters

The results of histological analyses showed normal morphology of a functional liver, spleen and kidney of test animal with exposure to natural environmental conditions (control group A; Figures 4 (a– c).

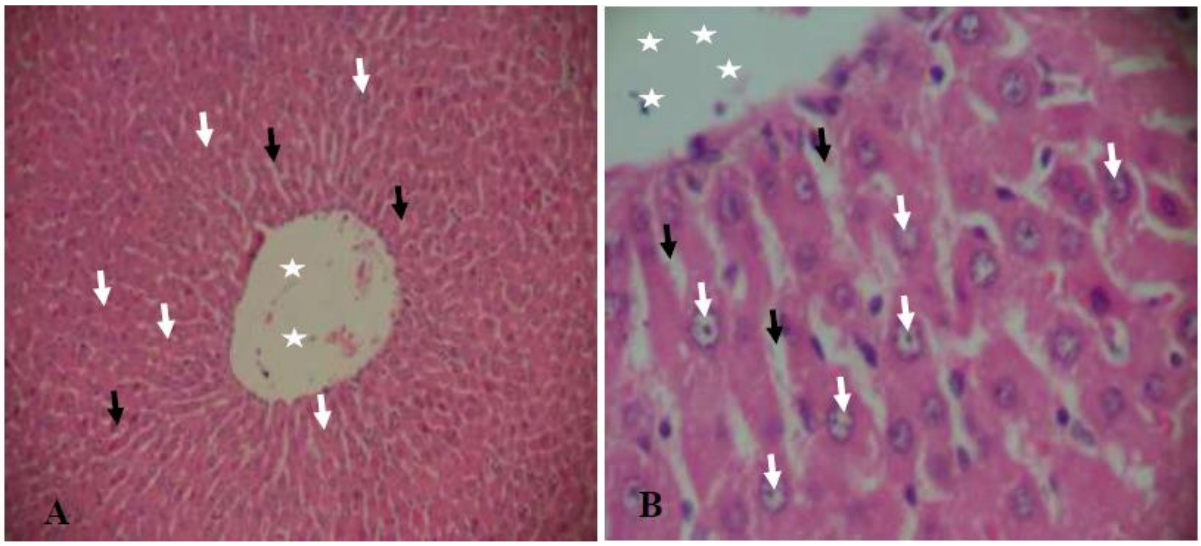


Figure 4. (a) Group A (Control) - Liver

Liver for Wistar Albino rat fed normal commercial diet shown normal morphology (Figure 4a).

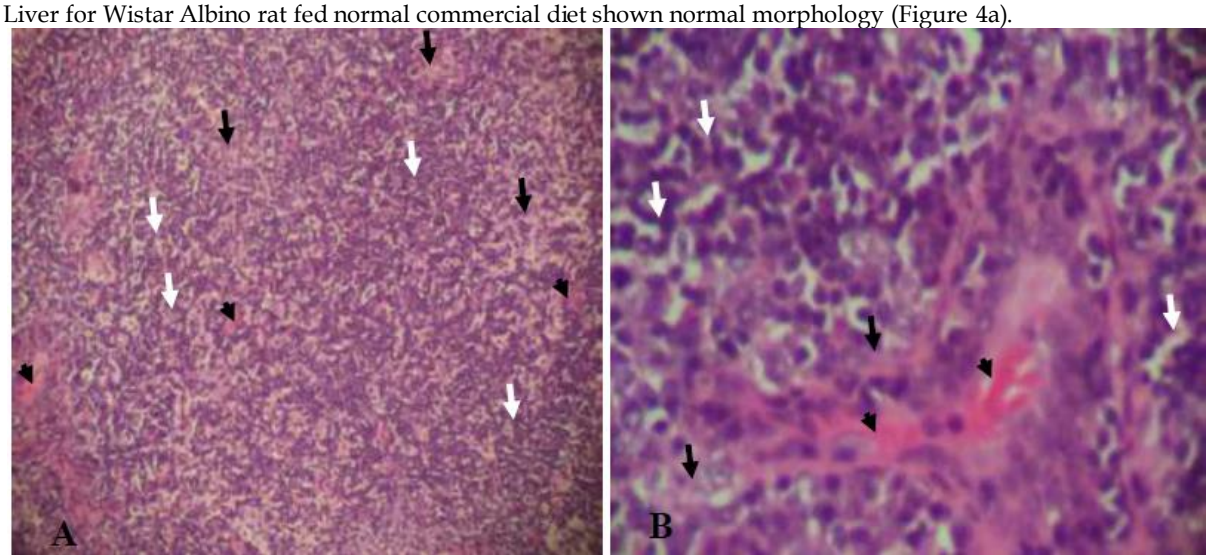


Figure 4. (b) Spleen for control rat group A

The Spleen of the control Wistar Albino rat fed normal diet and nutritional conditions showing normal morphology (Figure 4b). Black arrows = red pulp, white arrows = white pulp,

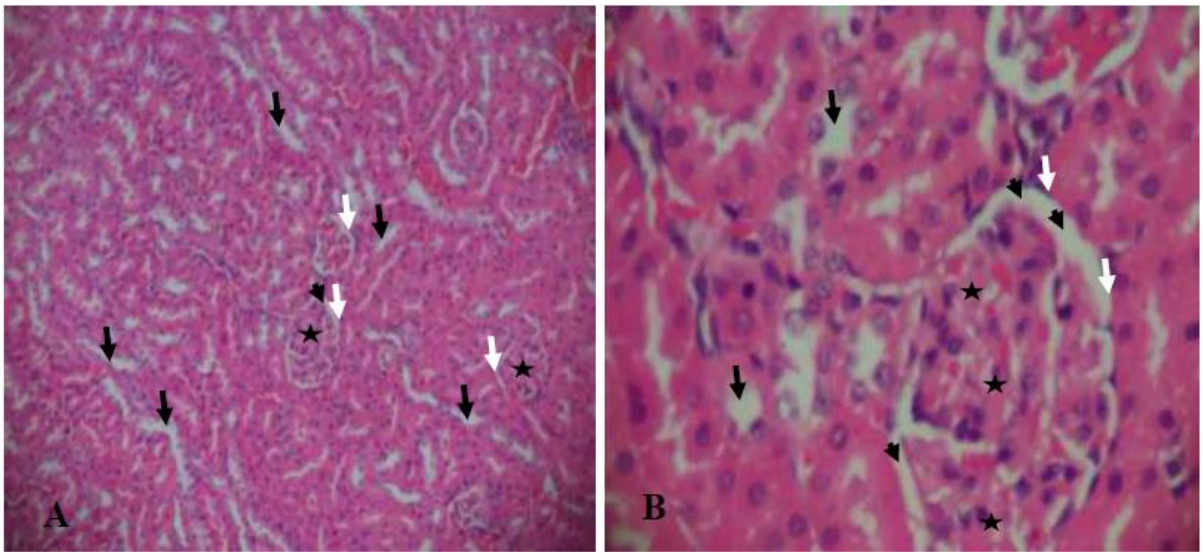


Figure 4. (c) Kidney for control rat (group A)
The kidney of the control Wistar Albino rat fed normal diet and nutritional conditions showing normal morphology (Figure 4c). Black stars= glomeruli, black arrows= kidney tubules, white arrows= glomerular capsule, black arrowheads=capsular spaces.

The conditions for liver, spleen, kidney in the test group B rats fed with soy flours from processing method one (PM1) is shown in Figures 5 12 (a – c). The normal morphological architecture of test animal is not unconnected with the anti-oxidants or phenolic compounds in the soy flours. The anti-oxidants in the soy flour have the capacity to reduce the hepatotoxic effect of carbon tetrachloride to cause damage to hepatoctyes.

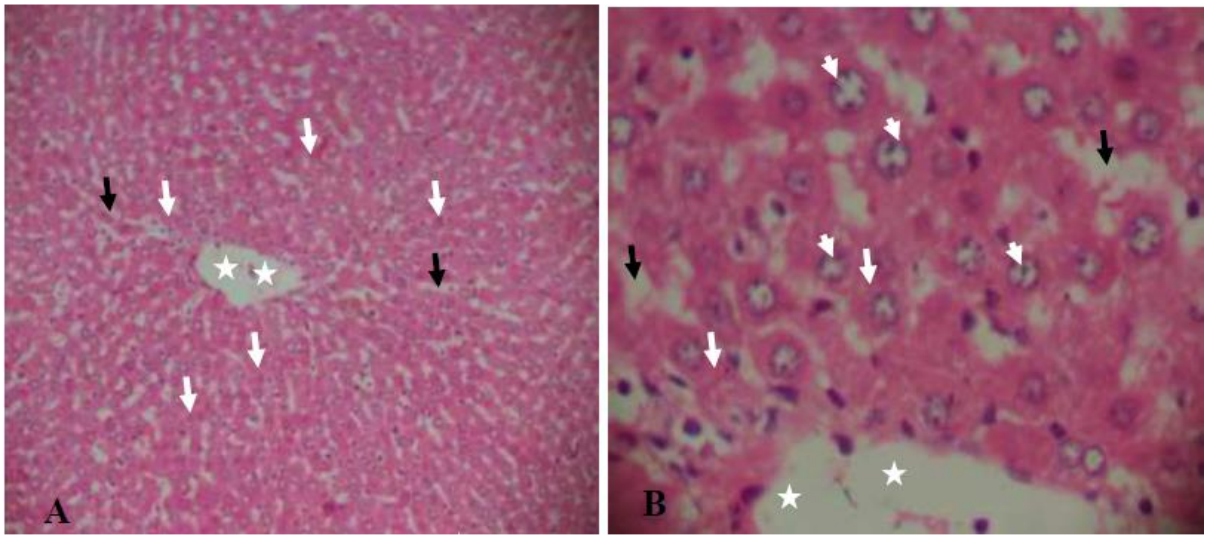


Figure 5. (a) Liver of test rat group B
The liver of Wistar Albino rat induced with CCl_4 and fed soy flour from processing method one (PM1), revealed intact nuclei.

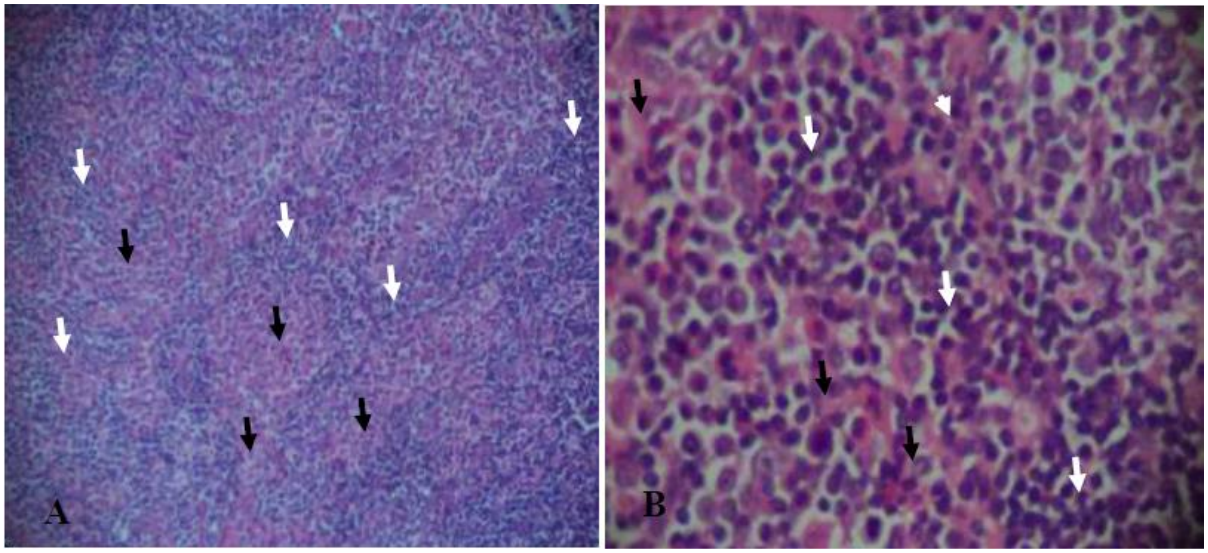


Figure 5. (b) Spleen of test rat group B
The spleen of Wistar Albino rat induced with CCl_4 and fed soy flour from processing method one (PM1) showing normal morphology with a clear distinction between the red pulp (black arrows) and white pulp (white arrows) (Figure 5b).

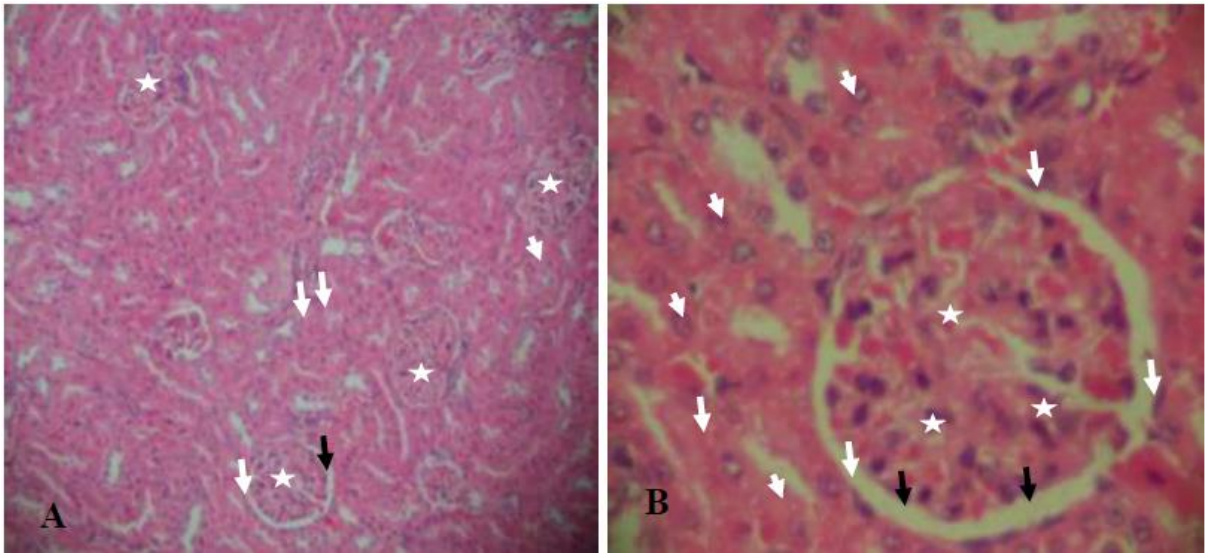


Figure 5. (c) Kidney of test rat group B
The kidney of Albino rats induced with CCL_4 and fed with soy flour from processing method one (PM1), showing normal morphology (Figure 5c).

For the liver of group C test rats fed with soy flours from processing method two (PM2) showed inflammation of the cells in the tissue, loss of tissue and necrosis (Figure 6 13 (a). The spleen of the rats in this group also showed atrophy as seen by increase interstitial spaces (Figure 13 (b). Similar result was obtained for kidney with the tissue degenerated (necrosis), lost of tissue architecture shown by the white arrow (Figure (c). The above result may be due to the presence of sodium bicarbonate ($NaHCO_3$) added to PM2 not being able to protect the rats against hepatotoxic effect of CCL_4.

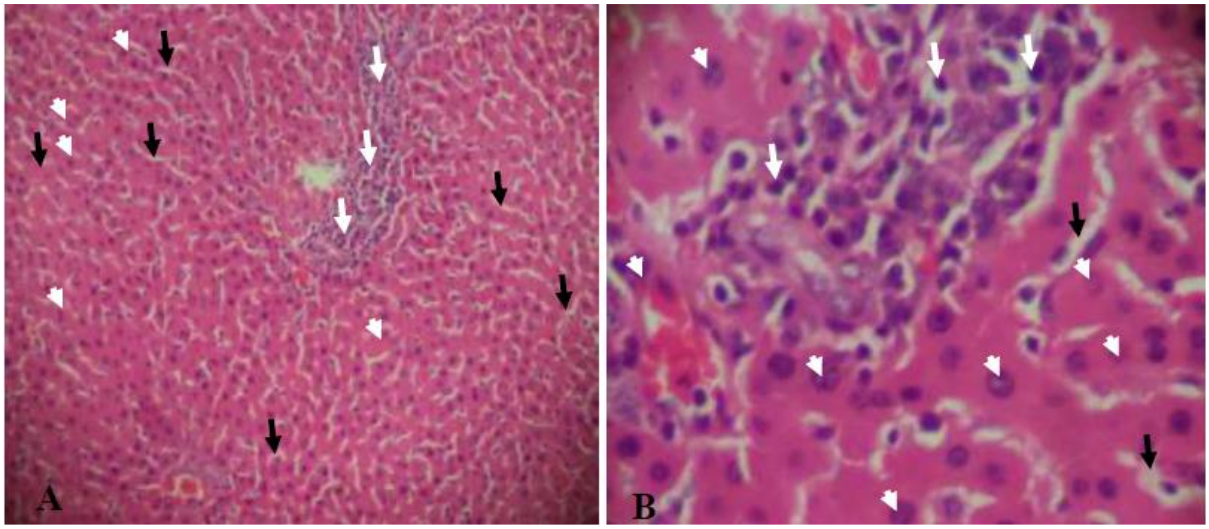


Figure 6. (a) Liver of test rat group C

The liver of Albino rat induced with CCl_4 and fed soy flour from processing method two (PM2), revealed inflammatory cells (white arrows). Notably, inflammatory cells (necrosis) replaced tissue (Figure 6a).

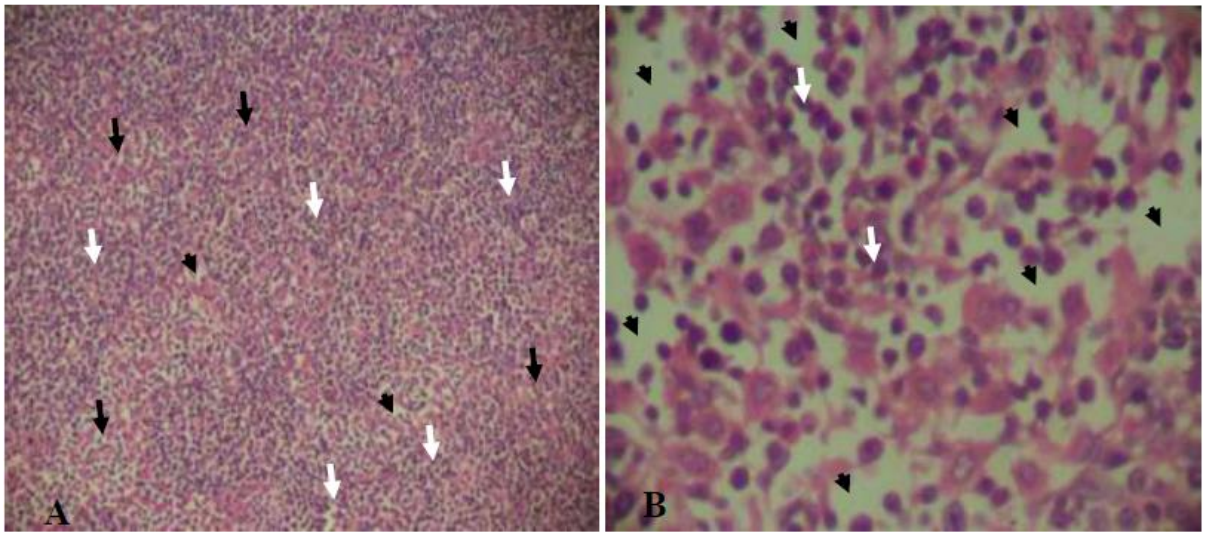


Figure 6. (b) Spleen of test rat group C

The spleen of albino rat induced with CCl4 and fed soy flour from processing method two (PM2), revealing deterioration (Figure 6b).

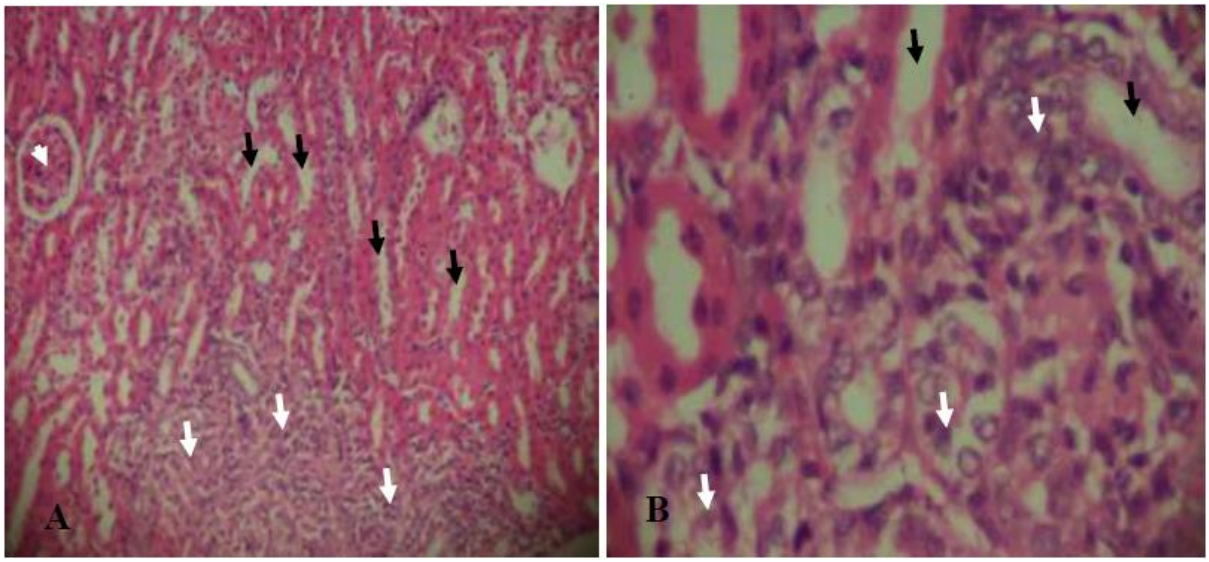
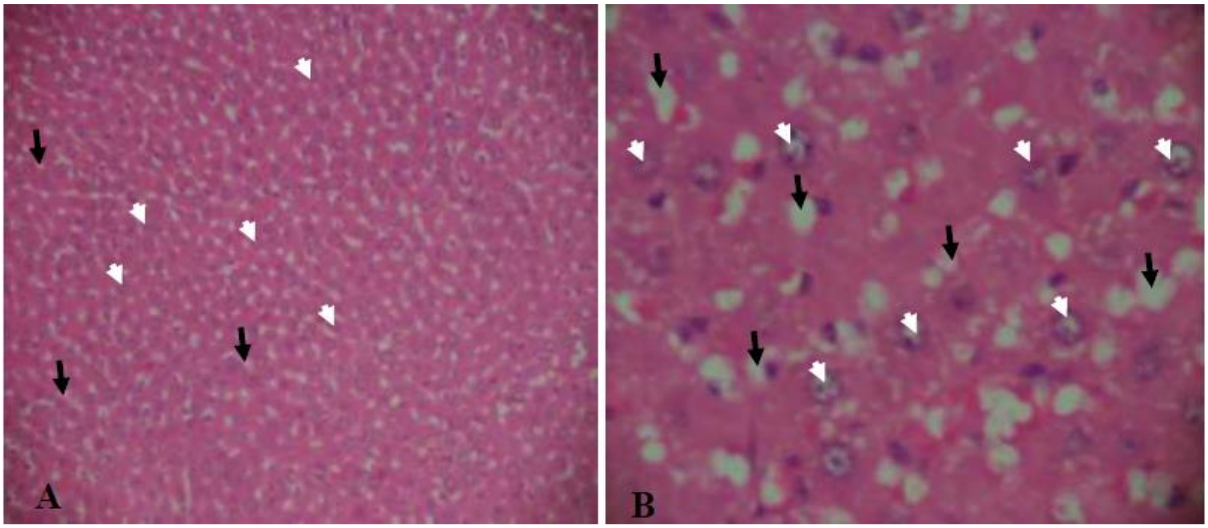


Figure 6: (c) Kidney of tested rat group C
The kidney of Wistar Albino rat induced with CCl_4 and fed soy flour from processing method two (PM2), showing deterioration of tissue.

The liver section of the rats in group D - induced with Carbon tetrachloride and fed soy flour from PM3 showed normal morphology. However, there was loss of cellular demarcation (Figure 7 14 (a). The spleen of rats in this group showed atrophy (Figure 7 (b) while the kidney (Figure14 (c) showed normal morphology implying that the soy flour protects the rats partially.

Figure 7. (a) Liver of test rat group D



The liver of Albino rat induced with CCl_4 and fed soy flour from processing method three (PM3), showing normal morphology with loss of cellular demarcations (mild hypertrophy).

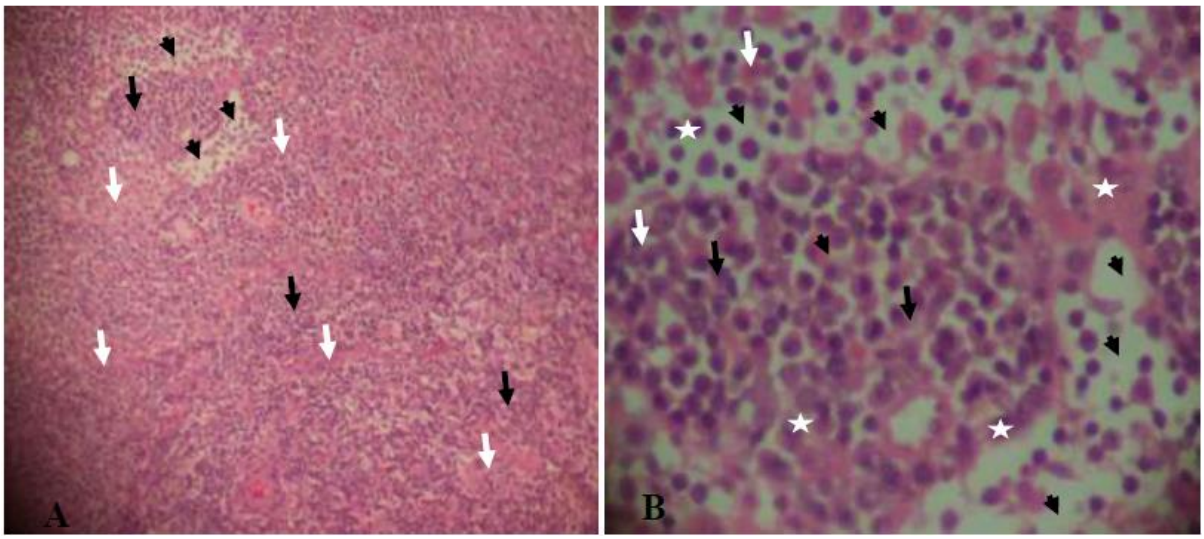


Figure 7. (b) Spleen of tested rat group D
The spleen of Albino rat induced with CCl_4 and fed soy flour from processing method three (PM3), showing deterioration.

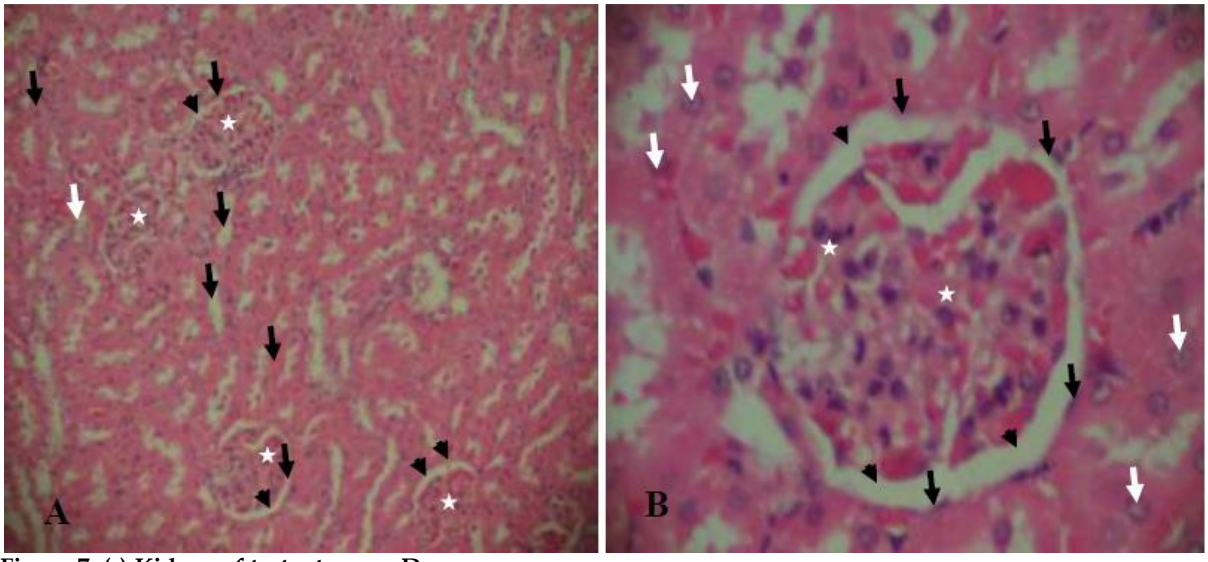


Figure 7. (c) Kidney of test rat group D

The kidney of Albino rat induced with CCl_4 and fed soy from processing method three (PM3), showing normal morphology. White stars= Glomeruli, White arrows= Cell nuclei.

Summarily, the increased concentration of WBC implies the diet was capable of boosting the immune system thus supporting antibacterial, antifungal, antiviral and immune-stimulative properties of the diet as reported by Morton (1987).

CONCLUSION

The body weight gain of Wistar Albino rats fed with soy flour from processing method one (PM1) had the highest weight, followed by that of processing method two (PM2). Liver enzymes and kidney parameters were however, within normal ranges. No adverse effect regarding haematological indices for Wistar Albino animal given soy flour from processing methods as increases and decreases in the above enzymes were still within normal ranges. The shape of the liver, kidney morphologically for rats given soy flour from PM1 and PM3 were perfectly intact. However, the spleen of rats in group D, liver, spleen and kidney of rats in group C showed inflammatory cell within the tissue, loss of tissue (necrosis) while the spleen showed atrophy. Processing methods (PM1, PM2 and PM3) had appreciable impact on the weight gain for Wistar Albino rats fed with soy flour from the different processing methods.

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