

Composite Flour Formulated from Roasted Cereal and Leguminous: Effects on Well-being of Young Rats

Séa Téhi Bernard^{1,*}, Rougbo N'djomon Paterne¹, Kouadio Natia Joseph¹,
Envrin Bogui Jacque Anicet¹, Soro Yadé René¹, Kouamé Lucien Patrice²

¹Laboratory of Biotechnology, Department of Biochemistry, UFR Biosciences,
Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire

²Laboratory of Biocatalysis and Bioprocesses, Department of food science and technology,
Nangui-Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

*Corresponding author: seatehi@yahoo.fr

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Abstract The purpose of this study was to investigate the effect of consumption of composite flour formulated from roasted maize (*Zea corn*), roasted soybean (*Glycine max*) and roasted groundnut (*Peanuts hypogaea*) on biochemical and hematological constants and histopathological effect on regulatory organs (kidney, liver and spleen) of strain Wistar rats aged to 50 ± 3 days. To do this, ten (10) young rats' males were fed with composite flour and control diet for a period of 15 days at the end of which their blood was collected for studying of biochemical and hematological constants. The kidneys, liver and spleen were also sampled for histopathological study. The results obtained show that contents of urea, creatinine, glucose, cholesterol and triglyceride are respectively 0.31 ± 0.09 ; 2.75 ± 0.50 ; 0.21 ± 0.10 ; 0.92 ± 0.12 and 0.57 ± 0.21 at the level of blood biochemical constants for rats fed with composite flour. For hematological parameters of young rats fed with composite flour, the contents are respectively 8.58 ± 1.10 ; 6.41 ± 1.51 ; 13.92 ± 1.03 ; 784.0 ± 164.96 ; 0.84 ± 0.31 and 2.18 ± 2.36 for white globule, red globule, hemoglobin, blood platelets, lymphocytes and neutrophils. Histopathological study revealed that no abnormalities were observed in the organs studied. In addition, the absence of significant differences in biochemical and hematological parameters in rats fed with control diet and composite flour suggest that the consumption of the composite flour does not had a negative impact on well-being of young rats. In summary, it appears from this study that the consumption of composite flour did not induce changes in the various biochemical and hematological constants and in the target organs of the metabolism studied. It would therefore be interesting to valorize this composite flour in human food in developing countries.

Keywords: biochemical parameters, composite flour, Hematological parameters, histological study, maize, soybean, groundnut

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1. Introduction

Adequate nutrition is a right for all and an essential condition for the child's proper physical, mental and psycho-emotional development and the quality of adults' life. Malnutrition remains a major public health problem in developing countries, particularly in South Asia and sub-Saharan Africa [1]. Protein needs are a crucial issue with growing concerns about food security and malnutrition in these countries [2]. About one billion people in the world have insufficient protein intakes, which affects negatively population growth and health [3]. Malnutrition can be due to a lack or excess of macronutrients (carbohydrates, fats or proteins) or micronutrients (vitamins and minerals) but also to the

toxic effect of these macro- and/or micronutrients in the body [1,2,3,4]. These forms of malnutrition affect populations throughout their life cycle, from childhood to adolescence, from adolescence to adulthood and among the elderly [1]. These nutritional deficiencies imply a critical need to find sustainable solutions to food insecurity and to resolve malnutrition problems through the formulation and supplementation of foods from local protein-rich resources [5]. Among the local protein-rich resources, the most commonly used in food formulations are soybean (*Glycine max*) and groundnuts (*Arachis hypogaea*) [6]. Their nutritional efficiency and high availability have made it possible to set up many food formulas. It is in this context that studies have led to formulate composite flour from flours of roasted maize (*Zea mays*), roasted soybean (*Glycine max*) and roasted groundnuts (*Arachis hypogaea*) [7]. A nutritional study

carried out with this composite flour allowed the young Wistar rats to grow well in terms of the zootechnical parameters evaluated [7]. However, no studies have been conducted on toxicological effect of the composite flour on blood constants and on vital organs such as liver, kidneys and spleen, which are the target organs of cellular metabolism. It is necessary to verify the toxicity of these foods formulated in order to avoid or prevent diseases that could occur in short or long term after consumption of composite flour. Thus, the purpose of this study was to evaluate the toxicological effect of consumption of composite flour formulated from roasted maize, soybean and groundnut using biochemical and hematological blood parameters on one hand and histological sections of organs on other hand of young Wistar rats.

2. Methods

2.1. Production of Maize, Soybean and Groundnut Flours

The grains used to make maize, soybean and groundnut flour were bought at Gouro markets in Adjamé, Abidjan Côte d'Ivoire. The grains were sent to the laboratory where they were transformed into flour according to the method described by [8]. To do this, the different grains have been cleaned and sorted to remove pieces of stones and wood debris. Then, the grains of maize and soybean were roasted at 120 °C for twenty minutes while those of groundnut were roasted at 80°C for twenty four hours using a ventilated oven. They were then grounded into flours using a blinder-type mixer. Finally, the flours obtained were sieved using a sieve of 150 µm of diameter and then stored at 4 °C in refrigerator in a clean bottles.

2.2. Formulation of Composite Flour and Diet Preparation

The composite flour was formulated by mixing for 100 g of food; 90 % of roasted corn flour, 5 % of roasted soybean flour and 5 % of roasted peanut flour. To the mixture was added 125 mL of distilled water and then the whole was cooked with an Electrolux brand hotplate for 10 minutes. The proximate composition of composite flour is shown in Table 1.

Table 1. Proximate composition of composite flour

Physicochemical parameters	Values
Dry matter (%)	89.44 ±0.13
Moisture (%)	10.56 ±0.13
Ash (%)	1.85 ±0.03
Total sugars (g/100g)	4.31 ±0.17
Total carbohydrate (%)	62.31 ±0.01
Starch (%)	52.19 ±0.2
Protein (%)	17.85 ±0.11
Fat (%)	5.83 ±1.72
Energy Value (Kcal/100g)	442.99±0.05

2.3. Toxicity Study

To determine the toxicity effect of composite flour on well-being of young rats, a nutritional assessment was

performed according to the method described by [9] for a period of 15 days using ten (10) albino young rats male of Wistar strains (*Rattus norvegicus*) with an average weight varied from 45 to 55 g and 50 ±3 day's old. These animals come from to the animals' barn of the UFR Biosciences of Félix Houphouët-Boigny University of Abidjan were divided into two groups of five (5) rats per group. The rats were housed individually in metal cages placed under a well ventilated laboratory with temperature (27 ±2°C), relative humidity (85 %) and an alternate 12 hours of natural light/ 12 hour of dark cycles. The animals were acclimatized for a period of 5 days before the start of the experiment. During the experiment, each young rats were fed with 30 g of corresponding diet between 7h30 and 8h30.

2.3.1. Blood Sampling

At the end of animal experiment, the animals were anaesthetized with chloroform and then sacrificed. Two (2) mL of blood were collected from dry tubes immersed in an ice tray for the determination of biochemical parameters and two (2) others mL were collected from purple tubes for the determination of hematological parameters. These analyses were carried out at the Medical Biochemistry Laboratory of the Medical Sciences UFR of Félix Houphouët-Boigny University, Abidjan.

2.3.2. Biochemical Parameter Assays

Biochemical analysis was performed using a centrifuge at 1480 rpm for 10 minutes to obtain the serum that was collected in Ependorfs tubes. It was stored at -20°C until the following biochemical parameters were determined: urea, creatinine, glucose, total proteins, cholesterol, triglyceride, HDL, LDL, phosphorus, calcium. The dosages were performed with a Hycl Lisa 300 PLC.

2.3.3. Hematological Parameter Assays

The hematological analysis was performed using an automatic hematological analyzer (Coulter STKS, Beckman). Parameters included: red blood cell count, white blood cell count, hematocrit level, hemoglobin concentration and platelet count were determined using methods described by [10,11]. The differential leukocyte count was performed with an optical microscope after staining, and in each case, 100 cells were counted

2.3.4. Histological Studies

At the end of our experimental period, the young rats were examined for general physical activity, mobility and agility. Subsequently, they were sacrificed. The organs of interest in this study (kidneys, liver and spleen) were excised and stored in a 10 % of formaldehyde solution (V/V) for 48 hours and washed with 70% of ethanol. The fabrics were then placed in small metal caskets, stirred by a magnetic stirrer, dehydrated with a series of alcohols from 70 % to 100 % alcohol and incorporated into paraffin using a coating machine. The paraffin blocks were cut using an ultra-rotating microtome, distributed on glass slides and dried overnight. Slides were observed under an optical microscope after being stained with hematoxylin and eosin (H&E) dyes [12]. The colored sections were examined under a Leica research microscope (DM

750) with a digital camera (Leica ICC 50) and digital photomicrographs of the colored sections were taken.

2.4. Statistical Analysis

Data accumulated was analyzed using statistical package for social sciences (SPSS) version 20.0 (IBM Statistics UK). A one-way analysis of variance (ANOVA) was used to test for the variations of different parameters observed while the mean differences of treatment groups were separated using Duncan Multiple Range Test (DMRT). All results were expressed as Mean \pm Standard error, while level of significance was placed at $P < 0.05$.

3. Results

3.1. Effect of Composite Flour on Biochemical Parameters of Young Rats

Table 2. Biochemical Parameters of Rats Fed with composite Flour and Control Diet

Biochemical parameters	Diets	
	Control diet	Composite flour
Urea (g/L)	0.27 \pm 0.07 ^a	0.31 \pm 0.09 ^a
Creatinine (mg/L)	3.25 \pm 0.50 ^a	2.75 \pm 0.50 ^a
Glucose (g/L)	0.48 \pm 0.19 ^a	0.21 \pm 0.10 ^b
Total proteins (g/L)	83.25 \pm 2.65 ^a	85.75 \pm 3.30 ^a
Cholesterol (g/L)	0.79 \pm 0.18 ^a	0.92 \pm 0.12 ^a
Triglyceride (g/L)	0.92 \pm 0.31 ^a	0.57 \pm 0.21 ^b
HDL (g/L)	0.28 \pm 0.06 ^a	0.24 \pm 0.06 ^a
LDL (g/L)	0.25 \pm 0.20 ^a	0.26 \pm 0.01 ^a
Phosphorus (mg/L)	80.25 \pm 2.63 ^a	87.75 \pm 3.15 ^a
Calcium (mg/L)	113.75 \pm 4.73 ^a	99.00 \pm 2.16 ^a

Values are mean \pm standard deviation of triplicate determinations. Values with different superscripts are significantly different from each other at the 5% level ($P < 0.05$) on the same line.

Table 2 shows the biochemical parameters serum of young rats fed with control diet and composite flour. Statistical analysis reveals on the one hand, that there are no significant difference at the content level of urea, creatinine, total protein, cholesterol, HDL, LDL, phosphorus and calcium of young rat's serum fed with composite flour and control diet at $P < 5\%$. On the other hand, statistical analysis reveals a difference significant at the content of glucose and triglyceride content of young rat serum fed with control diet and composite flour at $P < 5\%$. These content are higher in serum of rats fed with control diet than those fed with composite flour. The content of urea serum are respectively 0.27 \pm 0.07; 0.31 \pm 0.09 g/L for young rats fed with control diet and composite flour while the creatinine serum content are respectively 3.25 \pm 0.50 and 2.75 \pm 0.50 mg/L for young rat fed with control diet and composite flour. Glucose content of serum are respectively 0.48 \pm 0.19 and 0.21 \pm 0.10 g/L for young rat fed with control diet and composite flour. Concerning the total proteins of young rats' serum, the content are respectively 83.25 \pm 2.65 and 85.75 \pm 3.30 g/L for those fed with control diet and composite flour. For cholesterol serum content, the values obtained are respectively 0.79 \pm 0.18 and 0.92 \pm 0.12 g/L

for young rats fed with control diet and composite flour. The serum content of triglyceride is respectively 0.92 \pm 0.31 and 0.57 \pm 0.21 g/L for young rats fed with control diet and composite flour. The HDL content of serum are respectively 0.28 \pm 0.06 and 0.24 \pm 0.06 g/L for young rats fed with control diet and composite flour while the LDL content are respectively 0.25 \pm 0.20 and 0.26 \pm 0.01 g/L for young rats fed with control diet and composite flour. For serum mineral, the content of phosphorus are respectively 80.25 \pm 2.63 and 87.75 \pm 3.15 mg/L for young rats fed with control diet and composite flour while the calcium content are respectively 113.75 \pm 4.73 and 99.00 \pm 2.16 mg/L or young rats fed with control diet and composite flour.

3.2. Effect of Composite Flour on Hematological Parameters of Wistar Rats

The hematological parameters of young rats fed with control diet and composite flour are shown in Table 3. It appears from statistical analysis that there is no significant difference in the content of white globule, red globule, hemoglobin, lymphocytes, neutrophils, monocytes, eosinophils, basophils 1 and basophils 2 of young rats fed with control diet and composite flour. On other hand, at the level of blood platelets and hematocrit, the statistical analysis reveals a significant difference with higher concentration in to young rats fed with control diet in relation to those fed with composite flour. The concentration of white globule are 9.21 \pm 1.16 and 8.58 \pm 1.10 for young rats fed with control diet and composite flour respectively. For red blood, the concentration are respectively 7.75 \pm 0.69 and 6.41 \pm 1.51 for young rats fed with control diet and composite flour. Concerning the content of hemoglobin, it is respectively 14.37 \pm 0.82 and 13.92 \pm 1.03 for young rats fed with control diet and composite flour. Blood platelets which contains lymphocytes, neutrophils, monocytes, basophils 1 and 2 are respectively 406.50 \pm 75.42 and 784.0 \pm 164.96 in the blood of young rats with control diet and composite diet. For hematocrit concentration in young rats' blood, the values obtained are respectively 39.02 \pm 6.63 and 48.10 \pm 3.42 fed with control diet and composite flour.

Table 3. Hematological Properties of Rats Fed with Composite Flour and Control Diet

Hematological parameters	Diets	
	Control diet	Composite flour
White globule ($10^3/\mu\text{L}$)	9.21 \pm 1.16 ^a	8.58 \pm 1.10 ^a
Red globule ($10^6/\mu\text{L}$)	7.75 \pm 0.69 ^a	6.41 \pm 1.51 ^a
Hemoglobin (g/100mL)	14.37 \pm 0.82 ^a	13.92 \pm 1.03 ^a
Blood platelets	406.50 \pm 75.42 ^b	784.0 \pm 164.96 ^a
Lymphocytes (%)	1.00 \pm 0.01 ^a	0.84 \pm 0.31 ^a
Neutrophils (%)	1.00 \pm 0.01 ^a	2.18 \pm 2.36 ^a
Monocytes (%)	0.32 \pm 0.45 ^a	0.34 \pm 0.44 ^a
Eosinophils (%)	0.51 \pm 0.56 ^a	0.41 \pm 0.39 ^a
Basophils 1 (%)	1.00 \pm 0.01 ^a	0.90 \pm 0.19 ^a
Basophils 2 (%)	1.00 \pm 0.01 ^a	0.90 \pm 0.19 ^a
Hematocrit	39.02 \pm 6.63 ^b	48.10 \pm 3.42 ^a

Values are mean \pm standard deviation of triplicate determinations. Values with different superscripts are significantly different from each other at the 5% level ($P < 0.05$) on the same line.

3.3. Histological Study of Kidneys, Spleens and Livers

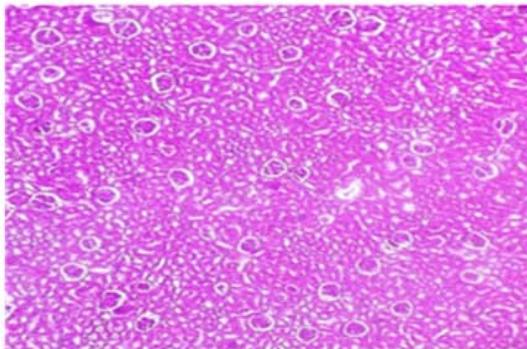
The Figure 1 displays the photograph of histological study carried out on kidneys, spleen and liver of young rat fed with different diets. It generally appears from observation of photography that no abnormalities or dysfunctions were observed in these organs. The Figure 1a and 1b which present the picture of kidney of young rat fed with control diet and composite flour show that glomerulus and filtration chamber are the same aspect. However, no degeneration of glomerulus and congestion of filtration chambers had observed in the structure of kidney.

About the spleen presented in Figure 1c and 1d, the photography does not show difference at level of white pulp, red pulp and capsule of young rats fed with control diet and composite flour. No dilatation, congestion, inflammation and proliferation are observed in the spleen of young rat fed with composite flour.

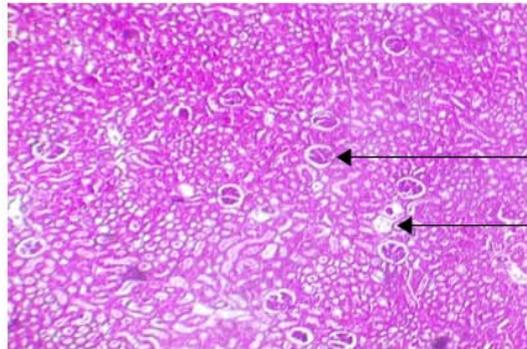
Concerning the liver of young rats fed with control diet and composite flour, the Figure 1e and 1f show that sinusoidal capillary, centrilobular vein and hepatocyte are the same aspect. No inflammation had observed at the level of sinusoidal capillary, centrilobular vein and hepatocyte of the young rats fed composite flour.

4. Discussion

Dietary changes may lead to series of reactions which can cause disruption of normal physiological activity bringing changes in biochemical constituents of the body fluid of animals' test [13]. Blood biochemical screening is a useful indicator for nutritional research [14]. Clinical pathological evaluation is being used as one of the safety assessment tools when some novel food sources are exploited for their appraisal as safe human food ingredient [15].

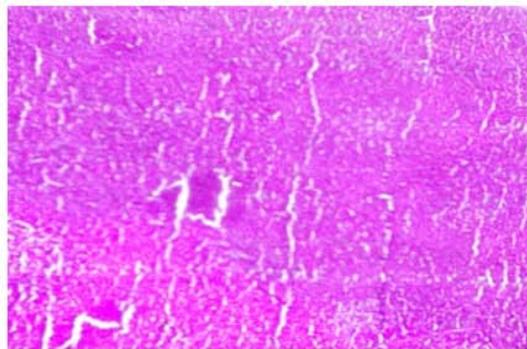


1a: Kidney of rats fed with composite flour, G=100

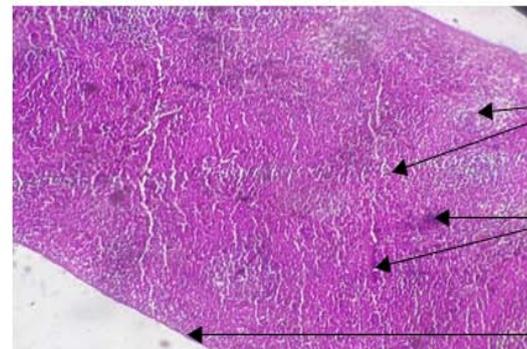


1b: Kidney Control diet, G=100

Glomerulus
Filtration chamber

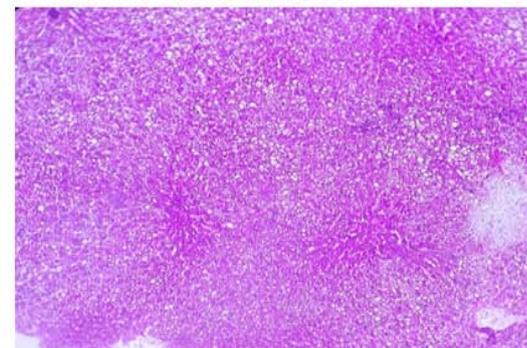


1c: Spleen of rat fed with composite flour, G=100

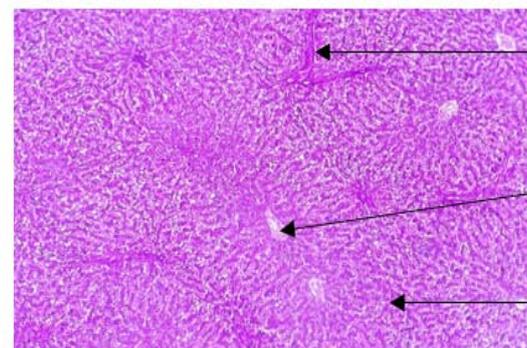


1d: Spleen Control diet, G=100

White pulp
Red pulp
Capsule



1e: Liver of rats fed with composite flour, G=100



1f: Liver Control diet, G=100

Sinusoidal capillary
Centrilobular vein
Hepatocyte

Figure 1. Photography of histological sections of kidneys, livers and spleens of young rats fed with composite flour and control diet.

At the end of our experiment, the young rats fed composite flour were physically healthy as the rats fed control diet. This well-being is confirmed by the lack of significant difference in the content of urea, creatinine, total protein, cholesterol, HDL, LDL, phosphorus and calcium values. This effect could be explained by the components used to formulate composite flour and the good treatment during formulation step. In fact, [16] show that high temperature could inhibit or deteriorate the food component whose consumption could affect negatively the biochemical constant of body. Also, the well-being could be explained by the role of proteins of serum which exerts a beneficial effect such as the maintenance of the osmotic pressure, the transport of molecules, the plasma purifying, strengthening the immune system and blood coagulation [17]. In addition, the rate of cholesterol, HDL and LDL of young rats show that the consumption of composite flour could protect the body from cardiovascular and coronary heart diseases [18,19]. In fact, cholesterol and triglyceride are both fatty substances found in blood, bile and brain tissue. They serve as a precursor to bile acids, steroids and vitamin D but the high content of serum could have lot of damage for the body [20]. This result confirms those obtained by Rougbo et al. [7] and suggests that the consumption of composite flour could have positive health effects.

Otherwise, the small content of glucose and triglyceride of serum constitute an advantage because many authors have revealed that very high serum glucose and triglyceride content could be the cause of many metabolic diseases such as diabetes, cardiovascular disease, coronary artery disease, etc. [21,22,23]. That way, the fact that serum blood sugar and triglyceride content of rats fed with composite flour are lower than rats fed with control diet indicates that consumption of this food may not have a negative impact on health.

Hematological parameters are those parameters that are related to the blood and blood-forming organs [24]. The hematological and serum examination is among the methods which may contribute to the detection of some changes in health status, which may not be apparent during physical examination but which affect the fitness of the animals [25]. In addition, hematological indices in animals are important to determine the toxicity risk since the changes in the blood system have a higher predictive value for human toxicity [26].

The absence of significant differences in the blood components of rats fed with composite flour and control diet suggest that consumption of composite flour did not induce an immune response in the rats' bodies. Indeed, lymphocytes, neutrophils, monocytes, eosinophils, basophils 1 and basophils 2 are leukocytes whose role is to help the body fight infections [27]. Statistical analysis of blood platelet and hematocrit levels in blood shows that they are significantly higher in young rats that consumed composite flour than those that consumed control diet $P < 5\%$. Indeed, blood platelets are a vital element of blood, in that they ensure vascular integrity and prevent bleeding [28]. An insufficient number of platelets or the presence of non-functional platelets may be responsible for bleeding and could constitute a health risk [29]. The function of lymphocytes is primarily its involvement in a variety of immunological functions, such as immunoglobulin

production and modulation of immune defense [30]. White globule cells are important in defending the body against infection [31]. The hematocrit content is in line with the recommendation which is comprised between 37.6 to 50.6 L/L for Wistar rats [32]. From all these analyses, we can deduce that the consumption of composite flour did not have a negative effect on blood components of young rats.

Histology is a discipline that aims to study the morphology and functioning of tissues. It therefore constitutes a fundamental basis for the diagnosis of pathology on a tissue scale [33]. The result of kidney photography are shown that no degeneration of glomerulus and congestion of filtration chambers had observed. This fact could suggest that the cells involved in renal filtration are normal and can purify the blood by eliminating the waste that comes from functioning of the body and maintain the chemical balance of blood [34].

About the spleen, the analysis of photography has shown that no dilatation, congestion, inflammation and proliferation have observed in to young rat fed with composite flour. Which means that spleen is in good condition and could maintain the body in good health. In fact, [35] showed that spleen is involved in elimination of old red blood cells from the blood. It contains a blood supply that can be very useful in the event of hemorrhagic shock and is also used for iron recycling.

In the liver of young rats fed with control diet, the result of histological study show that any inflammation had observed at the level of sinusoidal capillary, centrilobular vein and the hepatocyte of young rats fed composite flour. This fact suggest that consumption of composite flour has not negatively impact on the rats' liver. The liver is one of the most important organs of the body, since it provides many functions, in particular the synthesis and secretion of bile, the synthesis of proteins such as albumin, fibrinogen and coagulation factors. It is also involved in the metabolism of sugars and lipids, the synthesis of glycogen and the storage of vitamin B12 and iron [36].

5. Conclusion

At the end of this study, biochemical parameters reveals that consumption of composite flour formulated from roasted maize, soybean and groundnut have not induce any metabolic disease in to young Wistar rats. Regarding the hematological parameters, the results show that consumption of composite flour can protect the immune system and enhance its activities by defending the body against infection. About the histological section, the diagnostic of study organs have not reveal any degeneration, dilatation, congestion, inflammation and proliferation. The non-toxicity of composite flour shows that it can be used in infant nutrition in rural areas to solve the many problems of malnutrition.

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