GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Effects of sweet potato (*Ipomoea batatas*) flour enriched with moringa (*Moringa oleifera)* leaf powder on erythrocyte and leukocyte haematological parameters and liver and kidney histology of albino rats (*Rattus norvegicus*) in the Haut-Sassandra region Côte d'Ivoire

Traore Fakana Drissa *, Diomande Masse and Gbogbo Moussa

Agrovalorisation Laboratory, Department of Biochemistry and Microbiology, UFR Agroforestry, University of Jean Lorougnon Guede, Côte d'Ivoire.

GSC Biological and Pharmaceutical Sciences, 2023, 22(02), 099–110

Publication history: Received on 01 January 2023; revised on 13 February 2023; accepted on 15 February 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.2.0070

Abstract

Diabetes is a pandemic with expensive treatments. It would be interesting to use locally available and inexpensive plants. Sweet potato tubers and moringa leaves are among them. The aim is to evaluate the impact of sweet potato flour enriched with moringa leaf powder on the haematological and histological parameters of albino rats. Four formulations based on sweet potato flour enriched with 5%, 10%, 15% and 20% moringa leaf powder, respectively BBPM5, BBPM10, BBPM15 and BBPM20. A dose of 300 mg/kg bw of each of these formulations was administered daily by gavage to rats versus controls given distilled water for 28 days. Haematological parameters were determined using the automated system and the impact assessed from histological sections. A non-significant increase in haemoglobin, red blood cell count, haematocrit, GMV and blood platelet count was observed in all treated lots. In addition, a non-significant decrease in MCHF and MCHR was observed. The hepatic and renal cells of the rats remained intact with the absence of hyperchromatosis in all batches except the control and BBPM5. At the end of this work, haematological parameters, liver and kidney tissues were not affected. The BBPM15 formulation appears to be the most effective and should be recommended to diabetics.

Keywords: Sweet potato; Moringa; Enriched flour; Haematological and histological parameters; Albino rat

1. Introduction

Diabetes is a metabolic disease that has become a public health problem [1]. It is estimated that 5% of the world's population suffered from diabetes in 2014 and more than 41 million diabetics are expected in 2035 [1]. According to the WHO, 5.6% of men and 4.4% of women in the Ivorian population suffer from diabetes [2]. Treatment of diabetes has long been restricted to dietary changes, insulin injections and oral antidiabetic drugs [3] . The excessive cost of these anti-diabetic agents and the inadequate medical infrastructure, coupled with the lack of health care personnel in Africa, are leading people to turn to traditional medicine. Medicinal plants constitute a medical potential that is accessible, available and at low cost [4] . These plants constitute an inexhaustible resource that provides the majority of active ingredients in pharmaceutical products. However, many of the medicinal plants used have no scientific data on their efficacy and safety [5] . Indeed, for a rational use of these plants, work must be done to determine the possible harmful effects induced by their use and which could lead to other complications in the treatment of pathologies [6] . It is in this perspective that we undertook to study the effects of *Ipomea batatas* (concolvulaceae) flour enriched with *Moringa oleifera* (moringacea) powder, two plants from the Ivorian pharmacopoeia, used in the treatment of diabetes.

* Corresponding author: Traore Fakana Drissa

The objective of this work was to evaluate the effect of enriched flour on haematological parameters and its impact on the liver and kidney histology of albino rats in the Haut-Sassandra region (Côte d'Ivoire). More specifically, the effect of the formulations on erythrocyte and leukocyte parameters and then on liver and kidney tissues will be evaluated.

2. Material and methods

2.1. Animal Feeding

The experiment was conducted according to the protocol described in OECD Guideline 407 [7]. The animals were grouped into homogeneous groups according to their weight. Five (5) batches of five (5) rats were formed. Each batch consisted of male animals of approximately equal weight. The animals were tail-marked per batch and within each batch to identify them during the experiment.

Each group was placed in a plastic cage and maintained under standard laboratory conditions (temperature 25 ± 2° C) with a dark and light cycle (12h / 24h). Batch 1 represented the control rats which received distilled water by gavage (1ml/day). Batches 2, 3, 4 and 5 received BBPM5, BBPM10, BBPM15 and BBPM20 at 300 mg/kg bw of sweet potato flour solution supplemented with moringa leaf powder at 5%; 10%; 15%; 20% respectively by gavage. The volume administered was 1ml per day each morning for the duration of the treatment which is 28 days. Before the start of the actual experiment, one week was devoted to acclimatize the animals to this condition. During the experimental days, each group of rats was fed with water and standard food (kibble, IVOGRAIN) measured before and after each new administration. At the end of the experiment, the animals were sacrificed after fasting for 15 hours and blood samples were taken.

2.2. Blood sample collected

Two (2) blood samples were taken throughout the experiment. The first sample was taken prior to feeding the rats on day zero (0) to obtain initial values. Rats were sedated with ether and blood samples were collected through the tail into EDTA tubes from an incision in the tail tip. The second whole blood sample from each rat was collected on day 28 in the same EDTA tubes for blood count after sacrificing the rats by decapitation at the end of the experiment (28 days).

2.3. Determination of haematological parameters of wistar rats (Blood Count).

The blood count was performed immediately on EDTA tubes by the URIT-2900® automatic analyser.

2.3.1. Principle of the controller

The cell counting principle of the URIT-2900 is based on impedance variation. Thus, when 10 µL of whole blood is aspirated, the cells pass through a calibrated orifice. A direct current field is applied across the orifice. As the cell does not have the same conductivity as the surrounding medium, its passage through the orifice causes a change in the current between the two electrodes. This difference in current is recorded, which allows the cell to be counted each time it passes. Moreover, the current difference is proportional to the volume of the particle, which is measured at the same time as the count. The volume is therefore a criterion for differentiating blood cell populations, since platelets and red blood cells on the one hand and leukocytes on the other have distinct volumes most of the time.

In the white blood cell chamber, the sample remains for 10 seconds and then the size and number of leukocytes are determined by impedance measurement. In the red cell chamber, the size and number of erythrocytes and thrombocytes are also determined by the same method. From the measured values, the other parameters are calculated in the microprocessor of the machine. During the incubation time, the erythrocytes are dissolved under the influence of lysis and the haemoglobin is released and converted into methaemoglobin. A portion of the sample from this chamber is introduced into the haemoglobin flow cell. The haemoglobin concentration is measured spectrophotometrically.

2.3.2. Procedure

Prior to counting, the blood samples are thoroughly homogenised by successive gentle inversion to avoid the formation of micro clots. Manually, the sample tubes are presented vertically to the needle of the machine and the suction button is pressed. The sample tube is then withdrawn vertically after the aspiration needle is removed from the sample. The machine performs a complete analysis of the haematological parameters of a sample in 1 minute 35 seconds. The URIT-2900 is connected to a graphical printer that transcribes the results onto paper.

2.4. Making and observing histological sections of liver and kidney of wistar rats.

At the end of the experiments, the liver, kidney and heart were weighed and examined macroscopically. The shapes, sizes and colours of these internal organs were observed visually for signs of macroscopic damage. The organs (liver and kidneys) were then fixed in 10% formaldehyde to make histological sections. The method used to make the longitudinal sections was the paraffin embedding technique described by [8] . This technique was performed in three steps. The first step consisted of impregnating the organ parts contained in cassettes in a paraffin tray (Axel Johnson Labsystem, Sweden). The second step was to make thin sections on the paraffin blocks containing the organs using a Shandon AS 325 rotary microtome (Marshal Scientific, USA). The final step was to observe the histological sections of the liver and kidneys with a Photonic Microscope after staining with haematoxylin-eosin.

2.5. Determination of the rates of change.

The rate of change is calculated according to the following formula: rate of change=(final value - initial value x100)/(initial value)

2.6. Statistical analysis

All measurements were performed in triplicate and the means of the data were analysed statistically using XLSTAT 2014.5.03 One-way analysis of variance (ANOVA) was performed to compare the means. Differences were considered significant for values of $p \leq 0.05$. To separate the different sample means, multiple comparison tests (Tukey HSD) were performed.

3. Results

3.1. Influence of formulations on erythrocyte parameters

In terms of red blood cells (RBC), haemoglobin (HGB), mean corpuscular volume (MCV) and haematocrit (HCT), the results showed no significant difference ($p > 0.05$) between the rats fed the formulations and the rats of the control batch, both at the beginning and at the end of the trial. However, the results showed a non-significant ($p > 0.05$) decrease in mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCHC) and platelet count (PLT) as a function of time (Table 1).

The mean values of the red blood cell count before administration of the formulations ranged from 5.52±0.69 106/µL to 5.91±0.68 106/µL in all batches. Red blood cell counts at D28 ranged from 5.75±0.14 106/µL to 6.12±0.62 106/µL for rats fed the different formulations and 5.49±0.86 106/µL for the control lot. The rate of decrease of red blood cells is 0.54% for the control and 1.01% for BBPM15 while there is an increase in red blood cell count in rats of BBPM5, BBPM10 and BBPM20 with respective rates of 1.06%; 14.80% and 0.34% (Table 1 and 4. (Table 1 and 4).

At baseline, mean haemoglobin values ranged from 12.40±1.06 g/dL to 12.88±0.73g/dL in all batches of animals fed the formulations. Haemoglobin ranged at D28 from 12.08±0.27 to 12.68±0.90 for rats fed the different formulations and was 12.46±0.96 for the control lot. The rates of decrease in hemoglobin were 2.30% and 0.55% respectively for the rats of the BBPM10 batches while there is an increase in hemoglobin in the rats of the control, BBPM5 and BBPM20 batches with respective rates of 0.48%; 1.76% and 7.70% (Table 1 and 4).

These results revealed that the mean haematocrit levels of the different batches of animals at the initial time ranged from 31.70±0.7 to 32.50±1.36%. There was no significant difference between the haematocrit level of the control animals (30.94±2.89) and the animals of the other batches. The haematocrit level varied at D28 from 31.42± 0.53% to 32.74± 1.73% for the rats fed the different formulations and was 32.68±1.65% for the control batch. The rates of decrease in haematocrit were 1.23% and 0.88% respectively for the BBPM15 and BBPM20 rats while there is an increase in haematocrit levels in the control, BBPM5 and BBPM10 rats with respective rates of 5.60%; 0.70% and 0.98% (Table 1 and 4).

The GMV values before the start of the treatments ranged from 53.92 ± 8.58 fL to 63.26 ± 6.61 fL in all batches of rats present. There was no significant difference between the different batches of rats and the control rats (59.46 ± 9.75 fL). The GMV level varied at D28 from 57.48± 1.91 fL to 59.34± 1.42 fL for the rats fed the different formulations and was 60.6±3.92 fL for the control lot. The rate of decrease in GMVs was 6.3% for the BBPM20 rats while there was an increase in GMVs in the control, BBPM5, BBPM10 and BBPM20 rats with respective rates of 1.91%; 4.51%; 0.52% and 10.05% (Table 1 and 4).

The values of MCHC before the treatments ranged from 75.46 ± 7.70 g/dL to 152.00 ± 9.87 g/dL in all batches of animals. At the initial time, the MCHF values of the different batches were not significant compared to the control batch (49.56 ± 6.13 g/dL). The MCHF level varied at D28 from 34.7± 2.94 g/dL to 64.62± 9.81 g/dL for the rats fed the different formulations and was 37.62±6.74 g/dL for the control batch. The MCHF levels decreased for rats in the control, BBPM5, BBPM10, BBPM15 and BBPM20 batches by 24.09%; 14.30%; 70.60%; 0.70% and 76.85% respectively (Table 1 and 4).

The MCHT values before the formulations ranged from 36.76 ± 4.76 pg to 102.12 ± 8.66 pg in all batches of rats. At the initial time the MCHT values of the different batches were not significant compared to the control batch (25.66 ± 6.61 pg). The MCHT levels varied at D28 from 20.24± 0.50 pg to 36.10± 3.14 pg for the rats fed the different formulations and were 22.64±2.87 pg for the control lot. The MCHT levels decreased for rats in the control, BBPM5, BBPM10, BBPM15 and BBPM20 batches by 11.76%; 11.90%; 71.60%; 44.10% and 79.59% respectively (Table 1 and 4).

3.2. Influence of the formulations on the blood platelet content

The blood platelet concentrations of the different batches ranged from 266.57±57.87 103/µL to 320.6±94.91 103/µL at baseline and were not significant compared to the control batch (331.8±12.59 103/µL). Blood platelet concentrations ranged at D28 from 414.4± 32.09 103/µL to 587.8± 32.13 103/µL for rats fed the different formulations and that of the control lot was 520.6±98.28 10 3/µL. The platelet levels increased for the control, BBPM5, BBPM10, BBPM15 and BBPM20 rats by 56.90%; 75.07%; 111.71%; 83.09% and 65.87% respectively (Table 2 and 4).

3.3. Influence of formulations on WBC parameters

These results revealed that the white blood cell counts of the fasting and pre-treatment rats varied between 5.9 ± 0.89 and 9.58 ± 3.11 103/µL. At baseline, the mean WBC counts of the different batches were not significant ($p > 0.05$) compared to the control batch (5.98 ± 1.22 103/µL). The white blood cell count ranged at D28 from 6.76± 1.22 103/µL to 10.32± 4.84 10 3/µL for the rats fed the different formulations and was 8.26± 3.60 10 3/µL for the control lot. The rate of decrease in white blood cells is 6.11% and 28.60 respectively for the control and BBPM15 rats while there is an increase in white blood cell levels in the control, BBPM10 and BBPM20 rats with respective rates of 38.10%; 0.87% and 74.91% (Table 3 and 4).

The results revealed that the lymphocyte count of the rats, prior to any treatment, ranged from 4.14 ± 0.72103/µL to 7.08 ± 1.34 103/µL, which was not significantly different ($p > 0.05$) from the control lot (3.78 ± 1.77 103/µL). The lymphocyte count of the rats ranged at D28 from 5.08± 1.78 103/µL to 8.06± 1.67 103/µL for the rats fed the different formulations and was 6.04±3.58 103/µL for the control lot. Lymphocyte levels decreased by 28.24% for the BBPM15 rats and increased for the BBPM5, BBPM10 and BBPM20 rats by 59.78%, 1.00%, 20.60% and 80.19% respectively (Table 3 and 4).

Initially the values of the monocyte count of the different batches of rats ranged from 0.56± 0.18 103/µL to 0.92 ± 0.54 103/µL. These values of the mean monocyte count of the different batches of rats were non-significant (at $p > 0.05$) compared to the control batch (0.64± 0.24 103/µL). The monocyte count of the different batches of rats varied at D28 from 0.58± 0.18 103/µL to 0.70± 0.25 103/µL for the rats fed the different formulations and was 0.6±0.28 103/µL for the control batch. The monocyte count increased by 14.28% for the BBPM20 rats and decreased for the control; BBPM5; BBPM10 and BBPM15 rats by 6.25%; 14.28% and 20.45% and 36.90% respectively (Table 3 and 4).

The results revealed that the neutrophil count of the rats, before any treatment, ranged from 0.44 ± 0.11 103/µL to 0.62 ± 0.23 103/µL. The neutrophil count was non-significant ($p > 0.05$) compared to the control lot (0.46 ± 0.18 103/µL). The neutrophil count of the rats ranged at D28 from 0.34± 0.13 103/µL to 0.48± 0.36 103/µL for the rats fed the different formulations and was 0.6±0.19 103/µL for the control lot. The neutrophil count increased by 30.43% for the control rats and decreased for the BBPM5, BBPM10, BBPM15 and BBPM20 rats by 7.69%, 32.25%, 34.61% and 9.09% respectively (Table 3 and 4).

3.4. Tissue impact of enriched meals on the liver in rats

Figures 1a, 1b, 1c, 1d and 1e show the effect of enriched flours on rat liver tissue. In the control (Figure 1a) and treated batches (1b, 1c, 1d and 1e), the liver tissues are normal, with hepatocytes clearly visible. The liver cells of all rat batches retained their cellular integrity (intact cell membrane, visible nucleus, no cellular damage) in both the enriched meal-fed rats and the controls that received distilled water. No steatosis was observed by the accumulation of triglicerides in the hepatocytes. However, the control and BBPM5 batch had altered nuclei by condensation of nuclear content (Figure 1).

Table 1 Erythrocyte parameters of rats by batch

Constituents	batches of rats	Control	BBPM5	BBPM10	BBPM15	BBPM20
Red blood cells (10^6/µl)	D0	5.52±0.69a	5.63±0.43a	5.33±0.31a	5.91±0.68a	5.73±0.63a
	D28	5.49±0.86a	5.69±0.37a	6.12±0.62a	5.85±0.84a	5.75±0.14a
Haemoglobin (g/dL)	D0	12.4±1.06a	12.82±0.78a	12.88±0.73a	12.72±0.83a	12.46±0.49a
	D28	12.46±0.96a	12.68±0.90a	12.58±0.58a	12.65±1.09a	12.08±0.27a
Haematocrit (%)	D0	30.94±2.89a	32.5±1.36a	32.36±1.05a	32.3±0.86a	31.7±0.70a
	D28	32.68±1.65a	32.74±1.73a	32.68±1.24a	31.9±1.23a	31.42±0.53a
Mean corpuscular volume (fL)	D0	59.46±9.75a	55.8±5.97a	57.18±2.50a	53.92±8.58a	63.26±6.61a
	D28	60.6±3,92a	58.32±3.92a	57.48±1.91a	59.34±1.42a	59.26±2.28a
Mean corpuscular haemoglobin concentration (g/dL)	D0	49.56±6.13a	75.46±7.70a	120.42±3.16a	80.5± 7.90a	152±9.87a
	D28	37.62±6.74b	64.62±9.81b	35.3±1.92b	34.7±2.94b	35.18±1.88b
Mean corpuscular haemoglobin tenor (pg)	D0	25.66±6.61a	40.98±29.51a	70.28±75.40a	36.76±4.76a	102.12±8.66a
	D28	22.64±2.87b	36.1±3.14b	20.24±0.50b	20.52±1.27b	20.84±0.82b

a and b: mean followed by different letters within the same line are significantly different ($p < 0.05$); BBPM5= 95% sweet potato flour and 5% moringa leaf powder; BBPM10= 90% sweet potato flour and 10% moringa leaf powder; BBPM15= 85% sweet potato flour and 15% moringa leaf powder; BBPM20= 80% sweet potato flour and 20% moringa leaf powder; D0 = first day of the experiment D28= 28th day of the experiment

Table 2 Blood platelet parameters of rats by batch

Constituents	blood platelet (103/µl)	
batches of rats	D0	D28
Control	331.8±72.59a	520.6±98.28b
BBPM5	266.57±57.87a	466.7±51.18b
BBPM10	277.64±78.00a	587.8±32.13b
BBPM15	320.6±94.91a	587±24.39b
BBPM20	273.94±62.59a	414.4±32.09b

a and b: mean followed by different letters within the same line are significantly different ($p < 0.05$); BBPM5= 95% sweet potato flour and 5% moringa leaf powder; BBPM10= 90% sweet potato flour and 10% moringa leaf powder; BBPM15= 85% sweet potato flour and 15% moringa leaf powder; BBPM20= 80% sweet potato flour and 20% moringa leaf powder; D0 = first day of the experiment D28= 28th day of the experiment

Table 3 Leukocyte parameters of rats by batch

Constituents	batches of rats	Control	BBPM5	BBPM10	BBPM15	BBPM20
White blood cells (10^3/µl)	D0	5.98±1.22a	8.18±2.73a	9.1±4.15a	9.58±3.11a	5.9±0.89a
	D28	8.26±3.60a	7.68±2.97a	9.18±2.07a	6.76±1.22a	10.32±4.84a
Neutrophils (10^3/µl)	D0	0.46±0.18a	0.52±0.42a	0.62±0.23a	0.52±0.18a	0.44±0.11a
	D28	0.6±0.19a	0.48±0.28a	0.42±0.67a	0.34±0.13a	0.48±0.36a
Lymphocytes (10^3/µl)	D0	3.78±1.77a	6±0.26a	6.68±1.64a	7.08±1.34a	4.14±0.72a
	D28	6.04±3.58a	6.06±1.22a	8.06±1.67a	5.08±1.78a	7.46±1.83a
Monocytes (10^3/µl)	D0	0.64±0.24a	0.7±0.24a	0.88±0.59a	0.92±0.54a	0.56±0.18a
	D28	0.6±0.28a	0.6±0.19a	0.7±0.25a	0.58±0.18a	0.64±0.46a

a and b: mean followed by different letters within the same line are significantly different ($p < 0.05$); BBPM5= 95% sweet potato flour and 5% moringa leaf powder; BBPM10= 90% sweet potato flour and 10% moringa leaf powder; BBPM15= 85% sweet potato flour and 15% moringa leaf powder; BBPM20= 80% sweet potato flour and 20% moringa leaf powder; D0 = first day of the experiment D28= 28th day of the experiment

Table 4 Rate of change of hematological parameters

Rate of change (%)	Constituents	Control	BBPM5	BBPM10	BBPM15	BBPM20
	Red blood cells	-0.54	1.06	14.80	-1.01	0.34
	Haemoglobin	0.48	1.76	-2.30	-0.55	7.70
	Haematocrit	5.60	0.70	0.98	-1.23	-0.88
	Mean corpuscular volume	1.91	4.51	0.52	10.05	-6.30
	Mean corpuscular haemoglobin concentration	-24.09	-14.30	-70.60	-0.70	-76.85
	Mean corpuscular haemoglobin tenor	-11.76	-11.90	-71.20	-44.10	-79.59
	blood platelet	56.90	75.07	111.71	83.09	65.87
	White blood cells	38.10	-6.11	0.87	-28.60	74.91
	Lymphocytes	59.78	1.00	20.60	-28.24	80.19
	Monocytes	-6.25	-14.28	-20.45	-36.90	14.28
	Neutrophils	30.43	-7.69	-32.25	-34.61	-9.09

3.5. Tissue impact of enriched flours on the kidney in rats

Figures 2a, 2b, 2c, 2d and 2e show the effect of enriched flours on kidney tissue in rats. In the control (Figure 2a) and treated batches (Figure 2b, 2c, 2d and 2e), the kidneys show a normal appearance, with glomeruli and tubules clearly visible. The glomeruli and tubules are well vascularised and delineated with an absence of anomalies in all batches of rats both in the enriched meal fed rats and in the controls that received distilled water (Figure 2).

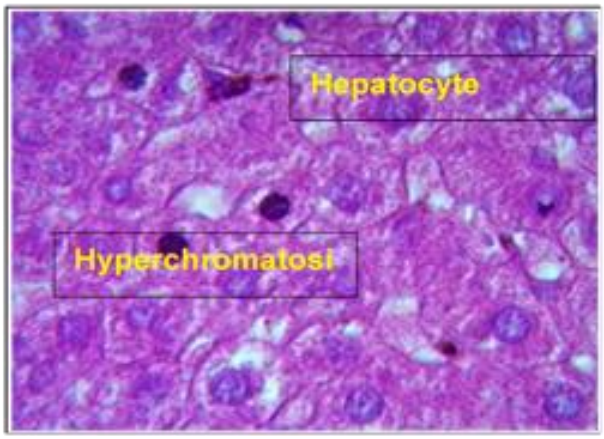

Figure 1a Control liver (GX400)

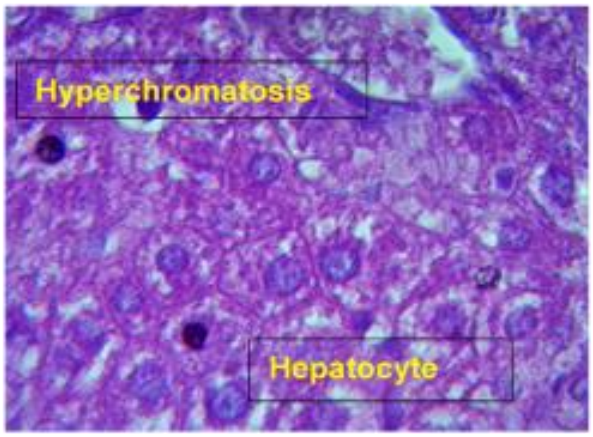

Figure 1b BBPM5 (GX400) liver

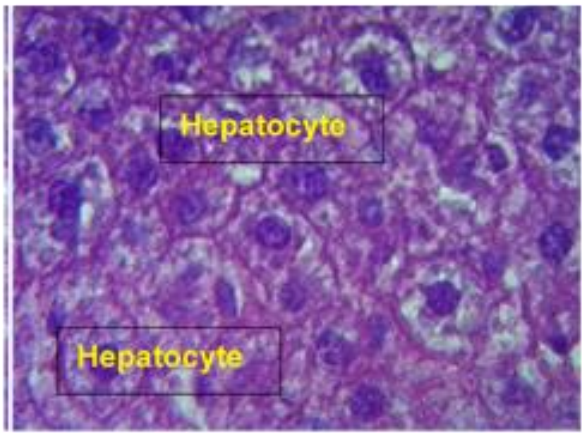

Figure 1c BBPM10 (GX400) liver

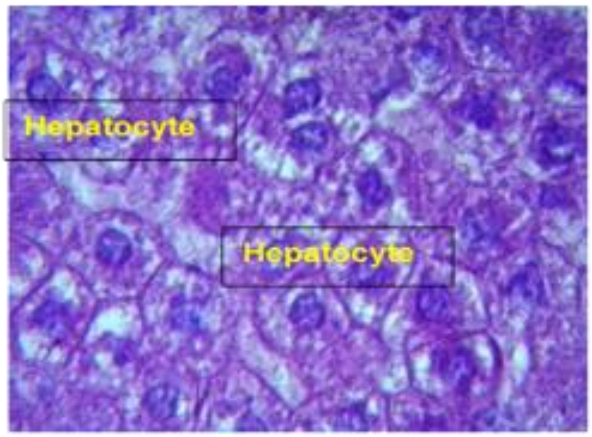

Figure 1d BBPM15 liver (GX400)

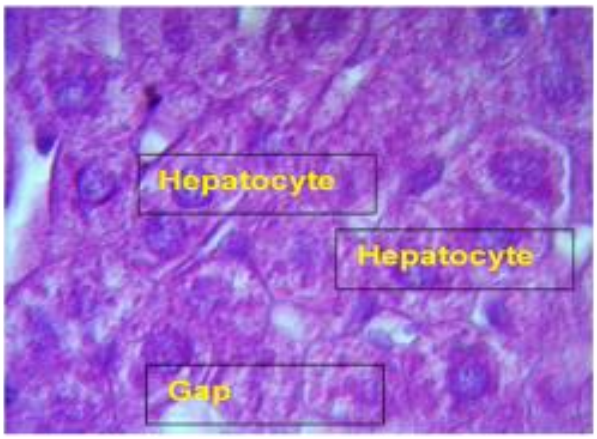

Figure 1e BBPM20 liver (GX400)

Figure 1 Histological sections of rat liver from the experiment

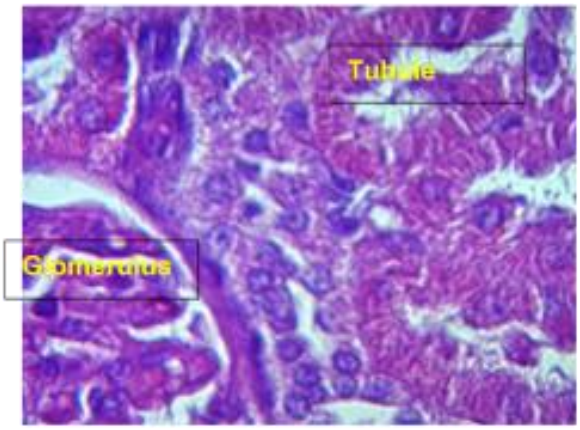

Figure 2a Kidney of the control batch (GX400)

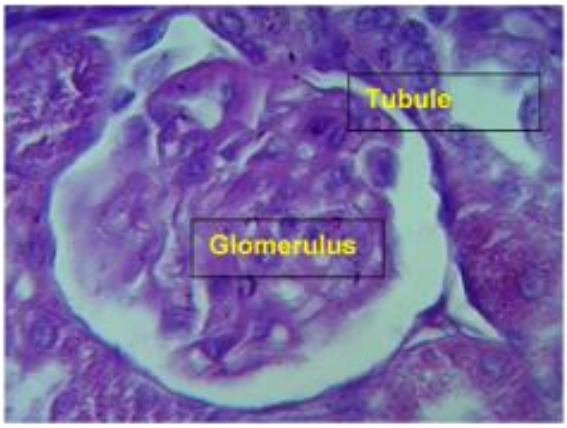

Figure 2b Kidney of BBPM5 (GX400)

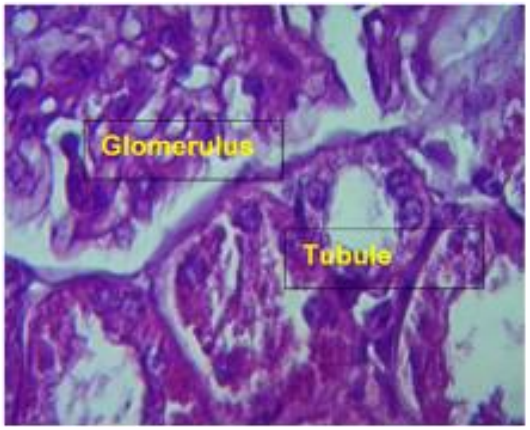

Figure 2c Kidney of BBPM10 (GX400)

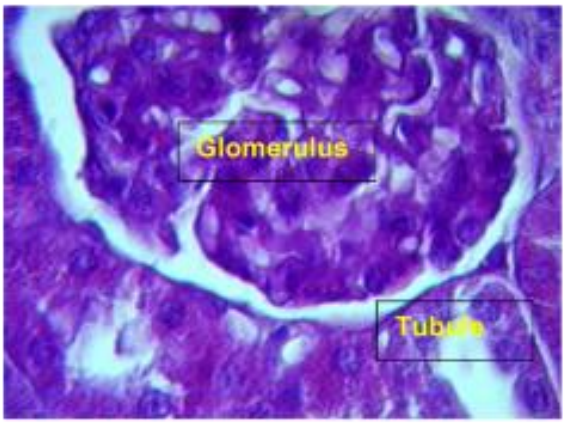

Figure 2d Kidney of BBPM15 (GX400)

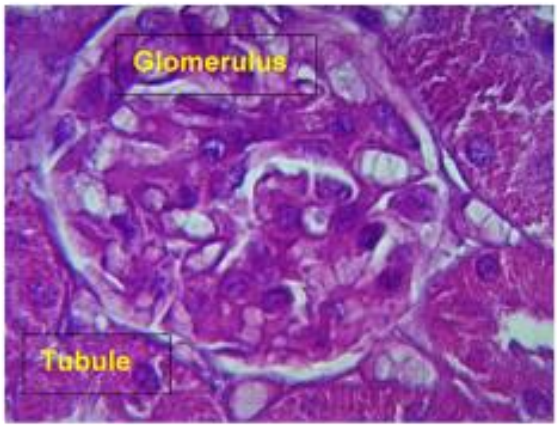

Figure 2e Kidney of BBPM20 (GX400)

Figure 2 Histological sections of experimental rat kidney

4. Discussion

4.1. Influence of the formulations on erythrocyte parameters

The mean values of the red blood cell count before administration of the formulations ranged from 5.52±0.69 106/µL to 5.91±0.68 106/µL. These values were in the same range as the red blood cell count at D28, which ranged from 5.75±0.14106/µL to 6.12±0.62106/µL for the rats fed the different formulations. The red blood cell count in this study was lower than that (7.07±0.79106/µL) in Bedou [9] using EABt extracts in rats. However, the control

(6.19±0.19106/µL) in the Bedou 2019 study is higher than that (5.49±0.86106/µL) in the present study. The highest value (6.12±0.62106/µL) of the present study was close to that of (6.6±0.5106/µL) of Onsiyor et *al.* [10] using aqueous extract of *Ageratum conyzoides* (EAqAc) on rats.

The red blood cell levels (8.75±0.51106/µL for male rats and 8.37±0.17106/µL for female rats) of Diaby et *al*. [11] using distilled water are higher than the levels obtained in this study. The lack of variation in red blood cells in all batches of rats compared to control rats could mean that our extract would not affect the red blood cells of treated rats.

The mean haemoglobin values ranged from 12.40±1.06 g/dL to 12.88±0.73g/dL in all batches at the beginning of the trial.These values are higher than the haemoglobin values at D28, which ranged from 12.08±0.27 g/dL to 12.68±0.90 g/dL after the rats were fed for 28 days. The hemoglobin levels are similar to the level (12.77±1.54 g/dL) in the study by Bedou [9] using EABt extracts in rats. These values are lower than the value (14.1±0.4g/dL) of the study of Onsiyor et *al.* [10] using aqueous extract of *Ageratum conyzoides* (EAqAc) and also lower than the haemoglobin level (14±2g/dL for both male and female rats) of Diaby et *al.* [11] receiving 4mg/kg of cadnium. The control level (12.46±0.96) in the present study is higher than the control level (11.17±0.61 g/dL) in the study by Bedou [9]. The lowest value (12.08±0.27 g/dL) in the present study is close to the value (12±0.9g/dL) obtained in the study by d'Onsiyor et *al.* [10]. These results show that the mean haematocrit levels of the different batches of animals at the initial time ranged from 31.70±0.7 to 32.50± 1.36%. These values are lower than the haematocrit levels at D28, which ranged from 31.42±0.53% to 32.74±1.73% for the rats fed the different formulations. These haematocrit levels are lower than the haematocrit level (38±0.6%) in the study by Onsiyor et *al.* [10]. on the effect of aqueous extracts of *Ageratum conyzoides* on rats. On the other hand the normal control rate (32.68±1.65%) is similar and that of the control animals (33.27±3.27%) in the study of Bedou [9] on *Bauhinia Thonningii* fruit. The haematocrit levels (51±6% for male rats and 48±3% for female rats) of Diaby et *al*. [11] receiving 4mg/kg of cadnium are higher than the level obtained in this study. The decrease in the number of red blood cells, haemoglobin and haematocrit levels is also due to excessive disruptions taking place in the metabolisms and haematopoiesis [12]; [13]. This is not the case in this study.

The GMV values before the start of the treatments ranged from 53.92± 8.58 fL to 63.26± 6.61 fL in all batches of rats present. These values are lower than the GMVs at D28 which ranged from 57.48± 1.91 fL to 59.34± 1.42 fL for rats fed the different formulations. The values (57.48± 1.91 fL to 59.34± 1.42 fL) in this study are similar to those (56.80±0.80 fL) of Bedou [9] on *Bauhinia Thonningii* fruit. The GMV levels of normal control rats (60.6±3.92 fL) in this study are similar to those (59.20±1.23 fL) of Bedou [9] study on the activity of *Bauhinia Thonningii* fruit on rats. These results are higher than the rate (54±2.1fl) of Onsiyor et *al.* [10] who worked on the impact of aqueous extract of *Ageratum conyzoides* on rats. The GMV levels (60±1fl for male and 62±2fl for female rats) of Diaby et *al.* [11] when given distilled water corroborate with the level obtained in this study. These values are higher than the GMV level (55±3fl for male and 56±1fl for female rats) of Diaby et *al.* [11] given 20mg/kg. The reduction in red blood cell count associated with a decrease in GMV level would indicate microcytic anaemia and conversely an increase in GMV level would indicate macrocytic anaemia.

The pre-treatment MCHC values ranged from 75.46 ± 7.70 g/dL to 152.00 ± 9.87 g/dL in all batches of animals. These values were higher than the MHCCs at D28 which ranged from 34.7± 2.94 g/dL to 64.62± 9.81 g/dL for the rats fed the different formulations. These values are similar to the CCMH level (36.20±0.99 g/dL) in the Bedou [9] study. The level of control rats (49.56±6.13 g/dL) in this study is similar to the level of control rats (33.20±2.53 g/dL) in the Bedou [9] study. The levels of HDAC (29±1g/dL for male rats and 30±11g/dL for females) of Diaby et *al.* [11] who received distilled water is lower than the level obtained in this study. The reduction in red blood cell count associated with a decrease in MHCC would symbolise hypochromic anaemia [14, [15].

The MCHT values before administration of the formulations ranged from 36.76± 4.76 pg to 102.12± 8.66 pg and are higher than the MCHT levels at D28 which ranged from 20.24± 0.50 pg to 36.10± 3.14 pg for rats fed the different formulations. These values are higher than the MCHT level (18.17±0.17 pg) in the Bedou [9] study. The MCHT level of the control (19.63±1.19 pg) was lower than the control (22.64±2.87 pg) in the Bedou [9] study. The MCHT levels (17±1pg, for male rats and 19±1pg, for female rats) of Diaby et *al.* [11] who received distilled water corroborate with the level obtained in this study.

4.2. Influence of the formulations on the blood platelet content.

The blood platelet concentrations of the different batches ranged from 266.57±57.87 103/µL to 331.8±12.59 10 3/µL at baseline and were lower than the blood platelet concentrations at D28, which ranged from 414.4±32.09 10 3/µL to 587.8±32.13 10 3/µL for the rats fed the different formulations. The platelet levels were lower than the Bedou 2019 platelet level (791.67±27.06 103/µL). The platelet levels (1255±210103/µL for male rats and 876±80103/µL for female

rats) of Diaby et *al*. [11] fed with distilled water are higher than the levels obtained in this study. The platelet levels of control rats (795.67±26.6710 3/µL) in the present study are higher than the platelet levels (520.6±98.28 103/µL) obtained in the study by Bedou [9]. The platelet levels are lower than the level (918±4.7 10 3/µL) of Onsiyor et *al.* [10] of the impact of aqueous extract of *Ageratum conyzoides* on rats.

4.3. Influence of formulations on WBC parameters

These results revealed that the white blood cell counts of the rats prior to any treatment ranged from 5.9 ± 0.89 to 9.58 ± 3.11 103/µL. Blood WBC counts ranged at D28 from 6.76± 1.22 103/µL to 10.32± 4.84 10 3/µL for rats fed the different formulations. These values are higher than the values before the rats were fed the formulations. The value (8.26± 3.60 10 3/µL) of the control batch fell between the minimum and maximum values of the treated batches. The minimum value is lower than the value (9.8±0.8 10 3/µL) of the normoglycaemic control [10] . The maximum value is close to the value (10±0.6 10 3/µL) obtained by Onsiyor et *al.* [11] with the aqueous extract of *Ageratum conyzoides* (EAqAc). The white blood cell counts (7±1.3103/µL for male rats and 6.29±0.8103/µL for female rats) of Diaby et al 2016 when given distilled water corroborated with the count obtained in this study.

The results revealed that the lymphocyte count of the rats, prior to any treatment, ranged from 4.14±0.72 to 7.08±1.34%. The lymphocyte count of the rats ranged at D28 from 5.08±1.78% to 8.06±1.67% for the rats fed the different formulations and was 6.04±3.58% for the control lot. These levels are lower than the lymphocyte count (70.88±0.65% for normal rats) of Bedou [9] before alloxane injection on rats.

Initially the monocyte count values of the different batches of rats ranged from 0.56±0.18% to 0.92±0.54%. These values of the mean monocyte count of the different batches of rats are non-significant (at $p > 0.05$) compared to the control batch (0.64± 0.24%). The monocyte count of the different batches of rats varied at D28 from 0.58± 0.18% to 0.70± 0.25% for the rats fed the different formulations and was 0.6±0.28% for the control batch. These levels are lower than the monocyte level (3.57±0.49% for normal rats) of Bedou [9] before alloxane injection.

The neutrophil count of the rats varied at D28 from 0.34±0.13% to 0.48±0.36% for the rats fed the different formulations and was 0.6±0.19% for the control lot. This rate is not significant ($p > 0.05$) compared to the control lot (0.46± 0.18%). These rates are lower than the neutrophyl rate (19.93±0.28% for normal rats) of Bedou [9] before alloxane injection. The haematopoietic system and blood cells are among the most sensitive targets of toxic compounds [16]. According to Olson et *al.* [17], data obtained from studies on the haematopoietic system in animals are predictive of human toxicity.

4.4. Impact of enriched meals on the liver of rats

The histopathological results obtained showed that enriched flours do not affect the structural architecture of rat liver tissue. The results of this study are consistent with the results of the study in which chronic treatment of healthy animals with total aqueous extracts of *Naudea lalifolia* and *Combretum glulinosum* would not result in histopathological changes in the livers. According to the experimental protocol for the investigation of side effects by long-term treatments as advocated by Guillouzo et *al.* [18]. These results are different from other studies prior to the present study. Namely, histological examination in cadmium-exposed rats revealed cellular disorganization, cyto-nuclear atypia and necrosis in liver and kidney tissues reported by Berroukche et *al.* [19] on the evaluation of the effect of cadmium sulfate in the presence of zinc in Wistar rats. However, the trigliceride granulations (steatosis) observed in some rats of all batches could be due to the granules distributed to the rats during the experimental period. According to Novikoff [20] , Hinton And Lauren [21], the lipid accumulation is due to an inhibition of the synthesis of apoproteins (deficient endoplasmic reticulum) involved in the transfer of lipids from the hepatocyte to the extracellular compartment and/or a deficiency in the transport of golgitic condensation vacuoles by microtubules. In addition, given the reduction in the number of mitochondria, a decrease in fatty acid catabolism (oxidation) may be involved. Furthermore, Martin and Feldmann [22] observed sections of rat livers treated with *Naudea lalifolia* extracts. These sections showed mitosis patterns and numerous hepatocytes with reactive hypertrophic nuclei, abundant and regularly cross-linked chromatin and a clearly visible membrane. Treatments of this type could therefore induce some increase in hepatocyte activity. Also, similar studies on animals treated with Tinospora bakis extracts have shown discrete microvacuolar steatosis lesions mostly located in the centrilobular regions. This would be a sign of fatty degeneration that could be induced by high doses of extracts of this plant as reported by Fortin et *al.* [23].

4.5. Impact of enriched meals on rat kidneys

The histopathological results obtained showed that enriched flours do not affect the structural architecture of the rat kidneys. The results of the present study are contrary to those of the study on the effect of lead acetate and cadmium

chloride toxicity on kidney and liver tissue in guinea pigs. Randa et *al.* [24] showed in this study that metals mainly damage the kidney and liver. Furthermore, the renal histopathology study by Kaplan et *al.* [25] not only confirmed but also showed that cadmium affects the proximal tubules and is transported by the blood to be deposited in the organs producing biochemical, histological or morphological effects that result in specific organ alterations [26].

5. Conclusion

In the present work, the effects of sweet potato flour enriched with Moringa powder on blood cells were evaluated through haematological, liver and kidney tissue analyses.

These analyses showed a non-significant increase in haemoglobin, red blood cell count, haematocrit and HSCC in all animals treated at 300 mg/kg body weight compared to the control animals. Furthermore, the results showed that there was a non-significant decrease in GMV, MCHR and platelet count at the same dose.

These formulations do not have a negative effect on the haematological and histological parameters of the liver and kidneys of treated rats. These sweet potato flours enriched with moringa powder can therefore be used without prejudice to help diabetics to treat themselves.

Compliance with ethical standards

Acknowledgments

We would like to thank all the team of the animal house of the biology and health laboratory of the University Jean Lorougnon Guede for providing us with rats for the experiments.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

The experimental protocol and animal handling procedures were conducted according to good laboratory practice .

References

[1] IDF, IDF Diabetes Atlas. 6th edition, 2014.

[2] W.H.O, World Health Organization. Country profiles for diabetes, 2016.

[3] Gbekley E.H., Karou D.S., Gnoula C., Agbodeka K., Anani K., Tchacondo T., Agbonon A., Batawila K., Simpore J. , Ethnobotanical study of plants used in the treatment of diabetes in the traditional medicine of the Maritime region of Togo. Pan Afr Med j. 2015, 20: 437.

[4] Okigbo R.N., Omodamiro O.D. , Antimicrobial effect of leaf extract of Pigeon pea (Cajanus cajan (L) Mill sp) on some human pathogen. J Herbs Spices Med Plants, 2006, 12: 117-27.

[5] Fleurentin J., Ethnopharmacology in the service of therapeutics: sources and methods. Hegel, 2012, 2: 12-8.

[6] Azzi R., Djaziri R., Lahafa F., Scckal F.Z., Bennehdi H., Belkaccm N., Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. J. Med. plants Res, 2012, 6: 2041- 2050.

[7] OECD, OECD Guideline for Testing of Chemicals. In 28-day repeated dose oral toxicity study in rodents, 2008, OECD 407. p. 9,

[8] Hould R., Histopathology and cytopathology techniques. Maloine edition, Paris, France, 1984,399 p,

[9] Bedou K. D. , evaluation of the inhibitory activity of the fruits of bauhinia thonningii (fabaceae) on two glycosidases and test for the treatment of diabetes in wistar rats: doctoral thesis of the felix houphouët- boigny university Pharmacology of Natural Substances, 2019, 168p.

[10] Onsiyor E. J., Akaffou N. A., Zahoui O. S. and Traore F. , Antidiabetic effects of aqueous extract of Ageratum conyzoides (Asteraceae) in rats rendered diabetic by partial pancreatectomy and evaluation of their

hematological parameters Int. J. Biol. Chem. Sci. , 2019, 13(3): 1621-1628, ISSN 1997-342X (Online), ISSN 1991-8631 (Print).

[11] Diaby Vandjiguiba, Yapo Adou Francis, Adon Arsène Mousan, Dosso Mireille, Djama Allico Joseph., Renal, Hepatic and Splenic Biotoxicity of Cadmium Sulphate in theWistar Rats. International Journal of Environmental Science and Toxicology Research, 2016 ,4(6):103-110.

[12] Moussa M.A., Biological and physiological studies on the effect of the gramoxon and stomp herbicides on Nile tilapia (Oreochromis niloticus). In Faculty of Science Zoology Department. Cairo University, 1999, p. 200.

[13] Khaled S. A. and Houda A., Effect of cadmium in drinking water on growth, some haematological and biochemical parameters of chicken. European Journal of Experimental Biology, 2013, 3(5):287-291.

[14] Wagner A., The role of the laboratory in the exploration of iron metabolism, in Revue de l'ACOMEN, 2000, pp 23-51.

[15] Ayed A., Classification of Anemias. In Anemias. Edited by S.T.d.B. Clinique, Centre d'Expertise de Medecine Aeronautique de Tunis, 2015, p. 25.

[16] Mukinda J.T. et Syce J.A., Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents, J Ethnopharmacol, 2007 : 112 (1):138-44.

[17] Olson H.G., Betton D., Robinson K., Thomas A., Monro G., Kolaja P., Lilly J., Sanders G., Sipes W., Bracken M., Dorato K., Van Deun P., Smith B. & Heller A., Concordance of the toxicity of pharmaceuticals in humans and in animals. Regular Toxicology Pharmacology, 2000, 32(1) : 56-67.

[18] Guillouzo A., Clerc C., Malledant Y., Chesne C., Ratanasavanh D., gugen-guillouzo C. Models for the study of hepatic cytoprotection, 1989.

[19] Berroukche A., Slimani M., Kahloula K., Kafi H. and Cheikh A., Evaluation of the activity of cadmium, in the presence of zinc, on the structures of flow regulating tissues in the Wistar Int. rat. J. Biol. Chem. Sci, 2014, 8(4): 1796-1807.

[20] Novikoff A.B., Intracellular organelles and lipoprotein metabolism in normal and fatty livers. In The liver Biology and Pathobiology, Arias I.M., Popper H., Shachter D., Shafritz D.A. (Eds.), Raven Press N.Y., 1982 , 143-167.

[21] Hinton D.E. and Lauren D.J., Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In Biomarkers of environmental contamination, MCCARTHY J.F., SHUGART L.R. (eds.), Lewis Publishers, CRC Press, Boca Raton USA, 1990 , 17-57.

[22] Martin E., Feldmann G., Histopathology of the liver and biliary tract in adults and children. Ed. Masson, 1983, 357 p.

[23] Fortin D., Lo M., Maynart G., Plantes médicinales du Sahel - Ed. CECIIENDA 300 pges. Gastroenterol. Clin. Biol. -il, 1990, pp. 725-730.

[24] Randa, A. Hassan; Dawlat M. Amin; Nariman A. Rahmy; Hatem, M.E. and Dessouky, M.I. , Clinicopathological, histopathological and immunolog1ical studies on animals exposed to lead and cadmium under experimental conditions. New York Science Journal, 2012, 5(12):120-136.

[25] Kaplan M., İrfan H. A., Nurettin A., Tevfik A.z, Fulya O. P. G. S., Burcu T., Osman İ., The effect of melatonin on cadmium-induced renal injury in chronically exposed rats. Turkish Journal of Urology, 2009.

[26] Moussavou M. C. F., Study of cadmium accumulation mechanisms in Arabidopsis thaliana (ecotype Wassilewskija) and in a hybrid larch (Larix xeurolepis) by molecular and developmental approaches. University Of Limoges. Environmental Sciences / Biology - Science - Health. In Biology - Science - Healthspecialty: Environmental Biology, 2010, 251 p

[27] Organisation for Economic Co-operation and Development (OECD) (1998). Series on the principles of good laboratory practice and verification of compliance with these principles. ENV/MC/CHEM (98). 17 : 22-23.